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Investigating the Effect of 17α-Methyl Testosterone Hormone on Hue and Color Measurement of Scatophagus Argus

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Abstract 17α-methyl testosterone is an androgen plays role in sexual cycle and affects increasing growth and changing secondary attributes. In the present study, the effect of three different doses of this hormone (300, 400 and 1000 mg/kg/kg) on secondary sexual characteristics (Hue and value) of *Scatophagus argus* was investigated. The results of this research indicated that there is a strong relationship between the physiological condition of fish with the skin color and secondary sexual characteristics and by increase of dose, the glitter in the fish's tint was increased. Tint, the bottom of body in fish samples with doses of 0, 300 and 400 ml/kg had no significant difference ($P>0.05$), while in a dose of 1000 ml/kg of the allotment it had significant difference ($P<0.05$) and the maximum tint was observed in samples with 1000 ml dosage. These results showed a tendency to increase red tint, while in all investigated doses, significant differences in the rate of tint between tailfin and body fin ($P<0.05$). On the other side, the factor of tint in the tailfin of fish had significant difference too ($P<0.05$) and indicates a decreasing procedure toward red tint.

Keywords 17α-methyl testosterone, Secondary sexual characteristics, Color measurement, Tint, *Scatophagus argus*

1. Introduction

Scatophagus argus belongs to the scatophagidae family from the vernacular fish of the Persian Gulf. These fish are diadromous, they naturally live in seawater, but they can easily enter fresh waters. We can mention to its small head and couple short, but dynamic fins in its anatomy[12].

The color pattern on the body of this fish is changed by feeding on different nutrients and it is included of green, violet, black, yellow, brown and white in the ventral part.

If its habit of feeding known as a carnivore, violet and brown colors would be more revealing and if it's become an herbivore, green and yellow colors are revealed. This fish has a white belly. In female fish, this white stain is only in lower fins, however, in the male fish, it starts from the beneath of its gills and continues to its anus. They usually spawn in brackish water and in its genesis in freshwater, eggs hardly become larvae. The body of this fish shows the maximum coloring in relatively saltwaters[1]. The attractiveness and behavior of male fish are effective in being selected by the females, such behavior is observed in some other fish too. For example, in Guppy, the color of males, affects selection of females[6; 10; 11, 21]. During the couple selections, prior to mating, the females prefer males with relatively high attractive colors[20], especially the carotenoid color (orange, red and yellow)[7, 8]. Many of these specifications are considered as secondary sexual characteristics and are under the influence of the rate of blood hormones. It is specified that the color of a fish's body[14; 17) and development of Gonopodium in young males[9; 17] are controlled by Gonad androgens. Bayley et al.[3] Studied the rate of orange color proportioned to the body surface as a secondary characteristic under influence of androgens in Guppy fish[3]. Also in the studies of Nielsen and Baatrup[15] the orange color was investigated as secondary indicators and determining factors in success of mating.

2. Materials and Method

In order to perform this study, 4 different doses of 17α-methyl testosterone hormone (0, 300, 400 and 1000 mg/kg/kg) with three replications were considered[18]. Each replication was included of 3 samples. The commercial methyl testosterone pills of Abureyhan Pharmaceutics Company containing 25 mg/kg of active ingredient that after calculation of the rate of active ingredient, it has become

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powder and mixed with their food. The fish were fed by allotment containing hormone for 45 days. In order to feed them, the Biomar food of France with a diameter of 0.8 mm is used twice in a day in saturation level. (The ingredients of biomar food are included of protein: 56%, fat: 18%, Fiber: 0.4%, ash: 10.5%, moisture and other compositions: 15.1%). In order to keep the water quality, half of the water of the aquarium was changed every three days. The aquariums were fully fanned during the test period. easuring qualitative parameters of water were conducted during the test. The general results of measuring qualitative parameters of water are shown in table (1).

Table 1. Results of measuring qualitative parameters of water

No.	Measured parameter	Measured amount
1	Temperature	29±1
2	pH	8±0.5
3	Oxygen (mg/kg per Lit)	6.38±0.15

Figure 1. Scatophagus argus

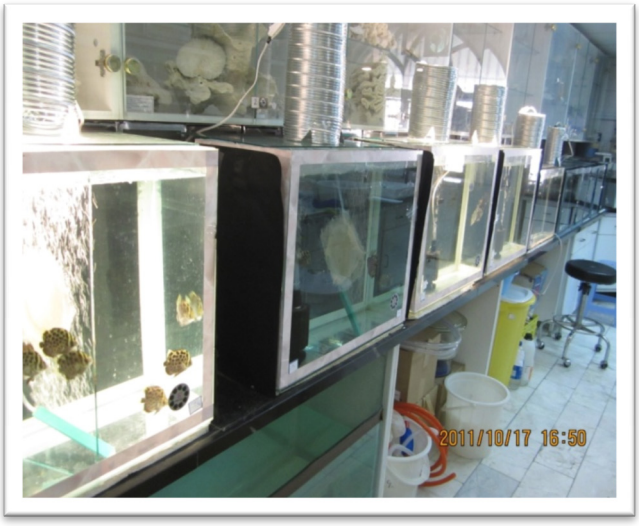


Figure 2. Treatments

At the end of the test, 3 fish from each tank (totaling 9 fish for each treatment) were selected for color analysis (fig. 1& 2). From each *Scatophagus argus* color measurement was conducted in two fields: side area of the body and the tailfin area. In order to measure the color rate, the color and appearance measurement system, made in England by Camera system CAM-system 500 were used that based on this proposed system (commission international de 1' Ecalairage, 1978, [CIE]), indicated three main components of L^*, a^* and b^* that 0 to +100 means black to pure white, -100 to +100 is green to red and 0 is indicative of gray and +100 and -100 for blue to yellow and 0 is gray. Before measuring, the device was automatically calibrated by help of 24 grid tile.

In order to measure chroma formulation 1 was used, also the rate of tint (Hue) and ECI is calculated from relations 2 & 3[18]:

$$\text{Chroma}= (a^2+b^2)^{0.5} \quad (1)$$

$$H^\circ = \arctan (b^*/a^*) \quad (2)$$

$$\text{ECI}+C_i^* (H_i-H_{mean}) \quad (3)$$

That H_{mean} is the mean of tint; C_i and H_i are the Chroma and respective tint related to each measurement.

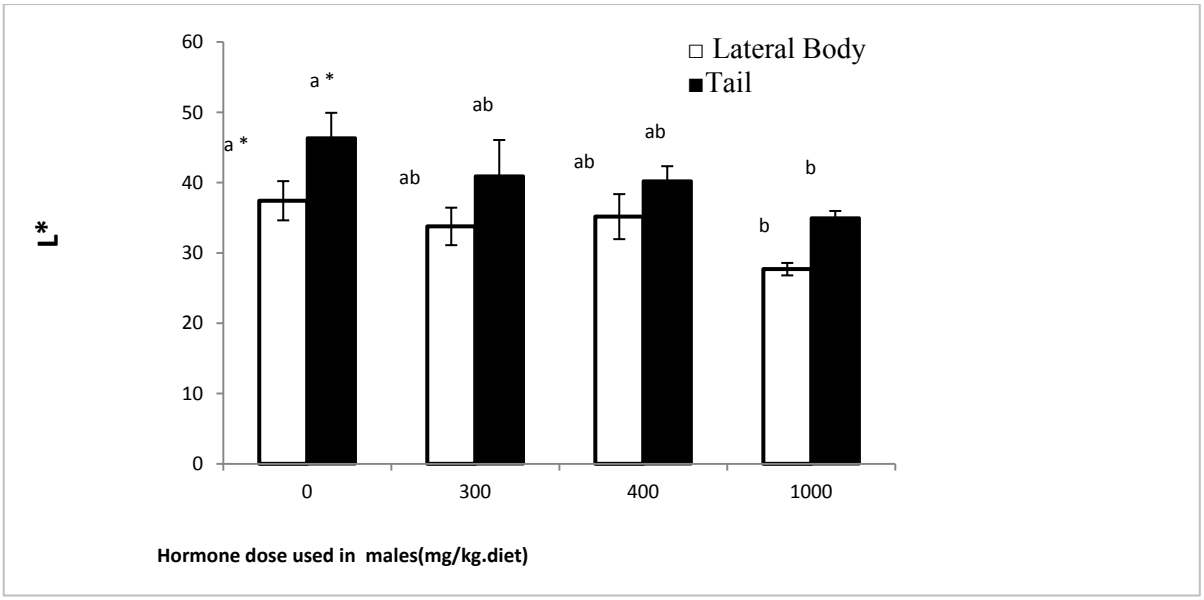
By considering the CIE[4], color is a 3D characteristic which is included of one part light and two color parts called Chroma and tint. Colors can be distinguished from each other by specifications of these three components. Tint is the dominant wavelength and is indicated as the tint angle in a^*-b^* plate that increases around its axis counter clockwise, that 0° is red, 90° is yellow, 180° is green and 270° is blue. Chroma refers to the rate of saturation of a color and indicates that how much gray light is mixed with pure white color. Chroma changes from neutral to shiny and refers to the distance on the axis perpendicular to a^*-b^* plate. A color which is included of tint and Chroma is included of two correlated components which cannot be considered separately in a^*-b^* plate. Instead, it is as two vertical components in which tint is orientation angle and Chroma is length of color vector. ECI indicates the image of each color vector along the group's mean group and indicates its color in the mean color. This factor is composed of two factors of tint and chroma and since it is a numeric quantity, classical statistical analysis about them is used[18].

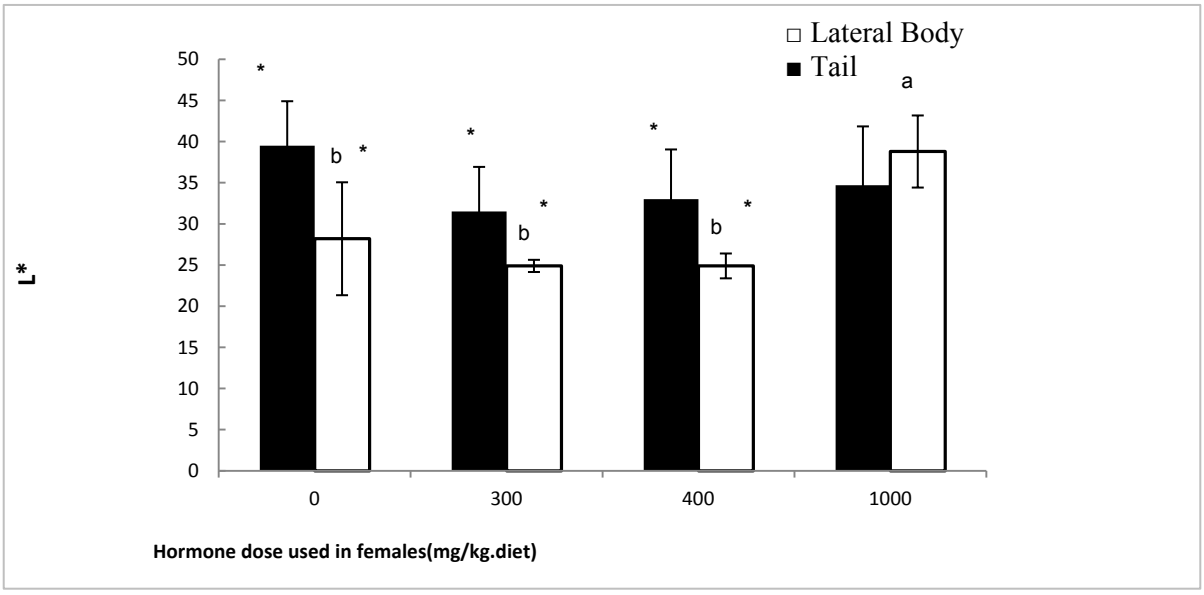
In this study, circle distribution is used for analysis of angular data (tint) (18: 24). In this way that Rayleigh test was used to investigate the normal state of circular data. The difference in tint rates between measuring points and measured groups was conducted by Watson-Williams method and in case at least one of the measured groups was abnormal, Watson's non-parametric test was used. Statistical differences between amounts of light, ECI and Chroma in different doses in the body or tail area was measured by one-way variation analysis method and in case of significance of that was categorized by Duncan test. In order to compare difference of different doses between the body and tail, two-way variation analysis is used. Classic statistics were done by Excel, SAS and comparison of angular amounts with Oriana Version 3.00.

Table 2. Rate of mean and standard deviation of tint in samples of *Scatophagus argus* in different doses of 17α-methyl testosterone hormone in two areas of body and tailfin

Doses	Body Part	Mean and standard deviation tint in males	P	Mean and standard deviation tint in females	P	Mean and standard deviation and ECI in females
0	Body	299.648 (2.105[b]	<0.05	242.185±25.704	0.5>	4.08 (0.72[b]
	Tail	16.070 (4.322[a]		13.038±33.522		2.63 (2.77[c]
300	Body	304.705 (1.644[b]	<0.05	217.066±15.016	0.2>	5.71 (2.33[b]
	Tail	16.358 (1.512[ab]		247.423±22.024		9.42±7.72[cb]
400	Body	303.836 (4.400[b]	<0.05	212.035±16.086	0.5>	5.68 (1.70[b]
	Tail	12.632 (1.064[b]		248.720±32.365		6.60 (5.63[b]
1000	Body	317.730 (4.051[a]	<0.05	200.84±4.451	<0.02	8.35 (1.57[a]
	Tail	345.386 (5.784[c]		11.08±6.781		18.44 (2.74[a]

*similar letters are indicative of lack of significant difference among treatments.





Antennas are indicative of standard deviation.
L*: -100 to +100 for blue to yellow and 0 for Gary.

Figure 3. Effect of different doses of 17α-methyl testosterone hormone on light on the body and tail area of *Scatophagus argus*, the * is indicative of significant difference between body and tail area in each day and English letters are indicative of significant difference in each part of the body and tail (P<0.05)

3. Results

As it is shown in table (2), by the increase of hormone dosage, significant differences in light rate (L^*) of the body and tailfin of the fish were observed. Also, the light rate of the body of fish in 1000 mg/kg dosage had the maximum rate compared to three other doses and didn't have significant differences in light between different doses at the tail area of the fish ($P<0.05$). At all hormone doses, the rate of light in tail area has been significantly more than body area ($P<0.05$).

The maximum rate of tint is gaining an inside area of the fish's body in 1000 mg/kg dosage which had significant difference to other doses ($P<0.05$). By increasing of hormone dose, the rate of tint in tailfin indicated a significant decrease.

As it is observed in table (2), about tint, the body of a fish in doses 0, 300 and 400 mg/kg didn't have significant differences, while in 1000 mg/kg dosage, the difference is significant ($P<0.05$) and the maximum tint was observed in fish with 1000 mg/kg dosage. This result is indicative of the tendency to increase the red tint, while at all studied doses, significant difference is observed in the tint between tailfin and body ($P<0.05$). On one side, the factor of tint in the tailfin area of the fish had significant difference ($P<0.05$) and indicates the decreasing procedure in a red tint, because as it is observed, the area of 0 (360) degree is an area with red color.

By considering the lack of significant differences among treatments in the rate of tint, ECI amounts were calculated. Generally, the amounts calculated for ECI indicate an increasing procedure, while the change procedure in the body part in different doses didn't have significant difference ($P<0.05$). However, the amounts of ECI in 1000 mg/kg had maximum ECI amount. The data related to tailfin indicated a similar procedure. It means that the dosage of 1000 mg/kg had the maximum rate and dosage 0 mg/kg had the minimum rate and doses, if 1000 and 400 mg/kg both with control had significant difference ($P<0.05$). In this part of the body we have the total procedure of increase in ECI rate, while in the fish with 1000 mg/kg significant difference was observed in the tint rate among the body and tailfin area ($P<0.05$) and by considering the related ECI, the tailfin area indicated an increase of pigments and increase of ECI that in this part increase toward red tint was observed.

In figure (3), the effect of different doses of 17α-methyl testosterone hormone on the light of the body and tail area of *Scatophagus argus* is indicated.

4. Discussion and Conclusions

In fish investigated in the present study, an interesting similarity was observed in the change procedure for tint and the light rate. In the area of tailfin of the control fish, the highlight was observed, in this dosage fewer tints is toward red and more tint is toward yellow, but in a dosage of 300 mg/kg the light rate decreased suddenly which was due to tendency to red tint. In higher doses also the tendency was toward increase of red tint and in 1000 mg/kg red tint was dominant and in this dosage that in this dosage also indicated an increase in doses 300 and 400, but it was also less than control treatment which can be due to change in melanin, drozopetrin and cartenoeid pigments, but determining its definite factor requires measuring the change process in skin pigments. In the side part of the body, by the increase of tendency from 270 tint (blue) which the light is decreased due to melanin pigments and other pigments, but in 1000 mg/kg, by the increase of the tendency of tint toward red tint, the light would increase too. This issue happened with the decrease of tendency in blue tint and its creating pigments. Color changes due to the effect of hormone was less in the body area in a way that only in 1000 mg/kg dosage it caused significant differences ($P<0.05$). Pavlidis et al.[18] related the low amount of light factor as a result of high rates of melanin and the pigment percentage of cartenoeid in the skin. The results of other studies also indicated an increase in red color being exposed to methyl testosterone or androgen materials. In male mature Guppy fish, the areas with orange color were influenced after being exposed to high densities of acetyl phenol or estradiol[2: 23]. In the study of Pikulkaew and Wongsathein[19] the intensity of red color in the tailfin indicated a decrease under specific densities of materials similar to Di Chlorodiphenyl-trichloroethane (an estrogen material). Bayley et al. In 2002 concluded on young fish that the fish exposed to anti-androgens of vinclosolin and p^1, p, Chlorodiphenyl-trichloroethane (DDT) -DDE and flotamid indicate a reduction in the orange color. Devasurenda et al. (2007) are reporting increases in the rate of drozopetrin that creates red spots in this fish by use of density of 250 mg/kg of food from one analogue androgen and they attributed the creation of orange spots on this fish under the influence of androgens.

drozopetrin and cartenoeid play role in the creation of chroma of orange spots, thus the existence of these two pigments can be studied in the fish. The factor of light also refers to the amount of light that reflects the subjective color or passes it through itself and this issue would cause distinction between light and dark colors.

The fish can change their color by distributing and gathering pigments in chromatophore quickly (physiological color change) or by changing the rate of pigments or the number of chromatophore (morphological color change)[22]. Vast and slow expansion of pigments and especially the red color in this study during the test period is indicative of this fact that a change of color in studying fish was a morphological type and its reason was changed at the rate of pigments or the rate of chromatophore. Joaki Larsson et al.[13] have concluded the same result about Guppy fish exposed to androgen materials and increases in red color.

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