

## EFFECT OF HORMONAL TREATMENT ON PRODUCTION OF MONOSEX MALES TILAPIA (*Oreochromis niloticus*)

Magouz, F. I.\*; M. A. El-Hairy\*\*; A. E. Abdel-Khalek\*\* and A. F. Ebada\*\*

\* Animal Production Department, Faculty of Agriculture, Kafr El-Sheikh, Tanta University.

\*\* Animal Production Department, Faculty of Agriculture, Mansoura University.

### ABSTRACT

A set of 2400 of Nile tilapia fry (*Oreochromis niloticus*) with average initial weight of 0.02g, about six days of age was collected from a private fish farm in Kafr El-Sheikh Governorate. The fry were randomly divided into 24 similar groups and stocked in the experimental aquaria (100 fry in each). Experimental groups (24) were assigned to the  $17\alpha$ -MT hormonal treatments at concentrations of 0, 20, 40, 80, 160 and 320 mg/kg feed for 21 and 56 days (2 groups/treatment). Two basal diets were formulated to contain 45 and 35% crude protein (CP). All experimental fish groups were fed on the basal diet contained 45% CP in a powder form during the first 7 weeks, while the fish were offered a diet containing 35% CP in a 1mm pelleted form during the other 18 weeks of the experimental period.

The optimum level of  $17\alpha$ -MT hormone used in tilapia sex reversal is 40 mg/kg feed and increasing  $17\alpha$ -MT level over 40 mg/kg feed resulted in decreasing percentage of male produced. Both of hormonal treatment and treatment periods did not significantly affected the growth performances of experimental tilapia fish. There were no significant differences among the treated fish groups, while a significant increase was found in the survival rate in the short treatment period (21 days). Hormonal treatment resulted in abnormal structure of the tilapia fish gonads including semeniferous tubules degeneration and atrophy of the interstitial tissue.

**Key words:** Tilapia, monosex,  $17\alpha$ -methyltestosterone.

### INTRODUCTION

The process of sex differentiation in teleosts is diverse and labile (Francis, 1992) making hormonal sex reversal possible in many species of fish. Hormonal induction of sex reversal may serve as available tool for understanding the process of sex differentiation, and to produce mono-sex populations for the aquaculture industry. Sex reversal has been successfully achieved at present in 47 species belonging to 15 families by using one of 31 natural or synthetic steroids (Pandian and Sheela, 1995).

In tilapia species, sex reversal is very important and considered one of the successful methods for overcoming the problem of over population caused by the uncontrolled reproduction. Culture of all male tilapia produced by oral administration of synthetic androgens is the most commonly used in the developed countries and increasingly gaining acceptance in the developing countries. Effective sex reversal treatments for tilapia species have been developed and the technique has been found by several authors to be feasible and economical in commercial production of all-male or nearly all-male fingerlings (McGeachin *et al.*, 1987; Guerrero and Guerrero, 1993; and Varadaraj and Pandian, 1991).

The aim of androgen sex reversal is to produce fast-growing all-male populations for the control of reproduction so that the desired marketable size can be attained in a shorter period of time and to obtain a more uniform fish crop after grow-out in ponds (Little, 1989). The percentage of male produced is dependent on many factors such as the potency of hormone, time of hormone treatment, hormone level, treatment period..... etc.

The objective of this study is to investigate the effect of using different dosages of  $17\alpha$ -methyltestosterone(MT) for two treatment periods (21 and 56 days) on the percentage of sex reversed offspring, growth performance, chemical analysis of fish body and histostructure of gonads in *Tilapia nilotica*(*Oreochromis niloticus*).

## **MATERIALS AND METHODS**

The present work was conducted in the Fish Research Laboratory, Faculty of Agriculture, Kafr El-Sheikh, Tanta University in cooperation with the Physiology Laboratory, Animal Production Department, Faculty of Agriculture, El-Mansoura University.

The experimental system consisted of 24 equal glass aquaria (80x35x40 cm) with about 102 L total water volume in each. Each aquarium was filled with dechlorinated tap water at a constant level of 40 liter. One half of the water in each aquarium was replaced daily by fresh water after removing the accumulated excreta and wasted feed. The experimental aquaria were supplied with compressed air through air compressor (100 liter volume). Water temperature was controlled thermostatically by electric heater and maintained at  $25\pm 1^{\circ}\text{C}$ .

A set of 2400 of Nile tilapia fry (*Oreochromis niloticus*) with average initial weight of 0.02 g / fish and about six days of age was collected from a private fish farm in Kafr El-Sheikh Governorate. The fry were randomly divided into 24 similar groups and stocked in the experimental aquaria (100 fry of each). Twelve groups were assigned to the hormonal treatments (2 groups / treatment) for 21 days, while the other twelve fry groups were treated with hormonal treatments for 56 days.

For 25 weeks as an experimental period all treatment groups were fed on a basal diet containing 45% crude protein during the first seven weeks of the experimental period. The level of protein was reduced in the diet during the other 18 weeks of the experimental period to be 35%. Dietary ingredients were collected from the local market for diet formulation as shown in table (1).

The diet was offered to fish in a powder form during the first seven weeks, thereafter in 1 mm pelleted diet during the other 18 weeks. Composition, chemical analysis and calculated metabolizable energy (ME) of the experimental diets are shown in table (1).

**Table 1: Composition, chemical analysis and calculated metabolizable energy (ME) of the experimental diets.**

Ingredient	Diet	
	(0-7 weeks of age)	(8-25 weeks of age)
Fish meal	42.0	26.0
Soybean meal	30.0	25.0
Yellow corn	22.7	32.0
Wheat bran	-	10.0
Gluten	-	1.7
Sunflower oil	5.0	5.0
Vit. And min. Mix*	0.3	0.3
<b>Chemical analysis %</b>		
Dry matter	86.0	85.3
Crude protein	45.5	35.0
Ether extract	10.4	11.8
Crude fiber	2.7	3.5
Ash	6.4	5.3
Nitrogen free extract	35.0	44.5
ME (Kcal/kg)**	4111.4	4083.9

\* Vitamin and mineral mixture produced by Pfizer Co. I.U. Vit. D., 4.0 g; Vit. E., 0.8 g; Vit. K<sub>3</sub>, 0.4 g; Vit. B<sub>1</sub>, 0.6 g; Vit. B<sub>2</sub>, 4.0 g; Vit. B<sub>6</sub>, 4.0 g; Vit. B<sub>12</sub>, 1.6 g; Pantothenic acid, 8.0 g; Nicotenic acid, 0.4 g; Folic acid, 20 mg; Biotin, 200 g; Choline chloride, 4.0 g; Copper, 0.4 g; Iodine, 12.0 g; Iron, 22.0 g; Manganese, 22.0 g; Zinc and Selenium, 40.0 mg.

\*\* Metabolizable energy (ME) calculated using 4.5 kcal/g protein, 8.1 kcal/g fat and 3.49 kcal/g, carbohydrate according to Pantha (1982).

Synthetic hormone, 17- $\alpha$  methyltestosterone (Sigma Chemical Co.) was administered at concentrations of 0, 20, 40, 80, 160 or 320 mg/kg formulated diet for both 21 and 56 days as hormonal treatment periods. Solution containing the previous hormonal doses was prepared using 95% ethanol. The treated diet was prepaid by the alcohol evaporation method (Macintosh and Little, 1995) the diets were stored in airtight plastic bags and were kept refrigerated. The diets were offered to the fry six times daily at a rate of 50, 40, 35, 30, 25, 20, 15, 10, 8, 6 and 3% of body weight during the (1-3), (4-5), (6-7), (8-9), (10-11), (12-13), (14-15), (16-17), (18-19), (20-21), (22-25) weeks of age, respectively. Body weight and length of *Tilapia niloticus* fish and feed intake of the fish were biweekly measured or determined. Hence, the calculation of total body weight gain was recorded by subtracting the initial body weight from the final body weight. The following equation was used to calculate the specific growth rate (SGR%) of fish:

$$\text{SGR} = \frac{\log(\text{final body weight}) - \log(\text{Initial body weight})}{\text{time}}$$

The chemical analysis of the experimental diet was carried out according to the methods of the A.O.A.C (1985).

Specimens of gonads were taken from 5 fish at 25 weeks of age to prepare for the histological examination using the routine method after Bancroft and Stevens (1982).

The prepared sections were examined by means of a research microscope to determine different changes in gonads.

Data obtained were subjected to statistical analysis according to the method outlined by Snedecor and Cochran (1980). Means were compared using Duncan multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### Effect of hormonal treatment on:

#### Male percentage:

The present results revealed significant ( $P < 0.05$ ) differences among hormonal treatments studied (Table 2). The third treatment with the dose of 40 mg.kg<sup>-1</sup> feed (T3) showed the highest values of male percentage, followed by T2 (20 mg.kg<sup>-1</sup> feed), each of T4 (80 mg.kg<sup>-1</sup> feed) and T5 (160 mg.kg<sup>-1</sup> feed), and T6 (320 mg.kg<sup>-1</sup> feed), while the control treatment (T1; 0 mg.kg<sup>-1</sup> feed) showed the lowest values (Fig. 1). The higher male percentages (86.8-100%) produced from all treated groups as compared to the lowest percentage (49.7%) obtained from the untreated control group may indicate the beneficial effect of the hormonal treatment with 17 $\alpha$ -MT on sex reversal ratio.

It is worthy noting, however, that no consistent trend of male percentage was associated with doses of the hormonal treatment. Increasing level of 17 $\alpha$ -MT above 40 mg/kg diet resulted in decreasing percentage of male produced (Table 2 and Fig.1).

**Table 2: Male percentage achieved by different doses of 17- $\alpha$  MT after 21 and 56 days of hormonal treatment at 25 weeks of age.**

Treatment Period (day)	Hormonal doses (mg/kg feed)						Overall Mean
	0 (T1)	20 (T2)	40 (T3)	80 (T4)	160 (T5)	320 (T6)	
P <sub>1</sub> (21 d)	47.5	99.3	100	94.1	93.4	88.4	91.6
P <sub>2</sub> (56 d)	52.5	96.7	100	93.8	91.2	85.2	90.4
Overall mean	49.7 <sup>E</sup>	98.2 <sup>B</sup>	100 <sup>A</sup>	93.7 <sup>C</sup>	92.3 <sup>C</sup>	86.8 <sup>D</sup>	91.0

A, B,.....D Mean values having different superscripts within the same row are significantly ( $P < 0.05$ ) different.

Standard errors of means for treatments, periods and their interaction were 0.08, 0.05 and 0.11, respectively.

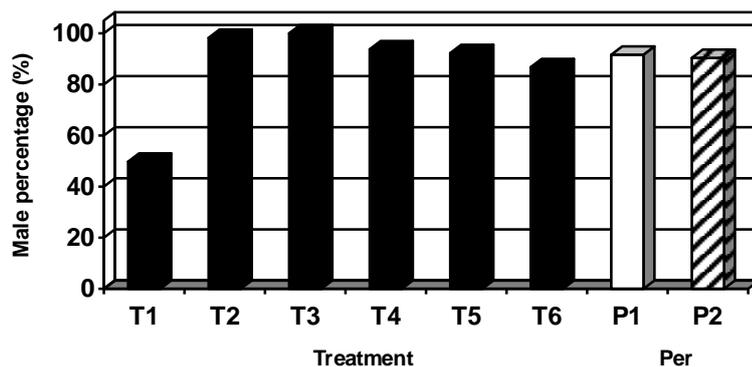


Fig. (1): Effect of different hormonal doses and treatment periods on male percentage of tilapia fish.

The percentage of males produced by the hormonal treatment with synthetic aromatizable androgene ( $17\alpha$ -MT) used in this study was almost higher than the successful results (>95% males) obtained using doses of 30-60 mg kg<sup>-1</sup> feed of  $17\alpha$ -MT (Magouz *et al.*, 1997). However, McGeachin *et al.* (1987) obtained 96-99% males using treatment with 60-120 mg  $17\alpha$ -MT kg<sup>-1</sup> feed for 22 days in *O. aureus*. The reduction in male % resulting from increasing the hormonal dosage are in accordance with the results of Piferrer *et al.* (1993) using high level of  $17\alpha$ -MT (400  $\mu$ g/L) for both of short (21 days) and long (56 days) treatment period which caused marked reduction in male percentages in Salmon fish as compared to the lower dosages.

Concerning the treatment period, no significant differences in male percentages produced were obtained for both hormonal treatment period (21 and 56 days) (Fig. 1). This may indicate the effective role of the hormonal treatment by  $17\alpha$ -MT at the early stage of age. Age and size of fry at the beginning of the hormonal treatment for monosexing are important as they are linked with gonadal differentiation (Eckstein and Spira, 1995). Shelton *et al.* (1978) recommended the use of *O. niloticus* fry with an age of two weeks after hatching to achieve a high percentage of males.

It is of interest to note that male percentage values were almost higher in all treatments with short treatment than long period (Table 2 and Fig. 1), however the interaction of dosages treatment x treatment period was not significant. Yet, T3 showed the same male percentage at both treatment periods, being 100% (Table 2).

#### Growth performance:

The present results revealed that the final body weight of the control group recorded the highest value being 657.8 g/aquarium compared with all treated groups, which ranged between 465.3 and 631.2 g/aquarium (Table 3), however such differences were statistically not significant.

In spite of the insignificant differences in body weight, the fish in T3, treated with 40 mg  $17\alpha$ -MT/kg diet had the highest final weight (631.2

g/aquarium) among the treated groups, while those treated with a dose of 320 mg  $17\alpha$ -MT/kg diet in T6 gave the lowest final weight, being 465.3 g/aquarium (Table 3). Throughout the experimental weeks, the average body weight of Nile tilapia fish hormonally treated for 21 days showed similar trend of change in all groups (Fig. 2), being insignificantly higher in T1, T3 and T5 than in T2, T4 and T6.

**Table 3: Initial body weight of tilapia (g/100 fry), final body weight (g/aquarium), weight gain, specific growth rate(SGR) and survival rate(SR) as affected by different doses of the hormonal treatment ( $17\alpha$ -MT) at both treatment periods.**

Treatment Period (day)	Items	Hormonal doses (mg/kg feed)						Overall mean
		0 (T1)	20 (T2)	40 (T3)	80 (T4)	160 (T5)	320 (T6)	
P1 (21 d)	Initial wt. (g/100 fry)	1.95	1.95	2.10	1.95	1.90	2.05	1.98
	Final wt. (g/ aquarium)	702.200	728.45	644.5	414.5	475.35	456.54	570.24
	Gain	700.4	726.7	642.4	412.8	473.6	454.4	586.4
	SGR	1.42	1.43	1.36	1.28	1.33	1.30	1.35
	SR%	32.0	28.9	31.4	24.4	21.8	26.0	27.4 <sup>B</sup>
P2 (56 d)	Initial wt. (g/100 fry)	1.80	2.05	2.00	2.15	1.85	2.15	2.00
	Final wt. (g/ aquarium)	613.30	492.00	617.80	414.5	510.45	475.05	549.04
	Gain	611.8	499.0	616.2	548.5	509.0	470.9	547.1
	SGR	1.40	1.33	1.38	1.28	1.35	1.30	1.35
	SR%	53.8	43.8	23.1	39.4	40.7	40.0	39.9 <sup>A</sup>
Overall mean	Initial wt. (g/100 fry)	1.88	2.00	2.05	2.05	1.88	2.10	
	Final wt. (g/ aquarium)	657.80	610.23	613.15	500.58	492.9	465.25	
	Gain	655.1	608.3	629.3	498.3	491.3	462.7	
	SGR	1.41 <sup>A</sup>	1.38 <sup>AB</sup>	1.37 <sup>ABC</sup>	1.31 <sup>ABC</sup>	1.34 <sup>ABC</sup>	1.30 <sup>C</sup>	
	SR%	42.7	36.1	27.2	31.6	30.9	32.3	

A, B, C, Mean values having the different superscripts within the same row are significantly different at  $P < 0.05$

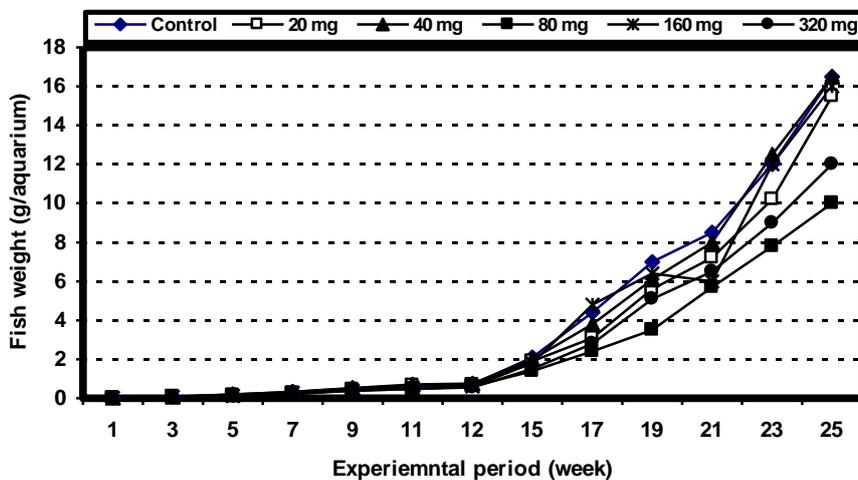
In spite of the insignificant effect of the hormonal dosage, the differences in average body weight among treated groups were pronounced from the 15<sup>th</sup> week up to the end of the experiment at 25 weeks of age (Fig. 2). Among all hormonally treated groups for 21 days, fish treated with 40 mg / kg feed showed the highest average body weight per aquarium (Fig. 2). This may be partly related to the lowest survival rate in this treatment, as will be discussed later, and, hence, the possibility of highest feed intake per fish and also the likelihood of less competition among fish within aquarium in T3 compared with other treatments.

Comparing the body weight changes between both treatment period, tilapia fish treated for 56 days (Fig. 3) and those treated for 21 days (Fig. 2), showed similar trend of change. The differences between treatment groups started to be pronounced from the 19<sup>th</sup> week of the experimental period.

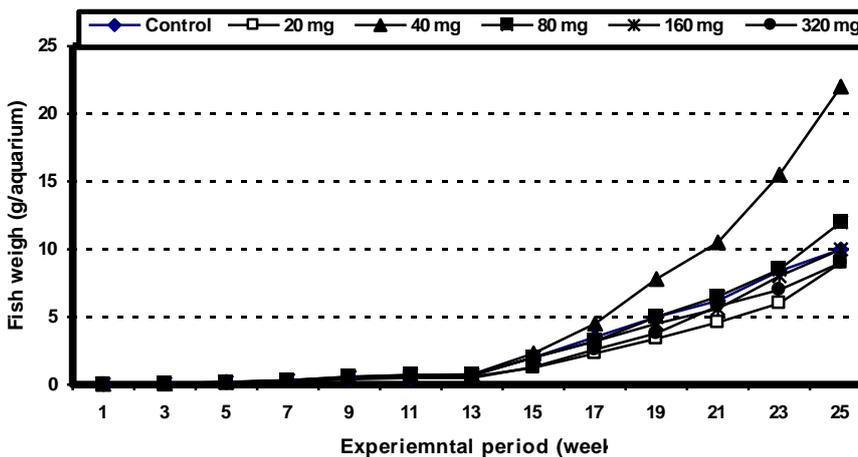
In agreement with previous studies, no significant effect on growth of fish was reported during the treatment period of sex reversal hormone using different doses of  $17\alpha$ -MT. Magouz *et al.* (1997) indicated that the level of 30-60 mg  $17\alpha$ -MT kg<sup>-1</sup> feed had no effect on the growth rate of treated tilapia. Similarly, Cruz and Mair (1994) and Rinchar *et al.* (1999) reported no effect of the hormonal treatment on growth rate of monosex reversal fish. Also, El-

Harairy (2000) found that the growth was hindered in the hormonally treated Nile tilapia fish despite the lower number of fish in each aquaria.

Concerning the effect of the hormonal treatment on average total weight gain of Nile tilapia fish, no significant differences were found among the experimental treatments, although fish in T1 showed the highest values, followed by T3, T2, T4 and T5, while T6 showed the lowest values (Table 3).



**Fig. 2:** Changes in body weight of fish at different experimental weeks in response to different doses of the hormonal treatment for 21 days.



**Fig. 3:** Changes in body weight of fish at different experimental weeks in response to different doses of the hormonal treatment for 56 days.

The effect of treatment period and its interaction with the hormonal treatment was also not significant. Regardless of the insignificant differences in total weight gain, it is of interest to note that the lower doses (20-40 mg/kg<sup>-1</sup> feed) of the hormonal treatment were almost more effective on total weight

gain which was higher in the short than in the long treatment period. The opposite was observed for the higher doses 80-320 mgkg<sup>-1</sup> diet.

Administration of androgens has been reported to accelerate growth in fish (Yamazaki, 1976 and Lone and Matty, 1980). The present results contrasted the results of Lone and Matty (1980), who reported that 17 $\alpha$ -MT induces better growth of fish. Meanwhile, they have also suggested a retardation of growth with 17 $\alpha$ -MT when the dosage increased from 5 to 10 ppm, which is in agreement with increasing dosage of the hormonal treatment in the present study above 40 mgkg<sup>-1</sup> diet.

Similarly, detritus and detrimental effects of 17 $\alpha$ -MT at higher dosage have also been reported. The tendency of retarded growth rate at the higher dosage has been attributed to catabolic action of 17- $\alpha$  MT and decrease in appetite of the organism (Lone and Matty, 1980).

On the other hand, the effect of interaction between the hormonal dosage and treatment period on total weight gain was not significant (Table 5). However, the lower doses (20 and 40 mgkg<sup>-1</sup> feed) resulted in higher total gain with the short than long treatment period. The opposite was observed for the higher doses (80-320 mg kg<sup>-1</sup> feed) (Table 3). There were significant differences ( $P < 0.05$ ) among the experimental treatment groups in the specific growth rate (SGR). The highest SGR was recorded in the control group being 1.41 followed by 1.38 and 1.37 for groups treated with 20 and 40 mg 17 $\alpha$ -MTkg<sup>-1</sup> feed, respectively (Table 3). Such difference was significantly higher in the control and the T2 than in the T6. The SGR of fish treated with 320 mg 17 $\alpha$ -MTkg<sup>-1</sup> feed (T6) recorded lower value (1.30) as compared to those treated with 20 mg 17 $\alpha$ -MTkg<sup>-1</sup> feed (T2).

Ridha and Lone (1990) reported no significant effect on relative and specific growth rates of *O. spilurus* stocked in brackish water and treated with MT for 34 days at 50 mg/kg treatment level. Similar results were also reported by Cruz and Mair (1994) on *O. niloticus* fish. The treatment period of 17 $\alpha$ -MT had statistically insignificant effect on specific growth rate (SGR). Concerning the effect of interaction between the hormonal treatment period, no significant differences were observed. Generally, higher specific growth rates were obtained for the lower dosages (up to 40 mg) during the short period of treatment, while, the lower specific growth rates were recorded for the dosage above 40 mg.

#### **Survival rate:**

There were insignificant differences among treatments, however, treatment period showed a significant effect on the survival rate, being higher during the long period (56 days) than the short period (21 days) (Table 3).

It is worthy noting that the fry treated with 40 mg 17 $\alpha$ -MTkg<sup>-1</sup> diet showed the lowest survival rate on the basis of over all mean for the long and short treatment periods (Table 3).

Values of SR% showed marked reduction in all treatment groups at the beginning of the experiment up to the 5<sup>th</sup> week of experiment period in fish treated for either 21 days or 56 days. The drop was, however, more pronounced during the first three weeks in the treated groups compared to

the control one. Masculinization with the male heterogametic species may lead to lower SR% of males (Pandian and Sheela, 1995).

The control group had the highest SR as compared to all treated groups during the first nine weeks of the experimental period. McGeachin *et al.* (1987) suggested that increasing the hormonal dosage resulted in decreasing SR% of *O. aureus* fry fed MT.

It is well established that fry of most species raised in aquaria usually exhibited high losses during the first few weeks of life (Hara *et al.*, 1986; Rizk, 1997 and El-Harairy, 2000).

Jensen and Shelton (1979) reported that bacterial diseases may cause high mortality, especially during the earlier age of fry. However, Pandian and Sheela (1995) suggested that in most fish species, using synthetic hormone for sex reversal resulted in a higher mortality. In contrast, the results obtained by Magouz *et al.* (1997) on Nile tilapia fry indicated that the relatively low levels of hormone (30-60 mg MT kg<sup>-1</sup> feed) had no effect on the SR of treated fish. Furthermore, Dan and Little (2000) found no effect of hormone treatment using the doses of 30-60 mg MT kg<sup>-1</sup> feed, on the SR of monosex Nile tilapia fry introduced in Northern Vietnam.

Concerning the effect of interaction between the hormonal treatment and period of treatment on SR%, it was revealed that the short period of treatment had lower values than the long period in almost all treatment groups.

It is worthy noting that, fry in T3 treated with 40 mg 17 $\alpha$ -MTkg<sup>-1</sup> showed the highest SR% among the treated groups for 21 days. However, with the hormonal treatment period of 56 days dosage of 40 mg 17 $\alpha$ -MT showed the lowest SR%, which was reflected in the lowest SR% on the basis of overall mean for both treatment periods (Table 3 ).

#### **Histological examination of gonads:**

The histological examination of the control and different treated groups revealed differentiated gonads (tests and ovaries) in fish of the control group in both male and female (Plates 1 and 2, respectively).

Gonadal sex differentiation in fishes is a complex and labile process, controlled ultimately by specific sex-determining gene (Conover and Fleisher, 1986 and Shapiro, 1988). Gonadal differentiation is highly sensitive to sex-including substances produced by the somatic elements of developing gonads and perhaps, by other tissue (Yamamoto, 1969 and Adkins-Regan, 1987). In sex reversal males, the gonads had abnormal structure, which tended mainly to be testes (Plate 3). In treated groups (T2, T4, T5 and T6), some individuals showed ovaries having immature follicles (Plate 4).

In fish, the different biopotential germ cells would differentiated into spermatogonia in response to andro-inducers, whereas, those same cells would yield oogonia in the presence of gyro-inducers (Evans, 1993). The present histostructure of gonads seems to support a role for synthetic 17 $\alpha$ -MT as a sex steroid hormone in induction of monsex tilapia fish.

**Plate 1: Section in testes of tilapia fish showing the normal structure of the control group with normal seminiferous tubules and interstitial tissue. (X 400 H&E)**

**Plate 2: Section in ovary of tilapia showing the normal structure of the control group with normal developmental stages of ovarian follicles. (X 400 H&E)**

**Plate 3: Section in gonads of sex reversed tilapia fish showing female like oocyte elements(a) and male like seminiferous tubules (b) scattered through the gonadal tissue. (X 400 H&E)**

**Plate 4: Section in the tests of Nile tilapia fish showing the degenerated seminiferous tubules with clear intraluminal cellular depress in T5. (X 400 H&E)**

**Plate 5: Section in the tests of Nile tilapia fish showing interrupted basement membrane in T3. (X 400 H&E)**

**Plate 6: Section in the tests of Nile tilapia fish showing atrophy and necrotic cells of the interstitial tissue T4. (X 400 H&E)**

Steroids including androgens are important to be present before gonadal differentiation to sex detecting in fish (Evans, 1993). Exogenous androgens may postpone this process by some unknown mechanism resulting in masculinization (Rothbard *et al.*, 1990). Most compelling, however, are the numerous studies showing reversal of expected sexual phenotype in many fish species by the judicious application of exogenous androgens to masculinize during the sensitive period before or during gonadal differentiation (Hunter and Donaldson, 1983).

The treated fish were almost characterized by small, thin filiform sterile gonads as compared to large well developed gonads (ovaries or testes) in the control fish. This is in accordance with the results of Ali and Rao (1989) in carp (*Cyprinus carpio*) treated with 300 or 400 ppm  $17\alpha$ -MT.

In all treatments of the present study, some sex reversed fish individuals showed sterility and had undeveloped testes. Spermatogenesis cells at different stages of development were separated from the basement membrane of the seminiferous tubules and were located centrally within their lumen (Plate 4). Ruptured basement membranes were observed (Plate 5). Within the interstitial tissue of the testes, numerous necrotic cells were noticed compared with the control. Similar observations were reported recently by El-Harairy (2000) in tilapia fish treated with high dosage of  $17\alpha$ -MT for different treatment periods.

The administration of steroid hormones has also been reported to cause sterility in Rainbow trout (Yamazaki, 1983). Also, some individuals in treated groups showed male-female gonads. Female like oocyte elements were observed separately scattered through the testicular tissue of sex reversal males in T5 and T6 treated groups (Plate 3). It is speculated that the steroids stored within the egg do not allow differentiation into either male or female sex during the initial period (Saad and Rezek, 1999).

In light of the present findings, the hormonal treatment with  $17\alpha$ -MT resulted in some alteration in gonadal differentiation into testes in most treated groups. Generally, degeneration in the seminiferous tubules, atrophy and necrotic interstitial cells were detected as a result of the hormonal treatment in all treated groups of tilapia fish.

From the foregoing results it could be concluded that low dose of 20 mg  $17\alpha$ -MT.kg<sup>-1</sup> feed caused intersexuality (sterility), while the high doses of 80-320 mg  $17\alpha$ -MT.kg<sup>-1</sup> feed resulted in paradoxical included males and females of tilapia fish. However, the optimum recommended dose of hormone would be 40 mg  $17\alpha$ -MT.kg<sup>-1</sup> feed for treatment period of 21 days, which produced 100% males.

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**تأثير المعاملة الهرمونية على إنتاج ذكور وحيدة الجنس من أسماك البلطي النيلي**  
فوزى إبراهيم معجوز\* ، مصطفى عبد الحليم الحرايرى\*\*، عبد الخالق السيد عبد الخالق\*\* ، عادل فتحى عباده\*\*  
\* قسم الإنتاج الحيوانى - كلية الزراعة - كفر الشيخ - جامعة طنطا.  
\*\* قسم الإنتاج الحيوانى - كلية الزراعة - جامعة المنصورة.

تمت هذه التجربة لدراسة تأثير التغذية بمستويات مختلفة من هرمون 17-ألفا ميثيل تستوستيرون المخلق صناعيا على قلب الجنس و خصائص النمو فى زريعة البلطي النيلي (عمر حوالى أسبوع) .

اشتملت هذه الدراسة على 240 أصبغية من فقس الأسماك الحديث وقد قسمت هذه الأسماك إليست مجموعات حسب الجرعة المستخدمة وقد اشتملت على فترتين للمعاملة الهرمونية (21 ، 56 يوماً) ، واحتوت كل مجموعة على حوضين (مكررتين) وكل حوض به 100 سمكة . غذيت مجموعة واحدة من المجموعات التجريبية على عليقة خالية من الهرمون واعتبرت المعاملة المقارنة ثم غذيت الخمس مجموعات الأخرى على عليقة تحتوى على 20، 40، 80، 160 و 320 ملليجرام 17-ألفا ميثيل تستوستيرون/كجم عليقة لمدة 21 يوم ، 56 يوم ثم غذيت الأسماك بعد إنتهاء المعاملة على عليقة خالية من الهرمون لمدة تتراوح من 140-154 يوم حسب فترة المعاملة. وكانت أهم النتائج المتحصل عليها كما يلي :

1. إن إضافة هرمون 17-ألفا ميثيل تستوستيرون بالمستويات المختلفة 80، 40، 20، 160، 320 قد أنتجت نسبة عالية من الذكور تتراوح نسبتها ما بين 88،4 إلى 100% ذكور وظهرت المعاملة بمستوى 40 ملليجرام 17-ألفا ميثيل تستوستيرون أعلى نسبة قلب فى الجنس معنويا(100%) عند كل من فترتى المعامله الهرمونية (21 ، 56 يوماً) وكانت أقل هذه النسب فى أسماك مجموعة المقارنة (50%) وظهرت نتيجة 50% ذكور ، ولم تلاحظ أى فروق معنوية بين فترتى المعاملة .
2. وجد أن إضافة هرمون 17-ألفا ميثيل تستوستيرون بالمستويات والفترات المختلفة للمعاملة تحت الدراسة لم تؤثر معنويا على وزن الأسماك أو معدل النمو الكلى فى الأسماك ولم توجد أى فروق معنوية بين المعاملات وبين الفترات بالنسبة لنمو الأسماك.
3. بالنسبة لمعدل النمو النوعى وجد أن إضافة هرمون 17-ألفا ميثيل تستوستيرون بالمستويات المختلفة فى الدراسة لم يؤدى الى اية فروق معنوية بين المعاملات و بين الفترات.
4. أبتت التجربة أنه لم توجد أى فروق معنوية بين المعاملات فى معدل حيايته السماك ولكن كانت هناك فروق معنوية بين فترتى المعاملة وكان أعلى معدل نفوق للمعاملات الهرمونية للفترة الطويلة (56 يوم).
5. أظهر الفحص الهستولوجى حدوث تغيير غير طبيعى فى تركيب الغدد الجنسية للذكور الناتجة من المعاملة والتي كانت معظمها عقيمة مقارنة بمجموعة المقارنة.