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Sex reversal, mortality rate and growth performance of platy *Xiphophorus variatus* (Poeciliidae) treated by methyltestosterone

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Abstract. The red platy *Xiphophorus variatus* Meek, 1904 is a commercially important ornamental species. To produce mono-sex population, the effects of different dose rates of synthetic androgen 17-alpha methyltestosterone (MT) i.e. 10, 20, 50, 80, 100 and 200 mg of MT kg^{-1} of feed on sex reversal, mortality rate and growth performance were determined. MT was administrated orally by using pellet dry starter and Ethanol Alcohol, diet to newborn fry platy for 40 days in glass aquaria. The fries were also kept for twelve weeks after treatment to monitor their growth performance; at the end of the experiment the sex ratio was determined by secondary sexual character and macroscopic examination of gonads. Growth performance was monitored by recording the morphometric characteristics. Wet body weight and total length of fish on 120 day-old platy were measured. The results revealed that ≥20 mg MT receiving treatments showed a significantly higher male proportion than the other treatment and the control experiments. Dose rate of 50 mg MT kg^{-1} of feed resulted in maximum male population with 19.17±2.88% loss of fish. While 200 mg MT kg^{-1} of feed showed 35.83±4.31% mortality, it is concluded that high doses of the MT have negative effect on survival rate. The maximum growth in mean body weight i.e. 1974±219 mg and length i.e. 49.48±2.06 mm and specific growth rate were observed in the MT 10 mg kg^{-1} treated groups than the other levels of MT treated groups, and hence 10 mg kg^{-1} is considered as optimum doses for attaining higher growth rate.
Key Words: aquarium fish, Poeciliidae, dose rate, masculinization, alcohol dry method.

Introduction. Studies on the application of steroid hormones in fish have drawn the attention of aquaculturists and researchers to control unwanted reproduction and divert energy for somatic growth in the economically important edible fishes (Piferrer & Lim 1997; Mousavi-Sabet 2011; Mousavi-Sabet et al 2012; Mousavi-Sabet & Ghasemnejad 2013). Also, many species of ornamental fishes, 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 swordtail (*Xiphophorus hellerii*), guppy (*Poecilia reticulata*), balloon molly (*P. latipinna*), sailfin molly (*P. velifera*), and platy (*Xiphophorus variatus*), as well as the egg-layers such as dwarf gourami (*Colisa lalia*), Siamese fighting fish (*Betta splendens*), rosy barb (*Barbus conchonius*), convict cichlid (*Cichlasoma nigrofasciatum*), and red Australian rainbow (*Glossolepis incisus*). The technique of sex manipulation by hormone treatment is also of interest to the aquarists as the price of some species of aquarium fish depend on their sex. Males of most of the ornamental fishes are colorful and have a higher commercial value than females. In Poeciliidae, males are more attractive than females due to their colorful body. In Anabantidae, the Siamese fighting fish male has attractive colour and large fins. 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, 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 in breeding 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 populations, because the males grown 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. The most commonly employed technique for mono-sex production is administration of steroids to undifferentiated fish through diets. 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).

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). One of the most important species in livebearers ornamental fish culture is the platy. In this study the platy 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 platy.

Material and Method. The sex reversal of newborn fry platy was achieved by oral administration of 17-alpha methyltestosterone through feed. A stock of platy, 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±1°C) and exposed to a 12 (light):12 (dark) photoperiod, where oxygen concentration was kept above 5.5 mg L^{-1} by continues aeration (Mousavi-Sabet et al 2012). The experimental stocking density was adjusted to eight fish per aquaria at a sexual ratio of two male and six females (Garcia-Ulloa & Garcia-Olea 2004; Mousavi-Sabet et al 2012; Mousavi-Sabet & Ghasemnejad 2013). 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 (Mousavi-Sabet et al 2012). All of the platy fries used in these treatment studies were obtained from these broodstocks. In order to prevent fries from being eaten by their parents, the ripe females were kept in plastic nets (Mousavi-Sabet et al 2012; Mousavi-Sabet & Ghasemnejad 2013). 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, 10, 20, 50, 80, 100 and 200 mg of MT kg^{-1} of feed

(each in three replicates), were set out, MT was added to diet by Alcohol (Ethanol 96°) dry method (Elmdoust 2003; Mousavi-Sabet 2011; Mousavi-Sabet et al 2012), 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, Niruza brand) six times per day, for 40 days (10% body weight [BW]). The important water quality parameters were fixed and recorded, e.g.: temperature (26±1°C), dissolved oxygen (5.7±0.4 mg L^{-1}), hardness (185±10 mg L^{-1}) and pH (7.7±0.5).

The study was done in Bahar ornamental fish farm in Tehran, from February to March 2014. The important growth parameters such as BW and total length were recorded at 120 day-old platies (80 days after the end of hormonal treatment). Specific growth rate (SGR) were calculated by SGR = 100 (lnW1 – lnW0) $(t)^{-1}$, where W0 and W1 were wet body weight of fish at the start and end of the experiment (Mousavi-Sabet et al 2011). Differences among the groups in terms of sex ratio of the offspring were determined on the basis of secondary sex character and macroscopic gonad examination. The secondary sex character used included: males have a developed gonopodium, a specialized reproductive fin found only in male fish (Piferrer & Lim 1997; Amiri-Moghaddam et al 2010). Differences in mortality and differences in body weight and length between groups were tested by one-way analysis of variance by ranks (SPSS ver. 16.0) followed by the Duncan non-parametric multiple comparison procedure.

Results and Discussion. Obtained results showed that each hormone treated group (except 10mg MT kg^{-1} feed) gave a mean male/female ratio that deviated significantly from the normal 1:1 ratio (Table 1), while the control groups and the 10 mg MT kg^{-1} feed treatment showed normal 1:1 ratio. Four groups (50, 80, 100 and 200 mg MT kg^{-1} feed) of the experimental trial gave almost all male populations. Also, we achieved 72.22% masculinization in platy by feeding with 20 mg MT kg^{-1} feed for 40 days. The sex ratio observed in 10 mg MT kg^{-1} feed treatment groups was 56:57 (male:female), while in the 20 mg MT kg^{-1} feed treatment groups was 78:30 (male:female), and in the 50 mg MTkg^{-1} feed treatment groups was 92:09 (male:female). These results were interpreted as an indication that increasing concentrations of MT caused an increasing in the number of males.

Table 1

Effects of treatment with MT on sex ratio in *Xiphophorus variatus*

Dosage MT (mg kg^{-1} feed)	*Treatment duration (days)*	*Sex distributions (male:female; M:F)*	*Sex ratio (%) (M:F)*
0 (Control)	40	59:56 (*n* = 115)	51.30:48.70
0 (Ethanol group)	40	55:58 (*n* = 113)	48.67:51.33
10	40	56:57 (*n* = 113)	49.56:50.44
20	40	78:30 (*n* = 108)	72.22:27.88
50	40	92:09 (*n* = 101)	91.09:08.91
80	40	94:03 (*n* = 97)	96.91:03.09
100	40	96:00 (*n* = 96)	100.00:0.00
200	40	77:00 (*n* = 77)	100.00:0.00

The goal of the present study was to find an optimum dose of MT for masculinization and improving the growth performance in platy, because it is one of the most important species in livebearer ornamental fish culture. In *Xiphophorus* genus, 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; Amiri-Moghaddam et al 2010; Mousavi-Sabet et al 2012; Basavaraja et al 2014) succeeded in inducing masculinization of livebearers 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 platy. However, the present results are consistent with reported results from studies with such fish species as *Sciaenochromis ahli*, *Cyprinus carpio*, *Cichlasoma nigrofasciatum*, *Xiphophorus hellerii* and *Poecilia reticulata*, all of which were treated with synthetic hormone (Farahmand

1993; George & Pandian 1996; Elmdoust 2003; Mag & Bud 2006; Mousavi-Sabet 2011; Mousavi-Sabet et al 2012; Mousavi-Sabet & Ghasemnejad 2013).

Mousavi-Sabet et al (2012) successfully masculinized the *P. reticulata* by 200 mg MTkg^{-1} of feed in 40 days. Mousavi-Sabet & Ghasemnejad (2013) made a mono-sex population of swordtail *X. hellerii* by 40 mg MT kg^{-1} of feed in 40 days. George & Pandian (1996) obtained 82% male population in *P. reticulata* at dose rate of 200 mg MT kg^{-1} of feed in 20 days and Elmdoust (2003) showed a successful masculinization in *Sciaenochromis ahli* (Cichlidae) with 60 mg MT kg^{-1} of feed in 30 days. Mag & Bud (2006) showed that 200 mg 17-alphamethyltestosterone kg^{-1} food administered 5-24 days prior to parturition and, as well, 150 mg 17 alpha methyltestosterone kg^{-1} food can be used in production of all-male guppy stocks. Mousavi-Sabet (2011) obtained 95.49% male population in *C. nigrofasciatum* by using 100 mg MT kg^{-1} 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^{-1} of diet. Other authors have used the higher dose rates to achieve sex reversal. In *Oreochromis* sp., Romerio et al (2000) obtained 98% male population at dose rate of 60 mg MT kg^{-1} 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^{-1} 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^{-1} of feed. Other results showed the over dose such as more than 100 mg MT kg^{-1} 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 2002) and for black crappie, *Pomoxis nigromaculatus* (Al-Ablani & Phelps 1997). Also lower doses of the hormone (1-4 mg kg^{-1}) are used to investigate the aggressive behaviors in *Betta splendens* (Forsatkar et al 2013). However, this dose is lower than that used for the induction of sex reversal in *C. carpio* (100 mg of MT kg^{-1} feed; Gomelsky et al 1994), but higher than that used in European seabass, *Dicentrarchus labrax* (0.5-5 mg 17α-methyl-dehydrotestosterone kg^{-1} feed; Chatain et al 1999) or the euryhaline tilapia, *O. mossambicus* (10 mg of MT kg^{-1} feed; Ron et al 1995).

Table 2 shows the survival and growth rates of the both control groups and MT-treated platy in terms of total body length and body weight. Significantly higher growth rate was observed in lowest concentration treated fish (10 mg MT kg^{-1} feed) than other treatments and control groups. When considering mortality, no significant different was observed among control group, ethanol group and dose 10 mg, but mortality rates in doses 20, 50, 80, 100 and 200 mg MT/kg feed were significantly different among these treatments and significantly higher than the other groups. Control, ethanol group, and dose rates of 10 mg MT kg^{-1} feed exhibited 4.17±2.04, 5.83±1.79 and 5.83±1.91 percent in mortality rate, respectively. Also, in dose rates, 20 and which were more than 20 mg hormone kg^{-1} feed, mortality rate were significantly higher than others. These results were interpreted as an indication that increasing concentrations of MT caused an increasing in the mortality rate in platy.

Table 2

Effects of treatment by MT on the survival rate and growth indices (mean ± SE) in *Xiphophorus variatus*

Dosage MT (mg kg^{-1} feed)	*Survival rate (%)*	*Mortality rate (%)*	*Body weight (mg)*	*Total length (mm)*	*SGR (%)*
0 (Control)	95.83±2.04	4.17±2.04	1750±108	43.09±2.45	3.73±0.11
0 (Ethanol group)	94.17±1.79	5.83±1.79	1742±144	43.52±3.11	3.72±0.12
10	94.17±1.91	5.83±1.91	1974±219	49.48±2.06	3.83±0.17
20	90.00±2.12	10.00±2.12	1889±192	46.77±2.92	3.79±0.14
50	84.17±2.68	15.83±2.68	1711±135	42.90±2.83	3.70±0.10
80	80.83±2.88	19.17±2.88	1759±162	43.34±2.55	3.73±0.13
100	81.67±3.15	18.33±3.15	1793±180	43.86±3.18	3.75±0.16
200	64.17±4.31	35.83±4.31	1805±176	45.19±3.43	3.75±0.19

At the end of the experiment (120th day), total body length and weight were significantly increased in 10 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 platy (especially when it used 80 and more than 80 mg kg^{-1} feed), but it has the ability to increase total body weight and length at the low tested concentrations.

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), Mousavi-Sabet (2011), Mousavi-Sabet et al (2012), and Mousavi-Sabet & Ghasemnejad (2013), in present study, different dose rates of MT significantly effected on the growth of platy, and lower (10 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^{-1} feed and 10.9 g, by dose 50 mg MT kg^{-1} 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 (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, *Pomoxis nigromaculatus* and largemouth bass, *Micropterus salmoides* females grow faster and larger than males (Arslan 2002).

Conclusions. As conclusion in the present study and about the platy masculinization, best selected dose rate which resulted in maximum male population with the least loss of fish was the 50-80 mg MT kg^{-1} of feed. In addition, the dose 10 mg MT kg^{-1} of feed showed the best growth performance in the fish. Based on previous reports, the effective doses of 17a-methyltestosterone in some livebearers (e.g. *P. reticulata*) were higher compared to egg-layer fish species (e.g. Cichlids). The obtained information provides a useful method for sex reversal with lower mortality and commercial scale production of platy by using 17a-methyltestosterone.

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