FEMINIZATION OF NILE TILAPIA *Oreochromis niloticus* (L.) BY DIETHYLSTILBESTROL GROWTH AND GONADOSOMATIC INDEX

Feminización de la tilapia del nilo *Oreochromis niloticus* (L.) mediante dietilestilbestrol. Crecimiento e índice gonadosomático

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ABSTRACT. Sex-reversal by exogenous hormones is the most common technique used to generate monosex populations of Nile tilapia (*Oreochromis niloticus*). However, this technique has provoked a negative perception in recent years. Because of this, alternative techniques have been developed, including the production of YY males. Although the final product (for sale) is not administered hormones, the first part of this technique still requires the feminization of XY fry by use of estrogens, including some of a synthetic nature, such as diethylstilbestrol (DES), an estrogen that has shown particularly excellent results in related species. The aim of this study was to evaluate the effect of increasing concentrations of DES (100, 200, 300, and 400 mg kg^{-1}) during the fry stage on the sex proportion, growth and gonadosomatic index (GSI) of Nile tilapia. The 400 mg kg^{-1} concentration was the one that produced the highest proportion of females (91 %). However, increasing the concentration of DES provided through diet does not guarantee a 100 % feminization rate. Additionally, the growth, survival and GSI, showed a significant decrease ($p < 0.05$) in all groups fed with DES compared to the control group. It is possible that the anabolic effect of DES observed in other species is not present in Nile tilapia.

Keywords: Anabolic effect, gonadal development, sex-reversal, survival

RESUMEN. La reversión sexual a través de hormonas exógenas es la técnica más usada para obtener poblaciones monosexo de tilapia del Nilo (*Oreochromis niloticus*). Sin embargo, esta técnica ha generado una percepción negativa en los últimos años. Debido a lo anterior, se han desarrollado técnicas alternativas incluyendo la producción de machos YY. Aunque con esta técnica el producto final (para vender) no recibe hormona, la primera parte de esta técnica aun requiere la feminización de alevines XY a través de estrógenos, incluyendo algunos de naturaleza sintética como el dietilestilbestrol (DES), el cual ha mostrado excelentes resultados en especies relacionadas. El objetivo del presente trabajo fue evaluar el efecto de concentraciones crecientes de DES (100, 200, 300, 400 mg kg^{-1}) durante el periodo de alevín en la proporción de sexos, crecimiento e índice gonadosomático (IGS) de la tilapia del Nilo. La concentración de 400 mg kg^{-1} fue la que arrojó la mayor proporción de hembras (91 %). Sin embargo, incrementar la concentración de DES proporcionada a través de la dieta no garantiza una feminización del 100 %. Adicionalmente, el crecimiento, la supervivencia y el IGS, mostraron un descenso significativo ($p < 0.05$) en todos los grupos alimentados con DES, en comparación con el grupo control. Es posible que el efecto anabólico del DES observado en otras especies no este presente en la tilapia del Nilo.

Palabras clave: Desarrollo gonadal, efecto anabólico, reversión sexual, supervivencia

INTRODUCTION

The Nile tilapia *Oreochromis niloticus* (L.) is one of the most economically important species worldwide in finfish aquaculture (Nonglak *et al*. 2012). 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 result in a wide range of fish sizes at harvest instead of the larger and more uniform fish expected from the original stocking (Mair *et al*. 1997, Tariq-Ezaz *et al*. 2004). Production of a monosex, all-male population in tilapia culture eliminates sex behavior and therefore uncontrolled reproduction, allowing the production of marketable-sized fish (Varadaraj 1989, Ponzoni *et al*. 2005). All-male tilapia show better growth than females, and for many years, the production of all-male tilapia has been recognized as the most effective technique to increase tilapia production (Mair *et al*. 1997, Müller and Hörstgen 2007, Nonglak *et al*. 2012).

Sex-reversal by feeding fry with different hormones is the most common method used to produce all-male populations. However, the use of this method is increasingly being criticized. The accumulation of hormones in wild populations of fish in their natural environment, as well as the increasing number of consumers that are interested in environmentally friendly production techniques and do not want to eat products that have been treated with hormones (Müller and Hörstgen 2007, Nguyen *et al*. 2007, Leet *et al*. 2011), have led to the search for alternative techniques for the production of all-male tilapia populations. One viable alternative, on a commercial scale, is the production of genetically male tilapia (GMT^{TM}) based on crosses between YY males and XX females (Mair *et al*. 1997, Müller and Hörstgen 2007).

The initial development in the production of YY males requires the feminization of the XY genotypes during their sexually undifferentiated stage and the identification of these newly created sex reversed females (XY females) through a progeny test (Mair *et al*. 1997). However, unlike masculinization where it is feasible to obtain close to 100 % males by feeding the fry with different hormones, feminization of genotypically XY *O. niloticus* has proven to be more complicated.

Although there have been numerous published attempts to optimize feminization by varying parameters such as hormone type, hormone Concentration, treatment start time, duration of treatment and stocking density, this method still has some limitations (Rosenstein and Hulata 1994, Piferrer 2001). Development in recent years of YY male technology at the Universidad del Papaloapan has led to attempts to optimize the feminization process. One alternative for optimizing feminization in Nile tilapia is the use of the synthetic hormone diethylstilbestrol (DES). Application of DES in high concentrations has been associated with improved growth and feminization rates of 100 % in related species (Varadaraj 1989).

The present work was undertaken (1) to determine whether high proportions of sexually undifferentiated XY *O. niloticus* fry could be sex reversed to females using DES and (2) to describe the effect of DES on the gonadosomatic index and growth after normal sex differentiation in cultivated tilapia.

MATERIALS AND METHODS

Area of study

The present work was conducted at the Universidad del Papaloapan, located in Loma Bonita, Oaxaca, Mexico, at the following coordinates: 18° 06' N and 95° 53' W, at a height of 30 m above sea level. The weather in this area is warm and humid, with abundant rain during the summer. The mean temperature and average annual rainfall is 25 °C and 1845.2 mm, respectively (FAM 2014).

Broodstock

The Nile tilapia broodstock used in this research were produced using breeders from the Centro Acuicola de Temazcal (Oaxaca, Mexico) and the Sistema Cooperativo Integral (Veracruz, Mexico). This broodstock has been reared for a year 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).

Preparation of hormonal treatment

The synthetic hormone diethylstilbestrol (DES, Sigma Aldrich Chemical Co., St Louis, MO, USA) was added to commercial fish meal (< 0.35 mm, 53 % protein, 15 % lipids) using the alcohol evaporation method described by Guerrero (1975). In short, DES was dissolved in 95 % ethanol, sprayed over the food and kept overnight at room temperature to allow the alcohol to evaporate. Four levels of DES hormone-treatments were added to the food: 100, 200, 300 and 400 mg kg^{-1}. The food for the control group was treated in exactly the same manner with the exclusion of the added hormone.

Fry production

The Nile tilapia broodstock were stocked at a ratio of 1:3 (male:female) in two 3 m diameter outdoor concrete tanks (28-30 °C) supplied with fertilized water. Fry were collected 14 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 ($\sim$ 0.2 g weight and 8 mm length) were pooled, transported to a closed system and randomly divided into 15 glass aquaria of 85 L at an initial stocking density of 1 fry L^{-1}. Each treatment was carried out in triplicate. During the period of treatment, a photoperiod of 12 L: 12 D was measured using an automatic timer, and water temperature was thermostatically controlled and adjusted at 25 $\pm$ 0.5 °C.

Experimental conditions

Fry were fed with the food supplemented with hormones for 20 d at 1 h intervals. The feed rate during the hormonal treatment was adjusted to 20 % of the total body weight. Random samples of 25 % of the fry per replicate were collected every 10 d. Mean wet weight was obtained using a digital scale ($\pm$ 0.01) and total length was recorded from a digitized image using a imaging software (ImageJ version 1.36). Once the hormonal treatment was completed, the fry were fed with untreated commercial diet containing 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 the juveniles from each treatment were counted, weighed and measured for the calculation of the survival rate and to determine the average weight and length. Juveniles were then transferred to outdoor 3 m diameter concrete tanks supplied with fertilized water and reared to sexual maturity (five months of age). During this period, the juveniles were fed six times a day with untreated commercial diet (44 and 40 % protein), followed by a commercial diet at 35 % protein three times a day and subsequently a commercial diet with 25 % protein until the end of the experiment. The water temperature and dissolved oxygen were monitored daily during all the post-treatment part of the experiment. Total length and wet weight were registered every 21 d for 60 random individuals per treatment.

Evaluation of sex ratio and gonadosomatic index

The sex of 60 fish per treatment was determined by external examination using methylene blue at 1 % to highlight the differences in the papilla structure. Each fish was classified as male or female. All fish were weighed, measured and sexed again by removing the gonad and the spermatic/ovarian ducts. Gonads were classified as ovaries or testes. The extracted gonads were weighed using a digital scale (± 0.01) to calculate the gonadosomatic index (Sturm 1978) using the following formula:

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

Statistics

Data were analyzed using statistical analysis software (Statistical version 7.0). Differences in total length and wet weight were analyzed using

a one-way analysis of variance. The percentage of survival during the fry stage was arcsine transformed and analyzed using a one-way analysis of variance. The proportion of females identified in each treatment was tested against the 1:1 expectation using a chi square test at a probability of 0.1 % ($p < 0.001$). Differences in the gonadosomatic index between the different treatments were arcsine transformed and analyzed using Kruskal-Wallis nonparametric analysis.

RESULTS

Fry

The mean wet weight and total length of the treated and control fry during the DES treatment is shown in Table 1. The wet weight and total length were significantly ($p < 0.05$) higher at 20 and 30 d of age in the control group than in the DES-treated groups (Table 1). In the DES-treated groups, the wet weight showed no significant differences, whereas the total length registered differences only at 30 d of age, with the concentration of 300 mg kg^{-1} showing a significantly ($p < 0.05$) larger length than that of the concentrations of 100 and 200 mg kg^{-1}. Survival at 30 d of age was significantly lower in the concentration of 100 and 200 mg kg^{-1} than in the concentrations of 300 and 400 mg kg^{-1} and the control group. No significant differences were detected between the control group and the concentrations of 300 and 400 mg kg^{-1} (Table 1).

Sex-reversed females were produced in all DES treatments. The results showed that only the concentrations of 200 and 400 mg kg^{-1} produced progeny with a significantly higher ($p < 0.001$) proportion of females than the predicted 1:1 sex ratio (Table 2). The proportion of females was higher in the concentrations of 100 and 300 mg kg^{-1} than that observed in progeny without hormonal treatment in our laboratory (54 to 58 %); however, no significant differences were detected from normal sex ratios (Table 2). Significant differences ($p < 0.05$) were observed in the gonadosomatic index, with the fish of the control group showing a significantly ($p < 0.05$) higher value than the fish of the DES-treated groups. No significant differences were observed among the fish of the DES-treated groups (Table 2).

Survival and growth

Final survival was significantly lower ($p < 0.05$) in the DES-treated groups than in the control group (Table 2). The mortality in the groups fed with DES was close to 50 %, except for the concentration of 300 mg kg^{-1}. Significant ($p < 0.05$) differences were observed in the post-treatment growth, with the fish of the control group being significantly heavier (Figure 1a) and larger (Figure 1b) than the fish in the DES-treated groups throughout this part of the experiment. In the groups fed with DES, the wet weight showed significant differences ($p < 0.05$) only at 51 and 72 d of age, with the concentration of 300 mg kg^{-1} showing a significantly higher value (Figure 1a) than that of the concentrations of 100 and 200 mg kg^{-1}. As observed with the wet weight, the total length also showed significant differences ($p < 0.05$) only in the early days of post-treatment culture, with the concentrations of 300 and 400 mg kg^{-1} showing higher values than those observed in the groups treated with concentrations of 100 and 200 mg kg^{-1} at 51, 72 and 93 d of age (Figure 1b).

DISCUSSION

The aim of most of sex-reversal treatments is to control the sex phenotype in fish species of commercial interest (Devlin and Nagahama 2002, Singh *et al.* 2012, Singh 2013). Over the years, these treatments have provided some physiological insight as to the regulation of sex differentiation by steroid hormones (Singh 2013). Currently, it is widely accepted that endogenously synthesized steroids play an important role in the gonadal sex differentiation of many fish species. Therefore, it is possible that exogenous steroid treatments can disrupt the natural differentiation process by overriding the normal developmental pattern of gene expressions and physiological regulations leading to sex reversion,

Table 1. Mean survival, wet weight (WW) and total length (TL) (± S.E.) (n = 3) of Nile tilapia (*Oreochromis niloticus*) fed different doses of diethylstilbestrol. Values in each row superscripted with different letters indicate significant differences between doses ($p < 0.05$).

	Hormone dosage (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
10					
WW	0.06 ± 0.01^a	0.05 ± 0.02^a	0.05 ± 0.01^a	0.05 ± 0.01^a	0.05 ± 0.01^a
TL	1.31 ± 0.02^a	1.27 ± 0.02^a	1.22 ± 0.01^a	1.24 ± 0.01^a	1.26 ± 0.01^a
20					
WW	0.22 ± 0.01^a	0.14 ± 0.02^b	0.14 ± 0.01^b	0.14 ± 0.02^b	0.15 ± 0.01^b
TL	2.25 ± 0.03^a	1.92 ± 0.03^b	1.84 ± 0.02^b	1.85 ± 0.02^b	1.95 ± 0.02^b
30					
WW	0.63 ± 0.01^a	0.26 ± 0.02^b	0.25 ± 0.01^b	0.31 ± 0.02^b	0.31 ± 0.02^b
TL	3.12 ± 0.05^a	2.36 ± 0.04^c	2.34 ± 0.03^c	2.56 ± 0.02^b	2.51 ± 0.03^{bc}
Survival (%)	95.5 ± 2.18^a	85.0 ± 0.60^b	76.7 ± 4.05^c	94.2 ± 1.76^a	92.9 ± 0.33^a

Table 2. Percentage of survival, percentage of females and gonadosomatic index (GSI) of Nile tilapia (*Oreochromis niloticus*) fed different doses of diethylstilbestrol. Data collected at the end of experiment (five months of age). NFE = Number of fish evaluated. Values in each column superscripted with different letters indicate significant differences between doses ($p < 0.05$).

			Sex proportion (%)		
Hormone dosage (mg kg^{-1})	NFE	Survival (%)	Male	Female	GSI
Control	60	92	61	39	1.52 ± 0.21^a
100	60	55*	38	62	0.74 ± 0.13^{ab}
200	60	50*	33	67**	0.39 ± 0.08^b
300	60	73*	36	64	0.35 ± 0.08^b
400	60	57*	9	91**	0.45 ± 0.08^b

Percentage of survival significantly different from the control group: *$P < 0.05$. **Significantly different from the expected 1:1 distribution ($p < 0.001$).

even after the initiation of the natural differentiation process (Devlin and Nagahama 2002).

Current trends in both the market and research being conducted show an interest toward decreasing the use of hormones during sex-reversal treatments that produce monosex populations for commercial purposes. One of the most important alternative techniques is the production of YY males (Mair *et al.* 1997). Although the first part of this technique requires the use of hormones to feminize XY fry to obtain XY females, the final product (for sale) is not administered hormones, which significantly reduces the concentration of hormones in farm effluents.

Feminization, therefore, is one of the critical stages of YY male technology. Production and identification of XY females ensures an adequate number of YY males (Mair *et al.* 1997). Hamdoon *et al.* (2013) reported an increase in the proportion of females of Nile tilapia as the concentration of DES increased. In our case, a similar increase was observed, with a higher proportion of females registered for the concentration of 400 mg kg^{-1}(91 %). However, as in previous reports for Nile tilapia (Potts and Phelps 1995, Hamdoon *et al.* 2013), 100 % females was not possible to attain using the different concentrations of DES.

The effectiveness of DES to feminize is strongly related to the biology of the particular species and the breeding techniques applied. Additionally, the interaction between genotypic factors (parental and sex determination factors) and the environment (especially temperature) could affect the rate of feminization obtained. These traits are complex and can have different degrees of interaction based on the genetic constitution and the relative

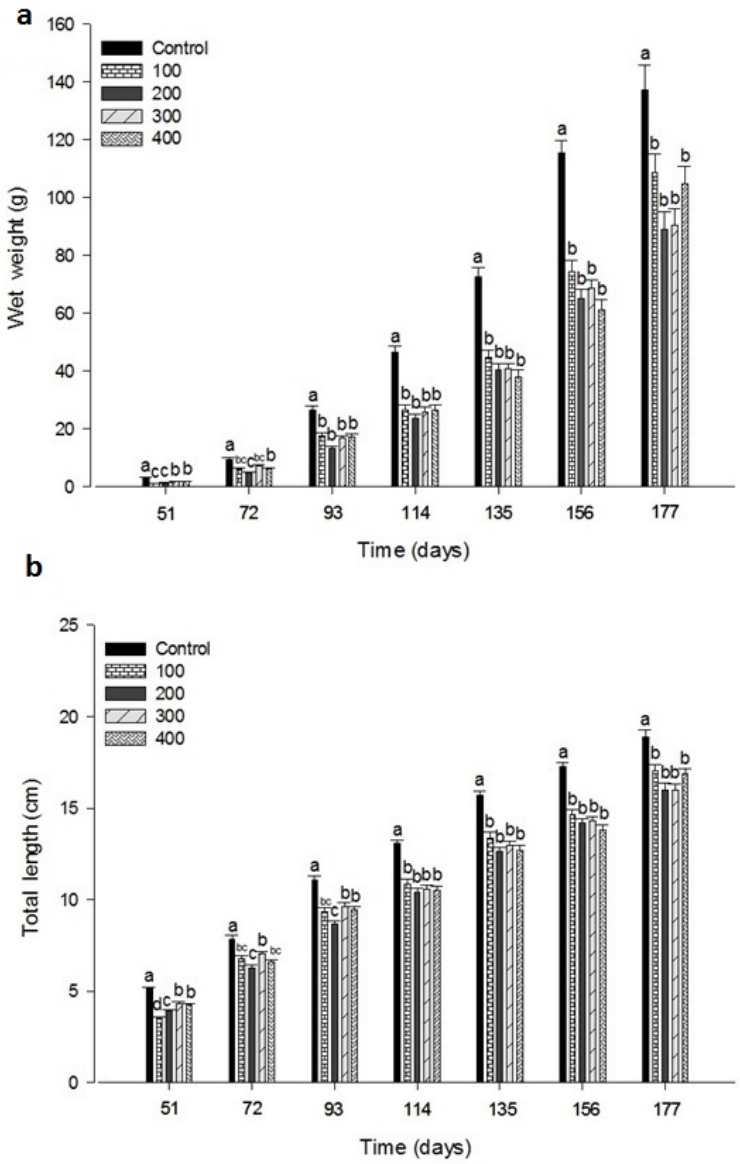

Figure 1. Growth at five months of age of Nile tilapia (*Oreochromis niloticus*) fed with diethylstilbestrol at different doses. (a) wet weight, (b) total length. The vertical bars represent the mean ± S.E. Values in each column superscripted with different letters indicate significant differences between doses ($p < 0.05$).

strength of the sexual factors in each species (Mylonas *et al.* 2005).

The proportion of females observed in the concentrations of 100, 200 and 300 mg kg^{-1} was lower than that observed in previous reports for Nile tilapia and related species at similar concentrations (Varadaraj 1989, Hamdoon *et al.* 2013). This could be related to low levels of hormone uptake or the interaction with environmental variables such as water temperature. However, a genetic parental effect could also be responsible for the low proportion of females observed using these concentrations of DES.

An important parental effect in Nile tilapia may be responsible, in accordance with the model of sex determination proposed by Baroiller *et al.* (2009), for the low proportion of females observed because of the lower parental sensitivity to feminization. It is possible that the females or males used as breeders could have displayed a genetic tendency to produce more males than females at normal rearing conditions. The low proportion of females observed in the control group (39 %) supports this. For Nile tilapia, Potts and Phelps (1995) report a proportion of females similar to that obtained in our experiment using 100 and 200 mg kg^{-1} of DES (> 60 %) but a lower proportion using 400 mg kg-1 of DES (80 %). On the other hand, for their study of Nile tilapia, Hamdoon *et al.* (2013) reported a proportion of females close to 90 % when using only 100 mg kg^{-1} of DES. These results support the idea that a parental genetic effect could be responsible for the proportion of females obtained due to a lower sensitivity to the hormonal treatment.

Although water temperature during hormonal treatments has been suggested as a factor responsible for deviations from expected sex ratios (Wang and Tsai 2000), in this experiment, water temperature during the fry stage (25 °C) was lower than that normally used in our laboratory (28-29 °C). Therefore, it is unlikely that the temperature could have influenced the proportion of females observed. Previous reports in Nile tilapia (Wessels and Hörstgen-Schwark 2011, Alcántar-Vázquez *et al.* 2014) have shown a significant deviation from the expected 1:1 sex ratio in both control and treated groups reared at 28 °C during a feminization or masculinization treatment.

Some natural and synthetic steroids have been shown to be growth promoters when administered at low concentrations. However, a decrease in growth induced by exposure to synthetic steroids has also been reported in several species of teleost, especially at high concentrations (Blázquez 2001, Piferrer 2001). The reduction in growth observed in the groups fed with DES compared to the control group is consistent with this. Although no significant differences were observed between the DES-treated groups, final weight and length were higher in the groups exposed to the lowest concentration of DES. A similar reduction in growth was observed by Hamdoon *et al.* (2013) after feeding fry of Nile tilapia with different concentrations of DES for 40 d.

In our case, the decrease in growth compared to the control group was observed in all groups fed with DES despite the increase in DES concentration. These results do not agree with those reported by Varadaraj (1989) in the Mozambique tilapia (*O. mossambicus*). In this case, the growth of DES-fed juveniles increased significantly compared to that of the control group as the concentration of DES increased up until a concentration equivalent to 500 mg kg^{-1}. This difference in growth could probably be caused by a differential physiological response to an anabolic effect of DES between species. It is possible that the anabolic effect of DES (originally observed in chickens and cattle) is present in the tilapia of Mozambique but not in the Nile tilapia. Herman and Kincaid (1988) have shown that different metabolic pathways may be responsible for this increase/decrease in growth. Additionally, Ridha and Lone (1995) reported that estrogens usually show no anabolic effect in most teleosts, so the Mozambique tilapia could be one of few species of fish responding to the anabolic effect of DES.

A decrease in growth has also been observed using DES in other freshwater species, such as the European catfish (*Silurus glanis*) (Król *et al.* 2014). In this case, the negative effect on growth was not observed when the natural estrogen estradiol-17β was used. Similar results have been observed in our laboratory in early trials comparing estradiol-17β, 17α-ethinylestradiol and DES at the same concentrations. Currently, estradiol-17β is the estrogen that shows better growth and survival than the other two synthetic hormones but results in a lower feminization rate. The Nile tilapia is probably more susceptible to synthetic estrogens whose effect at the physiological level is more powerful.

Piferrer (2001) reports that sex-reversal through estrogens (natural or synthetic) could pro-

voke adverse effects on survival. However, this depends on a number of factors including the type of estrogen, the concentration used, the timing and the duration of the hormonal treatment. The timing of optimum hormonal treatment is based on the timing of gonadal differentiation into ovaries and testes (Lin *et al*. 2012). Additionally, the sensitivity at the physiological level of the species determines the magnitude of the negative effects on survival, especially if a particular threshold is exceeded (Piferrer 2001, Lin *et al*. 2012). Hamdoon *et al*. (2013) reported a decrease in survival in Nile tilapia groups fed with DES for 40 d at two different concentrations, 50 and 100 mg kg^{-1}, whereas Varadaraj (1989) reported no significant mortality in the Mozambique tilapia when using DES in high concentrations (equivalent to 500 and 1000 mg kg^{-1}). In other freshwater species, Zhong *et al*. (2005) reported a reduction in survival in groups of Chinese minnow (*Gobiocypris rarus*) fed with DES at three different concentrations for 30 d. In this case, survival decreased close to 50 % as the concentration of DES increased. In our work, survival showed a similar decrease ($\sim$ 50 %) in the groups fed with DES to that of the control group; however, this reduction was high immediately after the application of lower concentrations of DES rather than decreasing gradually as the concentration of DES increased. This probably could have been caused by the inhibition of the endocrine (liver-derived) and autocrine/paracrine local insulin-like growth factor I system. Shved *et al*. (2009) mentions that estrogens have recently been shown to inhibit this system in several species of fish. The same author reported in tilapia that exposure to 17α-ethinylestradiol during early development distinctly affected the IGF system in tilapia immune organs and that this provokes interference with the antigen presentation capacity of the immune system, leading to an altered susceptibility to infections. Probably, DES, as well as 17α-ethinylestradiol, both synthetic estrogens, can provoke an increase in mortality associated with an impaired ability to fight infections in Nile tilapia. This could explain the high mortality observed in all the groups fed with DES compared to that of the control group.

The GSI is an indicator of sexual maturity of the fish and consequently of their health and nutritional status. Recent studies have shown that based on an observed reduction of GSI as well as morphological and histological changes undergone by the gonads, continuous exposure to synthetic compounds, including hormones, can induce a decrease in gonadal development (Linderoth *et al*. 2006, Marchand *et al*. 2008, Louiz *et al*. 2009).

In our work, gonadal examination of the fish fed with DES at different concentrations in comparison with the control group showed a significant reduction in the values of GSI and an increase in the proportion of alterations of gonadal structure. Reduction of the size of one of the two gonads or abnormal growth in one or both gonads was observed in approximately 20 % of all fish analyzed, especially the males. Similar results and abnormalities have been reported by those using DES in other freshwater species (Zhong *et al*. 2005, Paul-Prasanth *et al*. 2011). Piferrer (2001) mentioned that the presence of these abnormalities could be caused by the administration of estrogens through diet, especially at high concentrations. Song *et al*. (2014) reported negative effects in the GSI and serious atrophy of the gonads in several treatments in goldfish (*Carassius auratus*) using individual and binary mixtures of estrogens.

The negative effect of DES in the GSI observed in our experiment could have been the result of a direct effect on tissue development and gonadal structure. Haux and Norberg (1985) and Washburn *et al*. (1993) reported adverse effects during development on the functioning of the liver of fish treated with estrogen hormones. Because the liver and gonad work closely through the action of steroid hormones, poor development of the gonad in treated fish could have an origin in the effects of estrogens on the liver. It is probable that although the DES concentrations used did not produce all females, these concentrations can be considered high at the physiological level, provoking a negative effect on the gonadal development, especially at the morphological level and with regard to functionality.

Finally, Milnes *et al.* (2006) reported that exposure of males to estrogens can result in the reduction of testicular growth or testicular atrophy due to testicular lesions such as fibrosis and histological alterations. This could explain the high presence of abnormalities of the gonads, especially in males fed with DES.

CONCLUSIONS

Although the feminization rate was higher than in previous reports on Nile tilapia that were administered a high concentration of DES, reduction in gonadal development represents an important obstacle to the use of this synthetic hormone in future feminization treatments. The XY females produced during feminization treatments are key players in the successful development of YY males. An XY female with impaired gonadal growth has a reduced probability of being selected as a breeder. Alternative techniques of feminization need further investigation to assess whether DES has any possibility of being used in the development of Nile tilapia YY male technology.

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