Masculinization, mortality and growth rates of swordtail *Xiphophorus hellerii* (Poeciliidae) affected by methyltestosterone

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**Abstract.** The red swordtail *Xiphophorus hellerii* Heckel, 1848 is a commercially important ornamental species. Since male swordtail has more commercial value than female, the effects of different dose rates of synthetic androgen 17-alpha methyltestosterone (MT) i.e. 5, 10, 20, 40, 80 and 200 mg of MT kg\(^{-1}\) of feed on masculinizing, mortality rate and growth performance were determined. MT was administrated orally by using pellet dry starter and Ethanol, diet to newborn fry swordtail 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 sex characteristics, specifically the growth of the sword extension. Growth performance was monitored by recording the morphometric characteristics. Wet body weight and total length of fish on 120 day-old swordtails were measured. The results revealed that ≥10 mg MT receiving treatments showed a significantly higher male proportion than the other treatment and the control experiments. Dose rate of 40 mg MT/kg of feed resulted in maximum male population with 14.17±3.97% loss of fish. While 200 mg MT/kg of feed showed 41.67±5.15% mortality, it is concluded that high doses of the MT have negative effect on survival rate. The maximum growth of males in mean body weight i.e. 2192±226mg and length i.e. 51.06±2.11mm and specific growth rate were observed in the MT 5 mg kg\(^{-1}\) treated groups than the other levels of MT treated groups, and hence 5-10 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). 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 helleri*, 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 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 colourful and have a higher commercial value than females. In Poeciliidae, male red swordtail *Xiphophorus helleri* is more attractive than female due to their long sword behind the caudal fin on the ventral side. In Anabantidae, the siamese fighting fish, *Betta splendens* 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).
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 swordtail, 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 populations, 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. The most commonly employed technique for monosex 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).

One of the most important species in ornamental fish culture is the swordtail. 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 swordtail 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 swordtail.

Materials and Methods. The sex reversal of newborn fry swordtail was achieved by oral administration of 17-alpha methyltestosterone through feed. A stock of X. hellerii, 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 by continuous aeration (Mousavi-Sabet et al 2012). The experimental stocking density was adjusted to eight fish per aquaria at a sexual ratio of two males and six females (Garcia-Ulloa & Garcia-Olea 2004; Mousavi-Sabet et al 2012). 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 swordtail fries used in these treatment studies was 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). 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, 5, 10, 20, 40, 80 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 et al. 2012), and each trial was stocked with 40 fish. The fish 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), hardness (185±10 mg/L) and pH (7.7±0.5). The important growth parameters such as BW and total length were recorded at 120 day-old swordtails (80 days after the end of hormonal treatment). Specific growth rate (SGR) were calculated by $SGR = 100 \left( \frac{ln W1 - ln W0}{t} \right)^{-1}$, where $W0$ and $W1$ were wet body weight of fish at the start and end of the experiment (Mousavi-Sabet et al. 2011). Differences between groups in terms of sex ratio of the offspring were determined on the basis of secondary sex characteristics (specifically the growth of the sword extension). The secondary sex characteristics used included: males have a developed gonopodium, a specialized reproductive fin found only in male fish; growth of the sword extension in caudal fin of males (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 for Windows) followed by the Duncan non-parametric multiple comparison procedure.

**Results and Discussion.** Obtained results showed that each hormone treated group (except 5mg MT/kg feed) gave a mean male/female ratio that deviated significantly from the normal 1:1 ratio (Table 1), while the control groups and the 5mg MT/kg feed treatment showed normal 1:1 ratio. Three groups (40, 80 and 200 mg MT/kg feed) of the experimental trial gave all male populations. Also, we achieved 91.59% masculinization in *X. hellerii* by feeding with 20 mg MT/kg feed for 40 days. The sex ratio observed in 5mg MT/kg feed treatment groups was 55:59 (male:female), while in the 10mg MT/kg feed treatment groups was 76:29 (male:female), and in the 20mg MT/kg feed treatment groups was 98:09 (male:female). These results were interpreted as an indication that increasing concentrations of MT caused an increasing in the number of males.

<table>
<thead>
<tr>
<th>Dosage MT (mg/kg feed)</th>
<th>Treatment duration (day)</th>
<th>Sex distributions (male:female; M:F)</th>
<th>Sex ratio (%) (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>40</td>
<td>46:63 (n =109)</td>
<td>42.20:57.80</td>
</tr>
<tr>
<td>0 (Ethanol group)</td>
<td>40</td>
<td>51:61 (n =112)</td>
<td>45.54:54.46</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>55:59 (n =114)</td>
<td>48.25:51.75</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>76:29 (n =105)</td>
<td>72.38:27.62</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>98:09 (n =107)</td>
<td>91.59:08.41</td>
</tr>
<tr>
<td>40</td>
<td>40</td>
<td>102:1 (n =103)</td>
<td>99.03:00.97</td>
</tr>
<tr>
<td>80</td>
<td>40</td>
<td>94:00 (n =94)</td>
<td>100.00:00.00</td>
</tr>
<tr>
<td>200</td>
<td>40</td>
<td>70:00 (n =70)</td>
<td>100.00:00.00</td>
</tr>
</tbody>
</table>

Table 1

The goal of the present study was to find an optimum dose of MT for masculinization and improving the growth performance in *X. hellerii*. One of the most important species in ornamental fish culture is the swordtail. 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; Amiri-Moghaddam et al. 2010; Mousavi-Sabet et al. 2012) 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 *X. hellerii*. However, the present results are consistent with reported results from studies with such fish species as *Sciaenochromis ahli*, *Cyprinus carpio*, *Cichlasoma nigrofasciatum* 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 et al (2012) successfully masculinized the guppy by 200 mg MT/kg of feed in 40 days. 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-alphamethyltestosterone/kg food administered 5-24 days prior to parturition and, as well, 150 mg 17 alpha methyltestosterone/kg food can be used 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 rates 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 17α-methyl-dehydrotestosterone /kg feed; Chatain et al 1999) or the euryhaline tilapia, Oreochromis mossambicus (10 mg of MT /kg feed; Ron et al 1995).

Table 2 shows the survival and growth rates of the both control groups and MT-treated X. hellerii in terms of total body length and body weight. Significantly higher growth rate was observed in lowest concentration treated fish (5mg MT/kg feed) than other treatments and control groups. When considering mortality, no significant different was observed among control group, ethanol group and dose 5mg, but mortality rates in dose 10, 20, 60, 80 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 5 mg MT/kg feed exhibited 9.17±2.11, 6.67±1.64 and 5.00±2.15 percent in mortality rate, respectively. Also, in dose rates, 10 and which were more than 10 mg hormone/kg 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 swordtail.

Table 2

<table>
<thead>
<tr>
<th>Dosage MT (mg/kg feed)</th>
<th>Survival rate (%)</th>
<th>Mortality rate (%)</th>
<th>Body weight (mg)</th>
<th>Total length (mm)</th>
<th>SGR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>90.83±2.11</td>
<td>9.17±2.11</td>
<td>1816±144</td>
<td>47.31±1.15</td>
<td>4.52±0.10</td>
</tr>
<tr>
<td>0 (Ethanol group)</td>
<td>93.33±1.64</td>
<td>6.67±1.64</td>
<td>1871±195</td>
<td>46.77±1.09</td>
<td>4.52±0.16</td>
</tr>
<tr>
<td>5</td>
<td>95.00±2.15</td>
<td>5.00±2.15</td>
<td>2192±226</td>
<td>51.06±2.11</td>
<td>4.73±0.19</td>
</tr>
<tr>
<td>10</td>
<td>87.50±1.48</td>
<td>12.50±1.48</td>
<td>2155±203</td>
<td>50.84±1.63</td>
<td>4.68±0.17</td>
</tr>
<tr>
<td>20</td>
<td>89.17±3.08</td>
<td>10.83±3.08</td>
<td>1915±112</td>
<td>47.65±1.75</td>
<td>4.55±0.09</td>
</tr>
<tr>
<td>40</td>
<td>85.83±3.97</td>
<td>14.17±3.97</td>
<td>1892±157</td>
<td>46.29±2.14</td>
<td>4.53±0.11</td>
</tr>
<tr>
<td>80</td>
<td>78.33±4.41</td>
<td>21.67±4.41</td>
<td>1935±178</td>
<td>46.71±1.90</td>
<td>4.59±0.15</td>
</tr>
<tr>
<td>200</td>
<td>58.33±5.15</td>
<td>41.67±5.15</td>
<td>1813±188</td>
<td>46.03±2.04</td>
<td>4.52±0.16</td>
</tr>
</tbody>
</table>
At the end of the experiment (120th day), total body length and weight were significantly increased in 5-10 mg MT treated groups compared to other treatments and the controls (Table 2). Also this indicates that MT has a negative effect on the survival rate of X. helleri (especially when it used 80 and more than 80 mg/kg 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) and Mousavi-Sabet et al (2012), in present study, different dose rates of MT significantly effected on the growth of X. helleri, and lower (5-10 mg) MT treatments 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).

Conclusion. As conclusion in present study and about the X. helleri masculinization, best selected dose rate which resulted in maximum male population with the least loss of fish was the 20-40 mg MT/kg of feed. In addition, the dose 5 mg MT/kg of feed showed the best growth performance in the fish. Based on previous reports, the effective dose of 17a-methyltestosterone in some livebearers (e.g. Poecilia reticulata) was higher compared to egg-layer fish species (e.g. Cichlids), while about the studied livebearer species X. helleri it is not true. The obtained information provides a useful method for sex reversal with lower mortality and commercial scale production of swordtail by using 17a-methyltestosterone.

References


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