

Masculinization of Blue Hap (*Sciaenochromis ahli*) Treated with 17 α -methyltestosterone

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ABSTRACT

The objective of this study was to determine the efficacy of two procedures i.e. feeding and immersion followed by feeding, for masculinizing Blue Hap. Two experiments (experiments I and II) were conducted. In experiment I, feeding hormonal treatment was applied on post-yolk sac stage fry (10 days after fertilization at 27°C). Dosages of 30mg (group A) and 60mg (group B) of 17 α -methyltestosterone per kg of food were used during 60-day periods (40 fry per treatment). The control group i.e. group C, received no hormone. In experiment II, one day post hatching larvae were exposed to an immersion treatment in 17 α -methyltestosterone at 1000 μ g/l up to 2 h (16 larvae per treatment). Later, at the first day of post-yolk sac stage, the treatment was followed by oral procedure which was divided in 2 groups: group D and group E. In group D, 30 mg 17 α -Methyltestosterone per kg of food was used during a 60-day period. In group E, fry received 60 mg of hormone per kg of food during the same period. The control group i.e. group F, did not receive any hormone. Change in sex proportion within each experiment as well as between experiments was analyzed by chi-square test ($p < 0.05$). In experiment I, 60 mg feeding treatment significantly ($p < 0.05$) increased the proportion of the males (85.7%) in comparison to the control group (46.67%). Lower male proportion (60%), but still significant ($p < 0.05$), was evaluated in 30 mg feeding treatment. In experiment II, in both groups (D and E), although sex ratio was different from the theoretical 1:1 sex ratio, fish skewed toward sterility rather than masculinization because of high hormonal doses. In conclusion, this study confirms that it is possible to achieve high rates of hormonal masculinization in Blue hap.

Keywords: Masculinization, Blue hap, 17 α -methyltestosterone

INTRODUCTION

Blue hap (*Siaenochromis ahli*) is one of the mouth brooder cichlids (Cichlidae) which shows sex dimorphism at the time of maturation. Males have one to several yellow spots on their anal fin and metallic blue color while females are darker without any spots on their anal fin (Sandford and Gina, 2004). More bright and attractive color in males makes them more preferable than females for ornamental fish customers. Therefore the production of all male populations would be more profitable for ornamental fish culturists.

In the late 1930's and early 1940's, it was shown that gonadal sex of fish could be influenced by exogenous steroid hormones and it would be desirable to enhance the expression of sex associated with morphological, physiological, or etiological characteristics that could be advantageous under certain circumstances (Hunter and Donaldson, 1993; Yamazaki, 1983). This phenomenon is due to the biopotentiality of pre-meiotic germ cells in early gonadal development (Piferrer, 2001).

One of the common techniques for producing mono-sex populations is steroid induced sex inversion (Hunter and Donaldson,

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1993) in which androgens and estrogens are used as masculinizing and feminizing agents, respectively (Wasserman and Afonso, 2003).

Several methods of steroid administration have been used for sex reversal including injection, feeding and immersion of fry (Gale *et al.*, 1999). Oral and immersion treatments are more common (Pandian and Sheela, 1995; Diaz and Neira, 2005). Successful endocrine sex reversal requires that the steroid used mimics natural induction initiated by genetic sex-determining factors and is administered at a sufficient dose and duration during a critical period of gonadal differentiation, called labile period, and the steroids-induced development of gonadal sex does not spontaneously revert (Piferrer, 2001).

Sex reversal with synthetic androgens is nowadays one of the most frequently applied techniques to produce mono sex male populations in some species. This technique is efficient both at experimental and commercial scales (Guerrero, 1975; Rothbord and Yaron, 1987; Vera Cruz and Mair, 1994; Melard, 1995). The most widely used androgen for sex reversal in fish is a synthetic androgen, namely, 17 α -methyltestosterone (MT) (Pandian and Sheela, 1995).

Oral administration of 17 α -methyltestosterone has been effective in producing all male populations in tilapia (Wassermann and Afonso, 2003). To the best of our knowledge, there is no report in the literature about the masculinizing potency of MT in blue hap. The object of this study was to determine whether 17 α -methyltestosterone can be used to produce high percentage of male populations of blue hap to obtain more metallic blue coloration of the fish.

MATERIALS AND METHODS

Rearing Condition

This study was carried out at Behinehkaran Aquaculture Company in Karaj-Iran. Breeders were kept in 10000 liter thermo regulated tanks at 26 $\pm$ 2°C in recirculation system. The reproductive status was checked daily by looking for dilation of bucco-

pharyngeal cavity of females due to their mouth brooding behavior. Eggs, larvae and post yolk sac i.e. free swimming fry were removed by washing the mouth cavity and divided into different groups. Each group was reared in a 30 l tank at a temperature of 26 $\pm$ 2°C (thermo regulated recirculation system) during the treatments.

Preparation of Hormone Treated Diet

The 17 α -methyltestosterone used in this study was obtained from Abooreyhan Institute in Tehran, Iran, and is identified under number L00027306.

Hormone treated diet was prepared by dissolving 30mg and 60mg of 17 α -methyltestosterone in 500 ml of 95% ethanol per each kg of formulated diet (commercial Beh Parvar Rainbow trout food in Tehran, Iran with 45% protein). The hormone solutions were sprayed to the diet slowly and were mixed continuously. The control diet was prepared with the same method but without any hormone. All the hormone treated diets were prepared at once before starting the treatments.

Hormone Immersion

Stock solution of hormone was prepared by dissolving 30 mg of 17 α -methyltestosterone in 3 ml of 95% ethanol. The stock solution was added to 30 l tank (1000 μ g of hormone per 1 liter) with one day post hatch (DPH) larvae. After 2 hours larvae were transferred to clean water.

Experimental Designs

Two experiments were designed in this study (Table 1).

Experiment I

In experiment I, post yolk-sac stage fry (10 days after fertilization) was divided into two groups: A and B. Each group consisted of 40

Table 1. Experimental design including experiments I and II.

Experiment	Treated fish	Immersion duration (h)	Immersion solution concentration ($\mu\text{g l}^{-1}$)	Hormonal feeding treatment duration	Hormone dosage in food (mg kg^{-1})	Repetition	Group
I	Free swimming fry at their first day of exogenous feeding	-	-	60	30	2	A
					60	2	B
	Control Free swimming fry at their first day of exogenous feeding	-	-	-	0	2	C
II	1DHP larvae	2	1000	60	30	2	D
					60	2	E
	Control 1DHP larvae	-	0	-	0	2	F

fry and was reared in a 30 liter tank. The sex reversal treatment was applied on group A with concentrations of 30 mg of 17α -methyltestosterone per kg of food in a 60-day period. In group B, 60 mg of 17α -methyltestosterone per kg of food were used in the same period. A control group (group C) was fed simultaneously by the control food.

Experiment II

In the first part of experiment II (Table 1), one day post hatch larvae were immersed in a solution containing 17α -methyltestosterone at 1000 $\mu\text{g per l}$ concentration for two hours. Then immersed larvae were divided into two groups: D and E. At their first day of post yolk sac stage, these two groups were fed by 30mg and 60mg of 17α -methyltestosterone per kg of food, respectively. This oral administration continued for 60 days. The control group was immersed in the same alcohol solution concentration without any hormone and, then, was fed with the control food during the same period. (Table 1)

Sexing and Statistical Analysis

Sex ratio in the treated and the control groups were determined by two different

procedures: color observations and histology examinations.

At 70 DPH (10 days after finishing the treatments), a random sample of 15 fish was taken from each treatment for color observations, in which the fish with metallic coloration were considered as male and the fish with common blue coloration were considered as females. After color observations, sample fish were fixed in 10% formalin solution; then, tissues were processed and stained by Hematoxylin-Eosin to sex the fish. Changes in sex proportion within each experiment as well as between the experiments were analyzed by chi-square test ($p < 0.05$).

RESULTS

Sex ratio in the control groups from both experiments was not statistically different ($p < 0.05$) from the theoretical 50:50 sex ratio (Tables 2 and 3).

Experiment I

In experiment I, based on histological observations (Table 2), the sex ratio in the group B (60 mg of 17α -methyltestosterone per kg of food) skewed towards male

**Table 2.** Effects of different 17 α -methyltestosterone on sex proportion of Blue hap in experiment I.

Sex	Group A (30 mg of hormone kg ⁻¹ of food)		Group B (60 mg of hormone kg ⁻¹ of food)		Group C (control)	
	Repetition1	Repetition2	Repetition1	Repetition2	Repetition1	Repetition2
Sexed fish	15	15	15	15	15	15
Male (n)	9	10	12	13	7	8
Female (n)	2	2	1	1	8	7
Inter sex	4	3	1	0	0	0
Sterile	0	0	1	1	0	0
Metallic blue individuals	10	10	13	13	0	0
Male (%)	60	66.6	85.7	86.6	46.67	53.33
Survival (%)	97.5	100	95.0	97.5	97.5	95.0
Mean body size (cm)	3.54	3.52	3.90	3.92	3.10	3.12

(85.7%) and was significantly ($p < 0.05$) higher than the sex ratio in the control group (46.67%). Lower, but still significant ($p < 0.05$), male proportion (60%) was evaluated in group A (30 mg of 17 α -methyltestosterone per kg of food) (Figures 1, 2, and 3 and Table 2).

In experiment I, numbers and percentages of males evaluated in different groups by color observations were significant and a little higher than those evaluated by histology examinations. There was no distinguishable difference in color between the two sexes of the fish in the control group.

Experiment II

In experiment II, based on histological observations (Table 3), the sex ratio was different from the theoretical 1:1 sex ratio ($p < 0.05$), but the fish in both groups D and E were skewed toward sterility rather than masculinization. Male proportions in group D and E were 60% and 40%, respectively, while 33.33% of the fish in group D and 53.33% in group E were sterile.

In experiment II, numbers and percentages of males evaluated in different groups, by color observations were still significant (Table 3).

Table 3. Effects of different 17 α -methyltestosterone on sex proportion of Blue hap in experiment II.

Sex	Group D (immersion followed by 30 mg of hormone kg ⁻¹ of food)		Group E (immersion followed by 60 mg of hormone kg ⁻¹ of food)		Group F (Control)	
	Repetition1	Repetition2	Repetition1	Repetition2	Repetition1	Repetition2
Sexed fish	15	15	15	15	15	15
Male (n)	8	9	5	6	7	7
Female (n)	1	1	1	7	8	8
Inter sex	1	0	1	0	0	0
Sterile	5	5	8	8	0	0
Metallic blue individuals	11	13	10	11	0	0
Male (%)	53.33	60	33.33	40	46.6	46.6
Survival (%)	100	100	100	100	100	100
Mean body size (cm)	3.8	3.9	4.1	4.0	3.0	3.0

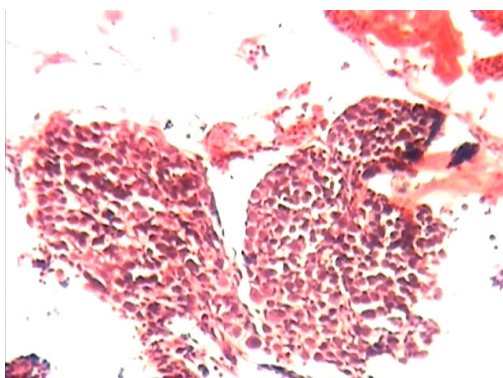


Figure 1. Testicular tissue of a male individual in experiment I, group B at 70 DHP.

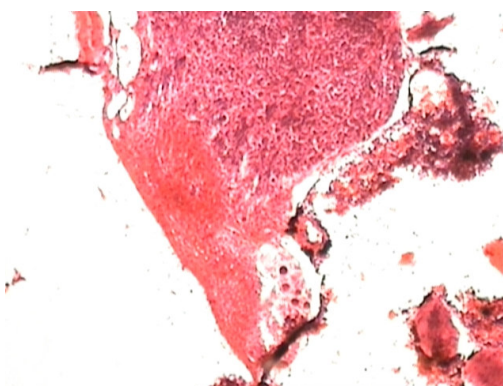


Figure 2. Gonadal tissue of an intersex individual in experiment I, group A at 70 DPH. Oögonia are obvious at the bottom of testicular tissue.

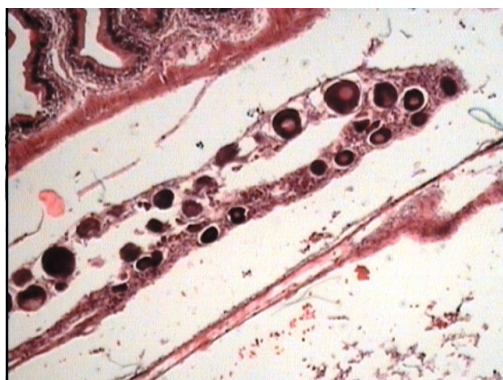


Figure3. Ovary of an individual from experiment I, group A at 70 DHP.

DISCUSSION

To the best of our knowledge, there are no records on sex reversal in Blue hap in the

literature. Therefore, studies on other cichlid species, especially Nile tilapia (*Oreochromis niloticus*), were used as a guide. In Cichlidae suitable 17α -methyltestosterone dose is up to 40 mg per kg of food (Pandian and Sheela, 1995). Thirty mg 17α -methyltestosterone per kg of food in a 40-day period can lead to masculinization in Nile tilapia (Jonson, *et al.*, 1983). In the current study, higher doses were used because of longer period of gonadal development and maturation in Blue hap in comparison to Nile tilapia. It is shown that androgen immersion treatments at 1800 $\mu\text{g/l}$ for four hours are effective in sex ratio, up to 14 days post hatch in Nile tilapia (Wassermann and Afonso, 2003). Exogenous steroid treatments can be more effective if they are used earlier than gonadal differentiation and treatment period can be shortened to two hours (Piferrer and Donaldson, 1992). Therefore, in the present study, the larvae were treated at one DPH about two hours and lower immersion hormonal solution concentration was used because immersion treatments were followed by oral hormone administrations.

In experiment I, all hormonal treatments were capable of masculinizing Blue hap. The higher masculinization rate was achieved by group B with higher hormonal dose (60 mg hormone per kg of food) in comparison to group A (30 mg hormone per kg of food). Besides, the most intersex individuals among all groups were in group A, which shows that, although the fish in this group skewed toward masculinization, better results can be obtained by using higher doses. On the other hand, it can be concluded from the results that 100% of male populations were not achieved in group B, because the labile period was not affected efficiently by the hormonal treatments.

In experiment II, lower male proportions were achieved and high proportions of sterile fish were observed. Since high doses of 17α -methyltestosterone can cause sterility in treated fish (Farahmand, 1995), the fish in experiment II skewed toward sterility rather than masculinization.



Considering that oral hormonal treatment doses were the same in both experiments, high sterility in experiment II in comparison with experiment I shows that initial hormonal immersion administration is responsible for high proportions of sterility in both treatments in experiment II. Accordingly, it can be concluded that initial hormonal administration is very efficient in sex reversal in Blue hap. Moreover, this high efficiency of hormonal immersion at one DPH shows that labile period is much closer to one DPH than 10 DPH. Therefore, by using hormonal immersion administration at one DPH, less oral hormonal administration is needed and high proportions of male and less sterile fish can be obtained.

Low mortality rates observed in this study can be due to natural resistance of Blue hap to stress of hormonal treatments, low density of the fish in the tanks, good rearing conditions during the experiments or a combination of these factors.

In salmon fishes, growth rate and testosterone levels of plasma rises during smoltification (Wedemeyer, 1996). No statistical test was used to analyze growth rates in different groups in this study but mean sizes of the treated fish was a little more than the control fish. This phenomenon can be due to metabolic effects of 17α -methyltestosterone.

Early maturing fishes do not usually grow to large sizes because producing gonadal products is very energy consuming (Bone *et al.*, 1996). Therefore, it can be concluded that 17α -methyltestosterone can increase growth at early stages of gonadal development. In this stage, gonads do not consume much energy in comparison with late stages and metabolic virtue of 17α -methyltestosterone can affect other organs in the body, but, at late stages of gonadal development, most of the gained energy is used to produce gonadal productions.

In the present study, developmental stages in the treated fish were not compared with those of the control groups, but, the treated fish had more expanded gonadal tissues.

Although in this study hormonal treatments were effective in producing high percentage of male fish, further studies are needed to determine the labile period in order to optimize hormonal treatments. Furthermore, 17α -methyltestosterone can be naturally converted to 17β estradiol (E_2) via aromatase (Devlin and Nagahama, 2002); the non-aromatizable synthetic androgen 17α -methyl dihydrotestosterone (MDHT) has recently become the preferred androgen for fish sex reversal (Henry *et al.*, 2003). A non-aromatizable androgen avoids paradoxical feminization that occurs when aromatizable androgens are used in high doses (Hunter and Donaldson, 1983; Piferrer *et al.*, 1993).

In conclusion, this study confirmed the possibility of achieving higher rates of hormonal masculinization in Blue hap.

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نر سازی در ماهی هاپ آبی (*Siaenochromis ahli*) با استفاده از هورمون $\alpha 17$ -متیل

تستوسترون

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چکیده

هدف از انجام این مطالعه بررسی اثر دو روش (غذا دهی و غذا دهی در پی غوطه وری) برای نر سازی در ماهی هاپ آبی بوده است. در این مطالعه دو آزمایش (I و II) طراحی شد. در آزمایش I، تیمار تغیر جنسیت روی بچه ماهیان نر (۱۰ روز پس از بارور شدن تخمها در ۲۷ درجه سانتیگراد) اعمال شد. برای این منظور دوز های ۳۰ (گروه A) و ۶۰ میلی گرم هورمون $\alpha 17$ -متیل تستوسترون به ازای هر کیلوگرم غذا (گروه B) در طول دوره ۶۰ روزه به کار گرفته شد. در هر تیمار ۴۰ بچه ماهی نر وجود



داشت. در آزمایش II، لاروهایی که یک روز از تفریخ آنها می گذشت در معرض تیمار غوطه وری با هورمون $\alpha 17$ -متیل تستوسترون با غلظت ۱۰۰۰ میکروگرم در هر لیتر به مدت دو ساعت (۱۶ لار و به ازای هر تیمار) قرار گرفتند سپس در اولین روز جذب کیسه زده، تیمار با روش غذا دهی هورمونی پیگیری شد. برای این منظور ماهیان غوطه ور شده به دو گروه (D و E) تقسیم شدند. در گروه D، ۳۰ میلی گرم و در گروه E ۶۰ میلی گرم هورمون $\alpha 17$ -متیل تستوسترون در یک دوره ۶۰ روزه به کار گرفته شد. تغییر در تعداد ماهیان نر در هر تیمار از طریق آزمون مربع کای ($P < 0.05$) مورد تجزیه و تحلیل قرار گرفت. در آزمایش I، تیمار غذا دهی ۶۰ میلی گرم (گروه B) به صورت معنی داری ($P < 0.05$) تعداد ماهیان نر را (۸۵/۷٪) در مقایسه با تیمار شاهد (گروه C، ۴۶/۶۷٪) افزایش داد. مقادیر کمتر ماهی نر (۶۰٪) اما معنی دار ($P < 0.05$) در تیمار غذا دهی ۳۰ میلی گرم (گروه A) مشاهده شد. در آزمایش II با اینکه نسبت ماهیان نر و ماده از نسبت ۱:۱ به صورت معنی داری ($P < 0.05$) فاصله پیدا کرد، اما به دلیل استفاده از دوز های بالای هورمونی، جمعیت ماهیان به سمت عقیمی پیش رفت. با توجه به نتایج این آزمایش، امکان دست یابی به مقادیر بالای ماهیان نر با استفاده از تیمار های هورمونی $\alpha 17$ -متیل تستوسترون در ماهی هاپ آبی وجود دارد.