World Applied Sciences Journal 18 (7): 868-872, 2012
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2012.18.07.6496

Esffect of Different Administration of 17α-Estradiol on Gonadal Sex Differentiation in *Astatotilapia latifasciata*

[1]*Mehdi Yousefian,* [1]*Atena Osia Laghab,* [1]*Eiman ahmadi,*
[2]*Mehrdad Irani and* [3]*Changiz Makhdoomi*

[1]Department of Fishery, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran
[2]Department of Animal Sciences, Islamic Azad University, Qaemshahr branch, Iran
[3]Fish Restocking Center of Shahid Rajaei-Sari, Iran

Abstract: The effect of estrogen on gonadal sex differentiation in *Astatotilapia latifasciata* was examined by daily oral administration of 17α-estradiol at a dosage of 750 mg/kg diet, artemia enrichment and submerges of larvae at a dosage of 200 mg/l. When estrogen were applied to larvae for 30 days from 7 days after hatching a complete sex reversal from male to female was obtained. In the affected ovaries, oocytes developed quite similarly to those in controls. From the results, it was concluded that 17α-estradiol is capable to induce a complete masculinization of genetic females at a low dosage level (750 mg/kg) and all the three techniques are proper in sex reversal inducing in *Astatotilapia latifasciata.*

Key word: Masculinization · Aquarium Fish · Estrogen · Sex Reversal

INTRODUCTION

In recent years ornamental fish especially a family of cichlid such as *Astatotilapia latifasciata* has attained great economic importance, as they might be excellent fish for rearing in aquaria. They can feed easily, growth rapidly, propagate in freshwater and brackish water. The cichlid tolerates high salinity and different environmental conditions. The application of sex reversal technology in ornamental fish culture, previously was reviewed by Piferrer and Lim [1].

Among cichlid fish, the *Astatotilapia latifasciata* become mature and start to breed when they are still very small. The male of this fish are more beautiful than female.

The class of cichlid fishes exhibits a large variety of adaptation responses to match the vast array of existing ecological habitats. One of the most intriguing phenomena is probably the large number of reproductive strategies developed by these species. Several factors including season, temperature, social circumstance, age, genetics [2], physiology and biochemical status of fish [3] and other variables related to reproductive capacity and opportunity. Environmental factors also influencing developing of gonad and reproduction behavior [4].

It is now well established that phenotypic sex in fish may depend on external factors, although the effect of such factors will differ from one species to another [5, 6]. This plasticity of gonad development in fish, which contrasts with the more stable patterns found in higher vertebrates, has given rise to a number of exciting questions concerning both its adaptive significance [7, 8] and the underlying genetic and physiological regulations involved. In practice with regardless of this, in order to make sex reverse in fish, sex hormone is used. Generally, the synthetic hormones are administered for only 30-45 days period, from the fish larvae hatched or take their first feed and hence hormonal residues will have disappeared from the flesh of the fish long before they are mature. In using of the hormones there is not a fixed formula for administration of hormone and it is differ from one fish to another even at the same species. In fact, the levels of two hormones, estradiol-17β and 17α-methyltestosterone, which are the most commonly used for sex differentiation or/and reversal, are un-measurable quantities [9]. Therefore it is necessary to have a test for each fish species that we would like to make sex modification or reversal. It should be noted, that the pattern of gonad development [10] and sex differentiation and also the way of gonad formation is highly variable

Corresponding Author: Mahdi Yousefian, Department of Fishery, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran.

from one species to the next, but within a given species, the growth rate and water temperature is an important factor.

Several comprehensive reviews dealing with the morphological and histological description of sex differentiation and sex inversion [11] have already been published. Feminization of catfish can be produced by direct synthetic hormonal treatment that is efficient and straightforward [12]. In blue banded goby, sex reversal in pairs of *Lythrypnus dalli* behavioral and morphological changes have been studied [13], sex change in the protandrous black porgy, *Acanthopagrus schlegeli* have been done [14].

Besides of morphological and histological description of sex differentiation, studies of the molecular mechanisms for sex differentiation in fishes have been focused but only on a small number of fish species (medaka, trout and tilapia), in which genetic all-males or all-females populations (respectively produced from YY males or XX females) are available. These studies, mainly based on candidate gene approaches, have clearly demonstrated the key role of steroids and particularly estrogens in female differentiation [15, 16].

The purpose of the present investigation was to produce all female *Astatotilapia latifasciata* in order to stock aquarium fish with mono-sex. This was done by means of sex hormone 17α-estradiol treatment during the stage of gonad differentiation.

MATERIALS AND METHODS

The fry of *Astatotilapia latifasciata* were collected, 4 days after hatching, from propagation center of ornamental fish farm of Sari. They were fed on a commercial dry food for rainbow trout culture until 7 days at the start of each experiment. They were separated into one control and three experimental groups of 75 fry each experiment carried out in 3 replicates. The larvae of each experiment were kept separately in a glass aquarium with 20 liters of well-aerated water at 24±0.5°C. The fish group of the experiment 1 was administered orally with 17α-estradiol at a dosage of 750 mg/kg diet for periods of one month from 7 days after hatching. For preparing the hormone diet, 17α-estradiol was dissolved in absolute alcohol and was added to the food. The fish group of the second experiment was administered orally with *Artemia salina* enriched with the same dosage of hormone and at the same time after hatching. The diet was given to the fish three times a day. The fish group of the third experiment was submerged to water bath hormone at a dosage of 200 mg in 1 liter of water for 2 hours daily.

At the end of the treatment 20 fish of treated and 20 fish of control They were separated into one control and three experimental groups of 75 fry each were killed and the sex were analyzed histologicaly. For histological observations, the gonads of experimental and control fish were fixed in Bonin's fluid. The gonads embedded in paraffin were cut serially at 5 μm in thickness and stained with hematoxylin and eosin. The sex of fish were confirmed after two months of age, when male are recognized by their yellow-reddish color at abdominal sides and the female are detected by week pink in lower sides of body.

RESULTS

The sex differentiation in *Astatotilapia latifasciata* histologically recognizable by 30 days after hatching. The ovarian differentiation is characterized by the development of some germ cells into the formation of oocytes (Fig. 1), while the testicular differentiation is marked by the appearance of testis (Fig. 2). In order to achieve a complete feminization of genetic males by exogenous estrogen, the treatment should be carried out to cover this particular stage of sex differentiation extending up to 30 days after hatching.

In experiment one, the diet containing 17α-estradiol at the dose of 750 mg/kg of diet was given to 75 juvenile fish during the period from 7 to 30 days after hatching. The sex distribution of these fish at the end of the estrogen administration, the gonads of the treated fish could be divided into two types in respect to their histological aspects: those of one type had many different stages of oocytes and those of another possessed only a fewer oocytes, mainly at stage one and two. The former was seen to be in an initial phase of ovarian differentiation and the formation of the ovarian cavity was not complete. It seems some characters of male gonad phenotype will remain in sex reverse fish. In experiment two and experiment three submerging of larvae administration of estrogen by *Artemia salina*, the same results were obtained. Only in submerging techniques 2 percent male without any changes remained in treated group.

Besides histological testing, the effect of hormone was confirmed by the phenotype of female. The male *Astatotilapia latifasciata* showed yellow-reddish color at abdominal sides while the female are detected by week

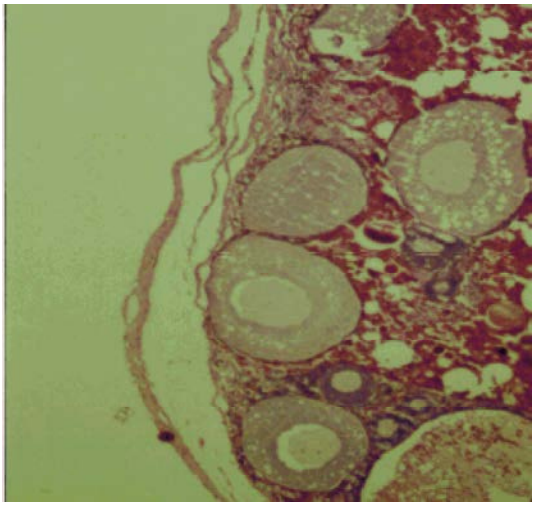

Fig. 1: Cross section of the treated ovary, 30 days after hatching (X 560).

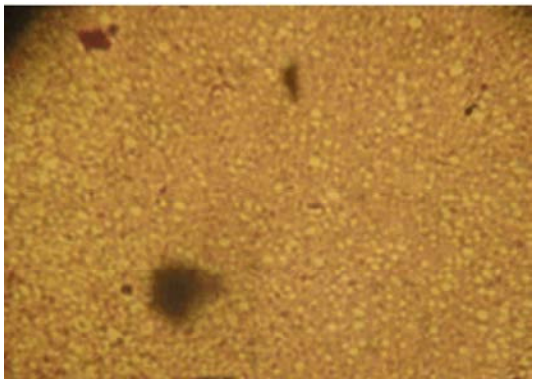

Fig. 2: Cross section through testis in control group, 30 days after hatching (X 560).

pink in that places. The treatment group and controls were kept up to 60 days when the male in control group displayed body coloration peculiar to mature males on their abdominal body sides. The gonads of these fish were distinctly the testes showing an active spermatogenesis and in females developed oocytes.

DISCUSION

The present study illustrated that 17α-estradiol administered to the fry of *Astatotilapia latifasciata* by different methods has an evident masculinizing potency when given at the dose of 750 mg/kg diet or 200 ml of hormone per one liter of water. Treating the fish with hormones has their best effect only if applied at proper time of gonad differentiations [17]. Therefore applying 17α-estradiol in the present study was done at proper time. Duration of administration also is very important. With respect of clear differences between male and female and observing not male phenotype after two month in treated group, it illustrated that 30 days duration for hormone therapy was effective in this fish species. Gonadal sex differentiation in both sexes of the cichlid becomes detectable morphologically to occur about 20 days after hatching [18]. Our results also confirmed by the earlier working with the fish of *Xiphorus helleri* with the same dosage and time of treatment gave 100 percent female [19]. By using lower dose however the duration should be longer. In the results obtained by Clemens and Inslee [20] who achieved a functional masculinization of genetic females of the cichlid *T. mossambica* by applying the androgen most effectively at 30 pg per g diet for 69 days following hatching.

Occasionally in monosex production some sex-reversed fish will back to its original genetic forms, but in the present study due to proper amount of dosage, duration and proper time of applying hormone, this phenomenon have not been observed. Considering the cichlid fish have a wide range of sex chromosomal system and environmental factors such as social status have influence in sex type formation, therefore *Astatotilapia latifasciata* may be have positive reaction to any hormonal treatment. The studies with an African cichlid fish, *Astatotilapia burtoni*, changes in both testosterone and 11-ketotestosterone (11-KT), an androgen specific to teleost fish, depend on male social status [21]. They characterize circulating plasma concentrations of testosterone and 11-KT in socially dominant (territorial) and socially subordinate (non-territorial) males. Territorial males have significantly higher circulating levels of both forms of androgen, which is another defining difference between dominant and subordinate males in this species. In tilapia, sex is determined by major genetic factors located on sex chromosomes, minor modifiers located on the autosomes, rearing temperature during the critical period of sex differentiation and by the interaction between these genetic and environmental factors [15]. The co-existence of major genetic factors on sex chromosomes similar to mammals as well as environmental influences, similar to what is found in amphibians and reptiles, makes the cichlid fish as a model to study sex differentiation. In rearing the *Astatotilapia latifasciata* aquarium with special temperature or special condition may have effect on sex reverse of this fish as well.

In the present study no number of fry died exceed than control. In the experiment of Jensen and Shelton [22], three naturally occurring estrogens, estriol, estrone and 17-Beta estradiol, were each administered at three concentrations for 3 or 5 week periods to fry of *Tilapia aurea*, 8-11 mm long, estrogen treatment neither affected survival nor altered growth of fries.

CONCLUSION

In conclusion, it may be said that there is a potential to change sex of *Astatotilapia latifasciata* with a dosage of 750 mg/kg of diet. Due to high successful feminization, we conclude that any adverse condition intrinsic, acquired or inherited, will tends to increase the capacity for hormone effect and cause in sex-reversal in this fish. Also, further investigations on higher or lower doses and/or treatment durations of hormones will conducted to determine the possible effects and to find the optimum concentration for producing all-female population of *Astatotilapia latifasciata* and other cichlid fish.

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