Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

ALL-MALE MONOSEX NILE TILAPIA (*OREOCHROMIS NILOTICUS*), PROS AND CONS

Abdelhamid*, A. M.; A. I. Mehrim*, M. F. I. Salem and H. A. E. Yosuf***

*** Dept. Anim. Prod., Fac. Agric., Almansoura Univ., Egypt**

**** Cent. Lab. Aquacul. Res., Ministry of Agriculture, Egypt**

ABSTRACT

To throw light onto pros and cons of using testosterone (for sex reversal of newly hatched fry of Nile tilapia); whether concerning fish safety (at the marketable size), i.e. fish growth and hormone residues; or concerning water and waste water pollution with the hormone residues, the present study was undertaken. The results showed that the hormone treated (HT, T_1) fish were superior in body weight and total body length comparing with the normal [hormone not treated (NT,T_2)] fish. Atrophy was happened in gonads of HT group; since there were decreases in values of gonads' diameter, volume, weight, specific gravity, and gonado-somatic index comparing with NT group. The histological examination of testis of (HT) showed abnormal structure and degeneration of seminiferous tubules. Generally, these histological findings were confirmable with those of the anatomical findings which showed slight atrophy in testes compared with testis in NT group. The HT fish group reflected significant increases of plasma values of cholesterol, total protein, and globulin, also there were non significant increases in plasma total lipids and albumin in comparison with NT fish group. Water analysis for testosterone reflected low levels. Yet, the highest levels were neither in the treatment pond nor in the branchial drain from the treatment pond but were in the fattening pond of HT fish and the main drain. Blood plasma of the HT fish group contained lower level of total testosterone hormone and higher level of progesterone hormone comparing with the NT fish group. The HT fish gave lower testosterone concentration and higher progesterone level in the gonads comparing with the control fish gonads. Testosterone level in the whole fish body was lower in HT fish than in NT fish. Thus, it is recommended to use 17-α-methyltestosterone in producing all male monosex (sex reversal) Nile tilapia to obtain more fish production with quick capital cycle and economic as well as environmental friendly product which is safe for human consumption.

Keywords: Monosex – Testosterone – Nile tilapia – Safety.

Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

INTRODUCTION

All-male monosex Nile tilapia fish produced by hormone treatment is a newly introduced technique to the local market. It is quickly outspreading for its economical advantages (high growth of fish with better feed conversion). Yet, there is a great debate concerning the safety of this technique on fish consumers and fish hatchery waste water, which may be drained into surface water. Particularly, this hormone, 17-α-methyltestosterone which used for sex reversal of tilapia, has carcinogenic effects (Abdelhamid, 2005-a) by mishandle (Nemat Allah, 2002). Although the precautional steps which are followed in hatcheries concerning the handle of this expensive hormone, and without any scientific study, the Egyptian General Authority for Fish Resources Development was responsible for the ministerial decision No. 2655 year 2003 which forbid the use of this hormone in the fish hatcheries for the public health and which considered its use is conflicting with the law no. 124 year 1983 (articles no. 17 & 55) (Abdelhamid, 2003-b and 2005-b). Therefore, the present study was designed to investigate and give lights on the positive and negative effects of the commercial using of this hormone to sex reversal of Nile tilapia fry. The evaluation was carried out through comparing hormone-treated and not-hormone treated fish at the same age concerning body measurements, indices, blood profile, hormone residues and histology of the gonads.

MATERIALS AND METHODS

This study was carried out during May/June 2008 in the Department of Animal Production, Faculty of Agriculture, Al-Mansourah University. Hormone treated all-males and non-treated mixed sex of Nile tilapia fish from the over-wintered fingerlings (of the last season 2007) were collected from Dr. Salah Ibrahim Fish Hatchery, Tolombat 7, Kafr El-Sheikh governorate. Five water samples also were collected (from the same fish farm). One sample from each of the concrete pond of sex reversal (feeding newly hatched fry the hormonized diet at a level of 60 ppm 17-α-methyltestosterone for 21 days), the branched drain (waste water of the concrete ponds), main drain, rearing earthen pond of the sex reversed all male, and rearing earthen pond of the sex non-reversed tilapia fish. All fish collected were individually weighed and measured (Abdelhamid, 2003-a). Their gonads were separated, weighed, measured and gonado – somatic index was calculated after Tseng and Chan (1982). Blood samples were withdrawn from the hearts into heparinized clean test tubes for plasma separation (by centrifugation for 20 min.) for biochemical analyses. Plasma parameters were colorimetrically tested using commercial kits for total protein according to Doumas et al. (1981) albumin, (Ratliff and Hall, 1973), globulin (by difference), total lipids (Schmit, 1964), and cholesterol (Stein,

ISSN: 1687-1146 ISBN: 12154/2002

Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

1986). Fish as well as gonads were extracted into methanol for testing the hormone level and its residues. Testosterone was detected in plasma and extracts of fish and gonads as well as in waste water via Apparatus Immulite (Siemens USA) FDA approved using commercial kit for chemiluminescence's technique for hormonal assay. Whereas progesterone was determined in plasma and gonads extract using the same Apparatus Immulite (Siemens USA) FDA approved for hormonal assay using chemiluminescence's technique. Samples of gonads were preserved in 10% neutralized buffered formalin saline solution for histological study (Bancroft et al., 1990). Thereafter, the slides were ready for examination under light microscope and histopathological changes in gonads were recorded. All numerical data collected were tested statistically by calculating the t-test for the statistical comparison between two means (Sachs, 1976).

RESULTS AND DISCUSSION

Growth performance: Table (1) revealed that hormone treated all males Nile tilapia have significantly ($P \leq 0.01$) higher fresh (wet) body weight (by 177%) and total body length (by 43.26%) means as well as lower standard errors than the hormone not treated (males plus females) fish, although both groups were from the same hatch. The superiority of all-male monosex Nile tilapia over the both sexes (not sex reversed) fish is due to the advantage of tilapia males which characterized by higher growth rate than females (Little and Edwards, 2004). Riley et al. (2004) said that the different growth rates observed between male and female tilapia may be a result of gonadal steroid hormones eliciting direct and antagonistic effects on production of insulin-like growth factor-1. The superiority of male than female (mixed sexes) in growth may also due to methyltestosterone (MT) used in sex reversal, which is a synthetic active androgen acts as synergist for insulin, increases protein anabolism (Kutsky, 1973). It is so, led to better growth in the 17-α-methyltestosterone treated fish. Since, routine metabolism corrected for metabolic body mass, was positively correlated with the biologically active metabolite of testosterone, 11-ketotestosterone, but not with testosterone itself (Ros et al., 2004). Meanwhile, Zaki (2004) found that the most rapidly growing fish were MT treated- sex reversed Nile tilapia. While normal male and normal female Nile tilapia produced by separation of sexes come second and third, respectively; however, mixed sex tilapia grew at the lowest rate.

ISSN: 1687-1146 ISBN: 12154/2002

Table (1): Effect of 17-α-methyltestosterone treatment for production of all-male monosex Nile tilapia on their body weight and total length.

Items	NT (control)	HT
Weight (g)	110.1** ± 8.83	305.0** ± 5.00
Length (cm)	17.8** ± 0.56	25.5** ± 0.29

** Means ± SE in the same row have significant difference.

n= 3 $P \leq 0.01$.

The following Fig. (1) illustrates some morphological and anatomical differences between monosex Nile tilapia treated fish with 17-α-methyltestosterone (T_1) and untreated Nile tilapia with 17-α-methyltestosterone (T_2, as a control group). From this Fig. (1), there are differences between both groups T_1 and T_2 in length and size from the morphological side. Monosex tilapia (T_1) appeared bigger and longer than untreated Nile tilapia (T_2). Many researchers are in agreement with these findings. In tilapia culture, males are preferred because they grow almost twice as fast as females, resulting in higher production and profit for the farmers (Hanson et al., 1983). However, Mair and Little (1991) added that the culture of monosex tilapia, preferably all-male populations, is important for the production of higher yields and more uniformly-sized fish. Moreover, during the critical period of differentiation, treatments with exogenous sex hormones often induce sex reversal. Consequently, endogenous androgens and estrogens have been assumed to act as the natural inducers of testicular and ovarian differentiation, respectively. Al-ablani and Phelps (2002) emphasized that monosex populations of fish are often desirable as a method to control reproduction and to take advantage of sex-linked differences in growth rates.

It is well known that anabolic steroids may produce fish with increased weight gains and muscle deposition (Sambhu and Jayaprakas, 1997). Since it increased the feed digestion and absorption rate causing increase in body weight (Yamazaki, 1976). It increased the proteolytic activity of the gut leading to increase the growth rate (Lone and Matty, 1981). It may also promote the release of growth hormone (Higgs et al., 1976). Little et al. (2003) came to the same conclusion, where sex-reversed tilapia grew better and economic than the non sex-reversed fish. Recently, El-Saidy (2005) revealed that the growth in weight and length was higher significantly in mono-sex male compared with mono-sex female and normal mixed sex Nile tilapia. He added that the mean values of mixed sex group

Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

were significantly higher than those of monosex female.

Gonads: It is clear from Table (2) that there was a positive (by 69.3%) and significant ($P \leq 0.01$) effect of hormone treatment on the mean of gonads length comparing with the non treated group of fish. Therefore, there were no significant ($P \geq 0.05$) effects on gonads' diameter, volume, weight, specific gravity and gonado-somatic index (GSI), although the mean values of these parameters (gonads' diameter, volume, weight, specific gravity and GSI) were lower in HT group (by 28.57, 3.49, 46.77, 43.52 and 79.82%, respectively) than the NT one. Testis showed slight atrophy in monosex tilapia fish T_1 (Fig. 2) compared with testis in the control group (T_2), which appeared in normal structure (Fig. 3). While, ovary in untreated Nile tilapia (T_2) group showed maturing ovary, which contains mature eggs (Fig. 4). This means that MT treatment for sex reversal may be negatively affected the growth of gonads during their evolution phase (hormone treatment period). Methyltestosterone is a synthetic active androgen which acts as antagonist for the estrogens (except in low concentrations), progesterone, norethandrolone, methylcholanthrene and α-norprogesterone. It also acts as synergist for insulin, other androgens, and estrogens (in low concentrations) (Kutsky, 1973). It has physiological functions including controls secondary male sex characteristics, maintains functional competence of male reproductive ducts and glands, increases protein anabolism, maintains spermatogenesis, inhibits gonadotrophin, increases male sex behavior, and increases closure of epiphyseal plates. Abdelhamid et al. (1998) reported mean value of GSI in hormone not treated Nile tilapia similar to that found herein, being 2.69 - 2.98%. El-Saidy (2005) also reported that GSI of mono-sex male Nile tilapia had significantly the lowest mean values (0.43%) compared with others (1.72% for female mono-sex and 1.75% for normal mixed sex).

Fig. (1): Shows differences in length and size between monosex Nile tilapia treated with 17-α-

Fig. (2): Shows slight atrophy in testes of monosex Nile tilapia

Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

methyltestosterone (T_1, above) and untreated Nile tilapia with 17-α-methyltestosterone (T_2, control group, beneath).

Fig. (3): Shows normal structure of testes in untreated Nile tilapia, with 17-α- methyltestosterone (T_2, as a control group).

treated with 17-α-methyltestosterone (T_1).

Fig. (4): Ovary of untreated Nile tilapia, with 17-α-methyltestosterone (T_2, as a control group) showing mature ovary contains mature eggs.

Table (2): Effect of 17-α-methyltestosterone treatment for production of all-male monosex Nile tilapia on their gonads length, diameter, volume, weight, specific gravity and GSI.

Items	NT (control)	HT
Length (cm)	4.43** ± 0.07	7.5** ± 0.55
Diameter (cm)	1.12^{NS} ± 0.21	0.8^{NS} ± 0.06
Volume (cm)	21.50^{NS} ± 0.85	20.75^{NS} ± 0.04
Weight (g)	2.63^{NS} ± 1.41	1.40^{NS} ± 0.27
Specific gravity	0.12^{NS} ± 0.19	0.0671^{NS} ± 0.01
GSI	2.26^{NS} ± 1.20	0.46^{NS} ± 0.08

NS: not significant at P ≥ 0.05. n=3

** Means ± SE in the same row have significant difference at P≤ 0.01.

ISSN: 1687-1146 ISBN: 12154/2002

Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

Histology of the gonads:

Testis: The histological examination of monosex Nile tilapia treated with 17-α- methyltestosterone (T_1) showed abnormal histological structure of testis with degeneration of seminiferous tubules and depressing some cells intraluminally (Fig. 5). Testis in the same fish group (T_1) at high magnification showed depression of seminiferous tubules, degeneration of spermatogenetic layer of some seminiferous tubules and disappearance regions of some seminiferous tubules (Fig. 6). Generally, these histological findings in testis of monosex Nile tilapia treated with 17-α-methyltestosterone (T_1) are confirmable with those of the anatomical findings (Fig. 2) which showed some slight atrophy in testes compared with other testis in T_2 group. These abnormal alterations in testis of monosex Nile tilapia (T_1) may be due to hormonal treatment of tilapia with 17-α-methyltestosterone in fry stage. However, the histological examination of testis from untreated Nile tilapia with 17-α- methyltestosterone (T_2, control group) showed typical bean shape, normal structure of testis and normal semineferous tubules with sperm and fibrous connective tissue (Fig. 7). Moreover, at high magnification of transverse section in testis from the same fish group (T_2), it showed normal structure of semineferous tubules, lumen (which contains primary and secondary spermatocytes) and interstitial tissue (Fig. 8). These histological findings in the present study are in agreement with those reported by El-Harairy (2000) in tilapia fish treated with 17-α- methyltestosterone. Also, Magouz et al. (2000) found that hormonal treatment resulted in abnormal structure of the tilapia fish testis including semeniferous tubules degeneration and atrophy of the interstitial tissue. Ebada (2004) reported that the use of 17-α-methyltestosterone with tilapia fish caused sterility, in term of separation of the spermatogenetic layers from the basement membrane of the semeniferous tubules of the testes. Besides, there were some semeniferous tubules free from spermatogenetic layers and the interstitial was almost free from lyding cells. The last author added that there were no sperms in the lumen of the semeniferous tubules in the treated fish. Moreover, Mehrim et al. (2006) emphasized the same findings in all examined testes which were not affected by the treatment of aflatoxin-B_1 but the drastic effects in gonads were found to be from the hormonal treatment with 17-α-methyltestosterone for producing monosex tilapia fish during fry stage in the commercial fish hatchery.

Ovary: The histological examination of ovary from untreated Nile tilapia with 17-α- methyltestosterone (T_2, control group) showed normal structure of ovarian lamellae, which contains oocytes at various stages of oogenesis (Fig. 9). Moreover, at high magnification of transverse section in ovary from the same fish group (T_2), it showed primary oocyte at the yolk vesicle (YV)

ISSN: 1687-1146 ISBN: 12154/2002

stage (Fig. 10) and primary oocyte with nucleus (N) at the yolk globules (YG) stage (Fig. 11). These histological findings in ovary of Nile tilapia fish are conformable with those of the anatomical findings (Fig. 4) which showed maturing ovary contains mature eggs. Also, from scientific point of view, these maturing alterations in ovary of Nile tilapia are due to spawning period of this species in Egypt (March to May) whenever the fish samples were collected. Similar observations in tilapia fish oocytes development stages were reported by many researchers. During a secondary growth period (vitellogenesis) the oocytes enlarged to 964µm, chiefly by rapid incorporation of large amounts of exogenous hepatically derived vitellogenin. Major developmental events can be divided into six phases: oogenesis, primary oocyte growth, cortical alveolar stage, vitellogenesis, maturation and ovulation (Tyler and Sumpter, 1996). Similar structures were reported by Hussein (1984). It is possible that the small vesicles located close to the oocyte periphery are cortical alveoli since their size, shape and distribution are very similar to those of alveoli at stage 4. Stage 5 is arguably the most important phase of oocyte development, since during this phase, vitellogenesis occurs, resulting in extensive oocyte growth. In accordance with Bromage and Cumaranatunga (1988), there was little change in the follicular layer of rainbow trout oocyte between stages 4 and 5 apart from a more cuboidal appearance to the granulosa.

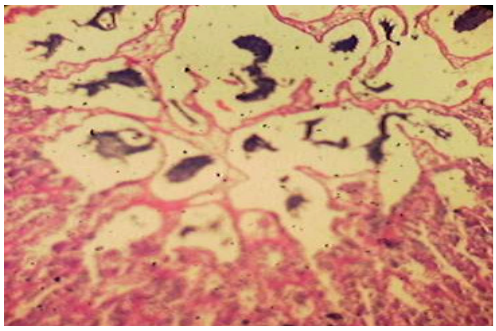

Fig. (5): Transverse section in testis of monosex tilapia treated fish with 17-α- methyltestosterone (T_1) showing abnormal histological structure of testis with degeneration of seminiferous tubules and depressing some cells intraluminally (X 60, H&E stains).

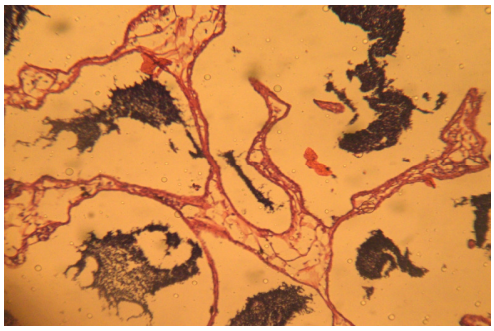

Fig. (6): Transverse section in testis of monosex tilapia treated fish with 17-α- methyltestosterone (T_1) showing depression of seminiferous tubules, degeneration of spermatogenetic layer of some seminiferous tubules and disappearance regions of some seminiferous tubules (X 120, H&E stains).

ISSN: 1687-1146 ISBN: 12154/2002

Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

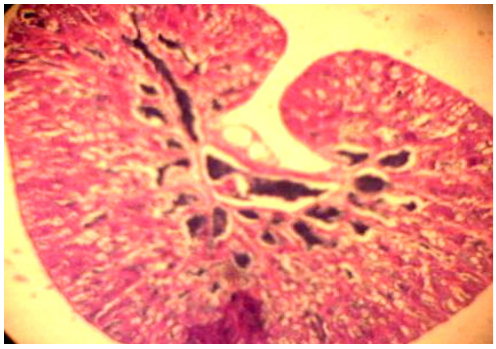

Fig. (7): Transverse section in testis of untreated Nile tilapia with 17-α-methyltestosterone (T_2, control group) showing typical bean shape, normal architecture of testis and normal semineferous tubules with sperm and fibrous connective tissue (X 60, H&E stains).

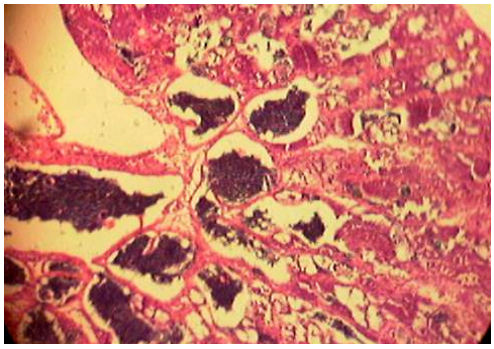

Fig. (8): Transverse section in testis of untreated Nile tilapia with 17-α-methyltestosterone (T_2, control group) showing normal structure of semineferous tubules, lumen which, contains primary and secondary spermatocytes, and interstitial tissue (X 120, H&E stains).

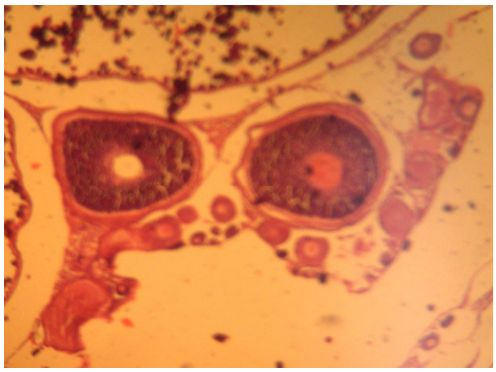

Fig. (9): Transverse section in ovary of untreated Nile tilapia with 17-α-methyltestosterone (T_2, control group) showing normal structure of ovarian lamellae, which contains oocytes at various stages of oogenesis (X 60, H&E stains).

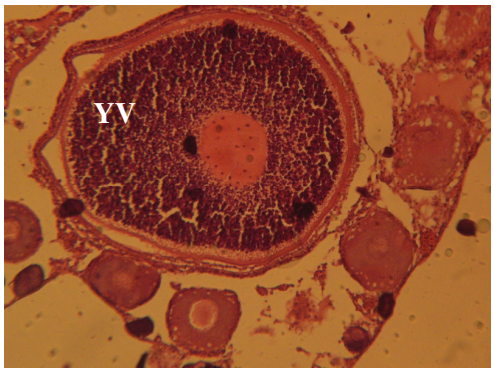

Fig. (10): Transverse section in ovary of untreated Nile tilapia with 17-α- methyltestosterone (T_2, control group) showing primary oocyte at the yolk vesicle (YV) stage (X 120, H&E stains).

ISSN: 1687-1146 ISBN: 12154/2002

Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

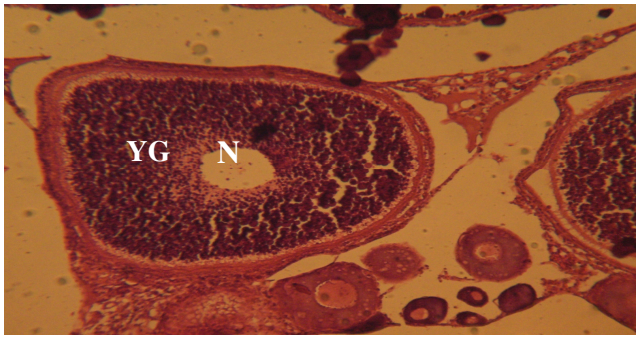

Fig. (11): Transverse section in ovary of untreated Nile tilapia with 17-α-methyltestosterone (T_2, control group) showing primary oocyte with nucleus (N) at the yolk globules (YG) stage (X 120, H&E stains).

Blood biochemical parameters: Table (3) illustrated the values of some biochemical parameters in blood plasma of Nile tilapia fish, hormone treated (HT) and not treated (NT). From this table, it is clear that total lipids and albumin values did not differ ($P \geq 0.05$) between HT and NT groups of fish; yet, there were significant ($P \leq 0.05$) differences between both groups concerning cholesterol, total protein and globulin levels in favor of HT fish. The increases are 210.8, 45.7 and 59.2%, respectively. Also, the non significant increases in total lipids and albumin mean values are 38.5 and 26.3%, respectively, in favor of HT comparing with NT fish. Moreover, the significantly increased protein herein was associated also with significantly increased globulin level, which is responsible for good immune system (Abdelhamid, 2003-a). In accordance with the present results, Sunny et al. (2002) reported increases in protein bands in the testosterone treated plasma of *O. mossambicus* confirmed the anabolic role of steroids in teleost. It is also recognized that fish lipids are healthy for humans, particularly for patients of blood circular system disorders (Abdelhamid, 2003-a).

Table (3): Effect of 17-α-methyltestosterone treatment for production of all-male monosex Nile tilapia on total lipids, cholesterol, total protein, albumin and globulin

ISSN: 1687-1146 ISBN: 12154/2002

Items	NT (control)	HT
Total lipids, (mg/dl	$402.3^{NS} \pm 36.98$	$557.3^{NS} \pm 47.13$
Cholesterol (mg/dl)	27.9* ± 15.39	86.7* ± 5.237
Total protein (g/dl)	2.19* ± 0.234	3.19* ± 0.162
Albumin (g/dl)	$0.95^{NS} \pm 0.120$	$1.20^{NS} \pm 0.054$
Globulin (g/dl)	1.25* ± 0.115	1.99* ± 0.118

NS: not significant at $P \geq 0.05$. n=3

*Means ± SE in the same row have significant difference at $P \leq 0.05$.

Hormonal residues follow-up:

In farm water and waste water: Testosterone levels in the farm water and waste water were 0.21, 0.18, 0.33, 0.39, and 0.19 ng/ml, in treatment concrete pond, branchial drain, main drain no. 7, fattening earthen pond for hormone treated fish, and fattening earthen pond for hormone not-treated fish, respectively. Its level was at the highest in the fattening pond of treated fish (0.39 ng/ml). The branchial drain and fattening pond of non treated fish contained 0.18 and 0.19 ng/ml, respectively, and the treatment pond's water contained also low level, being 0.21 ng/ml. Metabolism of MT is possible in bacteria and fungi (Jankov, 1977). In an outdoor pond, the hormone will be rapidly break down by the combination of light, temperature, and microbial degradation (Phelps et al., 1999). Fitzpatrick et al. (1999) detected MT in the water of containers in which tilapia were fed an MT-treated feed durian a treatment period of four weeks. The concentration decreased to background level by one week after the end of treatment. However, the concentrations of MT slightly decreased near the drain of the pond, moreover, the concentrations of MT detected in the environment were not significantly above background levels (Phelps et al., 2000). Contreras-Sánchez et al. (2001) reported that the detected levels have no evidence that it represent a health or environmental risk. Sex ratios of the groups fed control food while being maintained in the tanks that had contained the MT-treatment groups were not different from control fish (Contreras-Sánchez et al., 2002). Nemat Allah (2002) mentioned that one of the disadvantages of hormone sex reversal is the possibility of water contamination with hormone; so that, the treatment must be done in separate concrete ponds. However, this hormone will be destructed via direct sun rays during 24 hours. Moreover, the use of this hormone is restricted only for sex reversal, but not as growth promoter for its non economic (useless).

In fish blood: This test revealed no significant differences in total testosterone and progesterone between both groups of fish. Yet, the level

of testosterone in HT fish blood was lower by 16.8% than in NT group. But the progesterone level was higher by 45.7% in HT than NT fish (Table 4). These results are in accordance with those given by Nemat Allah (2002), where the obtained levels are of the natural produced (endogenous) hormone and there is no residual effect of the synthetic (exogenous) hormone used for sex reversal.

Table (4): Effect of 17-α-methyltestosterone application in production of all-male monosex Nile tilapia on plasma total testosterone and progesterone concentrations.

Items	NT (control)	HT
Total testosterone (ng/ml)	$2.56^{NS} \pm 0.51$	$2.13^{NS} \pm 0.80$
Progesterone (ng/ml)	$0.05^{NS} \pm 0.02$	$0.07^{NS} \pm 0.04$

NS: means **±** SE in the same row did not differ significantly.

n= 3 ($P \geq 0.05$).

In fish gonads: Table (5) shows that total testosterone level in gonads was insignificantly lower in HT by 53.8% than the control (NT). But the progesterone concentration was higher by 30% in HT gonads than NT. These data are in accordance with those of Nemat Allah (2002) which confirmed that the exogenous male sex hormone treatment leads to suppress the release of gonadotropin hormone, so lowers the production of natural sex hormone from the gonads of hormone treated monosex all-males Nile tilapia. Exogenous sex steroids markedly alter sex differentiation in fish. The endocrine and molecular mechanisms involved in these changes remain unclear. To further clarify the mechanism of androgen-induced testicular differentiation, the treated female tilapia with methyltestosterone (MT at a dose of 50 µg/g diet) were examined for the expression of P_{450} cholesterol-side-chain-cleavage, 3 β-hydroxysteroid dehydrogenase, and cytochrome P_{450} aromatase (P_{450} arom) in the gonads. The MT treatment resulted in 100% masculinization. Untreated fish showed normal ovarian differentiation with strong expression of all three steroidogenic enzymes. In gonads of MT-treated fish, expression of all three steroidogenic enzymes was attenuated within 15 days and completely disappeared within 30 days of treatment. The results indicated that exogenous androgen treatment suppresses the expression of key steroidogenic enzymes, including P_{450} arom throughout sex differentiation in tilapia, thus masculinizing the animal. Whether the absence of aromatase or the presence of androgens is responsible for testicular differentiation remains to be determined (Bhandari et al., 2006).

Egypt J. Basic Appl. Physiol., 8 (1):41-57 (2009)

Table (5): Effect of 17-α-methyltestosterone application in production of all-male monosex Nile tilapia on gonadal total testosterone and progesterone concentrations.

Items	NT (control)	HT
Total testosterone (ng/g)	$9.276^{NS} \pm 3.486$	$4.287^{NS} \pm 0.348$
Progesterone (ng/g)	$0.922^{NS} \pm 0.544$	$1.220^{NS} \pm 0.412$

NS: means ± SE in the same row did not differ significantly.

n= 3 ($P \geq 0.05$).

In fish carcass: The values of testosterone in fish carcass were 0.0236 and 0.0833 ng/g fresh weight in fish carcass from HT and NT Nile tilapia fish, respectively. It is very pronounced that HT fish contained 71.67% lower testosterone concentration than NT fish. This means that the exogenous hormone was totally metabolized and the endogenous hormone production was inhibited by the negative effect of the HT on the gonads. Hines et al. (1999) demonstrated that changes in the steroidogenic profile occur during early transitions of gonadal development. Notably, steroid biosynthetic capacity precedes gonadal differentiation. They added that, bimodality of levels of testosterone and different steroid metabolism later in development may reflect the onset of sexual divergence. Phelps (2001) registered rapid metabolism and excretion of MT by a fish treated early in its life history, combined with the extended period needed to produce a marketable size fish results in safe consumer product. Therefore, almost every tilapia grower now uses hormone-treated fingerling tilapia. This basically reduces the time it takes to get the tilapia through the growing stage and hopefully allows for slightly higher profits from the sale of the fish since a farmer can grow more each year (Hickling, 2006). Nemat Allah (2002) mentioned that during the treating fry with the low level of the male sex hormone, more than 90% of the hormone quantity are concentrated in the viscera (liver, spleen, kidneys and intestine), whereas 10% of the hormone go to the muscles. Moreover, Nemat Allah (2002) added that, after the 28 day-treatment period, the hormone is quickly disappeared. After 4 days (0.5 g body weight), 1% only is found in the viscera. So, after one month of the hormonized diet withdrawal, it seems obviously that there are no any traces of the synthetic hormone in the fish body (3 cm length). After 4 months of the withdrawal (catch and marketable size), the level of normal (endogenous) male sex hormone in the monosex Nile tilapia was 11 ng/g comparing with 37 ng/g in the normal male (not sex reversed) male. For knowledge, the effective treating dose of the male sex hormone for human being 80 mg (1333 times as the dose required for fish sex reversal). He attributed the lower male sex hormone level in monosex than the normal male to the depression of hormone production from pituitary

ISSN: 1687-1146 ISBN: 12154/2002

gland, which stimulates the sex hormone production from the gonads (gonadotrophen hormone releasing hormone). This means that the hormone found in the monosex is the natural (endogenous) hormone only. In this connection, all-males monosex are much lower in their fertility (due to lower number and viability of their sperms) comparing with the non treated males.

REFERENCES

Abdelhamid, A.M. (2003-a). Scientific Fundamentals of Fish Production and Husbandry. Al-Mansoura Univ. Press. 2nd Ed., Deposit No. 15733/ 2003.

Abdelhamid, A.M. (2003-b). Egyptian legislations governing the fisheries in Egypt from a view point of environment, administration and production. Proc. 1st Inter. Conf. Fish Wealth and Food Security in Arab and Islamic Countries. 22 – 24 Oct., Al-Azhar Univ., 14 p.

Abdelhamid, A.M. (2005-a). Carcinogens. Darannashr for Universities. Cairo, Deposit No. 1949/2005.

Abdelhamid, A.M. (2005-b). Legislations controlling fisheries in Egypt. Al-Saiad, 33: 4.

Abdelhamid, A.M.; Khalil, F.F. and El-Barbary, M.I. (1998). Effect of using graded levels of gibberellic acid in diets differing in the crude protein levels on performance and chemical composition of Nile tilapia fingerlings. Egypt. J. Aquat. Biol. & Fish., 2(4): 221-233.

Al-ablani, S.A. and Phelps, R.P. (2002). Paradoxes in exogenous androgen treatments of bluegill. Journal of Applied Ichthyology, 18: 61- 64.

Bancroft, J.D.; Sterens, A. and Turner, D.R. (1990). Theory and Practice of Histological Technique. 3rd Ed., Churchill, Livingston, Edinburgh, London, Melbourne and New York.

Bhandari, R.K.;. Nakamura, M.; Kobayashi, T. and Nagahama, Y. (2006). Suppression of steroidogenic enzyme expression during androgen – induced sex reversal in Nile tilapia (*Oreochromis niloticus*). Gen. Comp. Endocrinol., 145(1): 20 – 24.

Bromage, N. R. and. Cumaranatunga, P. R. T (1988). Egg production in the rainbow trout. In Recent Advances in Aquaculture (Muir, J. F. and Roberts, R. J., eds.), pp. 65–138. London: Croom Helm.

Contreras-Sánchez, W.M.; Fitzpatrick, M.S. and Schreck, C.B. (2001). Fate of methyltestosterone in the pond environment: detection of MT in pond soil from a CRSP site. In: A Gupta, K. McElwee, D. Burke, J. Burright, X. Cummings, and H. Egna (Editors), 18th Annual

Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon, USA, 5 p.

Contreras-Sánchez, W.M.; Fitzpatrick, M.S. and Schreck, C.B. (2002). Fate of Methyltestosterone in the pond environment: Impact of MT-contaminated soil on tilapia sex differentiation. PD/A CRSP 18th Annual Technical Report, Oregon, USA.

Doumas, B.T.; Bayse, D.D.; Carter, R.J.; Peters, T. and Schaffer, R. (1981). Clin. Chem., 27: 1642.

Ebada, A.F.E.A. (2004). Studies on tilapia fish. Ph.D. Thesis, Al-Mansourah Fac. of Agric., Egypt.

El-Harairy, M.A. (2000). Utilization of androgen for the production of monosex males tilapia (*Oreochromis niloticus*). J. Agric. Sci. Mansoura Univ., 25: 4949-4956.

El-Saidy, D.M.S.D. (2005). Growth performance and feed utilization of mono-sex (male or female) and normal mixed sex Nile tilapia, *Oreochromis niloticus* (L.) cultured in tanks. Minufiya J. Agric. Res., 30(3): 877 – 889.

Fitzpatrick, M.S.; Contreras-Sanchez, W.M.; Milston, R.H.; Hornick, R.; Feist, G.W. and Schreck, C.B. (1999). Detection of MT in aquarium water after treatment with MT food. In: K. McElwee, D. Burke, M. Niles, and H. Egna (Editors), Sixteenth Annual Technical Report. Pond Dynamics/Aquaculture CRSP, Oregon State University, Corvallis, Oregon. USA, pp. 81 – 83.

Hanson, T. R.; Smitherman, R. O.; Shelton, W. L. and Dunham, R. A. (1983). Growth comparisons of monosex tilapia produced by separation of sexes, hybridisation, and sex reversal. P: 570–579 in: Fishelson, L. and Yaron, Z. (eds.) Proceedings of the First International Symposium on Tilapia in Aquaculture. May 8–13, Tel Aviv University, Nazareth, Israel.

Hickling, C.F. (2006). The cultivation of Tilapia. http//www.aquanet.com/features/tilapia/perfectfish.htm.

Higgs, D.A.; Donaldson, E.M.; Dye, H.M. and McBride, J.R. (1976). Influence of bovine growth hormone and L-thyroxine on growth, muscle composition and histological structure of the gonads, thyroid, pancreas and pituitary of coho salmon (*Oncorhynchus kisutch*). Res. Board Can., 33: 1585 – 1603.

Hines, G.A.; Boots, L.R.; Wibbels, T. and Watts, S.A. (1999). Steroid levels and steroid metabolism in relation to early gonadal development in the tilapia *Oreochromis niloticus*. Gen. Comp. Endocrinol., 114(2): 235 – 248.

Hussein, K. A. (1984). Gonadal maturation and fecundity of a fish with partial spawning (*Tilapia nilotica* L.). Arabian Journal of Science and Research, 2 : 683–699.

ISSN: 1687-1146 **ISBN: 12154/2002**

Jankov, R.M. (1977). Microbial transformation of steroids. V. Aromatization of the ring A of androstane steroids by *Mycobacterium phlei.* Glas. Hem. Drus. Beograd, 42(9-10): 655 – 668.

Kutsky, R.J. (1973). Handbook of Vitamins and Hormones. Van Nostrand Reinhold Company, New York, Cincinnati, Toronto, London, Melbourne.

Little, D. and Edwards, P. (2004). Impact of nutrition and season on pond culture performance of mono-sex and mixed-sex Nile tilapia (*Oreochromis niloticus*). Aquaculture, 232: 279 – 292.

Little, D.C.; Bhujel, R.C. and Pham, T.A. (2003). Advanced nursing of mixed-sex and mono-sex tilapia (*O. niloticus*) fry, and its impact on subsequent growth in fertilized ponds. Aquaculture, 221: 265 – 276.

Lone, K.P. and Matty, A.J. (1981). The effect of feeding androgenic hormones on the proteolytic activity of the alimentary canal of carp *Cyprinus carpio* L.J. Fish Biol., 18: 353 – 358.

Magouz, F.I.; El-Harairy, M.A.; Abdel-Khalek, A.E. and Ebada, A.F. (2000). Effect of hormonal treatment on production of mono-sex males tilapia (*Oreochromis niloticus*). J. Agric. Sci. Mansoura Univ., 25: 7577 – 7590.

Mair, G.C. and Little, D.C. (1991). Population control in farmed methyl-5-androsten-3-17-diol through rearing tilapia. NAGA, ICLARM Q. 14 (3) : 8–13. Water. Current Science, 56: 412–413.

Mehrim, A. I.; Abdelhamid, A. M.; Abou-Shousha, A.; Salem, M.F.I. and El-Sharawy, M.A.M.M. (2006). Nutritious attempts to detoxify aflatoxic diets of tilapia fish: 2- Clinical, biochemical and histological parameters. Journal of the Arabian Aquaculture Society, 1: 69-90.

Nemat Allah, B.R. (2002). Positive and negative sites of Monosex tilapia culture. Mansoura Univ., J. of Agric. Sci. (Special issue), Feb., pp: 133 – 136.

Phelps, R.P. (2001). Sex reversal: the directed control of gonadal development in tilapia. Pages 35 – 60. In: D.E. Meyer (ed.). Proceedings for Tilapia Sessions from the 6th Centeral American Aquaculture Symposium, 22 – 24 August, Tegucigalpa, Honduras.

Phelps, R.P.; Arndt, J.T. and Warrington, R.L. (1999). Masculinization of tilapia fry by immersion in trenbolone acetate (TBA) at a production level. In: McElwee, K., Burke, D.n Iles, M. and Egna, H. (Editors) 16th Annual Technical Report, Pond Dynamics/Aquaculture (RSP) Oregon State Univ., Corvallis, Oregon, 2 p.

Phelps, R.P.; Fitzpatrick, M.S.; Contreras-Sanchez, W.M.; Warrington R.L. and Arndt, J.T. (2000). Detection of MT in pond water after treatment with MT food. Pages 57-59. In: K. McElwee, D. Burke, M.

Niles, X Cummings and H. Egna (eds.). Pond dynamics/Aquaculture Collaborative Research Support Program. 17th Annual Technical Report. Pond dynamics/ Aquaculture CRSP, Oregon State University, Corvallis, Or., USA.

Ratliff, C.R. and Hall, F.F. (1973). Laboratory Manual of Clinical Biochemistry, Vol. 1. Scott and White Memorial Hospital Publications Office, Temple, Texas.

Riley, L.G.; Hirano, T. and Grau, E.G. (2004). Estradiol –17 beta and dihydrotestosterone differentially regulate vitellogenin and insulin-like growth factor-I production in primary hepatocytes of the tilapia *Oreochromis mossambicus*. Comp. Biochem. Physiol. C, Toxicol. Pharmacol. 138(2): 177 – 186.

Ros, A.F.H.; Becker, K.; Canario, A.V.M. and Oliveira, R.F. (2004). Androgen levels and energy metabolism in *Oreochromis mossambicus*. J. Fish Biol., 65(4): 895 – 905.

Sachs, L. (1976). Statistische Methoden, Ein Soforthelfer. 3. Neubearbeitete Auflage. Springer – Verlag. Berlin, Heidelberg, New York.

Sambhu, C. and Jayaprakas, V. (1997). Dietary supplementation of testosterone propionate on growth performance of white prawn, *Panaeus indicus* (M. Edwards). Indian J. Experimental Biol., 35(12): 1353 – 1358.

Schmit, J.M. (1964). These, Lyon (cited from the pamphlet of the commercial kit).

Stein, E.A. (1986). In: Textbook of Clinical Chemistry, N.W. Tietz (Ed). W.B. Saunders, Philadelphia, pp: 879 – 886.

Sunny, F.; Lakshmy, P.S. and Oommen, O.V. (2002). Rapid action of cortisol and testosterone on lipogenic enzymes in a fresh mater fish *O. mossambicus*: short-term *in vivo* and *in vitro* study. Comp. Biochem. Physiol. B. Biochem. Mol. Biol., 131(3): 297 – 304.

Tseng, W.Y. and Chan, K.L. (1982). The productive biology of the rabbit fish in Hong Kong. J. World Maricul. Soc., 13: 313 – 321.

Tyler, C. R. and J. P. Sumpter (1996). Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries, 6: 287–318.

Yamazaki, F. (1976). Application of hormones in fish culture. J. Fish. Res. Board Can., 33: 948 – 958.

Zaki, M.A. (2004). Growth performance, feed utilization and carcass composition comparisons of mono-sex tilapia (*Oreochromis niloticus*) produced by various techniques. J. Agric. Sci. Mansoura Univ., 29: 4461 – 4471.

ISSN: 1687-1146 **ISBN: 12154/2002**