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RESEARCH PAPER

Feminization by 17α-Ethinylestradiol of The Progeny of XY-Female Nile Tilapia (*Oreochromis niloticus*). Effects on Growth, Condition Factor and Gonadosomatic Index

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Abstract

Production of YY-males is one of the most important alternatives to the commercial use of hormones for producing monosex populations in the culture of Nile tilapia. One of the final stages of YY-technology is the feminization of the progeny of XY-females in order to obtain YY-females, which when crossed with YY-males makes possible to obtain populations composed of 100% YY-males. The aim of the study was to evaluate the effects of four concentrations (100, 200, 300, 400 mg kg^{-1}) of estrogen 17α-ethinylestradiol (EE_2) on the sex ratio, growth, condition factor (CV) and gonadosomatic index (GSI) of the progeny of XY-females. Significant differences were observed in wet weight, total length and CV during the experiment; however, final values in EE_2-treated groups showed not significant differences with control group. The observed proportion of females deviated significantly from the proportion of females expected in the progeny of XY-females in all groups fed EE_2; however, only 90% feminization was attained. The group fed 200 mg kg^{-1} of EE_2 showed a significantly higher value of GSI in comparison to that observed in the control group. By increasing the concentration of EE_2 it is possible to increase the proportion of females without affecting growth or GSI.

Keywords: Sex-reversal, YY-females, gonadal development, YY-males.

Introduction

In mixed-sex cultures, Nile tilapia (*Oreochromis niloticus*) (Linnaeus, 1758) reach sexual maturity long before reaching commercial size (Jiménez & Arredondo, 2000; Arboleda-Obregón, 2005). Therefore, in commercial farming of Nile tilapia, reproduction during grow-out is a major problem, leading to the presence of fry and juveniles that overpopulate ponds, and ultimately resulting in a wide range of fish sizes at harvest instead of the larger and more uniform fish expected from the original stocking (Mair, Abucay, Skibinski, & Beardmore, 1997; Tariq-Ezaz, Myers, Powell, Mcandrew, & Penman, 2004). To avoid this, a series of techniques have been developed to produce a monosex, all-male population in tilapia culture. Production of all-male populations eliminates sex behavior and therefore uncontrolled reproduction, allowing the production of marketable-sized fish (Varadaraj, 1989; Ponzoni, Hamzah, Tan, & Kamaruzzaman, 2005). Additionally, all-male tilapia cultures show better growth than mixed cultures, and for many years the production of all-male populations has been recognized as the most effective technique to increase tilapia production under commercial culture conditions (Mair *et al.*, 1997; Müller & Hörstgen 2007; Nonglak, Boonanuntanasarn, Jangprai, Yoshizaki, & Nanakorn, 2012).

Hormonal sex reversal during fry period is the most commonly used method to produce all-male populations. However, in recent years this method has been increasingly contested since there is a growing concern worldwide about the accumulation of hormones in the environment (especially in marine waters) and a growing number of consumers who do not want to eat products that have been treated with hormones (Piferrer, 2001; Müller & Hörstgen, 2007; Leet, Gall, & Sepúlveda, 2011) and demand environmentally friendly production techniques.

One strong alternative, on a commercial scale, is the production of YY-males, which when combined with normal females (XX) produce progenies composed, in theory, of 100% genetic males. The first step in YY-technology involves using hormones to feminize sexually undifferentiated male fry (XY), which allows to obtain XY-females (Vera-Cruz, Mair, & Marino, 1996; Mair *et al.*, 1997). These females will be used to produce YY-males in a percentage of 25% when combined with normal males (XY).

Production of YY-males in recent years at the

Universidad del Papaloapan has led to attempts to optimize YY-technology. One alternative for optimizing the obtention of YY-male Nile tilapia is the feminization of the progeny of XY-females. This feminization will allow the production of two genotypes; XY-females and females with two Y chromosomes (YY-females). The YY-females will be used to produce populations composed of 100% YY-males when crossed with YY-males (Figure 1).

Feminization in our laboratory of progeny of normal females (XX) using the synthetic hormone 17α-ethinylestradiol (EE_2) has shown good results (>80%) in low concentrations. The application of EE_2 in higher concentrations could achieve successful feminization of the YY-genotype, which has been reported to be more difficult in comparison with the process for feminizing normal progeny (Abucay & Mair, 2004).

Material and Methods

Broodstock

XY-female and normal male (XY) Nile tilapia used in this research were produced at the Aquaculture Station of the Universidad del Papaloapan using broodstock from locally available strains obtained from the Centro Acuicola de Temazcal, Oaxaca, México and the Granja Unidad de Producción del Tesechoacan, Veracruz, Mexico. XY-females were produced by feeding fry at swim-up stage with 120 mg kg^{-1} 17β-estradiol-treated feed (53% protein) for 30 days. They were reared for 18 months in the aquaculture station of the Universidad del Papaloapan and fed twice a day with commercial pellets (32% protein, Nutripec, Agribrands Purina, Irapuato Gto. Mexico).

Hormonal Treatment

The synthetic hormone 17α-ethinylestradiol (EE_2, Sigma Aldrich Chemical Co., St Louis, MO, USA) was added to commercial fish meal (<0.35 mm, 53% protein, 15% lipids) using the method described by Guerrero (1975). In brief, selected concentrations of EE_2 were dissolved in 500 ml of 95% ethanol, sprayed over the food and maintained at room temperature for six hours to allow the alcohol to evaporate. Four levels of EE_2 hormone treatments were added to the food: 100, 200, 300 and 400 mg kg^{-1}. The control group's food was handled in exactly the same manner with the exception of the added hormone.

Fry Production and Experimental Conditions

O. niloticus broodstock were distributed at a male: XY-female ratio of 1:3 (Marín-Ramírez, Alcántar-Vázquez, Antonio-Estrada, Moreno-de la Torre, & Calzada-Ruiz, 2016) in two 3-m diameter outdoor concrete tanks (28-30ºC) supplied with fertilized water. Fry were collected 15 d later with a fine-mesh net after 90% of the water in the tanks had been siphoned. The recently hatched and sexually undifferentiated fry (~0.02 g wet weight and 8 mm length) were pooled, transported to a closed recirculating system and randomly divided into 15 acrylic aquaria of 85-L (three aquaria per treatment) at an initial stocking density of 1 fry L^{-1}. The water in the recirculating system was filtered using a mechanical filter (Hayward, Model S310T2, Hayward Pool Products Inc., Elizabeth, NJ, USA) and a bio-filter containing only plastic bio-balls (Aquatic Eco-System, Model CBB1, Pentair Ltd. Apopka, FL, USA).

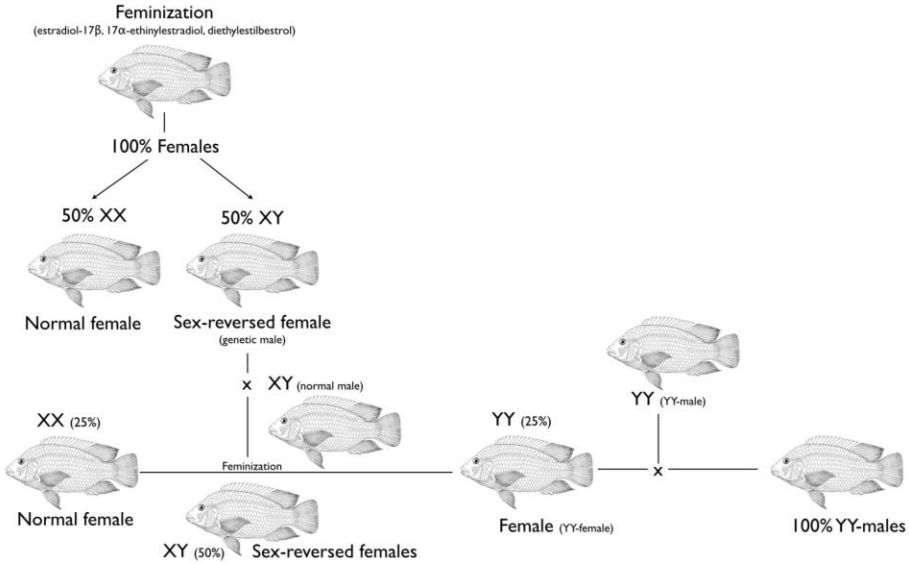

Figure 1. Diagram that represents the methodology applied to obtain XY-females and YY-females by feminization of XY and YY-genotypes. With information from Mair *et al.* (1997).

Hormonal treatment lasted for 20 d under a photoperiod of 12 L: 12 D and with water temperature adjusted thermostatically at 26.0±0.5 ºC. Fry were fed at 1-h intervals at a feed ratio of 20% of their total body weight. Water flow was closed in all aquaria for 20 minutes after the estrogen-enriched feeds were offered in order to encourage feeding. Random samples of 25% of fry per replicate were collected every 10 d. Mean wet weight was obtained using a digital scale (±0.01), and total length was recorded to the nearest 0.01 mm from a digitized image using an imaging software (ImageJ version 1.36). Once the hormonal treatment was completed, the fry were fed with untreated commercial diet (50% protein) for 10 more days until the fry period was finished. Water temperature and dissolved oxygen were monitored daily using a multiparameter display system (YSI model 655, Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA). The aquaria were siphoned daily to remove feces and dead fry.

At the end of the fry period, all juveniles from each treatment were counted, weighed and measured for the calculation of survival rate and to determine average weight and length. Juveniles were then transferred to 3-m diameter outdoor concrete tanks (maintaining replicates per treatment) supplied with fertilized water and reared to sexual maturity (four months of age). During this period, juveniles were fed six times a day with untreated commercial diet (44 and 40% protein) for approximately 30 d, followed by a commercial diet at 35% protein three times a day for another 30 d and subsequently a commercial diet with 25% protein until the end of the experiment. Total length and wet weight were registered every 21 d for 60 random fish per treatment (20 fish per replicate).

Fulton´s Condition Factor

The value of K was calculated throughout the experiment (at each sampling procedure) following Froese (2006):

$$K = 100 * [\text{fish weight (g)} / \text{fish length (cm)}^3]$$

Evaluation of Sex Ratio

The sex of 60 fish per treatment (20 fish per each replicate) was determined by removing the gonad. Gonads were classified as ovaries or testes.

Gonadosomatic Index

The extracted gonads were weighed using a digital scale (±0.01) to calculate the gonadosomatic index (Sturm, 1978) using the following formula:

$$GSI = [\text{Gonad weight (g)}/\text{Fish weight (g)}]\times 100.$$

Statistics

Differences in total length, wet weight and condition factor were analyzed using a one-way analysis of variance. The percentage of survival was arcsine transformed and analyzed using a one-way analysis of variance. The proportion of females identified in each treatment was tested against the 3:1 (male: female) expectation using a chi square test at a probability of 0.1% ($P<0.001$). Differences in gonadosomatic index between different treatments were analyzed using a Kruskal-Wallis nonparametric analysis (Zar, 1984).

Results

Fry Period

Mean wet weight, total length, condition factor and the percentage of survival of control group fry and EE_2-treated fry is shown in Table 1. Significant differences ($P<0.05$) were observed in wet weight and total length at 10 and 20 d of age. At 10 d of age wet

Table 1. Wet weight (WW), total length (TL), condition factor (CF) and percentage of survival (S) (±S.E.) (n=60 fish) of Nile tilapia (*Oreochromis niloticus*) fed different concentrations of 17α-ethinylestradiol for 20 days

	Hormone concentration (mg kg^{-1})				
Days	Control	100	200	300	400
0					
WW	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}
TL	0.80±0.01^{a}	0.80±0.01^{a}	0.80±0.01^{a}	0.80±0.01^{a}	0.80±0.01^{a}
CF	3.90±0.01^{a}	3.90±0.01^{a}	3.90±0.01^{a}	3.90±0.01^{a}	3.90±0.01^{a}
10					
WW	0.07±0.01^{c}	0.09±0.02^{a}	0.05±0.01^{d}	0.06±0.01^{c}	0.08±0.01^{b}
TL	1.21±0.03^{ab}	1.23±0.04^{ab}	1.16±0.04^{b}	1.20±0.04^{ab}	1.27±0.03^{a}
CF	4.28±0.10^{a}	5.00±0.12^{a}	3.20±0.12^{c}	3.48±0.09^{bc}	3.83±0.11^{b}
20					
WW	0.51±0.01^{b}	0.49±0.02^{bc}	0.47±0.01^{cd}	0.48±0.01^{bc}	0.64±0.02^{a}
TL	2.27±0.05^{b}	2.12±0.07^{c}	2.08±0.07^{c}	2.12±0.06^{c}	2.34±0.05^{a}
CF	7.48±0.10^{a}	5.20±0.11^{b}	5.22±0.09^{b}	5.11±0.12^{b}	5.00±0.11^{b}
S (%)	99.4±0.3^{a}	96.1±1.1^{b}	91.3±0.8^{c}	95.6±1.3^{b}	97.6±0.9^{b}

Percentage of survival significantly different from the control group $P<0.05$. Values in each row superscripted with different letters indicate significant differences between doses ($P<0.05$).

weight was significantly higher (P<0.05) in the concentration of 100 mg kg^{-1}, followed by the concentration of 400 mg kg^{-1}, while at 20 d of age the concentration of 400 mg kg^{-1} registered a significantly higher (P<0.05) value of wet weight, followed by the control group. A significantly higher (P<0.05) value of total length was shown for the concentration of 400 mg kg^{-1} at 10 and 20 d of age. Significantly lower (P<0.05) values of wet weight and total length were observed in the concentration of 200 mg kg^{-1} at 10 and 20 d of age.

Fulton´s condition factor was significantly higher (P<0.05) at 10 d of age in the control group and the concentration of 100 mg kg^{-1}. A significantly lower (P<0.05) condition factor was shown for the concentration of 200 mg kg^{-1}. At 20 d of age, the control group showed a significantly higher (P<0.05) condition factor in comparison to the EE_2-treated groups. No significant differences were observed between any of the EE_2-treated groups.

The percentage of survival at the end of the hormonal treatment was significantly higher (P<0.05) in the control group compared to the EE_2-treated groups. Between EE_2-treated groups, a significantly lower (P<0.05) percentage of survival was detected in the concentration of 200 mg Kg^{-1}.

Sex Ratio and Gonadosomatic Index

Sex-reversed females were produced in all EE_2-treated groups. The results showed that all concentrations of EE_2 produced progeny with a significantly higher (P<0.001) proportion of females than the 3:1 (male: female) sex ratio predicted for XY-females (Table 2). The proportion of females was higher in the concentration of 400 mg kg^{-1}; however, it was not possible to obtain a progeny composed of 100% females (Table 2). Significant differences (P<0.05) were observed in the gonadosomatic index, with the fish of the concentration of 200 mg kg^{-1} showing a significantly higher (P<0.05) value than the fish of the control group. No significant differences were observed between the fish of the EE_2-treated groups (Table 2).

Survival and Growth

Final survival was significantly lower (P<0.05) in the EE_2-treated groups than in the control group (Table 2). There were significant differences in wet weight and total length between groups throughout the entire post-treatment stage of the experiment (Table 3). A significantly lower (P<0.05) wet weight was observed in the concentration of 200 mg kg^{-1} at 41 and 62 d of age compared to the control group and the rest of the EE_2-treated groups. At 83 and 104 d of age, significantly higher (P<0.05) values of wet weight were registered in the fish of the control group and the concentrations of 400 and 100 mg kg^{-1}, respectively. At the end of the experiment (125 d of age), no significant differences were observed between the control group and all the EE_2-treated groups. However, a significantly lower (P<0.05) wet weight value was observed in the fish of the concentration of 400 mg kg^{-1} compared to the concentration of 100 mg kg^{-1}.

Total length was significantly higher (P<0.05) in the fish of the control group at 41 and 62 d of age compared to all the EE_2-treated groups. Between EE_2-treated groups, the concentration of 200 mg kg^{-1} registered significantly lower (P<0.05) values of total length. At 83 and 104 d of age, no significant differences were observed between the control group and the concentrations of 100, 300 and 400 mg kg^{-1}. The fish of the concentration of 200 mg kg^{-1} showed a significantly lower (P<0.05) value of total length. At the end of the experiment, similar to the findings for wet weight, no significant differences were observed between the fish of the control group and those of the EE_2-treated groups. However, a significantly lower (P<0.05) value of total length was observed in the group that received the highest concentration of EE_2 (400 mg kg^{-1}) in comparison to the group that received the lowest concentration of EE_2 (100 mg kg^{-1}).

Fulton´s Condition Factor

Significant differences were observed in the condition factor at 41 d of age, with a significantly lower (P<0.05) value in the control group in comparison to the EE_2-treated groups. Between the groups fed EE_2, a significantly lower condition factor was registered for the concentration of 200 mg kg^{-1}. At 62 d of age, a significantly higher (P<0.05) condition factor was observed in the concentration of 200 mg kg^{-1} compared to the control group and the

Table 2. Percentage of survival, percentage of males and females, and gonadosomatic index (GSI) of four-months-old Nile tilapia (*Oreochromis niloticus*) fed different concentrations of 17α-ethinylestradiol for 20 days

Hormone dosage (mg kg^{-1})	NFE	Survival (%)	Male (%)	Female (%)	GSI
Control	60	99	81	19	0.94 ± 0.23^{b}
100	60	87*	21	79^{1}	1.41 ± 0.28^{ab}
200	60	72*	11	89^{1}	1.89 ± 0.27^{a}
300	60	82*	16	84^{1}	1.39 ± 0.21^{ab}
400	60	88*	10	90^{1}	1.11 ± 0.21^{ab}

NFE = Number of fish evaluated per treatment (20 fish per replicate). * Percentage of survival significantly different from the control group P<0.05. [1]Significantly different from the expected 3:1 distribution (P<0.001). Values in each column superscripted with different letters significant differences between doses (P<0.05).

Table 3. Wet weight (WW), total length (TL) and condition factor (CV) (± S.E.) (n = 60 fish) at four-months-old of Nile tilapia (*Oreochromis niloticus*) fed with 17α-ethinylestradiol at different concentrations for 20 days

	Hormone concentration (mg kg^{-1})				
Days	Control	100	200	300	400
41					
WW	7.35±0.12^{a}	6.12±0.22^{a}	4.17±0.25^{b}	5.15±0.14^{a}	7.12±0.19^{a}
TL	4.89±0.07^{a}	4.16±0.10^{c}	3.79±0.12^{d}	3.93±0.11^{c}	4.33±0.11^{bc}
CF	6.28±0.10^{c}	8.50±0.15^{a}	7.66±0.18^{b}	8.48±0.13^{a}	8.77±0.11^{a}
62					
WW	13.30±0.45^{a}	11.82±0.55^{a}	8.79±0.55^{b}	11.45±0.55^{a}	12.19±0.54^{a}
TL	8.80±0.12^{a}	8.03±0.22^{db}	7.02±0.13^{e}	8.29±0.16^{cb}	8.18±0.18^{b}
CF	1.95±0.10^{b}	2.28±0.14^{ab}	2.54±0.09^{a}	2.00±0.11^{b}	2.22±0.14^{ab}
83					
WW	40.1±1.56^{a}	34.78±1.59^{ab}	30.89±1.34^{b}	32.72±1.37^{b}	36.29±1.51^{a}
TL	12.30±0.16^{a}	11.65±0.18^{a}	11.39±0.17^{b}	11.87±0.16^{a}	12.01±0.16^{a}
CF	2.09±0.10^{a}	2.08±0.09^{a}	2.02±0.07^{a}	1.88±0.09^{b}	2.12±0.08^{a}
104					
WW	52.43±2.58^{a}	50.63±0.02^{a}	39.89±2.92^{b}	48.38±2.48^{ab}	44.68±2.53^{b}
TL	13.35±0.81^{a}	13.10±0.31^{a}	11.85±0.37^{b}	13.15±0.26^{a}	12.33±0.34^{ab}
CF	1.66±0.12^{b}	2.07±0.9^{a}	2.08±0.10^{a}	2.09±0.11^{a}	2.15±0.15^{a}
125					
WW	82.85±2.26^{ab}	89.10±3.03^{a}	82.96±2.40^{ab}	80.66±2.40^{ab}	78.02±2.26^{b}
TL	15.93±0.15^{ab}	15.94±0.20^{a}	15.45±0.22^{ab}	15.57±0.17^{ab}	15.21±0.15^{b}
CF	2.03±0.8^{a}	2.22±0.11^{a}	2.22±0.09^{a}	2.12±0.08^{a}	2.19±0.07^{a}

Values in each row superscripted with different letters indicate significant differences between doses ($P<0.05$).

concentration of 300 mg kg^{-1}. At 83 and 104 d of age, a significantly lower ($P<0.05$) condition factor was registered for the concentration of 300 mg kg^{-1} and the control group, respectively. Finally, at the end of the experiment no significant differences were observed between the control group and the EE_2-treated groups.

Discussion

Feminization of sexually undifferentiated fry is one of the critical stages of YY-technology since it ensures the production of XY-females (sex-reversed females) that will be used in the production of YY-males. Therefore, the feminization of the progeny of females with XY-genotype will allow us to achieve two goals simultaneously; to obtain new XY-females (product of feminizing normal males) and YY-females (product of feminizing YY-males) (Alcántar-Vázquez, Moreno-de la Torre, Calzada-Ruiz, & Antonio-Estrada, 2014). The YY-females, sometimes called neo-females, will allow us to obtain monosex populations composed of 100% YY-males when crossing them with YY-males. This is the final step in YY-technology, as it allows the obtention of a sufficient number of YY-males in order to form broodstocks that can be crossed with normal females (XX) to obtain populations composed of 100% genetic males for commercial purposes.

In this work, an increase in the proportion of females by increasing the concentration of EE_2 was observed. Hopkins, Shelton, & Engle (1979) report a similar increase in the proportion of female blue tilapia (*Oreochromis aureus*) (Steindachner, 1864) as the concentration of EE_2 increased. In the same species, Mélard (1995) achieved 100% females using a diet with 200 mg kg^{-1} of EE_2. In this experiment it was not possible to obtain a complete feminization, probably caused by the use of XY-females which normally produce a percentage of males of approximately 75% compared with 50% males produced normally by XX-females. Additionally, it has been reported that the feminization of YY-males (present in approximately 25% of the progeny of XY-females, Mair *et al.*, 1997) may be more complicated than the feminization of XY-males (Abucay & Mair, 2004). This is supported by the findings of Shved *et al.* (2007) and Lázaro-Velasco (2014) after using EE_2 in balanced progenies of Nile tilapia. Both experiments achieve higher feminization rates at 120 mg kg^{-1} (83%) and 125 mg kg^{-1} (86.5%) when compared to that observed in our work at similar concentrations of EE_2 applied in our experiment. Finally, Genotte, Mélard, D´Cotta, Baroiller, and Rougeot (2014) report in Nile tilapia no sex-reversal of YY-embryos exposed to 6500 µg L^{-1} of EE_2 for 4 h, whereas sex-reversal rates in XY-embryos ranged from 0-60% (2000 µg L^{-1}). However, in spite of the above, we cannot rule out that the sex ratios obtained could be explained by the interaction of the three components that, according to Baroiller, D'Cotta, Bezault, Wessel, and Hoerstgen-Schwark (2009) govern sex in the Nile tilapia; a complex genetic sex determination system with a major determinant locus (sex chromosomes XX/XY), some minor genetic factors (parental factors) and the influence of the temperature (environmental factors) during the fry period.

It is considered that estrogens, either natural or synthetic, do not have a positive effect on the growth of teleosts, unlike androgens which are masculinizing hormones (Johnstone, Simpson, & Walker, 1979; Piferrer, 2001). However, a decrease in growth has been reported in several species of fish, especially when the estrogen concentration is increased (Piferrer, 2001; Marín-Ramírez *et al*., 2016). Marín-Ramírez *et al*. (2016) reported a reduced growth after feeding Nile tilapia fry with different concentrations of diethylstilbestrol for 20 days. Using the same synthetic estrogen, Król, Poblocki, Bockenheimer, and Hliwa (2014) report in the European catfish *Silurus glanis* (Linnaeus, 1758) a decrease in growth, while the natural estrogen estradiol-17β does not cause such decrease in growth. However, an increase in growth has been observed when using synthetic (diethylstilbestrol) or natural (17β-estradiol) estrogens in several species (Varadaraj, 1989; Chiba, Iwatsukis, Hayami, & Yamauchi, 1993). In the present work, no differences in growth were observed between the control group and the groups fed with EE_2, except for the highest concentration (400 mg kg^{-1}). Although no significant differences were detected, it was observed that the group fed with the concentration of 100 mg kg^{-1} recorded a higher wet weight compared to the control group.

These results and those observed during the fry period indicate that in Nile tilapia the synthetic estrogen EE_2 probably has an anabolic effect at physiological level during the sex differentiation period, which allows to achieve a higher growth rate rather than reduced as normally seen in other fish species (Piferrer, 2001). This anabolic effect has been reported in other species of the genus *Oreochromis* when using another synthetic estrogen (Varadaraj, 1989). Herman and Kincaid (1988) indicate that different metabolic pathways may be involved in both cases. However, in our case this anabolic effect was opposite (inverted) in the two growth stages, with the highest growth observed in the 400 mg kg^{-1} concentration of EE_2 during the fry stage, while during the post-treatment part of the experiment the 100 mg kg^{-1} concentration of EE_2 had the best results.

The growth rate obtained in this work becomes more relevant when we take into account that in the control group the proportion of males, which in Nile tilapia are characterized as having a higher growth rate than females, was over 80%, while in the groups fed with EE_2 the proportion of males only reached a maximum of 21%. The sex-reversed females obtained do not showed a reduction in growth rate, which could be expected in XY-females, associated with an increase of the physiological cost of producing eggs rather than producing sperm, especially in genetic males that were sex-reversed to phenotypic females (Hayward & Gillooly, 2011; Geffroy & Bardonnet, 2016). In this regard, Hayward and Gillooly (2011) report that gamete biomass in females is approximately two to four orders of magnitude higher compared to males in several animal species, including fish. This growth rate was probably caused, as noted previously, by an anabolic effect of the EE_2. However, we cannot rule out two other possibilities, the first being that the relation between growth and sex ratio is established for Nile tilapia before rather that during the sex differentiation period as observed for Diaz, Ribas and Piferrer (2013) in *Dicentrarchus labrax* (Linneo, 1758), in which case the growth rates observed in the phenotypic females (sex-reversed females) would be in accordance to that of the genetic males originally presented in the population. The second possibility is that final growth, particularly the wet weight, could be affected by the GSI obtained since female Nile tilapia normally have greater GSI than males as a result of having heavier gonads.

Condition factors including Fulton´s condition factor are an accepted criterion to assess the well being or fitness of a fish (nutritional status), defined by the amount of energy possessed (available fat content) by that fish to carry on several functions, including growth and reproduction (Jones, Petrell, & Pauly, 1999; Neff & Cargnelly, 2004; Gupta, Haque, & Khan, 2012). The condition factor also can be used to assess physiological status under a potential stressor (Hoque, Yusoff, Law, & Syed, 1998) such as a feeding fry with a synthetic estrogen. In our work, condition factor was highly variable during the fry period and initial stages of the juvenile period (41 d of age). Weatherley (1972) suggests that this could be caused by fluctuations in metabolic balance, patterns of maturation and the state of fullness of the alimentary canal. However, during the time of hormonal treatment it was possible to observe in general a lower condition factor in the fish of the EE_2-treated groups compared to the fish of the control group. Hoque *et al*. (1998) mention that this decrease could be interpreted as depletion of energy reserves stored as liver glycogen or body fat. A reduction in food intake could provoke such effect, especially at early stages of development. This reduction has been observed in several sex-reversal experiments carried out in our laboratory. Once hormonal treatment finished and fish reached the onset of sexual maturation (62 d of age), condition factor remained above 2.0 for almost all groups until the end of the experiment. Similar values have been reported in normally growing, healthy Nile tilapia and their hybrids of similar weight and length (Little, 1989; Fish Breeding Association, 2003; El-Saidy & Gaber, 2005; Crab, Kochva, Verstraete, & Avnimelech, 2009; Gupta *et al*., 2012). These results indicate that once the hormonal treatment ends, a compensatory growth takes place, caused probably by an increase in food intake and accumulation of energy in the form of available fat. The fact that final growth was statistically similar for the control group and the groups fed EE_2 support this.

Piferrer (2001) mentions that the application of estrogen, either natural or synthetic, can adversely

affect survival in the vast majority of teleosts, especially if a certain threshold is exceeded. This is related to a number of factors, among which are: the species, the type of hormone, its concentration and the duration of the hormonal treatment. Marín-Ramírez *et al*. (2016) report a decrease in survival in groups fed with diethylstilbestrol for 20 days at concentrations from 100 to 400 mg kg^{-1}. However, Varadaraj (1989) in the Mozambique tilapia (*Oreochromis mossambicus*) (Peters, 1852) reported no significant mortality using high concentrations of diethylstilbestrol (100 mg g^{-1} to 1000 mg g^{-1}). The effect of short-term exposure to different levels of EE_2 on survival and other parameters was investigated by Andersen, Holbech, Gessbo, Norrgren, and Petersen (2003) in the zebrafish (*Danio rerio*) (Hamilton, 1822) and by Van Aerle, Pounds, Hutchinson, Maddix, and Tyler (2002) in the Chinese minnow (*Pimephales promelas*) (Rafinesque, 1820). In both experiments, with the exception of one of all treatments evaluated, no significant decrease in survival or the appearance of malformations was observed using different water concentrations of EE_2 ranking from 10 to 15.4 ng L^{-1}. In Nile tilapia, Rougeot, Kanfitine, Prignon, Genotte, and Mélard (2008) report a reduction of survival of embryos reared for five days in two concentrations of EE_2 (100 and 500 µg L^{-1}). Shved *et al*. (2009) mention that exposure to EE_2 during early development negatively affects the IGF (insulin-like growth factor) system in Nile tilapia immune organs and could potentially interfere with the antigen presentation capacity of the immune system, thereby altering the susceptibility to infections during growth and reducing survival. In our work, it was observed that in all EE_2-fed groups there was a lower survival rate than in the control group. However, increasing the concentration of EE_2 did not provoke a decrease in survival as was observed in other experiments using synthetic estrogens (Rougeot *et al*., 2008; Marín-Ramírez *et al*., 2016). This increase in mortality could be associated with the Nile tilapia´s impaired ability to fight infections, which could explain the higher mortality observed in all the groups fed with EE_2 compared to that of the control group.

GSI indicates the sexual maturity of the fish, which also serves as an indicator of the health and nutritional status (Dadzie & Wangila, 1980; Zeyl, Love, & Higgs, 2014; Marín-Ramírez *et al*., 2016). Several studies have shown that, based on the observed reduction in the GSI as well as the morphological and histological changes suffered by the gonads, continuous exposure to synthetic compounds will cause a decrease in gonadal development (Linderoth *et al*., 2006; Marchand, Pieterse, & Barnhoorn, 2008; Louiz, Ben-Attiab, & Ben-Hassinea, 2009).

In the present study, GSI of fish from the control group was lower in comparison to that observed in the groups fed different concentrations of EE_2. However, significant differences were obtained only when compared to the concentration of 200 mg Kg^{-1}. The fact that the GSI was higher in all EE_2-fed groups probably indicates that EE_2 at concentrations used had no negative effects at physiological or morphological level on the development of Nile tilapia gonads. GSI values obtained also illustrate the change from males to females in the EE_2-treated groups. In general, Nile tilapia females have heavier gonads than males. The higher GSI observed could be explained by the fact that in the control group the proportion of females was less than 20%, while in the groups fed EE_2 the proportion was slightly higher than 80%. Production of oocytes, especially large ones like those produced by female Nile tilapia, will result in a GSI higher than that obtained in sperm-producing males. This is important since YY-females produced during feminization with EE_2 are key players in the successful development of YY-males at commercial level. A YY-female with impaired gonadal growth will have a reduced probability of being selected as a breeder.

In the present study, although 100% feminization was not achieved using EE_2 in high concentrations, the proportion of females obtained (in addition to the GSI, growth and survival observed in the groups fed EE_2) ensures an adequate production of XY- and YY-females which will be used as breeders in the production at commercial levels of YY-male Nile tilapia developed at the Universidad del Papaloapan.

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References

Abucay, S.J. & Mair, G.C. (2004). Methods of identifying males with YY genotype in *Oreochromis niloticus* L. In R.B. Bolivar, G.C. Mair & K. Fitzsimmons (Eds.), *Proceedings of the 6th International Symposium on Tilapia in Aquaculture* (pp. 104-109). Manila, Philippines, BFAR-ATA., 808 pp.

Alcántar-Vázquez, J.P., Moreno-de La Torre, R., Calzada-Ruiz, D. & Antonio-Estrada, C. (2014). Production of YY-male of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) from atypical fish. *Latin American Journal of Aquatic Research*, 42, 644-648. http://103856/vol42-issue3-fulltext-21

Andersen, L., Holbech, H., Gessbo, A., Norrgren, L., & Petersen, G.I. (2003). Effects of exposure to 17alpha-ethinylestradiol during early development on sexual differentiation and induction of vitellogenin in

zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology Part C*, 134(3), 365-374. http://dx.doi.org/10.1016/S1532-0456(03)00006-1

Arboleda-Obregón, D.A. (2005). Reversión sexual de las tilapias rojas (*Oreochromis* Sp), una guía básica para el acuicultor. *Revista Electrónica de Veterinaria*, 6, 1-5.

Baroiller, J.F., D´Cotta, H., Bezault, E., Wessels S. & Hoerstgen-Schwark, G. (2009). Tilapia sex determination: where temperature and genetics meet. *Comparative Biochemistry and Physiology Part A*, 153, 30-38. http://10.1016/j.cbpa.2008.11.018

Chiba, H., Iwatsukis, K., Hayami, K. & Yamauchi, K. (1993). Effects of dietary estradiol-17β on feminization, growth and body composition in the Japanese eel (*Anguilla japonica*). *Comparative Biochemistry and Physiology Part A*, 106 (2), 367-371. http://10.1016/0300-9629(93)90527-B

Crab, R., Kochva, M., Verstraete, W. & Avnimelech, Y. (2009). Bio-flocs technology application in over-wintering of tilapia. *Aquacultural Engineering*, 40, 105-112. http://dx.doi.org/10.1016/j.aquaeng.2008.12.004

Dadzie, S. & Wangila, B.C.C. (1980). Reproductive biology, length-weight relationship and relative condition of pond raised *Tilapia zilli* (Gervais). *Journal of Fish Biology*, 17, 243-253. http://10.1111/j.1095-8649.1980.tb02758.x

El-Saidy, D.M.S., & Gaber, M.A.M. (2005). Effect of dietary protein levels and feeding rates on growth performance, production traits and body composition of Nile tilapia, *Oreochromis niloticus* (L.) cultured in concrete tanks. *Aquaculture research*, 36, 163-171. http://doi:10.1111/j.1365-2109.2004.01201.x

Fish Breeding Association. (2003). Technical Handbook 2003. Fish Breeding Association, Israel, 106 pp.

Froese, R. (2006). Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *Journal of Applied Ichthyology*, 22, 241-253. http:// 10.1111/j.1439-0426.2006.00805.x

Genotte, V., Mélard, C., D´Cotta, H., Baroiller, J.F. & Rougeot, C. (2014). The sensitive period for male-to-female sex reversal begins at the embryonic stage in the Nile tilapia and is associated with the sexual genotype. *Molecular Reproduction and Development*, 81(12), http://1146-1158. http://10.1002/mrd.22436.

Guerrero, R. (1975). Use of androgens for the production of all-male *tilapia aurea* (Steindachner). *Transaction of the American Fisheries Society*, 2, 342-348. http://dx.doi.org/10.1577/1548-8659(1975)104<342:UOAFTP>2.0.CO;2

Gupta, N., Haque, M.M. & Khan, M. (2012). Growth performance of tilapia fingerling in cage in ponds managed by *Adivasi* households: An assessment through length-weight relationship. *Journal of Bangladesh Agricultural University*, 10(1), 149-155. http://dx.doi.org/10.3329/jbau.v10i1.12107

Hayward, A. & Gillooly, J.F. (2011). The cost of sex: quantifying energetic investment in gamete production by males and females. *PLoS One* 6, e16557. http://dx.doi.org/10.1371/journal.pone.0016557

Herman, R.L. & Kincaid, H.L. (1988). Pathological effects of orally administered estradiol to rainbow trout. *Aquaculture*, 72, 165-172. http://doi:10.1016/0044-8486(88)90156-1

Hopkins, D.K., Shelton, L.W. & Engle, R.C. (1979). Estrogen sex-reversal of *Tilapia aurea*. *Aquaculture*, 18, 263-268. http://doi:10.1016/0044-8486(79)90017-6

Hoque, M.T., Yusoff, F.M., Law, A.T., & Syed, M.A. (1998). Effect of hydrogen sulphide on liver somatic index and Fulton's condition factor in *Mystus nemurus*. *Journal of Fish Biology*, 52, 23-30. http://10.1111/j.1095-8649.1998.tb01549.x

Jiménez, B.M.L., & Arredondo, F.J.L. (2000). Manual técnico para la reversión sexual de tilapia. D.F., México, UAM-Iztapalapa., 36 pp.

Johnstone, R., Simpson, T.H., & Walker, A.F. (1979). Sex reversal in salmonid culture: Part III. The production and performance of all female population of brook trout. *Aquaculture*, 18, 241-252. http://doi:10.1016/0044-8486(79)90015-2

Jones, R.E., Petrell, R.J. & Pauly, D. (1999). Using modified length-weight relationship to assess the condition of fish. *Aquacultural Engineering*, 20, 261-276. http://dx.doi.org/10.1016/S0144-8609(99)00020-5

Król, J., Poblocki, W., Bockenheimer, T. & Hliwa, P. (2014). Effect of diethylstilbestrol (DES) and 17β-estradiol (E2) on growth, survival and histological structure of the internal organs in juvenile European catfish *silurus glanis* (L.). *Aquaculture International*, 22, 53-62. http://10.1007/s10499-013-9664-3

Lázaro-Velasco, A.J. (2014). *Efecto de la temperatura y hormonas exógenas en el desarrollo de la tilapia del Nilo* (Bachelor Thesis). Instituto Tecnológico de la Cuenca del Papaloapan, Oaxaca, México.

Leet, K.J., Gall, E.H., & Sepúlveda, S.M. (2011). A review of studies on androgen and estrogen exposure in fish early life stages: effects on gene and hormonal control of sexual differentiation. *Journal of Applied Toxicology*, 31, 379-398. http://10.1002/jat.1682

Linderoth, M., Hansson, T., Liewenborg, B., Sundberg, H., Noaksson, E., Hanson, M., … Balk, L. (2006). Basic physiological biomarkers in adult female perch (*Perca fluviatilis*) in a chronically polluted gradient in the Stockholm recipient (Sweden). *Marine Pollution Bulletin*, 53, 437-450. http://dx.doi.org/10.1016/j.marpolbul.2006.02.007

Little, C.D. (1989). *An evaluation of strategies for production of Nile tilapia (Oreochromis niloticus* L.*) fry suitable for hormonal treatment* (PhD Thesis). University of Stirling, Stirling, Scotland.

Louiz, I., Ben-Attiab, M. & Ben-Hassinea, O. (2009). Gonadosomatic index and gonad histopathology of *Gobius niger* (Gobiidea, Teleost) from Bizerta lagoon (Tunisia): Evidence of reproduction disturbance. *Fisheries Research*, 100, 266-273. http://10.1016/j.fishres.2009.08.009

Mair, G.C., Abucay, J.S., Skibinski, D.F. & Beardmore, J.A. (1997). Genetic manipulation of sex ratio for the large-scale production of all-male tilapia, *Oreochromis niloticus*. *Canadian Journal of Fisheries and Aquatic Sciences*, 54, 396-404. http://10.1139/f96-282

Marchand, M.J., Pieterse, G.M. & Barnhoorn, I.J. (2008). Preliminary results on sperm motility and testicular histology of two feral fish species, *Oreochromis mossambicus* and *Clarias gariepinus*, from a currently DDT-sprayed area, South Africa. *Journal of Applied Ichthyology*, 24, 423-429. http://10.1111/j.1439-0426.2008.01141.x

Marín-Ramírez, J.A., Alcántar-Vázquez, J.P., Antonio-

Estrada, C., Moreno-de la Torre, R., & Calzada-Ruiz, D. (2016). Feminization of Nile tilapia *Oreochromis niloticus* (L.) by diethylstilbestrol. Growth and gonadosomatic index. *Ecosistemas y Recursos Agropecuarios*, 3(7), 51-61.

Mélard, C. (1995). Production of a high percentage of male offspring with 17α-ethynylestradiol sex-reversed *Oreochromis aureus*. I. Estrogen sex-reversal and production of F2 pseudofemales. *Aquaculture*, 130(1), 25-34. http://10.1016/0044-8486(94)00313-D

Müller, B.A., & Hörstgen, S.G. (2007). A YY-male *Oreochromis niloticus* strain developed from an exceptional mitotic gynogenetic male and growth performance testing of genetically all-male progenies. *Aquaculture Research*, 38, 773-775. http://10.1111/j.1365-2109.2007.01712.x

Neff, D, B., & Cargnelli, M.L. (2004). Relationships between condition factors, parasite load and paternity in bluegill sunfish, *Lepomis macrochirus*. *Environmental Biology of Fishes*, 71, 297-304. htpp://10.1007/s10641-004-1263-8

Nonglak, P., Boonanuntanasarn, S., Jangprai, A., Yoshizaki, G., & Nanakorn, U. (2012). Pubertal effects of 17α-methyltestosterone on GH-IGF-related genes of the hypothalamic-pituitary-liver-gonadal axis and other biological parameters in male, female and sex-reversed Nile tilapia. *General and Comparative Endocrinology*, 177, 278-292. htpp://10.1016/j.ygcen.2012.03.008

Piferrer, F. (2001). Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture*, 197, 229-281. http://dx.doi.org/10.1016/S0044-8486(01)00589-0

Ponzoni, R.W., Hamzah, A., Tan, S. & Kamaruzzaman, N. (2005). Genetic parameters and response to selection for live weight in the GIFT strain of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 247, 203-210. http://dx.doi.org/10.1016/j.aquaculture.2005.02.020

Rougeot, C., Kanfitine, S.Y., Prignon, C., Genotte, V. & Mélard, C. (2008). Early sex reversal during embryonic development in the Nile tilapia. In Team of reproduction of fish of INRA (Eds.), *Proceedings of the 8th International Symposium on Reproductive Physiology of Fish (ISRPF)* (pp. 104-105). Saint-Malo, France, INRA, 348 pp.

Shved, N., Berishvili, G., Häusermann, E., D'cotta, H., Baroiller, J.F. & Eppler, E. (2009). Challenge with 17α-ethinylestradiol (EE2) during early development persistently impairs growth, differentiation, and local expression of IGF-I and IGF-II in immune organs of tilapia. *Fish and Shellfish Immunology*, 26, 524-530. http://dx.doi.org/10.1016/j.fsi.2009.02.003

Sturm, G.M. de L. (1978). Aspects of the biology of *Scomberomorus maculatus* (Mitchill) in Trinidad. *Journal of Fish Biology*, 13, 155-172. http://10.1111/j.1095-8649.1978.tb03423.x

Tariq-Ezaz, M., Myers, J., Powell, S., Mcandrew, B. & Penman, D. (2004). Sex ratios in the progeny of androgenetic and gynogenetic YY male Nile tilapia, *Oreochromis niloticus* L. *Aquaculture*, 232, 205-214. http://dx.doi.org/10.1016/j.aquaculture.2003.08.001

Van Aerle, R., Pounds, N., Hutchinson, T.H., Maddix, S. & Tyler, C.R. (2002). Window of sensitivity for the estrogenic effects of ethinylestradiol in early life-stages of fathead minnow, *Pimephales promelas*. *Ecotoxicology*, 11(6), 423-434. http://10.1023/A:1021053217513

Varadaraj, K. (1989). Feminization of *Oreochromis mossambicus* by administration of diethylstilbestrol. *Aquaculture*, 80, 337-341. http://10.1016/0044-8486(89)90180-4

Vera-Cruz, M.E., Mair, C.G. & Marino, P.R. (1996). Feminization of genotypically YY Nile tilapia *Oreochromis niloticus* L. *Asian Fisheries Science*, 9, 161-167.

Weatherley, A.H. (1972). Growth and ecology of fish population. London, England. Academic Press., 293 pp.

Zar, J.H. (1984). Biostatistical analysis. New Jersey, USA, Prentice Hall., 718 pp.

Zeyl, N.J., Love, P.O., & Higgs, M.D. (2014). Evaluating gonadosomatic index as an estimator of reproductive condition in the invasive round goby, *Neogobius melanostomus*. *Journal of the Great Lakes Research*, 4(1), 164-171. http://dx.doi.org/10.1016/j.jglr.2013.12.004.