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**All correspondence should be addressed to:**

Abbassa International Journal for Aquaculture, Abbassa – Abou Hammad – Sharkia – Egypt. Tel.: 0020553401028 – Fax: 0020553400498

## GENERAL INFORMATION

Abbassa International Journal for Aquaculture is Egyptian specific publication in aquaculture of the Egyptian society for water, aquaculture and environment. The journal is published in four volumes per year to include results of research in different aspects of aquaculture sciences. The journal publishes also special issues of advanced topics that reflect applied experiences of importance in aquaculture sector.

# EVALUATION OF TAMOXIFEN TO INDUCE MONOSEX IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) REARED IN HAPAS SUSPENDED IN CONCRETE PONDS

**Ahmed A. Abd El-Rahman[1]; Ashraf A. Ramadan[2] And Ahmed S. Diab[3]**

*[1] Reproductive Physiology And Hatchery; [2] Genetics And Breeding Department; [3] Fish Health Department, Central Laboratory For Aquaculture Research, Agricultural Research Center. Egypt.*

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***Abstract***

The present study aimed to replace 17α- methyltestosterone hormone by tamoxifen to produce all male Nile tilapia and investigate final weight, male % and survival rate of *Oreochromis niloticus* fry treated with 60 mg / kg diet of 17α- methyltestosterone (MT), tamoxifen (100 & 200 mg / kg diet) and control fish. Fish fry reared in hapas suspended in concrete ponds.

The results indicated that there were highly significant differences in final weight, male percentage (%) and survival rate of fry at all treated groups when compared to the control group. The finial weight and male percentage were high significantly to (7.03 ± 0.83, 5.07± o.15, 4.77±0.23 (g) and (92.67±0.58, 81, 67 ±0.49 & 85.33± 0.91 (%) in fish treated with 17α-methyltestosterone, 100 & 200 mg of tamoxifen (antiestrogen) / kg of diet ) respectively than in the control group (4.07+ 0.25 (g) and 60.33±0.61%). While, increased significant in survival rate for control (92.00±0.46%) than fry treated with 17α- methyltestosterone, tamoxifen (antiestrogen) 100 & 200 mg / kg diet were 88.97±0.45, 84.13±0.50 and 77.93±0.60 % respectively, after 105 days. That is to say, using of tamoxifen 200 mg/kg diet for male sex reversal of *Oreochromis niloticus* fry better than hormone of 17α-methyltestosterone because it was best and safety for human.

**Key words:** *Oreochromis niloticus*, Monosex, Tamoxifen, 17 α-methyltestosterone.

## INTRODUCTION

Monosex culture of the faster growing males can prevent the problem of overcrowding. Males can be produced either by manual separation of sexes, hybridization or by hormonal sex reversal using androgens. Techniques and disadvantages of each method were outlined by Mires (1983) and Fryer & Iles (1972). Growth and gonadal development in fish, as in all vertebrates, are controlled, in part, through the orderly release of hormones from the neuroendocrine system. Supplementation of diets with steroids accelerates fish growth (Sindho and pandian, 1984). Guerrero and Guerrero, (1988) reported that tilapia masculinized with MT grew faster than both females and untreated males. Several methods for production of monosex male populations for example hand sexing (Guerrero and Guerrero, 1975), hyperidization, (McAndrew, 1993), genetically male tilapia production (Mair *et al.*, 1997) chromosomes manipulation (Thorgaard, 1983) and sex reversal.

Sex reversal of newly hatched fry is generally accomplished via oral administration of 17α- methyltestosterone (MT), which has been incorporated into a starter fish feed at 60 mg MT / kg feed for 28 days (Popma and Green, 1990). Oral administration of 17α- methyltestosterone (MT), at a rate of 30 to 60 mg MT / kg feed to newly hatched tilapia fry (9-11 mm total length, 10 – 15 mg body weight) for a period of 21 to 28 day produces populations comprised of less than or equal to 5 % females (Tayamen and Shelton, 1978). Mickeal *et al.*, (2003) found that *Oreochromis niloticus* fish collected from different farms in Kafer El-Sheikh province treated with M.T showed an improved body weight than the fish obtained from Central lab of aquaculture

research. In addition the residues of the hormones in treated muscle were increased as the body weight increased (70-90 g).

The present study aimed to replacement of 17α- methyltestosterone hormone by tamoxifen in production of all male Nile tilapia and investigate final weight, male % and survival rate of *Oreochromis niloticus* fries treated with 17α- methyltestosterone, tamoxifen in comparison with control reared in hapas suspended in concrete ponds.

## MATERIALS AND METHODS

This study was conducted at the Central Lab of Aquaculture Research (CLAR), Abbassa, Abou-hammad, Sharkia, Egypt, to replace 17α- methyltestosterone by tamoxifen to produce monosex Nile tilapia (*Oreochromis niloticus*) and to investigate their growth performance. The fry were reared in hapas suspended in concrete ponds.

**Breeding systems:**

*Orochromis niloticus* broodstock were collected from the Central Lab for Aquaculture Research (CLAR), Abbassa, Abou-hammad, Sharkia, Egypt. Fish were stocked at a sex ratio of 1 male: 3 females at a rate of 60 females (190 g) and 20 males (170 g). Fish were stocked in two hapa (4 x 6 x 1.2 m) in an earthen pond. The water depth was maintained at about 60 cm. Water was changed (10%) every third day. The experiment lasted for 4 months during the spawning season from May to August.

Air temperature ranged between 25 to 30 $^{o}$C and other ecological conditions are presented in table 1. Fish were fed on a commercial diet (25 % crude protein) twice daily at a rate of 2.5% of body weight/day.

**Table (1):** Ecological conditions of earthen pond water.

| Oxygen (ppm) | Alkalinity mg ($CaCo_3$) / L | $NH_4$ mg / L |
|---|---|---|
| 7.56 ± 1.54 | 265.5 ± 18.5 | 0.075 ± 0.006 |

Female broodstock were checked for spawning after 14 days and fry and eggs were collected. Fry were stocked and numbered in nursery net enclosures (hapas) that made of plastic mosquito net (mesh pore 1 mm). Hapas are easy to manage; because fry cannot escape harvesting. Hapa size was 1 $m^2$ with 1 m depth in concert pond.

**Preparation of the control diets:**

1 kg a ground basal fish diet contained the ingredient at table 2 and their proximate analyses are at table 3, mixed with 500 ml ethanol 95%. The control food were allowed to dry and packed in clean plastic bags and kept in refrigerator at 4$^oC$ until use.

**Table (2):** Formulation of diet.

| Ingredient | (%) |
|---|---|
| **Fish meal (50%) crud protein** | 30 |
| **Rice bran** | 21 |
| **Wheat bran** | 16 |
| **Yellow corn** | 16 |
| **Soybean meal (45 %) crud protein** | 10 |
| **Molasses** | 2.75 |
| **Vegetable oil** | 1.9 |
| **Fish oil** | 1.05 |
| **Dicalcium phosphate** | 1 |
| **Methionen** | 0.15 |

| | |
|---|---|
| **Mineral mix** | 0.07 |
| **Vitamin mix** | 0.05 |
| **Vitamin C** | 0.03 |
| **Total** | 100 |



**Table (3):** proximate analyses of diet (percent).

| **Items** | **(%)** |
|---|---|
| **Protein** | 34.85 |
| **Lipids** | 6.2 |
| **Ash** | 7.7 |
| **Fiber** | 6.13 |
| **NFE** | 45.12 % |

**Preparation of 17α- methyltestosterone treated diets:**

Sixty mg of the hormone (17α- methyltestosterone) dissolved in 500 ml ethanol 95% and mixed with 1 Kg of the ground basal fish diet. The treated food were allowed to air draying and packed in clean plastic bags and kept in refrigerator at $4^{o}$C until use.

**Preparation of tamoxifen (antiestrogen) treated diets:**

To prepare diet containing 100 mg or 200 mg tamoxifen per each Kilogram, dissolutions of 100 mg and 200 mg of tamoxifen were prepared in 500 ml 95% ethanol. Each prepared alcoholic solution was added to one kilogram of the ground basal fish diet and mixed well then left to dry and packed in clean plastic bags and stored at $4^{o}$C until use.

**The experimental design:**

12,000 fry of Nile tilapia *(Oreochromis niloticus)*, at a yolk sac larvae stage were transported from reproductive hapas in Central Lab for Aquaculture Research (CLAR). And allotted in 12 hapas suspended in concrete ponds to study sex reversal of Nile tilapia fry to obtained monosex males. ch hapa (1 x 1 x 1 m) was stocked with 1,000 fry of Nile tilapia.

The experimental was divided to three groups each group consists of three hapa. the first group were fed on the basal diet representing the control diet. Second group were fed on 17α-methyltestosterone treated diets. Third and forth group were fed on diet containing 100 and 200 mg tamoxifen/ Kg diet, respectively at a rate 20% of the body weight for 30 days.

At the end of the treatment period, fish were fed on the basal diet until 45 days age at rate of 12% from body weight. After than the all fish in all groups received the basal diet (25% crude protein) tell to the end of the experiment (105 days).

On the day 105 posts hatching all fish group were weighted and numbered then sacrificed 100 fish from each hepa to determine of sex ratio.

Determination of sex ratio in fish groups by staining of the gonads with acetocarmine stain (Guerrero and Shelton 1978).

The growth rates were determined by using electrical balance and the survival rates were determined as follows:-

$$\text{Survival rate} = \frac{\text{Number of live fish} \times 100}{\text{Total initial number of fish}}$$

**Statistical analysis:**

Statistical analysis was performed using the analysis of variance (ANOVA) and Duncan's Multiple Range Test **(**Duncan, 1955). SAS program was used for statistically analysis according to Snedecor (1971).

## RESULTS AND DISCUSSION

The reproductive performance of Nile tilapia brood (Table 4) indicated that the mean number of eggs per female was 456, mean egg weight was 6.16 mg, mean number of fry per female was 383 and hatchability % was 84.



The effect of the tested diets is summarized in table (5). Highly significant differences were found in mean final body weight, male % and survival rate % between *Oreochromis niloticus* treated with 60 mg / kg diet of 17α- methyltestosterone (MT), tamoxifen (100 & 200 mg / kg diet) and untreated fries reared in hapas suspended in concrete ponds for 30 days. Highly significant final the weight (17.033 ± 0.83 g) for fry treated with 60 mg 17α-methyltesestrone / kg diets than fries treated with 100 mg tamoxifen (5.067±0.15 g) and 200 mg tamoxifen (4.767±0.23 g) while the control group was significantly the lowest to (4.067±0.25g) after 105 days. It seems that MT promotes growth rate and protein anabolism without producing toxic effect in *Oreochromis niloticus*. The present results are in agreement with those obtained by Abdel-Ghany (2003) who studied the effect of different doses of 17α-methyltestosterone (2,5,8 mg/kg diet) for 20 days on the growth of *Oreochromis niloticus* fingerlings. He showed that 17α- methyltestosterone was effective as a growth promoter for *Oreochromis niloticus* during (20 days) and the maximum growth effect was seen in fish received 2 mg/kg diet (19.4 g) vs. 16.32 g. and 14.95 g. in 5 and 8 mg MT treated group respectively. Also, Piferrer and Donaldson, (1992) found that the mean body weight of fries treated with 17β-estradiol or 17∝-ethynylestradiol was higher than those in the control group. On the other hand, El-Halawany (2002) studied the effect

of MT on *Oreochromis niloticus* fry at dose 60 mg / kg diet of 17α- methyltestosterone for 28 days the results showed an increase in the final weight of the treated fish (57.87g.) when equal final weight of the control group 46.33 g. The present results were nearly similar with those reported by Pruginin *et al.* (1975) who found after 57-day post-treatment period, fish (*O. niloticus*) that received 60 mg MT / kg of feed during the hormone treatment period was 13.2 % larger than the non-treated fish.



Male % of fries treated with 17α-methyltestosterone was significantly higher to 92.667±0.58% than fries treated with 200 mg tamoxifein (85.333±0.91%) and 100 mg tamoxifein (81.667±0.49%) when compared to the control group (60.333±0.61%). These results were expected as Guerrero and Guerrero (1988) obtained 99 % male population of *O. niloticus* fry treated with a hormone-containing food for 7 days and stocked at initial density of 1,000 / $m^2$ in fine mesh net hapas held in outdoor tanks having a 5 % daily water exchange. Also Phelps *et al*. (1992) reported that the percentages of males were 97.9 % ± 0.7 when *O. niloticus* fry treated with food containing 60 mg of MT / kg of diet for 28 days. Similarly Hines and Watts (1995) studied the effect of 17α-methyltestosterone and non steroidal chemicals (tamoxifen and arciflaveine) on *Oreochromis niloticus* sex reversal at doses of 5, 15, 50 and 100 mg/kg food for each treatment during 6 weeks, results showed that treatment with 17α- methyltestosterone produced male % 79, 91, 100 and none determined male % for 5, 15, 50 and 100 mg methyltestosterone, respectively.

Highly significant differences were found in survival rate % for untreated fries group (92±0.46%) compared with fry treated by 17α-methyltestosterone, 100 mg tamoxifein and 200 mg tamoxifein /kg diet (88.967±0.45, 84.133±0.50 and 77.933±0.60 % respectively). Results

obtained by Lovshin (1977) showed that the survival rate of Tilapia hybrids (female *O. niloticus* × male *O. hornorum*) ranged from 94 to 100 %. In contrast, Guerrero (1975) found no significant difference in survival rate between *O. aureus* fry treated with MT at the dose of 15, 30 and 60 mg /kg of feed for 120 days and the control. Also, Jensen and Shelton (1979) found that there were no significant differences between *O. aureus* reared for 5 weeks and fed on different doses of estrogen and the control, the survival rate ranged from 95 to 100 %. Moreover, Abd El-Aziz (2002) showed that there was no significant difference in the survival rates (80-82%) of *Oreochromis niloticus* fry treated with 30 mg/kg diet 17α- methyltestosterone at different duration (14, 21 and 28 days). While, higher the growth rate was higher to 1.25g in group treated for 28 days than those treated for 14 or 21 days (1.1 g) at 28 days old.



The study recommended that using of tamoxifen (100 % 200 mg/kg diet) for male sex reversal of Nile tilapia fry as it was best and safety for human than 17α-methyltestosterone hormone.

**Table (4):** Reproductive performances of *Oreochromis niloticus* brood tested.

| Items | No | Weight (g) |
|---|---|---|
| **Male** | 20 | 170 |
| **Female** | 60 | 190 |
| **Egg / female** | 456 | 0.006 |
| **Fry/female** | 383 | |
| **Hatchability (%)** | 84 | |

**Table (5):** Effect of 17α- methyltestosterone and tamoxifen on final weight, male % and survival rate of fries *Oreochromis niloticus*.

| Treatments | Initial weight (mg) | Final weight (g) Mean±SD | Male % Mean±SD | Survival rate % Mean±SD |
|---|---|---|---|---|
| **Control** | 8.35 | 4.07±0.25 [c] | 60.33±0.61 [d] | 92.**00**±0.46 [a] |
| **17α-methyl testestron (60 mg)** | 8.35 | 7.03±0.83 [a] | 92.67±0.58 [a] | 88.97±0.45 [b] |
| **100 mg Tamoxifein** | 8.35 | 5.07±0.15 [b] | 81.67±0.49 [c] | 84.13±0.50 [c] |
| **200 mg Tamoxifein** | 8.35 | 4.77±0.23 [bc] | 85.33±0.91 [b] | 77.93±0.60 [d] |
| **F value** | | 23.26 | 1304.76 | 113.123 |
| **Propability** | | 0.0003 | 0.0001 | 0.0001 |
| **nificant** | | *** | *** | *** |

± Means with the same letter in each column are not significantly differences ($p<0.05$)





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## استخدام التاموكسيفين للحصول على وحيد الجنس ذكورلأسماك البلطي النيلي المرباه في هابات معلقه في أحواض خرسانيه

**أحمد عبد الله عبدالرحمن[١] ، أشرف عبدالرحمن رمضان[٢] ، أحمد سعيد دياب[٣]**

*[١] قسم بحوث التفريخ وفسيولوجيا التكاثر، [٢] قسم بحوث التربيه والوراثة ، [٣] قسم بحوث صحة الاسماك– المعمل المركزي لبحوث الثروة السمكية، مركز البحوث الزراعية، وزارة الزراعه، مصر.*

### الملخص العربى

تهدف هذة الدراسة الى إحداث الانقلاب الجنسى لزريعة البلطى النيلى وزن ١٠ ملجرام لتحويلها لذكور باستخدام التاموكسفين بتركيز ١٠٠ و٢٠٠ ملجرام وهرمون ١٧ الفا ميثيل

تستسترون بتركيز ٦٠ ملجرام / ١ كيلوجرام علف (٤٠% بروتين) لمدة ٣٠ يوم فى هابات ١×١×١ متر معلقة فى حوض خرسانى ٢.٥×٢٥×١ متر بكثافة ١٠٠٠ زريعة لكل هابة وثلاث مقرارات لكل معاملة ومقارنتها بالمجموعة الضابطة لمقارنة معدل النمو والاحياء ونسبة الذكور دلت النتائج على:

وجود اختلافات معنوية فى نسبة الذكور والوزن النهائى للزريعة ومعدل الاحياء بعد ٣٠ يوم من المعالجة فى الاربع مجموعات.

وجود اختلافات معنوية بين زريعة البلطي النيلي المعامل بـ ١٧ الفا ميثيل تستسترون و زريعة البلطي المعالج بالتاموكسفين بتركيزية والمجموعة الضابطة في إستجابة النمو حيث زريعة البلطي المعامل بـ ١٧ الفا ميثيل تيستوستيرون سجلت أفضل النتائج في النمو ونسبة الذكورعن زريعة البلطي النيلي المعامل بالتاموكسفين بتركيزية والمجموعة الضابطة.



معدلات الاعاشة دلت على أفضل النتائج في المجموعة الضابطة ووجود اختلاف معنوية بين زريعة البلطي النيلي المعامل بـ١٧ الفا ميثيل تيستوستيرون و زريعة البلطي المعالج بالتاموكسفين بتركيزية. وزريعة البلطي المعامل بـ ١٧ الفا ميثيل تستسترون سجلت أفضل النتائج في معدلات الاعاشة عن زريعة البلطي النيلي المعامل بـ بالتاموكسفين بتركيزية.

نسبة الذكور كانت أعلى في زريعة البلطي النيلي المعامل بـ ١٧ الفا ميثيل تيستوستيرون (٩٢.٦٦٧±٠.٥٨%) عن زريعة البلطي المعالج بالتاموكسفين بتركيز ٢٠٠ ملجرام / كيلوجرام (٨٥.٣٣±٠.٩١%) اى بنسبة ٧% ولكن التاموكسفين أكثر أمنا للبيئة والانسان ولذلك تخلص هذه الدراسة إلى أنه يمكن استخدام التاموكسفين فى الانقلاب الجنسى للبلطى النيلى ذكور لانة أكثر أمنا للبيئة والانسان من استخدام الهرمونات.