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Research Article

Morphometric comparison of the growth curve in Nile tilapia (*Oreochromis niloticus*) sexually reverted to masculinized and feminized

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ABSTRACT. Aquaculture is an industry in development around the world that allows covering the nutritional demand with a high nutritional value. Within this industry, monosex crops of some species, such as the Nile tilapia (*Oreochromis niloticus*), stand out. In this study, the effect of the administration of 17α-ethinyl estradiol and 17α-methyltestosterone on the morphometry of Nile tilapia at different times was evaluated. Nile tilapia were raised under controlled conditions and divided into three groups: control: no hormone treatment; feminized: treated with 17α-ethynyl estradiol and masculinized: treated with 17α-methyltestosterone. The measurements were made at 5, 10, 15, 25, 35, 40, 50, 60 and 70 days after fertilization with the morphometric technique "truss protocol." The data were analyzed using a general linear model of repeated measurements and analysis of variance of comparison of means. The growth curve was made using the Gompertz logistic model. The results showed that the masculinized larva presented a higher growth rate and increased daily gain of weight 2.05^{-2} mg compared to the controls and feminized 1.67^{-3} mg and 7.57^{-4} mg, respectively. The sexual reversion of Nile tilapia with 17 α-methyltestosterone increases the growth curve of the masculinized organism and the uniformity of the final size.

Keywords: *Oreochromis niloticus*; morphometry; sexual reversal; 17 α-ethynyl estradiol; 17 α-methyltestosterone

INTRODUCTION

Aquaculture participates in the human diet with 59.4 million t worldwide, where Mexico contributes with 1.1% of the fishing and aquaculture production (Hasan & Halwart, 2009). Currently, there has been an increase in interest in the cultivation of masculinized Nile tilapia *Oreochromis niloticus* (El-Greisy & El-Gamal, 2012; Megbowon & Mojekwu, 2013), mainly due to its accelerated growth, good adaptation to different environmental conditions and its high level of nutritional quality (Chakraborty *et al*., 2011; Hasheesh *et al*., 2011; Junior *et al*., 2012). However, the traditional crop of tilapia, constituted mainly by heterosexual organisms, has some drawbacks such as early maturation, a high percentage of fertility and frequent spawning (Minton, 2009) That increases the presence of juveniles and the variation in the size and weight of the fish at the time of harvest (Ezaz *et al*., 2004). These drawbacks impose a decrease in their economic productivity. Several strategies have been developed to avoid this, producing monosex crops mainly of males (Juárez-Juárez *et al*.,

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2017). In the cultivation of masculinized organisms, a high and homogeneous rate of body growth is maintained (Junior *et al*., 2012), avoiding variations in the size of the harvest and uncontrolled reproduction (Megbowon & Mojekwu, 2013).

There are several strategies to obtain monosex crops, among which are the classification by manual sexing, hybridization of organisms, chromosomal and genetic manipulations, in addition to sexual reversion through the administration of synthetic sex hormones (Chakraborty *et al*., 2011; Megbowon & Mojekwu, 2013). Of these procedures, the hormonal sexual reversion is the one that best adapts to the production of large-scale masculinized tilapia. The hormones commonly used are 19 norethyltestosterone, fluoxi testosterone, 17α-methyltestosterone (Ong *et al*., 2012), mesterelone, androstenedione, trenbolone acetate (Golan & Levavi-Sivan, 2014) and 17α-methyldihydrotestosterone (Passantino, 2012). The most frequently used hormone in this field is 17α-methyltestosterone (MT) due to its easy handling and high effectiveness (95 to 100% of the men's organism) (Celik *et al*., 2011; Hasheesh *et al*., 2011).

The purpose of this study was to review the effect of the administration of androgenic and estrogenic hormones on the morphometry of Nile tilapia crops.

MATERIALS AND METHODS

Animals

Five breeding pairs of *Oreochromis niloticus* from the Aquaculture Center (CONAPESCA) of Zacatepec Morelos were obtained; they were housed by sex in the Aquaculture Production Experimental Plant of the Autonomous Metropolitan University Campus Iztapalapa, in 250 L tanks. The organisms were maintained at a temperature between 28 and 30°C, pH of 6.8 to 7 in the water, oxygen concentration of 3.5 to 5 mg $L^{-1,}$ and a photoperiod of 14 light: 10 dark. The fish received commercial feed (Purina®) two times a day. The food presented 53% protein and 15% fat in a particle meal less than 0.35 mm. The pairs were chosen at random, to obtain gametes, and the spawning was performed manually, later the male and female gametes were incubated in a McDonald's chamber with a volume of 10 L of water for artificial insemination.

Treatments

Once the artificial fertilization was carried out, approximately 200 eggs were incubated in each treatment of *O. niloticus* in 80 L tanks where under the immersion technique they underwent the experimental treatments and were assigned to one of three experimental groups: control: without hormonal treatment; feminized: 200 μg L^{-1} of 17α-ethinyl estradiol (Sigma-Aldrich E4876) for 18 days to obtain 100% of the females (Wassermann & Afonso, 2003); masculinized: 1,800 μg L^{-1} of 17α-methyltestosterone (Sigma-Aldrich M7252) for 4 h to obtain 100% of the males (Gilling *et al*., 1996).

Morphometric measurements

The morphometric technique proposed by Strauss & Bookstein (1982) "truss protocol" was used, which consists of a system of measurements of vertical, horizontal and oblique distances between specific pre-selected anatomical points. These data are identified on selected morphological characters with which the body is divided into functional units. The measurements were done to 15 larvae per treatment at 5, 10, 15, 25, 35, 40, 50, 60 and 70 days after fertilization, using a total of 135 larvae in the study. The values obtained were the following, the total length that was measured from the head to the caudal fin, the standard length from the head to the furcation with an ichthyometer, the height of the individuals from the ventral fin to the dorsal fin and the total weight registered with an analytical balance.

Statistical analysis

The results were analyzed with the general linear model of repeated measures, an analysis of variance of comparison of means was performed. The normality and homogeneity of the variance were determined by the Kolmogorov-Smirnov test and a comparison analysis of Tukey's means ($P < 0.05$) was performed using the JMP version 9 program.

To evaluate the growth curve, the Gompertz logistic model was used, which is expressed by the following equation:

$$yt = A \exp^{[-B \exp(-Kt)]}$$

where yt: the weight of the organism at the end of the measured time, A: weight at maturity, B: integration parameter that does not correspond to a biological character, K: maturity index or the degree of maturity precocity of the individual, and t: time.

RESULTS

In this study, it was observed that the organisms masculinized with 17α-methyl testosterone have higher values of the morphometric measurements studied, concerning the feminized ones with 17α-ethynyl estradiol and controls. The effect of the treatment of each group on the morphometric measurements was compared against the control treatment. The total length of the masculinized fish was higher and statisti-

Table 1. Effect of the evaluated treatment on the total length in *Oreochromis niloticus*. n: 15 individuals per time; a: expresses the literal that shows the greatest growth in comparison with the letter b and c. Different letters between columns show significant differences ($P < 0.0001$).

Time (days)	Feminized (cm)	Masculinized (cm)	Control (cm)	Standard error	*P*-value
5	0.81 ± 0.13^{b}	1.08 ± 0.13^{a}	0.81 ± 0.19^{b}	0.03	<0.0001
10	0.82 ± 0.12^{c}	1.17 ± 0.17^{a}	1.02 ± 0.08^{b}	0.03	<0.0001
15	0.72 ± 0.09^{c}	1.30 ± 0.15^{a}	0.92 ± 0.16^{b}	0.04	<0.0001
25	0.9 ± 0.11^{b}	3.48 ± 0.44^{a}	1.24 ± 0.12^{b}	0.16	<0.0001
35	1.04 ± 0.12^{b}	3.85 ± 0.19^{a}	1.37 ± 0.20^{b}	0.16	<0.0001
40	1.10 ± 0.09^{c}	4.36 ± 0.20^{a}	1.3 ± 0.21^{b}	0.44	<0.0001
50	1.21 ± 0.09^{b}	4.52 ± 0.19^{a}	1.60 ± 0.20^{b}	0.19	<0.0001
60	1.31 ± 0.09^{b}	4.90 ± 0.19^{a}	1.7 ± 0.20^{b}	0.20	<0.0001
70	1.41 ± 0.09^{b}	5.40 ± 0.20^{a}	1.84 ± 0.20^{b}	0.20	<0.0001

Table 2. Effect of the evaluated treatment on the total pattern in *Oreochromis niloticus*. n: 15 individuals per time; a: expresses the literal that shows the greatest growth in comparison with the letter b and c. Different letters between columns show significant differences ($P < 0.0001$).

Time (days)	Feminized (cm)	Masculinized cm)	Control (cm)	Standard Error	*P*-value
5	0.52 ± 0.12^{b}	0.82 ± 0.12^{a}	0.56 ± 0.09^{b}	0.03	<0.0001
10	0.59 ± 0.12^{b}	0.92 ± 0.15^{a}	0.58 ± 0.11^{b}	0.04	<0.0001
15	0.514 ± 0.08^{c}	1.05 ± 0.14^{a}	0.69 ± 0.17^{b}	0.04	<0.0001
25	0.68 ± 0.10^{b}	3.12 ± 0.37^{a}	0.94 ± 0.11^{b}	0.13	<0.0001
35	0.79 ± 0.14^{b}	3.60 ± 0.20^{a}	1.13 ± 0.20^{b}	0.16	<0.0001
40	0.87 ± 0.12^{b}	3.89 ± 0.20^{a}	1.25 ± 0.20^{b}	0.17	<0.0001
50	0.91 ± 0.12^{b}	4.2 ± 0.20^{a}	1.3 ± 0.19^{b}	0.18	<0.0001
60	0.94 ± 0.12^{b}	4.48 ± 0.23^{a}	1.4 ± 0.19^{b}	0.2	<0.0001
70	0.97 ± 0.11^{b}	4.92 ± 0.23^{a}	1.61 ± 0.19^{b}	0.22	<0.0001

cally significant ($P < 0.001$) in all the times of measurement in comparison to the feminized organisms and those of the control treatment. On the contrary, the feminized organisms showed a decrease in the total length concerning controls and masculinized (23 and 75%, respectively) (Table 1). The effect of the treatment is evident after 15 days of age.

The total pattern shown in (Table 2) indicates that the masculinized organisms present a significant increase concerning the feminized organisms and the control treatment, giving the most outstanding effect after 25 days of age ($P < 0.001$).

The treatment with 17α-methyltestosterone increases the height of the organisms from day 25 in comparison to the control and feminized treatments ($P < 0.001$) (Table 3). The heights recorded in the masculinized organisms for all measurement times show significant differences ($P < 0.001$).

The response of the hormonal treatments on the growth rate shows significant differences ($P < 0.001$) in the masculinized organisms, which present a more significant growth in proportion to the organisms of the control treatment and the feminized ones; in addition, it is observed that these organisms present a higher daily weight gain (Table 4).

DISCUSSION

The results indicate that the hormone 17α-methyltestosterone induces a positive effect in the increase of the morphometric measurements in comparison to the control and feminized organisms, which is an aquaculture production that could increase the yield of the Nile tilapia *Oreochromis niloticus* crops, maintaining the uniformity of the sizes of the masculinized organisms. The results of Oponda *et al.* (2017), who worked with *O. niloticus*, agree with the results we present and support that morphometric behavior ratifies the relationship with the growth rate, depending on the genetic line. The sexual dimorphism that teleost fishes present, indicates a greater response in the growth of the masculine phenotype in masculini-

Table 3. Effect of evaluated treatment on height in *Oreochromis niloticus*. n: 15 individuals per time, a: expresses the literal that shows the greatest growth in comparison with the letter b and c. Different letters between columns show significant differences ($P < 0.0001$).

Time (days)	Feminized (cm)	Masculinized (cm)	Control (cm)	Standard error	*P*-value
5	0.12 ± 0.04^{c}	0.25 ± 0.05^{a}	0.17 ± 0.04^{b}	0.04	<0.0001
10	0.18 ± 0.03^{b}	0.33 ± 0.05^{a}	0.20 ± 0.02^{b}	0.01	<0.0001
15	0.21 ± 0.02^{c}	0.29 ± 0.04^{a}	0.24 ± 0.04^{b}	0.01	<0.0001
25	0.23 ± 0.25^{b}	0.91 ± 0.02^{a}	0.28 ± 0.04^{b}	0.04	<0.0001
35	0.26 ± 0.06^{c}	0.96 ± 0.07^{a}	0.44 ± 0.05^{b}	0.04	<0.0001
40	0.23 ± 0.06^{c}	1.11 ± 0.07^{a}	0.49 ± 0.07^{b}	0.05	<0.0001
50	0.31 ± 0.06^{b}	1.14 ± 0.07^{a}	0.50 ± 0.07^{c}	0.07	<0.0001
60	0.43 ± 0.06^{b}	1.25 ± 0.07^{a}	0.50 ± 0.07^{c}	0.81	<0.0001
70	0.46 ± 0.06^{b}	1.413 ± 0.07^{a}	0.52 ± 0.07^{b}	0.07	<0.0001

Table 4. The treatment effect on *Oreochromis niloticus* weight. n: 15 individuals per time; a: expresses the literal that shows the greatest growth in comparison with the letter b and c. Different letters between columns show significant differences ($P < 0.0001$). DGW: daily weight gain.

Treatment	Growth rate	Standard error	*P*-value	Initial weight (g)	Final weight (g)	DGW (g)
Feminized	0.46^{b}	0.43	0.2832	0.01 ± 0.01^{b}	0.05 ± 0.02^{b}	7.57^{E-04}
Masculinized	3.38^{a}	0.41	<0.0001	0.02 ± 0.03^{a}	1.43 ± 0.18^{a}	2.05^{E-02}
Control	0.48^{b}	0.41	0.2538	0.01 ± 0.04^{b}	0.11 ± 0.06^{b}	1.67^{E-03}

zed organisms with phenotype and genotype XX in comparison to other species of the same family of teleost fishes, as the case of the flounder fish (Kobelkowsky, 2004), depending very much on the environmental conditions in which the rearing of organisms is found (Santos *et al*., 2013). The studies conducted by de Oliveira *et al.* (2016), who evaluated five generations of Nile tilapia, show for each generation an increase in the growth rate of 4%, which markedly improved the daily weight gain, higher yield of the size and fillet obtained from these organisms. Nebo *et al*. (2013) reported an increase in the diameter of muscle fibers (30 and 40 μm), which indicated an increase in anabolism and that was reflected in muscle hypertrophy of fish treated with androgenic hormones in the juvenile stage, around 10 to 15 days of age after hormonal treatment. It has been reported that in some aquatic species such as rainbow trout (*Oncorhynchus mykiss*) and some salmonids (Salmonidae spp.), the muscle represents more than 50% of the total of the individual and exhibits indeterminate growth, unlike other vertebrates. Among the factors that control the production and maintenance of muscle fibers are the steroid hormones (Koganti *et al*., 2017). In fish, muscle tissue harbors some hormone receptors that regulate its metabolism, such as alpha and beta estrogen receptors that are activated by the presence of steroid hormones (Nagler *et al*., 2007; Koganti *et al*., 2017). In this study, the feminized organisms showed a delay in growth; previous studies claim that the presence of 17β-estradiol significantly increases sexual maturation in some aquatic species such as salmonids and rainbow trout. What increases metabolic processes of high energy demand such as follicular development (Lubzens *et al*., 2010); which leads to an increase in water content in the muscle (Davidson *et al*., 2014), increased protein degradation, and reduced muscle quality (Cleveland & Weber, 2011). It has also been observed in fish that 17β-estradiol decreases the levels of insulin-like growth factor 1 (IGF-1) (Hanson *et al*., 2014), unlike other species such as rodents, bovines and pigs in which estrogens have anabolic effects (Kamanga-Sollo *et al*., 2010). The IGF-1-GH (growth hormone) system participates in the regulation of growth, differentiation and maintenance of skeletal muscle homeostasis. IGFs stimulate both myoblast proliferation and differentiation (Duan *et al*., 2010). So if 17β-estradiol inhibits IGF-1-GH signaling, then skeletal muscle development and body growth will be affected (Norbeck & Sheridan, 2011; Lerner *et al*., 2012).

In some fish, the presence of 17β-estradiol and some androgens present a negative and positive impact, respectively, in terms of the expression of the genes involved in body growth (Hanson *et al*., 2014). These effects are mainly related to their signaling towards

somatotropin and in turn with IGF-1, which is suppressed by the proteolytic mechanisms that cause estrogen in muscle (Lerner *et al*., 2012). Although sex steroid production increases significantly during sexual maturation, fish may be exposed to natural steroids at juvenile age; the increase of these during the sexual maturation contributes to the decrease of the energetic reserves that, instead of being used for the corporal development, are used for the gonadal development (Cleveland & Weber, 2015). There is also the possibility that estrogens produce an increase in muscle protein degradation in these fish (Cleveland & Weber, 2011), since they increase the availability of amino acids during hormonal exposure; while with androgen treatment, anabolic processes are positive and help preserve muscle protein, in addition to positively regulating the expression of IGF-1, IGF-2 and IGF-5 in the muscle of these fish (Bower & Johnston, 2010).

CONCLUSIONS

The use of steroids in aquaculture for the sexual reversion of Nile tilapia *Oreochromis niloticus* favors the growth curve of the masculinized organisms and uniformity of size. However, it is important to know the molecular processes related to muscle development in the presence of steroid hormones.

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