

# Effects of synthetic androgen (17 $\alpha$ -methyltestosterone) and estrogen (17 $\beta$ -estradiol) on growth and skin coloration in emperor red cichlid, *Aulonocara nyassae* (Actinopterygii: Cichliformes: Cichlidae)

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## Abstract

In recent years, the use of anabolic steroids in the coloration and growth of fishes, especially ornamental ones, has attracted great interest. In the ornamental fish industry, it is economically advantageous to produce some species with high commercial value and higher demand, depending on size, color, and sex. Therefore, the most commonly used steroids in this study—i.e., 17 $\alpha$ -MT and 17 $\beta$ -Es (E<sub>2</sub>)—were added to the diet of emperor red cichlid, *Aulonocara nyassae* Regan, 1922, which has not been previously hormone-treated and has high economic value amongst ornamental fishes. A 60-day study was conducted in a closed system, where the juveniles of the emperor red cichlid were acclimatized with the control/basal diet for 15 days. After which, 15 fish with a similar shade of color and about 5 months old were weighed individually (0.71 ± 0.01 g). All fish were placed into aquaria (30 L) in five different groups, in triplicate. Five different groups consisted of control (without hormone), 50 mg · kg<sup>-1</sup> 17 $\alpha$ -MT, 100 mg · kg<sup>-1</sup> 17 $\alpha$ -MT, 50 mg · kg<sup>-1</sup> E<sub>2</sub>, and 100 mg · kg<sup>-1</sup> E<sub>2</sub>. The fish were fed a diet twice a day (10:00 h, 17:00 h) for 60 days till satiation. During the entire trial period, a 12 h light–12 h dark photoperiod was maintained. Water temperature was measured daily and recorded. Growth parameters of experimental fish were calculated. The color measurement of fish skin ( $L^*$ ,  $a^*$ ,  $b^*$  values) from around the dorsal section was performed using a colorimeter (Konica Minolta CR 400). Significant differences were determined in the following parameters: weight gain (WG), specific growth rate (SGR), feed conversion rate (FCR), survival rate (SR), condition factor (CF), and sex reversal. The fish group fed with 17 $\alpha$ -MT displayed brighter coloration as compared to other groups. Color analysis (instrumental) in terms of  $L^*$ ,  $a^*$ , and  $b^*$  values showed that the group fed with 17 $\alpha$ -MT displayed brighter coloration compared to other groups ( $P < 0.05$ ). In terms of sex reversal, the fish in the 17 $\alpha$ -MT groups exhibited 100% masculinization, whereas in E<sub>2</sub> supplemented fish groups (50 and 100 mg · kg<sup>-1</sup>), the feminization rates were 88.88% and 93.33%, respectively. In conclusion, both hormones were found to have positive and negative effects for this fish species, but the 17 $\alpha$ -MT hormone was found to be more effective in reversing skin pigmentation, growth, and sexing, which is the main driver in the ornamental fish trade.

## Keywords

coloration, growth performance, ornamental aquaculture, sex reversal

## Introduction

In the recent past, the production of ornamental fish feed was mostly dependent on bycatch and other aquatic organisms being used as live feeds. However, due to the increasing pressure on natural resources, organized industrial sites have been established for the breeding and marketing of ornamental fishes. Nowadays, feed and aquarium equipment used in ornamental fish farming have emerged as an industry that appeals to the global market. In developed and developing countries, the ornamental fish-farming sector has developed with major commercial importance within the aquaculture industry (Sales and Janssens 2003; Hekimoğlu 2006; Dominguez and Botella 2014).

Nutrition is one of the most important elements for the sustainability of fish farming. In addition to carbohydrates, proteins, and fats in the structure of the feed used in nutrition, minerals, vitamins, and anabolic steroids are also used as additives.

Anabolic steroids contain androgen (male sex hormone) and estrogen (female sex hormone), which are commonly used as growth supplements in feed. Steroid hormones can be used in feed without any biological loss since they are resistant to catabolism by digestive enzymes. Thus, fish can be supplemented with hormones without incurring any handling stress compared to other invasive methods like injection, etc. Moreover, administering hormones to multiple fish individuals is a simple procedure (Hoşsu et al. 2003).

The wide use of growth promoters in animal husbandry using the anabolic sex steroids has attracted their application in the aquaculture industry to shorten the production cycle and lower the production cost (Gannam and Lovell 1991).

Anabolic agents (or anabolics) have been defined as substances that enhance the protein synthesis of livestock by positively affecting the nitrogen balance. Therefore, anabolic hormones enable lean meat production and rapid weight gain of fish and livestock with less feed by expediting muscle development. Most of these are steroidal male and female sex hormones (both natural and synthetic) and non-steroidal substances with anabolic effects. Furthermore, growth hormones, somatomedins, insulin, and thyroid gland hormones have also been documented to affect the growth rate positively (Mercure et al. 2001; Karabulut 2008).

Sex reversal by hormone treatment and its application in certain expensive aquarium fish, especially for which market value depends on the sex, have drawn the attention of many ornamental fish farmers and this situation has demanded the requirement of more studies on this subject. Studies related to color enhancement in ornamental fish farming have gained momentum in recent years and particularly the use of anabolic steroids is of great interest. Apart from sex reversal, steroids are also used for enhancing the growth or color of fish for visual appeal.

Cichlids, being one of the most visually appealing fishes in the industry, are amongst the aquarium fish, for which market value depends on sex. The emperor red

cichlid, *Aulonocara nyassae* Regan, 1922, attracts attention, especially due to its color in shades of red. The colors of the males are much brighter red with a noticeable difference compared to the females and their dorsal and anal fins are more pointed. Females also usually have grey and dark tones. In this study, the emperor red cichlid was used due to its importance in the aquarium industry and there is a lack of information on the effect of sex steroids on the growth and coloration of this species.

## Materials and methods

**Experimental design.** A 60-day study was conducted in a closed system, where the juveniles of emperor red cichlid (*A. nyassae*) were acclimatized with the control/basal diet for 15 days. After which, 15 fish with a similar shade of color and about 5 months old (unsexed) were weighed individually ( $0.71 \pm 0.01$  g) and placed into each aquarium (30 L) in five different groups, in triplicate. Five different groups consisted of control (without hormone),  $50 \text{ mg} \cdot \text{kg}^{-1}$   $17\alpha$ -MT,  $100 \text{ mg} \cdot \text{kg}^{-1}$   $17\alpha$ -MT,  $50 \text{ mg} \cdot \text{kg}^{-1}$   $17\beta$ -Es ( $E_2$ ), and  $100 \text{ mg} \cdot \text{kg}^{-1}$   $17\beta$ -Es ( $E_2$ ). The basal feed had the approximate composition of 46.5% crude protein, 8.2% crude fat, 3.3% crude fiber, 5.0% moisture, and 5.3% crude ash. The fish were fed twice a day (10:00 h, 17:00 h) for 60 days a diet till satiation. Experimental fish were weighed at the beginning of the experiment and the last day of the experiment (on the 60<sup>th</sup> day). Waste materials were siphoned out every day and replacement water was added. In each experimental treatment group, fish were acclimatized to aquarium and feeding conditions for a week. The temperature of the aquarium unit was set to have a water temperature of  $24 \pm 1^\circ\text{C}$  throughout the experiment by a room air conditioner. Aquaria were aerated using an air stone.

Hormones were added to the commercial diet and the proximate composition of the feed is provided in Table 1. The control/basal feed was without hormone

**Table 1.** Proximate composition of the experimental diets used for feeding emperor red cichlid, *Aulonocara nyassae*.

Chemical composition	Diet group				
	Control	50 mg 17 $\alpha$ -MT	100 mg 17 $\alpha$ -MT	50 mg E <sub>2</sub>	100 mg E <sub>2</sub>
Crude protein [%]	46.5	46.5	46.5	46.5	46.5
Crude lipid [%]	8.2	8.2	8.2	8.2	8.2
Crude fiber [%]	3.3	3.3	3.3	3.3	3.3
Moisture [%]	5.0	5.0	5.0	5.0	5.0
Ash [%]	5.3	5.3	5.3	5.3	5.3
NFE	31.7	31.7	31.7	31.7	31.7
17 $\alpha$ -Methyltestosterone [mg · kg <sup>-1</sup> ]	—	50	100	—	—
17 $\beta$ -Estradiol [mg · kg <sup>-1</sup> ]	—	—	—	50	100
Proximate analysis					
GE, [kJ · g <sup>-1</sup> ]	19.69	19.69	19.69	19.69	19.69

NFE = Nitrogen Free Extract =  $100 - (\text{moisture} + \text{crude protein} + \text{crude lipid} + \text{ash} + \text{crude fiber})$ ; GE = Gross energy =  $(\text{crude protein} \times 23.6) + (\text{crude lipid} \times 39.5) + (\% \text{ carbohydrates} \times 17.3)$  (Koshio et al. 1993); Vitamin A 30000 IU · kg<sup>-1</sup>, Vitamin D<sub>3</sub> 1500 IU · kg<sup>-1</sup>, Vit. E (D, L- $\alpha$ -tocopherol asetat) 60 mg · kg<sup>-1</sup>, Vit. B<sub>1</sub> 30 mg · kg<sup>-1</sup>, Vit. B<sub>2</sub> 90 mg · kg<sup>-1</sup>, Vit. C preparation (L-ascorbic monophosphate) 550 mg · kg<sup>-1</sup>.

supplementation. The test diets were prepared with supplementation of 17 $\alpha$ -MT (50 and 100 mg · kg<sup>-1</sup>) and E<sub>2</sub> (50 and 100 mg · kg<sup>-1</sup>) following the methods of Degani (1985) and Santandreu and Diaz (1994). The feed was then stored in tightly sealed plastic bags and placed in a freezer at -20°C. The control diet was sprayed with a steroid-free 95% ethyl alcohol solution, processed in the same way as the treated feed, and stored until the day of use.

During the entire trial period, a 12 h light–12 h dark photoperiod was maintained. Water temperature was measured daily and recorded as 26 ± 1°C, while pH, dissolved oxygen, and NH<sub>4</sub><sup>+</sup> values were evaluated weekly and maintained at 6.5–8.5, > 5 mg · L<sup>-1</sup> and 2–5 mg · L<sup>-1</sup>, respectively.

**Color measurements.** The color measurement of fish skin ( $L^*$ ,  $a^*$ ,  $b^*$  values) (Hunt 1977; CIE 1986; Nickell and Bromage 1998; Berns 2000) from around the dorsal section was performed using a colorimeter (Konica Minolta CR 400) at the beginning (0<sup>th</sup> day), 30<sup>th</sup> day, and the end (60<sup>th</sup> day) of the experiment. The values of the earlier mentioned variables can be explained as follows:  $L^*$  = lightness (also referred to as luminance); the lightness or darkness of a color. It ranges from 0 (pure black) to 100 (diffuse white),  $a^*$  = red to green; (+) positive: redness, (-) negative: greenness,  $b^*$  = yellow to blue (+) positive: yellowness, (-) negative: blueness. Chroma ( $C^*$ ) (calculated with  $a^*$  and  $b^*$  values) indicates the intensity and lightness (clarity) of the colors.

The hue ( $H_{ab}^{\circ}$ ), also calculated with  $a^*$  and  $b^*$  values, is an angle value representing the shade of redness, yellowness, greenness, and blueness of the skin color. Hue degree indicates red as it approaches 0°, yellow as it approaches 90°, green as it approaches 180°, and blue as it approaches 270°.

$\Delta E$  is the color difference value and was calculated with  $L^*$ ,  $a^*$ , and  $b^*$  measurements obtained at the beginning and the end of the trial (Hunt 1977; CIE 1986; Nickell and Bromage 1998; Berns 2000).

$$C^* = (a^{*2} + b^{*2})^{0.5}$$

$$H_{ab}^{\circ} = 180 + \tan^{-1}(b^* \cdot a^{*-1}) \text{ for } a^* < 0$$

$$H_{ab}^{\circ} = \tan^{-1}(b^* \cdot a^{*-1}) \text{ for } a^* > 0$$

$$\Delta E = [(L_t - L_0)^2 + (a_t - a_0)^2 + (b_t - b_0)^2]^{0.5}$$

Where  $L_t$  is lightness at the end of the experiment,  $L_0$  is lightness in the initial of the experiment;  $a_t$  is red to green degree at the end of the experiment,  $a_0$  is red to green degree in the initial of the experiment;  $b_t$  is yellow to blue degree at the end of the experiment,  $b_0$  is yellow to blue degree in the initial of the experiment.

**Growth parameters.** Growth parameters of experimental fish were calculated with the following equations:

The specific growth rate (SGR) [% · day<sup>-1</sup>], the weight gain ( $W_G$ ) [%], the survival rate ( $S$ ) [%], the Feed Conver-

sion Ratio (FCR), and Fulton's condition factor ( $K$ ) were calculated using the respective formulas:

$$SGR = 100 [\ln W_F - \ln W_0] \cdot t^{-1}$$

$$W_G = 100 (W_F - W_0) \cdot W_0^{-1}$$

$$S = 100 (N_F - N_0)$$

$$FCR = W_F \cdot W_G^{-1}$$

$$K = 100(W_W \cdot L_T^{-3})$$

where  $W_0$  and  $W_F$  are the initial and final body weight and  $t$  is the time (feeding period) in days;  $N_t$  is the number of fish at the end of experiment and  $N_0$  is the number of fish at the beginning of the experiment);  $W_F$  is the weight [g] of the food given and  $L_T$  is the total length [cm] of the fish.

**Sex parameters.** In the experiment, the sex reversal of the fish was determined by looking at the primary and secondary sex characteristics.

**Statistical analyses.** Statistical analyses were conducted with “Minitab Release 17 for Windows” software. Data were subjected to one-way analyses of variance (ANOVA), followed by the Tukey test at a significance level of 5% (0.05).

**Ethical note.** This research was approved by the Ethics Committee of Sinop University with Reference No. MYO-1901-16-22. All the procedures applied in this study took into account the importance of preventing or at least minimizing, any kind of animal discomfort or suffering.

## Results

At the end of the 60-day feeding period, it was observed that the fish group fed a higher dose of 17 $\alpha$ -MT (100 mg · kg<sup>-1</sup>) had a significantly positive effect ( $P < 0.05$ ) in terms of weight increase, weight gain, FCR, and SGR. Growth parameters in the group fed 17 $\alpha$ -MT (50 mg · kg<sup>-1</sup>) were also better compared to the group without 17 $\alpha$ -MT. However, no change was observed in the fish groups fed E<sub>2</sub>. The highest survival rate was observed in the control group (93.33 ± 2.45%) followed by 50 mg · kg<sup>-1</sup> 17 $\alpha$ -MT treated group (86.67 ± 7.35%) ( $P < 0.05$ ). The survival rates of the remaining fish groups (50–100 mg · kg<sup>-1</sup> E<sub>2</sub> and 100 mg · kg<sup>-1</sup> 17 $\alpha$ -MT) were low.

In terms of sex reversal, the fish in 17 $\alpha$ -MT groups exhibited 100% masculinization, whereas in E<sub>2</sub> supplemented fish groups (50 and 100 mg · kg<sup>-1</sup>), the feminization rates were 88.88% and 93.33%, respectively (Table 2). No intersex fish were found at the end of the experiment.

Color analysis (instrumental) in terms of  $L^*$ ,  $a^*$ , and  $b^*$  values showed that the group fed 17 $\alpha$ -MT displayed brighter coloration compared to other groups ( $P < 0.05$ ). In terms of Hue ( $H_{ab}^{\circ}$ ) values, the skin color of fish in 17 $\alpha$ -MT supplemented groups showed more intense

**Table 2.** Growth performance, feed conversion ratio (FCR), survival, conditions and sex ratio of experimental feeds fed for 60 days to emperor red cichlid, *Aulonocara nyassae*.

Parameter	Control	50 mg 17 $\alpha$ -MT	100 mg 17 $\alpha$ -MT	50 mg E <sub>2</sub>	100 mg E <sub>2</sub>
Initial weight [g]	0.71 ± 0.01 <sup>a</sup>	0.71 ± 0.02 <sup>a</sup>	0.71 ± 0.01 <sup>a</sup>	0.71 ± 0.01 <sup>a</sup>	0.71 ± 0.02 <sup>a</sup>
Final weight [g]	1.54 ± 0.13 <sup>c</sup>	1.92 ± 0.17 <sup>b</sup>	2.23 ± 0.19 <sup>a</sup>	1.62 ± 0.14 <sup>c</sup>	1.59 ± 0.11 <sup>c</sup>
Weight increase [g]	0.83 ± 0.09 <sup>c</sup>	1.21 ± 0.13 <sup>b</sup>	1.52 ± 0.11 <sup>a</sup>	0.91 ± 0.12 <sup>c</sup>	0.88 ± 0.07 <sup>c</sup>
Weight gain [g]	116.90 ± 13.4 <sup>c</sup>	170.42 ± 12.09 <sup>b</sup>	214.08 ± 18.4 <sup>a</sup>	128.17 ± 15.07 <sup>c</sup>	123.94 ± 14.3 <sup>c</sup>
SGR	1.29 ± 0.18 <sup>c</sup>	1.66 ± 0.19 <sup>b</sup>	1.91 ± 0.26 <sup>a</sup>	1.37 ± 0.16 <sup>c</sup>	1.34 ± 0.15 <sup>c</sup>
FCR	3.23 ± 0.71 <sup>a</sup>	2.32 ± 0.43 <sup>c</sup>	2.19 ± 0.31 <sup>c</sup>	2.93 ± 0.66 <sup>b</sup>	3.01 ± 0.53 <sup>b</sup>
K	1.11 ± 0.12 <sup>b</sup>	1.23 ± 0.15 <sup>a</sup>	1.09 ± 0.08 <sup>b</sup>	1.24 ± 0.13 <sup>a</sup>	1.27 ± 0.07 <sup>a</sup>
Survival rate [%]	93.33 ± 2.45 <sup>a</sup>	86.67 ± 7.35 <sup>a</sup>	73.33 ± 9.24 <sup>b</sup>	68.8 ± 11.21 <sup>bc</sup>	62.20 ± 12.4 <sup>c</sup>
Males [%]	28.89 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>	11.11 <sup>c</sup>	6.67 <sup>c</sup>
Female [%]	71.11 <sup>b</sup>	0 <sup>c</sup>	0 <sup>c</sup>	88.88 <sup>a</sup>	93.33 <sup>a</sup>

Values (mean ± standard error of data for triplicate groups) with different superscripts in the same row are significantly different (one-way ANOVA and Tukey multiple-range test,  $P < 0.05$ ). SGR = specific growth rate, FCR = feed conversion ratio, K = condition factor.

**Table 3.** Color parameters measured in fish skin of juvenile emperor red cichlid (*Aulonocara nyassae*) during the experimental period.

Color parameters and periods	Control	50 mg 17 $\alpha$ -MT	Group		
			100 mg 17 $\alpha$ -MT	50 mg E <sub>2</sub>	100 mg E <sub>2</sub>
<b>L*</b>					
Beginning of experiments	41.57 ± 0.11	41.57 ± 0.11	41.57 ± 0.11	41.57 ± 0.11	41.57 ± 0.11
30 <sup>th</sup> day	40.62 ± 0.13	37.60 ± 0.09	29.13 ± 0.05	47.65 ± 0.12	47.28 ± 0.08
60 <sup>th</sup> day	40.15 ± 0.07 <sup>b</sup>	26.11 ± 0.18 <sup>c</sup>	17.22 ± 0.24 <sup>d</sup>	54.34 ± 0.32 <sup>a</sup>	53.16 ± 0.29 <sup>a</sup>
<b>a*</b>					
Beginning of experiments	-2.11 ± 0.04	-2.11 ± 0.04	-2.11 ± 0.04	-2.11 ± 0.04	-2.11 ± 0.04
30 <sup>th</sup> day	-1.57 ± 0.08	7.89 ± 0.11	8.23 ± 0.09	-1.67 ± 0.14	-1.44 ± 0.17
60 <sup>th</sup> day	2.06 ± 0.03 <sup>b</sup>	8.17 ± 0.21 <sup>a</sup>	9.86 ± 0.15 <sup>a</sup>	-1.28 ± 0.04 <sup>c</sup>	-1.53 ± 0.06 <sup>c</sup>
<b>b*</b>					
Beginning of experiments	4.82 ± 0.09	4.82 ± 0.09	4.82 ± 0.09	4.82 ± 0.09	4.82 ± 0.09
30 <sup>th</sup> day	15.21 ± 0.24	9.74 ± 0.15	8.48 ± 0.05	4.89 ± 0.12	4.14 ± 0.16
60 <sup>th</sup> day	9.78 ± 0.06 <sup>a</sup>	5.12 ± 0.13 <sup>b</sup>	4.09 ± 0.09 <sup>b</sup>	5.36 ± 0.14 <sup>b</sup>	5.29 ± 0.19 <sup>b</sup>
<b>H°<sub>ab</sub></b>					
Beginning of experiments	113.64 ± 0.14	113.64 ± 0.14	113.64 ± 0.14	113.64 ± 0.14	113.64 ± 0.14
30 <sup>th</sup> day	95.89 ± 0.19	50.99 ± 0.11	45.86 ± 0.23	108.86 ± 1.36	109.79 ± 1.44
60 <sup>th</sup> day	78.11 ± 0.27 <sup>b</sup>	32.07 ± 0.48 <sup>c</sup>	22.53 ± 0.32 <sup>c</sup>	103.43 ± 2.14 <sup>a</sup>	106.13 ± 1.56 <sup>a</sup>
<b>C*</b>					
Beginning of experiments	5.26 ± 0.08	5.26 ± 0.08	5.26 ± 0.08	5.26 ± 0.08	5.26 ± 0.08
30 <sup>th</sup> day	15.29 ± 0.45	12.53 ± 0.37	11.82 ± 0.33	5.17 ± 0.12	4.38 ± 0.15
60 <sup>th</sup> day	9.99 ± 0.02 <sup>a</sup>	9.64 ± 0.24 <sup>a</sup>	10.67 ± 0.21 <sup>a</sup>	5.51 ± 0.01 <sup>b</sup>	5.51 ± 0.03 <sup>b</sup>
<b><math>\Delta E</math></b>					
0 <sup>th</sup> -60 <sup>th</sup> day	6.63 ± 0.35 <sup>d</sup>	18.57 ± 0.44 <sup>b</sup>	27.14 ± 0.41 <sup>a</sup>	12.81 ± 0.23 <sup>c</sup>	11.61 ± 0.29 <sup>c</sup>

Values (mean ± standard error of data for triplicate groups) with different superscripts in the same row are significantly different (one-way ANOVA and Tukey multiple-range test,  $P < 0.05$ ); L\* = Lightness (also referred to as luminance); the lightness or darkness of a color; a\* = red to green; (+) positive: redness, (-) negative: greenness, b\* = yellow to blue (+) positive: yellowness, (-) negative: blueness;  $\Delta E$  is the color difference value and was calculated with L\*, a\*, and b\* measurements obtained at the beginning and the end of the trial; Chroma (C\*) (calculated with a\* and b\* values) indicates the intensity and lightness (clarity) of the colors; Hue (H°<sub>ab</sub>), also calculated with a\* and b\* values, is an angle value representing the shade of redness, yellowness, greenness, and blueness of the skin color.

shades of red, while the skin colors of fish in E<sub>2</sub> supplemented groups were observed to be shades of grey-brown closer to the shade of yellow color. The maximal change in color difference ( $\Delta E$ ) was observed in fish groups supplemented with 50–100 mg · kg<sup>-1</sup> 17 $\alpha$ -MT (Table 3).

## Discussion

In the ornamental fish industry, it is economically advantageous to produce certain species that have high commercial value and more demand depending on size, color, and sex. Therefore, in this study, the most commonly used steroids (namely, 17 $\alpha$ -MT, and E<sub>2</sub>) were supplemented to the feed of emperor red cichlid.

Fish groups fed 17 $\alpha$ -MT hormone had a better growth rate. These results are in agreement with the studies on *Labidochromis caeruleus* (see Karsli et al. 2018), *Oreo-*

*chromis andersonii* (see As et al. 2012), *O. niloticus* (see Greisy and Gamal 2012; Ajiboye et al. 2015), and *Trichogaster lalius* (see Biswas et al. 2014). However, other studies reported that the hormone treatment negatively affected growth and development in a dose-dependent manner in zebra cichlids (George and Pandian 1996), *Betta splendens* (see Kirankumar and Pandian 2002), and *O. niloticus* (see Sreenivasa and Prabhadevi 2018).

In the presently reported study, fish groups fed E<sub>2</sub> had no significant changes in growth parameters, which is in agreement with the results studied on *Amatitlania nigrofasciata* (see George and Pandian 1996) and *Centropomus undecimalis* (see Carvalho et al. 2014). Similarly, studies on *Xiphophorus helleri* by Lim et al. (1992) and Tamaru et al. (2009) stated that the hormones did not have any adverse effect on the growth.

Apart from beneficial effects, the most pronounced negative effect of hormone supplementation is on the sur-

vival rate, which, in turn, is dose-dependent, as well as species and sex specific. In this study, the survival rate was found to be high in the control group and the fish group supplemented with the low dose ( $50 \text{ mg} \cdot \text{kg}^{-1}$ ) of  $17\alpha$ -MT. On the other hand, the survival rate was low in all fish groups supplemented with  $E_2$  and the fish group treated with a high  $17\alpha$ -MT ratio. It has been reported that the high concentration of  $17\alpha$ -MT and  $E_2$  hormones lower the survival rate of swordtail (Tamaru et al. 2009) and zebra cichlid (George and Pandian 1996). Moreover, in the study of Komen et al. (1989) on *Cyprinus carpio*, the survival rate of fish treated with 25, 75, 125  $\text{mg} \cdot \text{kg}^{-1}$   $E_2$  hormone varied between 51 and 69%, while the fish groups treated with 50 and 100  $\text{mg} \cdot \text{kg}^{-1}$   $17\alpha$ -MT hormone had survival rates of 28% and 39%, respectively. In several studies involving the use of  $17\alpha$ -MT, the survival rate was negatively correlated in a dose-dependent manner as evident in *Trichogaster lalius* (see Katare et al. 2018), *O. niloticus* (see Das et al. 2010), and *Poecilia sphenops* (see George and Pandian 1998). Basavaraju et al. (2008) supplemented *C. carpio* fry and adults with  $17\alpha$ -MT and determined that compared to the control, the survival rate of the fry was lower, while that of the adults was higher.

When the sex ratios were examined, it was determined that the masculinization rate was 100% in fish groups with  $17\alpha$ -MT hormone. The groups supplemented with  $E_2$  had a positive impact on feminization compared to the control group, although it was not as effective as the  $17\alpha$ -MT in masculinization. George and Pandian (1996) supplemented the zebra cichlid with  $17\alpha$ -MT and  $E_2$  and observed the highest male (55%) and female (100%) rates in groups fed 200–300  $\text{mg} \cdot \text{kg}^{-1}$  hormone additives. Katare et al. (2018) determined that the  $17\alpha$ -MT supplementation at 50–100  $\text{mg} \cdot \text{kg}^{-1}$  was insufficient for the masculinization of *T. lalius*. Furthermore, in a study carried out on swordtail, it was reported that the female rate in the control group was 74.5% and the optimum dosage for feminization (97%) was 400  $\text{mg} \cdot \text{kg}^{-1}$   $E_2$  (Tamaru et al. 2009). The optimum  $17\alpha$ -MT supplementation rate for masculinization was reported to be 60  $\text{mg} \cdot \text{kg}^{-1}$  in *O. niloticus* (see Das et al. 2010; Greisy and Gamal 2012), *O. andersoni* (see As et al. 2012), and *Poecilia reticulata* (see Chakraborty et al. 2012). Moreover, Phelps and Okoko (2011) observed the highest male rate (99.3%) in 30  $\text{mg} \cdot \text{kg}^{-1}$  MT supplemented fish group in *O. niloticus* and Kipouros et al. (2011) obtained 100% males in groups supplemented with 2, 3, and 4  $\text{mg} \cdot \text{kg}^{-1}$   $17\alpha$ -MT. On the contrary,  $17\alpha$ -MT supplementation did not cause any significant difference in rosy barb and dwarf gourami (Ramee et al. 2020), as well as in *C. carpio* (see Basavaraju et al. 2008). The findings of the presently reported study suggest that hormone supplementation positively impacts the production of all male fish, thereby fetching a higher market value and increased profitability. This will result in further improvement of the ornamental fish market with the help of mono-sex fish production.

The visual appeal and color of aquarium fish are important criteria for their marketability. In the presently

reported study, enhancement in coloration of fish caused by hormone treatment was statistically demonstrated with the applied method and supported by the results of analyses. According to color analyses conducted at the beginning of the study, hue ( $H^{\circ}_{ab}$ ) values indicated shades of grey-brown as the fish were at the early developmental stage. The color differences amongst groups were apparent according to the analyses carried out on day 30. At the end of the study, the desired coloration was determined in  $17\alpha$ -MT-supplemented fish groups and there were differences between these groups over control. However, no such significant improvement in coloration was seen in the groups supplemented with  $E_2$ . As male individuals of emperor red cichlid appear as bright red, the primary reason for enhanced coloration in fish groups supplemented with  $17\alpha$ -MT hormone may be attributed to efficient masculinization in these groups, as it caused production of 100% male population.

Dananjaya et al. (2020) supplemented *Carassius auratus* with various doses (0, 50, 100, 200, and 250  $\text{mg} \cdot \text{kg}^{-1}$ ) of annatto (*Bixa orellana*) and determined that, according to  $L^*$ ,  $a^*$ , and  $H^{\circ}_{ab}$  values, the efficacy of annatto increased in a dose-dependent manner. Ninwichian et al. (2020) administered three different natural sources of carotenoids (*Phaffia rhodozyma*, *Paracoccus* sp., *Haematococcus pluvialis*) to *C. carpio* and determined that the fish supplemented with natural carotenoid sources displayed a higher red color in terms of  $a^*$  and  $H^{\circ}_{ab}$  values. Moreover, Gouveia et al. (2003) used various synthetic and natural astaxanthin, *Chlorella vulgaris*, *Arthrospira maxima* (Spirulina), and *Haematococcus pluvialis* coloration supplements in Kawari and Showa fish, varieties of *C. carpio* and observed significant effects on the shade of red ( $a^*$  and  $H^{\circ}_{ab}$ ) according to colorimetric analyses. Larsson et al. (2002) administered  $17\alpha$ -MT hormone to *P. reticulata* and reported that an intense coloration was observed after the 17<sup>th</sup> day and the difference was statistically significant. Similarly, it has been reported that the  $17\alpha$ -MT hormone increased pigmentation which has a positive impact on coloration in *B. splendens* and *Xiphophorus hellerii* (see Jessy and Warghese 1987). When the physical color analysis results of the Karsli et al. (2018) study on electric yellow cichlid were evaluated, it was determined that the best coloration was obtained in  $17\alpha$ -MT groups, similar to this study. In contrast to these studies, it was reported that  $17\alpha$ -MT supplementation for 28 days did not affect coloration in *Xiphophorus hellerii* (see Koshio et al. 1993; Yanong et al. 2006).

## Conclusion

In the presently reported study, the juveniles of emperor red cichlid (*A. nyassae*) which were supplemented with 100  $\text{mg} \cdot \text{kg}^{-1}$   $17\alpha$ -MT displayed the highest growth and weight gain. On the other hand, group  $E_2$  was found to be effective in sex reversal, similar to group  $17\alpha$ -MT. When the results of growth, coloration, sex reversal, and survival rate pa-

rameters are analyzed in this study, they were found to be important in the ease of marketing in the ornamental fish trade. Especially, skin coloration is the leading factor that determines the market value of ornamental fish. The discoloration and faded color in aquarium fish cause adverse effects on marketing. Aquarium fish cannot synthesize color pigments sufficiently in the culture environment and thus supplementation of synthetic or natural color through feed should be provided. When the results of growth, coloration,

sex reversal, and particularly survival rate are evaluated, the most effective hormone dose for emperor red cichlid fish was determined to be  $17\alpha$ -MT at  $50 \text{ mg} \cdot \text{kg}^{-1}$ .

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