

Effects of 17 β -estradiol Hormone on Growth and Sex Differentiation in the Mozambique Tilapia (*Oreochromis mossambicus*, Peters, 1852)

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ABSTRACT

In this study, the effects of 17- β -estradiol hormone on the zootechnic performances and sex ratio of Mozambique Tilapia (*Oreochromis mossambicus*, Peters, 1852) fry were investigated in order to initially produce functional phenotypical females and then super males. To this end, different E2 concentrations (50, 100, 150 and 200 ppm) were tested over a period of 45 days in a closed recirculating system. The evaluations showed that the female ratio was significantly (p value= 0.04 and α =0.05) higher in all treatment groups ranging from 61.90% to 86.36% compared to the control group. Growth was significantly higher (F value=22.78 and p value=0.00) in the control group compared to the treatment ones.

Keywords: 17 β -estradiol hormone, Mozambique tilapia, *Oreochromis mossambicus*, sex difference

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INTRODUCTION

Mozambique tilapia (*Oreochromis mossambicus*, Peters, 1852) in mixed-sex cultures is known to reach sexual maturity long before reaching commercial size (Jiménez & Arredondo, 2000; Arboleda-Obregón, 2005). Therefore, in commercial farming of Mozambique tilapia, reproduction during grow-out is a major problem, leading to the overpopulation of ponds, and finally resulting in a wide range of fish sizes at harvest. As a solution to this problem, several techniques have been developed to produce larger and uniform fishes including all-male production (Mair et al., 1997; Tariq-Ezaz et al., 2004). Production of a monosex population in a tilapia culture through hormone sex reversal eliminates uncontrolled reproduction and allows the production of marketable-sized fish (Shreck, 1974).

The initial development of a monosex population requires the feminization of the XY geno-

types during their sexually undifferentiated stage and the identification of the “neo-females” (females XY) through a progeny test (Mair et al., 1997). The feminization process is related to the fact that sex determination is under genetic control in Tilapias, but the ultimate differentiation of the gonads in fishes depends on endocrine signals, i.e. estrogens and androgens (Yamamoto, 1969; Arcand-Hoy and Benson, 1998; 1998; Uguz et al., 2003; Hayes, 2005). Indeed, the genetically prescribed sex in fishes (Atlantic cod *Gadus morhua* Nile tilapia *Oreochromis niloticus*, Honeycomb grouper *Epinephelus merra*) could easily be overridden by the applications of exogenous steroids if they are applied at the appropriate time and dose during early development (Shreck, 1974; Hunter and Donaldson, 1983; Uguz et al., 2003).

Even if successful feminization attempts have been conducted in the genus “*Oreochromis*” (Mair and Santiago, 1994; Rosenstein and Hula-

ta, 1994; Vera-Cruz et al., 1996; Piferrer, 2001), more often they concern exclusively the Nile Tilapia (*Oreochromis niloticus*). There is very little information available about varying parameters such as hormone dose, treatment start time, duration of treatment and stocking density in Mozambique tilapia (*Oreochromis mossambicus*). Additionally, the global effects of estradiol-17β (E2) on growth and sex-differentiation in this species have been studied in relation to the small number of publications related to this fish. Therefore, the present study was undertaken to determine the effects of E2 on the growth and the feminization of the gonads of sexually undifferentiated XY *O. mossambicus* fry. This is a preliminary step towards our ultimate objective of developing a breeding program to produce a male genetic population through supermale (YY) broodstock in EGE University in Western Turkey.

MATERIAL AND METHODS

Animal Culture Condition

This study was carried out in accordance with Guidelines for animal Care and Use of laboratory Animals over a period of five months (20 weeks). It took place in three phases and in three different breeding environments located in the experimental aquaculture unit of the Faculty of Aquaculture of EGE University in Turkey:

- First phase: Reproduction of broodstock maintained in the laboratory for four weeks in polyester tanks with a total volume of 200 Liters of water incorporated in a recirculation system at the ratio of one male to 3 females;
- Second phase: Sexual inversion of the larvae for 45 days in glass aquaria 30cm x 30cm x 30cm in size with a total volume of 27 lt;
- Third phase: Growth of fries for 12 weeks in a recirculating fresh water system at 28°C with UV filtration under a 14L: 10D photoperiod with a total volume of 80 L with artificial aeration and a daily water renewal rate of around 20%.

A total of 5 treatment groups (0, 50, 100, 150 and 200 ppm doses of 17β-estradiol hormone) with 3 replications including 17 larvae per replication were settled. The experimental groups formed are presented in Table 1.

Feeding

In the current experiment, the fish were treated with a dose of 50, 100, 150 and 200 ppm 17β-estradiol (E2) delivered within the feed. Hormones were given to the fry starting from the time they

began exogenous feeding for a period of 45 days. The appropriate amount of hormone (diluted in 95% ethanol) was added to the trout starter feed (Çamlı yem, Turkey) using the spray method. The fish were fed ad libitum. Control fish were fed without the hormone treatment.

Routine Measurements and Weighings

The sampling method was used in the measurement and weighing of the experimental fish. A 1 mm precision measuring board and an electronic scale of 0.01 g sensitivity were used to assess the total length (L) and weight (W) of the fish at the beginning of this trial and after every month by sampling all living fish for each experimental group. Live fish and total height measurements were taken back to their environment. As it was planned that the female individuals should be used first for the observation of YY males, sexing was performed by observing the external shape of gonads by the naked eye according to Goto et al., (1999). At the end of the trial, 10 fish were chosen randomly and cut for gonad examination.

Parameters Studied in the Research

Gonadosomatic index (GSI)

Calculation of the gonadosomatic index of the groups was made using the following formula (Halver, 1989; Hopher, 1990). $GSI = (Gonad\ weight\ (g) \times 100 / Fish\ weight\ (g))$

Daily Live Weight Gain (DLWG)

The growth rate of fish showing the daily live weight gain was determined according to the following formula (Wooten, 1990). $DLWG\ g / day = (Wt - W0) / t$ where

Wt: fish weight at the end of the trial (g),

W0: Fish weight per experiment,

T: Trial period (days)

Specific Growth Rate (SGR)

Specific growth rate SGR. was calculated according to the following equation (Hoşsu et al., 2003; De Silva and Anderson, 1995):

$SGR\ (\% \text{ body wt. gain/day}) =$

$$\left[\frac{\log_n \text{ Final fish wt.} - \log_n \text{ initial fish wt.}}{\text{Time interval}} \right] \times 100$$

Table 1. Experimental design of the trial

Trial duration	17β-estradiol hormone concentration (ppm)				
	Control group (0)	A group (50)	B group (100)	C group (150)	D group (200)
30 days (28 °C)	K1 (17)	A1 (17)	B1 (17)	C1 (17)	D1 (17)
	K2 (17)	A2 (17)	B2 (17)	C2 (17)	D2 (17)
	K3 (17)	A3 (17)	B3 (17)	C3 (17)	D3 (17)
Number per treatment	51	51	51	51	51
Total number of larvae			255		

Condition Factor (K)

The weight-length relation of Fulton, which was used to determine the health of fish, was calculated using the following formula (Ricker, 1975). $K = W.100 / L^3$

Feed conversion rate

FCR = Amount of feed consumed (g) / Live weight gain (g) (Santinha et al., 1999).

Survival Rate (SR)

The survival rate of fish is calculated by the formula reported by Pechsiri and Yakupitiyage (2005). $SR \% = (N_s / N_i) \times 100$.

Ns: Number of fish at the end of the trial; Ni: Number of fish at the beginning of the trial.

Statistical Analysis

All results presented in this study are expressed as the mean \pm SEM. All values were analyzed by the 1-way analysis of variance followed by Newman-Keuls multicomparison test to analyze the difference of means using SPSS 15.0 (SPSS, Chicago, IL) statistical software and ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Water Quality Parameters

Water quality parameters such as temperature and dissolved oxygen averages are given in table 2. Statistically no differences ($p > 0.05$) were observed between groups regarding the average temperature that ranged from 28.07 ± 0.18 to 28.25 ± 0.18 °C respectively in the Control and 50 ppm group. Inversely, statistical analysis showed significant differences related to the dissolved oxygen quantity between the experimental groups. However, all observed values (from 4.88 ± 0.19 to 5.90 ± 0.19) were above the recommended 3 ppm in the Tilapia rearing.

Female Ratio and Survival Rate

The sex ratios and survival rates obtained from the experimental groups at the end of the trial are given in Table 3.

The application of 17β-estradiol to *Oreochromis mossambicus* fry during 4 weeks post-hatching was found to be significantly ($P < 0.05$) successful for the sex-reversal in this species. The proportion of females was higher in hormone-fed fish groups than in control fish ($P < 0.05$). The female proportion of the control group (K') was almost equal to the proportion of males (51.52%). A significantly high proportion of females were obtained in the 150 ppm and 200 ppm groups - 76.60 % and 86.36 % respectively. Our studies clearly showed that the female proportion is dose-dependent.

When we come to the survival rates, statistical analysis showed a significant difference between groups ($P < 0.05$). Conversely, this parameter was higher in the treatment groups than in the control group (apart from the group of fish fed with 200 ppm hormonal feed). This was calculated as 80.39% and 64.71% respectively in the 50 and 100 ppm groups. There was no significant difference in fish mortality between 150 ppm and control groups ($p > 0.05$). The lowest survival rate was obtained in the 200 ppm group (43.14%) and allows us to suggest that high dose of 17β-estradiol is fatal for *O. mossambicus*.

Growth Performances

The average height and weight values obtained from the measurements and weighings during the test are shown in figure 1. A strong correlation was observed between weight and length in all treatment groups. The ANOVA analysis revealed significant ($p < 0.05$) differences regarding these two parameters.

The average weight of the Control group (25.87 ± 1.02 gr) was significantly higher than those of the treatment groups (< 0.05) and

Table 2. Water parameters during the experiment

Average	A (50 ppm)	B (100 ppm)	C (150 ppm)	D (200 ppm)	K (Control)	F	p
Temperature (°C)	28.22 ± 0.46 a	28.25 ± 0.52 a	28.20 ± 0.59 a	28.18 ± 0.45 a	28.07 ± 0.20 a	0.145	0.96
Diss. Oxygen (mg/g)	5.90 ± 0.35 a	5.63 ± 0.52 a	5.76 ± 0.517 a	4.88 ± 0.40 b	4.98 ± 0.68 b	5.96	0.00

Table 3. Female proportion and survival rate in *O. mossambicus* treated with E2

Treatment groups	Female proportion (%)	Survival rate (%)
A (50 ppm)	63.41 ^b	80.39 ^c
B (100 ppm)	61.90 ^b	82.35 ^c
C (150 ppm)	76.60 ^c	68.63 ^b
D (200 ppm)	86.36 ^d	43.14 ^a
K (Control group)	51.52 ^a	64.71 ^b
P value (X ² testi)	0.033	0.00

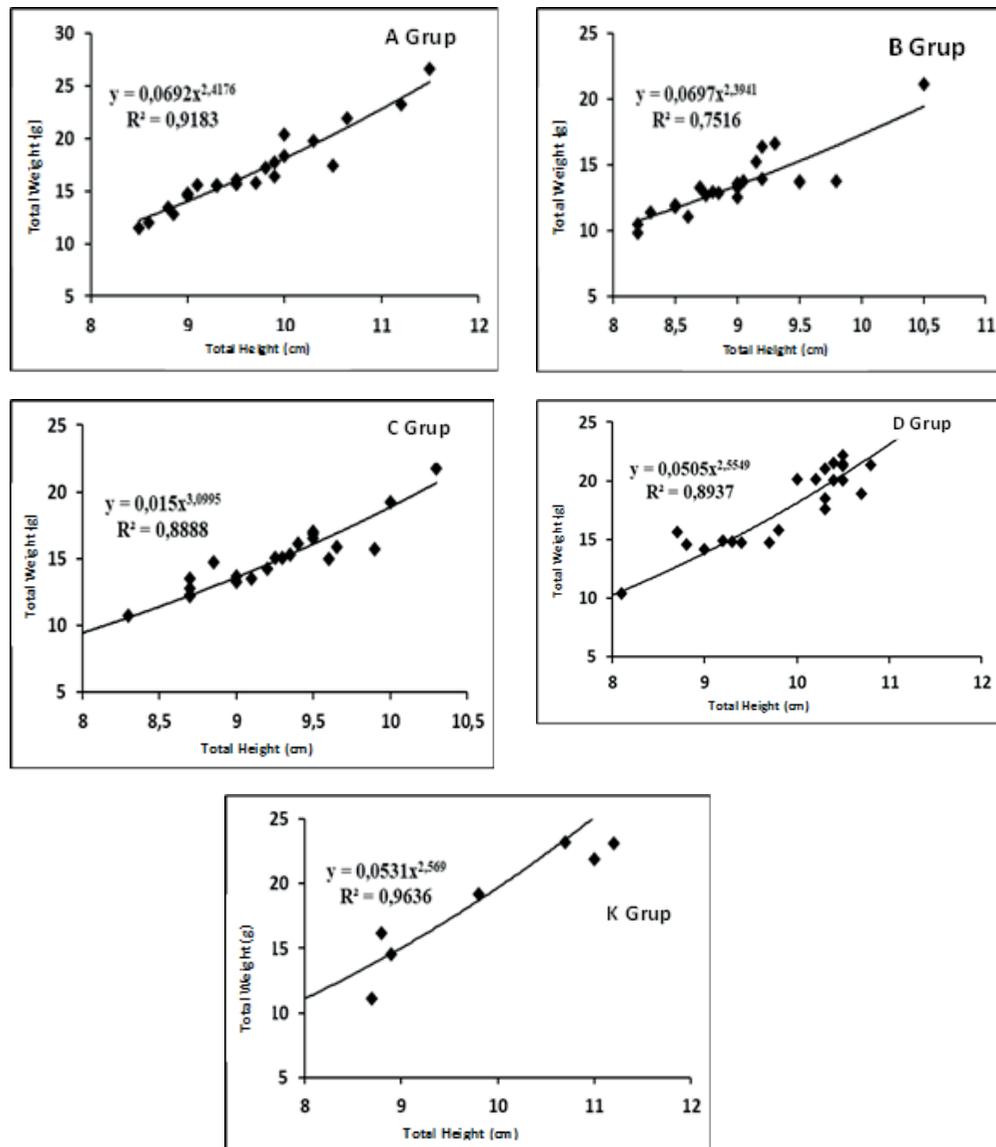


Figure 1. Weight and height relationship of treatment groups (n=7)

can be presented as follows: 13.44±1.02 gr for 100 ppm. 14.78±1.02 gr for 150 ppm. 16.65±1.02 gr for 50 ppm and finally 17.94±1.02 gr for 200 ppm groups. Therefore, it can be said that hormonal treatment with 17β-estradiol has an inhibitory effect on growth in Mozambique tilapia. The trend in average total length remained the same with a significantly ($p < 0.05$) higher value from the Control group (10.94±0.2 cm).

Table 4 shows the growth performances obtained in this experimentation. For all results, the corresponding values are significantly higher in the Control than in treatment groups ($p < 0.05$).

Feed Consumption

Figure 3 shows the variation of daily feed intake during the experimental period. Its analysis confirms the hegemony of the Control group over the others ($P < 0.05$). The highest daily feed intake was observed in this group. Feed consumption in the oth-

er experimental groups were relatively equal (Figure 2). Based on these results, it can be understood that hormonal treatment also diminishes feed intake (consumption) in *O. mossambicus*.

Meanwhile, there was no statistically significant difference ($P > 0.05$) between the groups in terms of condition factor (Figure 26). The condition factor values according to the groups are 1.80±0.04; 1.85±0.04; 1.88±0.04; 1.83±0.04 and 1.91±0.04 respectively for 50, 100, 150, 200 and 0 ppm (Control) groups.

The analysis of the FCR and FCE ratios revealed statistically significant differences between the values obtained in the different experimental groups ($p < 0.01$). However, it was determined that the values of the fish fed on a diet containing estradiol were better than those of the control group. In contrast, the results between the hormone treated groups weren't different from each other ($p > 0.05$). The lowest feed conversion ratio came from the

Table 4. Growth performances of *O. mossambicus* fed with feed containing different concentration of 17 β -estradiol (90 days)

Average	50 ppm	100 ppm	150 ppm	200 ppm	Control	F	p
Live weight (g)	16.6 \pm 3.79 bc	13.44 \pm 2.28 c	14.68 \pm 2.75 bc	17.84 \pm 4.34 b	25.77 \pm 8.87 a	22.78	0.00
Total Len. (cm)	9.77 \pm 0.83 b	8.97 \pm 0.52 c	9.20 \pm 0.54 b	9.88 \pm 0.95 b	10.94 \pm 1.6 a	14.85	0.00
DLWG	0.18 \pm 0.04 bc	0.15 \pm 0.03 c	0.16 \pm 0.03 bc	0.20 \pm 0.05 b	0.29 \pm 0.1 a	22.78	0.00
SGR	5.66 \pm 0.30 bc	5.43 \pm 0.18 c	5.53 \pm 0.22 bc	5.73 \pm 0.31 b	6.09 \pm 0.46 a	17.26	0.00
K	1.80 \pm 0.31 a	1.85 \pm 0.15 a	1.88 \pm 0.12 a	1.83 \pm 0.18 a	1.91 \pm 0.21 a	1.09	0.38
Feed cons. (g)	7.80 \pm 0.06 c	5.99 \pm 0.73 d	7.13 \pm 0.93 c	8.38 \pm 1.58 b	18.32 \pm 3.36 a	14.4	0.00
FCR	0.50 \pm 0.12 b	0.46 \pm 0.06 b	0.50 \pm 0.09 b	0.56 \pm 0.19 b	0.83 \pm 0.41 a	12.01	0.00
FCE	2.12 \pm 0.48 a	2.23 \pm 0.31 a	2.07 \pm 0.33 a	1.94 \pm 0.54 a	1.43 \pm 0.51 b	11.79	0.00

Table 5. Evaluation results of the trial group (8 months) (Different letters indicate that the difference between the groups is important ($p < 0.05$))

Treatment groups	A	B	C	D	Control	F Value	P Value
Body Weight (g)	57.5 \pm 10.5 a	47.4 \pm 17.8 a	52.5 \pm 19.0 a	57.6 \pm 16.2 a	58.8 \pm 16.2 a	0.75	0.56
Total Length (cm)	14.95 \pm 1.3 a	12.31 \pm 2.8 a	13.54 \pm 3.6 a	13.77 \pm 2.7 a	14.5 \pm 2.1 a	1.47	0.23
Condition Factor (F)	1.74 \pm 0.84 a	2.83 \pm 0.59 a	2.33 \pm 0.65 a	2.42 \pm 0.56 a	1.61 \pm 0.1 a	2.42	0.06
GSI	2.18 \pm 1.36 b	2.81 \pm 1.59 b	2.74 \pm 1.67 b	2.44 \pm 0.96 b	5.12 \pm 1.9a	3.15	0.02

fish fed on a 100 ppm estradiol diet (0.46 ± 0.04). As indicated above, the highest conversion rate was obtained from the control group (0.83 ± 0.04).

Histological Analysis

At the end of our experiment. after subjecting sex-reversed females to reproduction for 8 months, only a limited number of eggs and accompanying juvenile fishes were obtained. Subsequently, 10 individuals from each treatment group were sacrificed and subjected to gonadal examination and the GSI values excluded from the groups at the end of 8 months are presented in Table 5 together with the evaluation results.

The groups fed on the 17 β -estradiol diet did not differ from the control group ($p > 0.05$) in terms of body weight, height and condition factor according to table 5 analysis ($p > 0.05$). However, the analysis of GSI averages revealed a significant difference between the hormone fed fish and the control group fish ($p < 0.05$). In addition, the histological analysis showed significant problems (in terms of numbers and severity) such as structural defects of the gonads (asymmetry, defective eggs) and deformities of some internal organs such as the air sac.

Water quality during the course of the experiment was within the recommended parameters for Tilapia species as suggested by Philippart and Ruwet, 1982; Azaza et al., 2008 and Soltan et al., 2013. No statistical difference was observed in average water temperatures between all treatment groups ($p > 0.05$) which were

all around 28 °C (Table 2). Dissolved oxygen (Table 2) stayed within acceptable limits as well (DO- 4 mg/L or higher) as per the recommendations of Azim and Little, 2008; Iwama et al., 1997 and Rappaport et al., 1976. The comparison of the mean weight values between experimental groups (Table 5) revealed statistically significant differences ($P \leq 0.05$) and generally the estradiol hormone-fed fish groups were found to have a similar average body weight (apart from the 200 ppm group) but lower than the fish in the control group. The same observations were reported by Johnstone et al. (1979), Hunter and Donaldson (1983), according to whom estradiol hormone induces lower growth performances. Piferrer (2001) and Alcántar-Vázquez et al., (2015) suggested that, unlike androgen, estrogen has no effect on growth in fish. The best (lowest) feed conversion ratio (FCR) was 0.46 ± 0.06 and was recorded in the 100 ppm estradiol-fed fish group while the control group showed the worst (highest) FCR - 0.83 ± 0.41 . The FCR is considered as the best parameter for evaluating the acceptability of the feed and the final performance of the fish (Inayat and Salim, 2005). The FCR values obtained in our study were good and showed significant differences between the groups. All the fish fed on hormones were found to valorize feed better than those in the control group, although they consumed less. Our results are better than those of Malik et al. (2014) and Daudpota et al. (2016). Kalsoom et al. (2009), however, reported a higher FCR than ours in their study on *Catla catla* \times *Labeo rohita* hybrid. The plausible reason for such a good FCR of the present study may be due to the combination of the availability of appro-

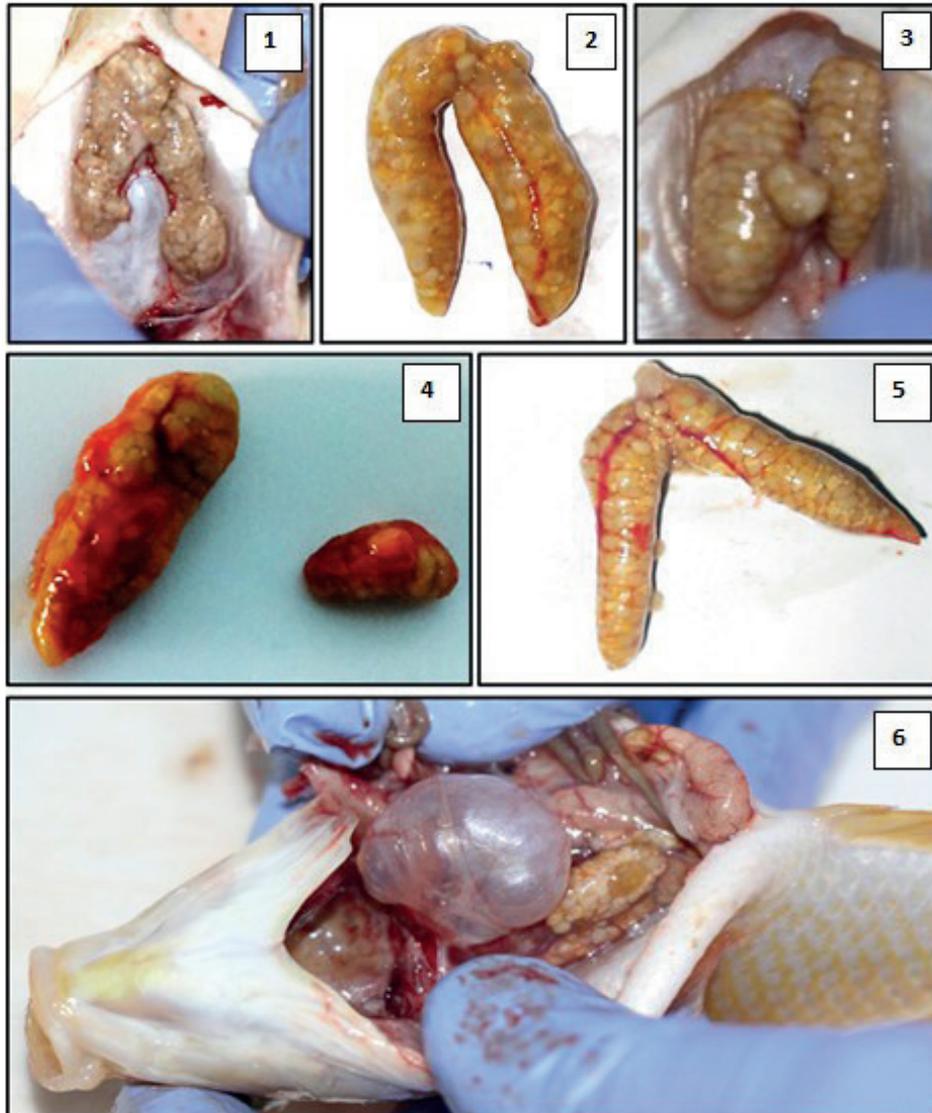


Figure 2. Deformations noticed during histological analysis: (1, 3, 4, 5: Asymmetric ovaries, 2: A female gonad full of loose and whitish eggs, 6: chick gonad and excessively blown airbladder

appropriate nutrient contents (protein rate of 50-55%) of the feed supplied, the optimized water quality and the great care provided in general. In the comparison of condition factor averages, it was seen that the K value of the estradiol-treated groups was slightly smaller than the control group even if the differences were not statistically significant ($P > 0.05$). As recommended for freshwater fish by Bagenal and Tesch (1978), Hile (1936) and Martin (1949), these values were ideal and in the range of 2 to 4. Ayode (2011) stated that K values above 1 indicates the healthiness and the so-desired isometric growth of the fish for commercialization.

Sex ratios were female-oriented and ranged from 51.52% (control) to 86.36% (200 ppm) and exceeding 76.60% in the 150 ppm estradiol hormone-fed fish group. The 50 and 100 ppm groups showed sex ratios of 63.41 and 61.90% female respectively. These differences were statistically significant ($P \leq 0.05$). The later gonad analysis of the fish showed the presence of intersex individuals in

the treatment group. The effect of estradiol concentration on sexual differentiation in *O. mossambicus* fish is clearly demonstrated in our experiment. These are in line with the results obtained by Alcántar-Vázquez et al. (2015) who reported in their work the use of *O. niloticus* fry of 10 mm total length on average since the size of the fish is very important for a successful sex reversal. The good sex reversal values observed in our study may be due to the initial length of the fry on the first feeding. As an example, VeraCruz et al. (1996) suggested that the initial length of fry for a successful feminization process of Tilapias should be less than 10 mm. In our study, the average initial length of Mozambique Tilapia pupils was 7 mm. Several works reported better results but with another estrogen hormones. Basavaraja et al. (1990) published a study in which 100% female individuals were obtained from *O. mossambicus* pups fed on a diet containing 50-100 ppm of diethylstilbestrol (DES). Similar results were reported in the Varadaj (1989) study. However, mortality also in-

creased significantly with higher hormone doses, exactly like in the present study. Wang et al. (2008) used 17- β -estradiol at different doses (50-200 ppm) for 60 days for the feminization of Coho salmon (*Lepomys macrochirus*) larvae fish. In the study conducted, larvae were fed with 17- β -estradiol supplemented feeds that led to 100% female populations. However, regarding the fish of the control group, the hormone-fed ones were found to grow more slowly during the experimental period, like in this study. In addition, the success of this experiment can be explained by the results of Abucay and Mair (1997) who explained that unlike in flow-through systems where they can escape, sex manipulations are likely to be more successful in re-circulation systems in which metabolites, or filtrates are kept within the system.

The GSI averages in all experimental groups were weaker than in the control group. Similar situations were described by Hatikakoty and Biswas (2004) and Ganie et al., (2013) in *O. mossambicus*. They found that most of the ovaries of fish fed with the same hormone diet remained blocked in the early maturation stage. This suggests that increasing estradiol hormone dosage prevents gonadic maturation. The GSI value found in the control group was higher than that of the experimental groups and the result was that the individuals belonging to this group could successfully deliver eggs and larvae. It has also been suggested that the increased rate of deformity of gonad structures of fish is related to the increasing dose of estradiol. The same conclusions were reported on Nile Tilapia (*O. niloticus*) by Alcántar-Vázquez et al. (2015), on sea bass (*Dicentrarchus labrax*) by Chatain et al. (1999) and on *Carassius auratus* by Beardmore et al. (2001).

CONCLUSION

This study aimed to assess the effects of 17 β estradiol on the sex differentiation and growth of *O. mossambicus*. The findings showed that the female ratio was clearly and positively dose-dependent. In addition, as reported in the literature, poorer results were obtained in terms of feed consumption and growth parameters of all of the fish that were subjected to estradiol administration compared to the control group. In contrast, the survival rate (apart from in the 200 ppm group) and feed valorization were observed to be better in hormone treated fishes. These results suggest that the best temperature, dose and duration of application of 17 β estradiol to Mozambique tilapia (*Oreochromis mossambicus*) should be applied at 28 °C, 100-150 ppm and 30 days of application for successful feminization. Furthermore, it was noticed that feminization experiment using 17 β estradiol hormone in *Oreochromis* gender on Nile Tilapia, almost no research has been conducted on Mozambique one. That is why we strongly recommended continuing or even repeating the same research in order to establish better and more reliable standards for fish aquaculturists, fish farmers or anyone involved in this field who might be interested in this practice.

Conflict of Interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics Committee Approval: This study was carried out in accordance with animal welfare and the ethics of trial. All procedures were performed in accordance Law on Veterinary and

Medical Activities and National Animal Welfare Act. Therefore ethical approval was not required.

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