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# FEMINIZATION OF NILE TILAPIA *OREOCHROMIS NILOTICUS* BY ORAL ADMINISTRATION OF SEX REVERSAL HORMONE "DIETHYLSTILBESTROLE"

(With 3 Tables and 3 Figures)

By

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## تأنيث البلطى النيلى بأستخدام هرمون الانعكاس الجنسى (داى ايثيل استلبستيرول)

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أجريت هذه الدراسة على أربعة مجموعات من زريعة البلطى النيلى رضعت تحت معاملات غذائية خاصة استخدم فيها هرمون الانعكاس الجنسى (داى ايثيل استلبستيرول) بجرعتين مختلفتين ولمدتى تغذية مختلفتين اضافة الى المجموعة الضابطة. وقد تم فى هذه الدراسة تحديد الجنس فى المجموعات المعالجة والضابطة وتم حساب النسبة الجنسية. كذلك تم دراسة الصورة الشكلية العامة لكل من الخصية والمبيض فى البلطى النيلى باستخدام المجهر الضوئى. ولقد أظهرت الدراسة النسيجيةأن الخصية مكونة من عدة فصوص بينها حواجز ليفية وكل فص يحتوى على خلايا منوية فى أطوارها المختلفة. وقد تميز المبيض بوجود حويصلات مبيضية فى مستويات مختلفة من النمو. هذا وأوضحت هذه الدراسة أن استخدام هرمون التأنيث يؤدى الى زيادة نسبة الاناث حيث تم الحصول على أعلى نسبة باستخدام ١٠٠ مج / كجم غذاء لمدة أربعين يوما. كذلك فقد بينت الدراسة أن استخدام هرمون التأنيث فى مرحلة التطور الجنسى تؤثر على متوسط أوزان الأسماك و معدلات نموها ونسبة الأسماك التى تبقى على قيد الحياة.

## SUMMARY

Induction of sex reversal in *Oreochromis niloticus* by diethylstilbestrol (DES) and its effect on growth promotion was studied at two different hormone doses, 50mg and 100mg/kg of feed for two different feeding duration of 25 and 40 days. Treated and untreated (control) groups were

sexed using the standard gonadal squash technique and sex ratio was calculated. The basic structure of both testis and ovary at the time of sexing was microscopically studied in semithin sections. The testis was found to be composed of lobules separated by connective tissue septa. Each lobule contained spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. The ovary displayed the presence of many ovarian follicles at different stages of development in addition to some oogonial cell nests. Both DES levels and feeding duration led to significant increases in female percentage as compared with the control group. The obtained results indicated that using 100mg/kg DES dose for 40 days feeding duration produced the highest percentage of females. In addition, the oral administration of DES variably affected individual fry weight, specific growth rate (SGR) and percentage of survival.

***Key words:*** *Nile Tilapia – Feminization – Sex Reversal Hormones*

## INTRODUCTION

Tilapias are of great potential importance in aquaculture in the tropics and subtropics including most of the areas suffering chronically from a lack of animal protein (Wohlfarth and Hulata, 1983). A major drawback in the world-wide culture of tilapias is the precocious maturation and uncontrolled reproduction resulting in overcrowding and stunted growth (Pandian and Vradaraj, 1990).

Many approaches for controlling reproduction and other various purposes have been tried and one of the most promising teqniques is the hormonal induction of monosex populations (Varadaraj, 1989; Ridha and Lone, 1995). Hormonal induction of sex reversal may serve as a valuable tool for understanding the process of sex differentiation and to produce monosex populations for the aquaculture industry (Pandian and Sheela, 1995).

Sex reversal is achieved by the administration of sex steroids during the period of gonadal differentiation. In fish, the gonadal differentiation takes place well after the fry has hatched and begun feeding (Purdom, 1993). The technology of sex reversal and production of all-females populations provides a new approach to fishery management (Struessmann et al., 1996). To feminize tilapia, a number of estrogens have been used with variable success. The most commonly used estrogens are estrone (Tayamen and Shelton, 1978), 17-β-estradiol

(Jensen and Shelton, 1979), diethylstilbestrol (Obi and Shelton, 1983) and 17-α-ethynylestradiol (Lahar, 1993; Rosenstein and Hulata, 1993). The effects of estrogenic hormones such as estrone, estriol, estradiol and diethylstilbestrol on primary and secondary sex characteristics differed widely in various treatments. Sexing according to external features is difficult in young fishes. The standard squash technique is a reliable method for sexing, but basic knowledge on gonadal morphology have to be established before applying this technique.

The present study was undertaken to determine: (1) the optimal dosage and duration of diethylstilbestrole (DES) required to produce the highest percentage of females and (2) the effect of the homone on the growth of tilapia. Such a study may contribute to develop a breeding program for producing monosex male genetic population through supermale (YY).

## MATERIALS and METHODS

**(1) Preparation of treatement diet:**

Two treatement feeds (45% protein) were prepared by the alcohol evaporation method (Guerrero, 1975). Diethylstilbestrol (DES, Sigma chemicals) was added at 50 and 100 mg / kg of feed. The control feed was prepared in the same manner but without DES.

**(2) Treatements:**

Fry for treatement (total length range of 9-11 mm) were collected from *Oreochromis niloticus* adult females. They were divided into 15 groups, 150 individuals/group. Three groups served as control and the rest for the different DES levels and different feeding duration (three for each treatment). Each group was stocked in 48 L glass aquaria.

Fry were fed at 10% of the body weight daily; the feed was divided into three rations given at 8.00, 12.00 and 17.00 O'clock. All control and treated groups were counted and weighed weekly until the end of feeding with treated diet. Specific growth rate (SGR) according to Watanaba et al. (1993) and number of survived fry were calculated. The amount of feed was adjusted weekly according to the changes in the weight. All troughs were cleaned every 2-3 days to reduce disease potential. After 40 days, all groups were transefered to 128 liters (L) aquaria and allowed to grow until sexing process (after 97 days). Normal levels of temperature, dissolved oxygen and pH were kept throughout the period of experiment.

**(3) Evaluation of treatments:**

At the end of the experiment, the tilapias were weighed, sexed using the standard squash technique (Guerrero and Shilton, 1974) then sex ratio was calculated. Testis and ovary samples were collected, fixed in paraformaldehyde-gluteraldehyde mixture (Karnovesky, 1965) and processed for semithin sectioning (1μm thick) and examined light microscopically.

**(4) Statistical analysis:**

The obtained results viz., the mean individual fry weight, mean specific growth rate, mean percentage of survived individuals and mean percentage of females were subjected to a two-way analysis of variance. A chi-square test was used to determine wether the observed sex ratio differs from the expected 1:1 ratio.

## RESULTS

The means of individual fry weight (gm), specific growth rate, percentage of survival and percentage of females for the control and treated groups are given in table (1), while analyses of variance are laid out in Table (2). As the analysis of variance revealed, there were significant reductions in the mean individual fry weight and the specific growth rate after 40 days of feeding on DES treated diet as compared with the control. At sexing time (97 days old), there were no significant differences in the mean weight and specific growth rate between both 50 and 100mg DES treatments for 40 days and the control groups. There were a significantly high percentage of survivals at 25 days feeding on 100mg DES/ kg as compared with the control, whereas a comparable reduction was observed at 40 days feeding on 50 mg DES / kg.

With regard to the percentage of females, the obtained results revealed that feeding *Oreochromis niloticus* tilapia with DES at 50 mg or 100 mg levels for either 25 or 40 days increased the percentage of females significantly than the control. The increase due to the later dose (100 mg DES/kg) was greater than that of the former one (50 mg DES/kg). Moreover increasing the feeding duration at each DES level caused slight increase in the percentage of females. The highest female percentage was obtained using 100 mg DES for 40 days (Fig. 1). A Chi square-test employed on the obtained sex ratios (Table 3) indicated significant deviations from the expected 1:1 ratio for all different DES treatments examined.

The differences between male and female gonadal squashes are illustrated in Fig. (2a & b). Using semithin sections, the testis (Fig.3a) appeared as consisting of testicular lobules showing few spermatogonia, many primary spermatocytes few secondary spermatocytes and spermatids. Spermatogonia had weakly stained cytoplasm and nuclei, the latter attained more deep staining as they approached their final forms (spermatids). Few Sertoli cells were hardly differentiated by their irregular nuclei and deeply stained nucleoli. The ovary (Fig.3b) showed many ovarian follicles at different stages of development in addition to some oogonia forming cell nests. The ovarian follicles were variable in size and structure. Small-sized follicles had only the oocyte with large pale centrally located nucleus and moderately stained cytoplasm. Medium-sized follicles were surrounded by one layer of flat cells and are characterized by the presence of Balbiani's body and few lipid droplets in their cytoplasm. Large follicles had large number of lipid droplets and deeply stained yolk granules.These follicles were surrounded by thin homogenous layer (zona pellucida) followed by one layer of collumnar cells and covered externally by a connective tissue theca.

## DISCUSSION

The observed reductions in specific growth rate with increasing feeding duration support the findings of Ridha and Lone (1993) who mentioned that strogens generally have no anabolic effects in most teloest fishes. In the same respect, Tayaman and Shelton (1978) found that *Oreochromis niloticus* fry treated with androgen (17α-methyltestosterone) had faster growth rate than those treated with oestrogens (diethylstilbestrol and estrone) indicating once more that estrogen had no anabolic effect in tilapia species. The higher values of mean weight after using either 50 or 100 mg DES for 40 days as compared with other groups can be attributed to the recorded low survival rate (as low rearing density allows fish to grow bigger).

The percentage of survivals at sexing time recorded a significant reduction as the period of DES treatment increased. This supports the suggestion of Purdom (1993) who stated that treating fish with sex steroids during the sexually indeterminate phase of life can lead to a significant fry mortality.

The organization of the testis in tilapia at the time of sexing (97 days old) resembled the unrestricted or lobular type of other teleost

fishes described by Grier et al. (1980), Billard (1990) and Mattei et al. (1993). No mature sperms have been seen inside the testicular lobules of tilapia at this age. This indicated that the testis was still in the early spermatogenic stage as described in bester by Mojazi Amiri et al. (1996). The ovarian morphology in the present study resembled basically that described in the bass, *Ecentrarcus labrax* (Mayer et al., 1988) and that of bullhead catfish, *Ictalorus nebulosus* (Rosenblum et al., 1987). A characteristic Balbiani's body as that seen by Beams and Kessel (1973) and Mayer et al. (1988) was clearly demonstrated in medium sized follicles.

With regard to the percentage of females, the present work proved that increasing either the DES level or duration of treatement resulted in an increase in the percentage of females. This increase was in all cases significantly higher than control. The highest female percentage (about 87%) was obtained using 100 mg DES/kg for 40 days. There are few reports of studies that used estrogens for sex reversal in fish which show that it is much more difficult to produce all-female population by direct administeration of the hormone. Although complete sex reversal was not achieved in the present study, the 87% females obtained in the 100 mg DES treated group compare well with those reported in the literature. In *Sarotherodon niloticus*, Tayamen and Shelton (1978) achieved 90% female population with DES at 100mg ppm for 25, 35 and 59 days. The sex reversing effect of DES in *Oreochromis mossambicus* (Varadaraj, 1989) was greater than in *Oreochromis aureus* (Hopkins et al., 1979) or *Sarotherodon niloticus* (Tayamen and Shelton, 1978). It can be concluded from the present work that a 100 mg DES /kg dose and a long treatement (40 days) might produce a higher monosex female population in *Oreochromis niloticus*.

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Table (1): Effect of feeding *Oreochromis niloticus* with different doses of Diethylstilbestrol for different feeding durations on individual fry weight, specific growth rate, percentage of survival and percentage of females.

| Treatement | Mean individual fry weight (gm) | | Mean specific growth rate % bw/day | | Mean percentage of survival | | Mean percentage of females |
|---|---|---|---|---|---|---|---|
| | At 40 days | At sexing (97 days) | At 40 days | At sexing (97 days) | At 40 days | At sexing (97 days) | |
| (1) Control | 0.236 | 2.387 | 0.559 | 2.447 | 48.223 | 22.887 | 57.073 |
| (2) 50 mg DES | | | | | | | |
| a-25 days | 0.237 | 1.717* | 0.569 | 1.757* | 52.890 | 29.777 | 70.290* |
| b-40 days | 0.153** | 2.203 | 0.364** | 2.260 | 43.997 | 11.780* | 75.863** |
| (3) 100 mg DES | | | | | | | |
| a-25 days | 0.203 | 1.290** | 0.482 | 1.323** | 57.557 | 43.333** | 81.863** |
| b-40 days | 0.166** | 2.343 | 0.388** | 2.423 | 50.447 | 18.667 | 87.393** |
| L.S.D. 0.05 | 0.041 | 0.664 | 0.099 | 0.677 | 11.431 | 8.124 | 9.465 |
| L.S.D. 0.01 | 0.057 | 0.944 | 0.141 | 0.963 | 16.250 | 11.548 | 13.455 |

* P< o.o5 ** P< o.o1

Table (2):Analysis of variance for individual fry weight, specific growth rate, percentage of survivals and percentage of females obtained from different DES levels at two different feeding durations in *Oreochromis niloticus*.

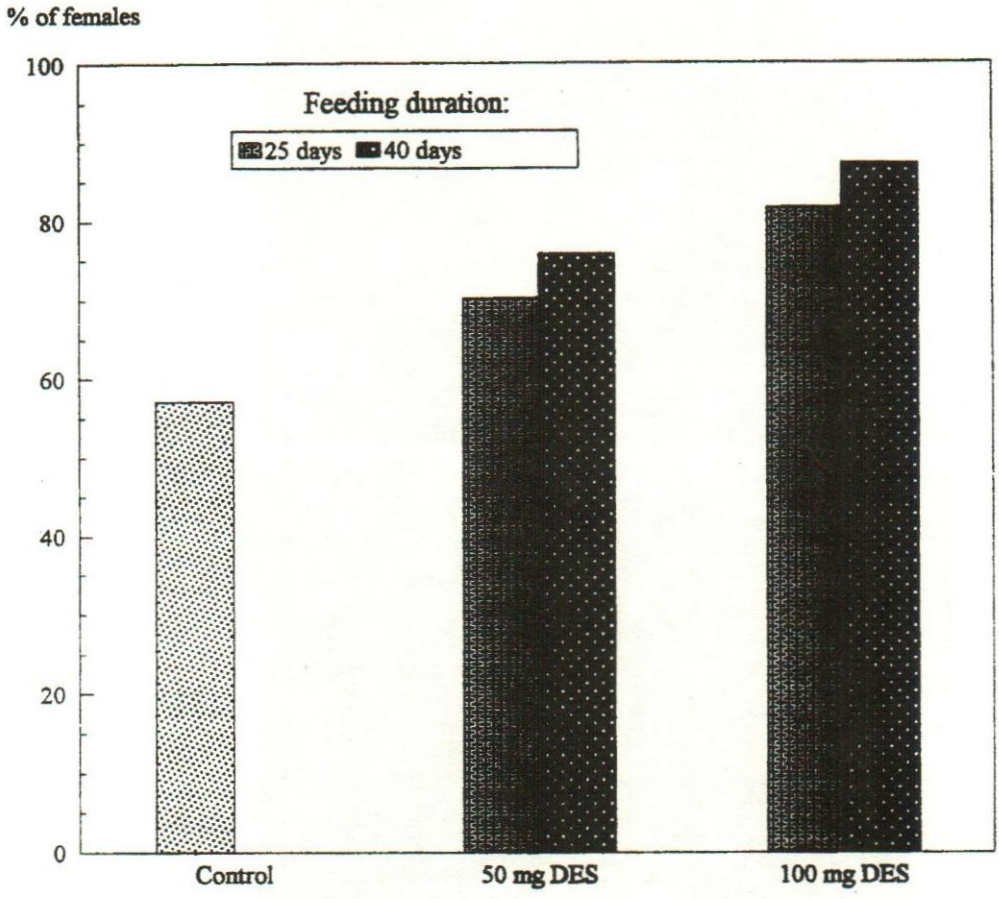
| Source | Degree of freedom | Mean individual fry weight | | | | Mean specific growth rate | | | | Mean percentage of survivals | | | | Mean percentage of females | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | At 40 days | | At sexing | | At 40 days | | At sexing | | At 40 days | | At sexing | | | |
| | | MS | F | MS | F | MS | F | MS | F | MS | F | MS | F | MS | F |
| Replicates | 2 | 0.002 | 2.21 | 0.430 | 1.61 | 0.013 | 2.20 | 0.440 | 1.60 | 129.295 | 1.64 | 197.621 | 4.96* | 113.297 | 2.10 |
| DES levels (A) | 2 | 0.004 | 4.74* | 0.527 | 1.98 | 0.025 | 4.11* | 0.539 | 1.95 | 64.333 | 0.82 | 174.754 | 4.39* | 1148.826 | 21.25** |
| Feed duration (B) | 1 | 0.007 | 7.94* | 1.186 | 4.46 | 0.045 | 7.41* | 1.285 | 4.64 | 128.053 | 1.62 | 910.080 | 22.86** | 61.64 | 1.14 |
| Interaction (AB) | 2 | 0.003 | 2.89 | 0.417 | 1.57 | 0.016 | 2.61 | 0.455 | 1.64 | 33.206 | 0.42 | 244.203 | 6.13* | 15.411 | 0.29 |
| Error | 10 | 0.001 | | 0.266 | | 0.006 | | 0.277 | | 78.828 | | 39.814 | 54.051 | | |

* P< 0.05 ** P< 0.01

Table (3): Effects of diethylstilbestrol (50 and 100 mg/kg diet) on sex differentiation of *Oreochromis niloticus*.

| DES dose (mg/kg diet) | Feeding duratio (days) | No. of recovered individuals | sex ratio | | | Chi-square values |
|---|---|---|---|---|---|---|
| | | | Males | Intersex | Females | |
| 0 | 40 | 103 | 44 | 0 | 59 | 2.19 |
| 50 | 25 | 134 | 36 | 4 | 94 | 25.88** |
| | 40 | 53 | 12 | 2 | 39 | 14.30** |
| 100 | 25 | 195 | 31 | 5 | 159 | 86.24** |
| | 40 | 84 | 11 | 0 | 73 | 45.76** |

Fig. (1): Percentage of females at sexing (97 days) resulting from different DES levels and different feeding durations



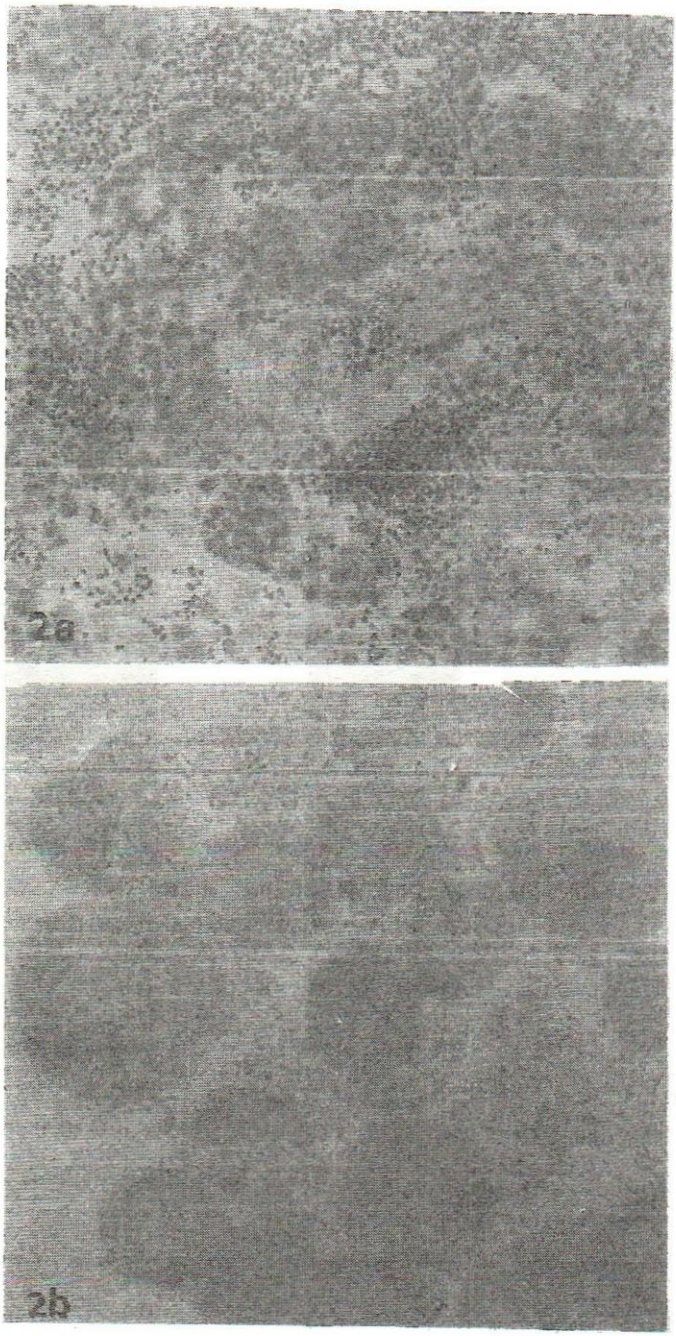



Fig. (2): Gonadal squashes of *Oreochromis niloticus* stained with acetocarmine. x 250

a: Testicular squash showing different generations of germ cells.

b: Ovarian squash showing variably-sized ovarian follicles.

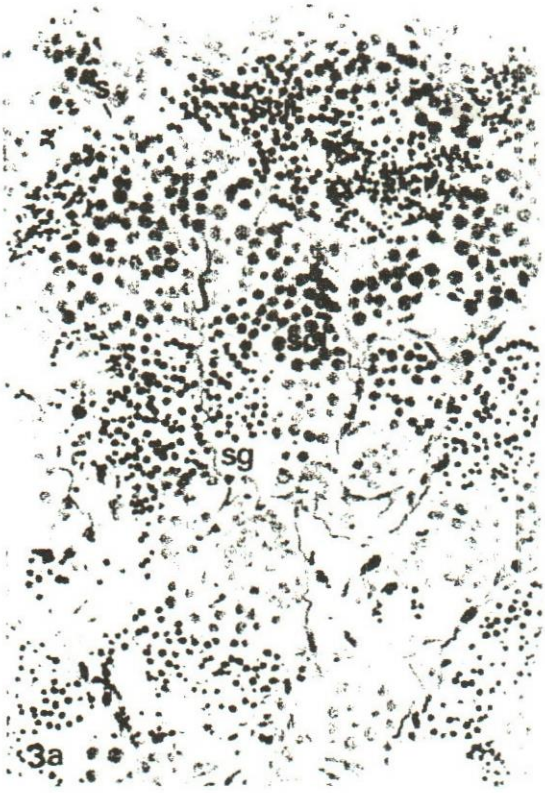



Fig. (3a): A semithin section in the testis of *Oreochromis niloticus*. Notice the presence of all generations of germ cell spermatogonia (sg), primary spermatocytes (scI), secondary spermatocytes (scII), spermatids (st) and Sertoli cells (s). Toludine blue, x 250.

Fig. (3b): A semithin section in the ovary of *Oreochromis niloticus* showing variably-sized ovarian follicles. Abbreviations: n (nucleus), b (Balbiani's body), ld (lipid droplets, yg (yolk granules), zp (zona pellucida), fc (follicular cells), T (connective tissue theca). x400