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UTILIZATION OF ANDROGEN FOR THE PRODUCTION OF MONOSEX MALES TILAPIA *(Oreochromis niloticus*)

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ABSTRACT

The efficacy of androgen hormone in producing monosexed males in Nile tilapia fry and the response of growth and survival rates to hormone treatment were studied. The influence of the androgen treatment on the histological features of the resulting gonads was also evaluated. The synthetic aromatizable 17α-methyltestosterone (MT) was supplemented in diet to newly hatched tilapia fry(<6days) at a level of 500 mg/kg feed for 21 and 56 days treatment periods ($G_1$and G_2),while the control group (G_3) was fed untreated diet. About 82% males were produced in groups 1 and 2. Treatment with MT had no significant effect on growth performance. However, MT treatment resulted in lowering the fish survival rate in treated groups (17.5±0.85 and 24.0±0.85 % for $G_1$and G_2, respectively),than that in the control group(33±0.87%). At the high level of MT used over 21 or 56 days,there were negative structural changes in the gonads.

Keywords: Tilapia, androgen, monosex, growth, survival, histology

INTRODUCTION

The sex differentiation process in teleosts is diverse and labile (Francis, 1992) making hormonal sex reversal possible in many fish species. Hormonal sex reversal may serve as an available tool to understand the process of sex differentiation, and to produce monosexed population for the aquaculture industry.

In tilapia species, sex reversal is considered one of the successful methods for overcoming the problem of heterogenity and overpopulation caused by dietary administration of synthetic androgens and is the most commonly method used in developing countries (Koman *et al*., 1989 and Rothbard *et al*., 1990). The percentage of male produced depends on the potency and dose of hormone and time and duration of treatment.

The success in inducing sex reversal in response to the dose of androgen used has been debated. While some authors reported increasing response with the increase of hormone level (Mc Geachin et al., 1987), others did not find such response (Piferrer *et al.*, 1993). Therefore, this study was designated to investigate the response to high dose of 17α-methyltestosterone (MT) for two different periods of treatment in terms of males produced, and histological features of gonads and also the growth performance of Nile tilapia fry.

MATERIALS AND METHODS

This study was conducted in co-operation with Fish Laboratory, Faculty of Agriculture, Kafr El-Sheikh. A set of 600 Nile tilapia *(Oreochromis niloticus*) fry with average initial weight of 0.02g of about 6 days posthatching were collected from a private fish farm. The fry were randomly divided into six similar sets with 100 fry each. Fish sets were stocked in glass aquaria

(80x35x40 cm) containing equal amount of water (40 liter). About 50% of total water volume was replaced daily by fresh and dechlorated tap water after cleaning and removing the debri from the bottom. All aquaria were supplied with compressed air through an air compressor. Water temperature was maintained at 25-26 ^{o}C by using an electeric heater.

A basal diet was formulated to contain about 45% protein for feeding the fry from the beginning of the experiment until the end of the 7th week ($Diet_1$). The protein level was reduced in the diet to be35% in the following 18 weeks until the end of the study ($Diet_2$). The dietary ingredients were collected from local market. The diet was offered to the fish in powder form during the first seven weeks, and in 1mm pelleted form during the rest of the experimental period. The ingredients and diets were chemically analysed according to A.O.A.C. (1984) methods. Composition and chemical analysis of the experimental diets are shown in table (1). Synthetic hormone, MT (Sigma chemical co.) was added to the basal diet (both diet 1 and diet 2) at the level of 0 and 500 mg/kg feed by the ethanol evaporation method (Macintosh and Little, 1995). The calculated amount of hormone was dissolved in ethyl alcohol (95%), mixed with the diet. The diet was then left uncovered for 3 h at room temperature for ethanol to evaporate. The treated diet was used in feeding the fry for 21d in two aquaria (G_1), for 56d in two aquaria (G_2), while the same basal diet was used without treatment, for feeding the fry in two other aquaria as control (G_3). The diets were offered to the fry four times daily at the rate of 30, 20, 10, 5 and 3% of body weight for 3, 3, 3, 2 and 14 weeks, respectively. Following the treatment period (21 or 56 d) fish in all aquaria were fed the basal untreated diet for eight further weeks (wk18-25). At the end of the 25th week, fish were hand-sexed to calculate the percentage of males produced in each group. Fish were weighed biweekly. For the histological examination, tissue samples were taken from the gonads, fixed in

Table (1): Composition and chemical analysis (%) of the experimental diets.

Ingredients	Diet	
	1	2
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. Mixture	0.3	0.3
Chemical analysis:		
Dry matter (DM)	86.0	85.0
DM basis:		
Crude protein	45.5	35.0
Ether extract	10.0	11.8
Crude fiber	2.7	3.5
Ash	6.4	5.2
Nitrogen free extract	35.0	44.5
ME(Kcal/kg)*	4111.4	4083.9

*** Metabolizable energy calculated using a value of 3.49, 8.1 and 4.5 Kcal/g for carbohydrate, fat and protein, respectively according to Pantha (1982).**

10% formaline solution, embedded in paraffin wax blocks, cut into 5-10 µm sections using a microtom, stained with heamatoxylin and eosin (H&E) and were microscopically examined. The data were statistically analyzed using one-way ANOVA according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Male percentage:

The percentage of males produced from treating Nile tilapia fry (*O. niloticus*) with high dosage (500 mg MT/kg feed) was about 82%, whether treatment lasted for 21 or 56 days (Table 2). This value was markedly higher than the control value (50%), the difference being statistically significant ($P<0.05$). Similarly, Joon *et al.* (2000) found that treatment of female Nile tilapia with 500 $mg.Kg^{-1}$ food aromatase inhibitor successfully masculinized <80% of the treated fish. The percentage of males produced resulting from treatment with the synthetic aromatizable androgen (MT) used is somewhat lower than the much more successful results (<95% males) obtained by other authors using lower doses (30-60 $mg.Kg^{-1}$ feed) of MT, (Magouz *et al.*, 1997). Similarly, McGeachin *et al.* (1987) obtained 96-99% males using treatment with 60-120 mg $MT.Kg^{-1}$ feed for 22 d in *O. aureus*.

The present results obtained herein, indicate, that the used high level of MT for both of short and long period caused paradoxical feminization and sterilization (Plate 1). This is in agreement with the findings of Piferrer and Donaldson (1991) and Piferrer *et al.* (1993) who reported that high doses of aromatizable androgens especially for long periods result in paradoxical feminization or sterilization in salmon fish. Similar results were noted by Carvalho and Foresti (1996) in Nile tilapia larvae aged <1 week which were treated with 30, 50 or 100 mg $MT.Kg^{-1}$ food over a period of 20, 40 or 60 d. Hypertrophic interstatial tissue and necrotic cells appeared in the testicular structure. Piferrer *et al.* (1993) suggested that higher MT doses than 400 µg/L resulted in fewer males in salmon fish. Age and size of fry at the beginning of 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 hatch to achieve a high percentage of males. This may provide a further explanation for the low percentage of males obtained in the present study using tilapia fry of about 6 days age.

Table 2. Least squares means* and standard errors of the traits studied.

Group	Means squares ± standard errors						
	Initial b.w. (g)	Final b.w. (g)	A.D.G. (g/d)	S.G.R.	F.C.R.	S.R.	Male %
G1	0.019±0.001	3.27±0.05	0.018±0.003	0.64±0.1	1.08±0.05	24.0±0.87	$81.5±2.1^{a}$
G2	0.018±0.001	3.96±0.05	0.022±0.004	0.66±0.1	1.07±0.05	17.5±0.85	$82.5±2.3^{a}$
G3	0.019±0.001	6.24±0.05	0.035±0.003	0.68±0.1	1.08±0.06	33.0±0.85	$50.0±2.3^{b}$

*** Means denoted by different superscripts differ significantly (P<0.05).**
A.D.G. = Average daily gain. **S.G.R. = Specific growth rate**
F.C.R. = Feed conversion ratio **SR = Survival rate.**

Growth performance:

As expected, initial body weight (I.b.w.) did not differ much among the different treatment groups studied (Table 2). It seems that treatment with MT adversely affected the average daily gain. The average daily gain (ADG) in groups 1 and 2 (G_1&G_2) were about 36 or 48%, respectively, lower than the control group (Table 2). Such differences were not statistically significant ($P<0.05$). It seems that growth was hindered in the hormonally treated fry (G_1 and G_2) despite the lower number of fry in each aquaria (that resulted from the lower SR in these groups compared to the control). These results may lead to a conclusion that the high dose of MT used affected both growth performance and survival of the treated fry. Data found in previous studies showed no significant effect on growth during treatment period of sex reversal hormone using lower doses of MT. Magouz *et al.* (1997) indicated that the low level of MT hormone (30-60 mg.Kg^{-1} feed) had no effect on the growth rate of treated tilapia. Similar results were also obtained by Cruz and Mair (1994) and Rinchard *et al.* (1999).

A similar rate of decrease in the final body weight was found in the present study as a result of the decrease in average daily weight gain in the two treated groups (G_1 & G_2), being 36 and 48%, respectively (Table 2). However, the differences in the final body weight between the two treated groups (1 & 2) and the control group (G_3) were not significant ($P>0.05$).

Concerning the body weight changes throughout the experimental weeks (Fig. 1), a similar trend of increase was observed in all experimental groups from the beginning of the experimental period until the 23rd week. Thenafter, body weight increased steeply during the last two weeks (wk 23-25) in all groups (Figure 1).

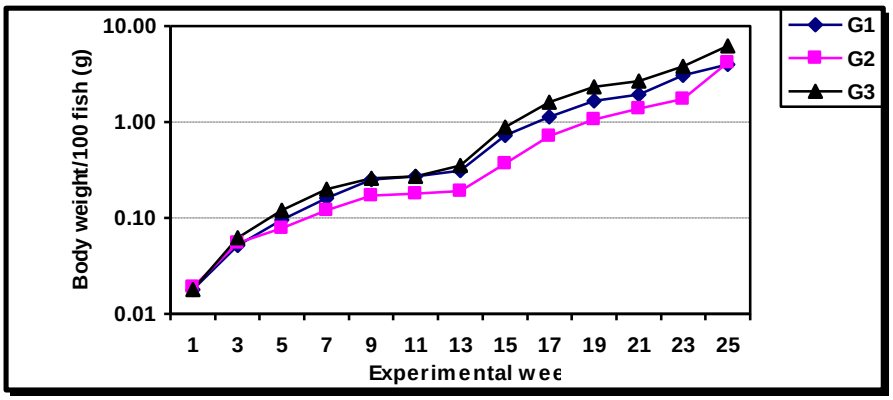

Fig. (1): Body weight changes (log scale) of fish at different experimental weeks as affected by hormonal treatment.

Both the specific growth rate (S.G.R.) and feed conversion ratio (F.C.R.), showed no significant differences ($P>0.05$) between the experimental groups studied (Table 2). Ridha and Lone (1990) reported no significant effect on relative and specific growth rate of *O. spilurus* stoked in

brackish water and treated with MT for 38 days at 50 mg/Kg treatment level. Similar results were also obtained recently by Cruz and Mair (1994) on *O. niloticus* fish.

Survival rate (SR%):

During the first five weeks of the experimental period, the survival rate (SR%) dropped sharply in all groups (Fig. 2). The drop was, however, more marked during the first 3 weeks in the treated groups compared to the control. The control group had consistently higher SR by about 15% than the two treated groups throughout the first 9 weeks of the experiment. It is well established that fish fry of most species raised in aquaria usually exhibit high losses during the first few weeks of life (Hara *et al.*, 1986 and Rizk, 1997). However, the noticeably larger drop in SR in the hormonally treated groups (G_1 and G_2) can be attributed to the high dose of MT used. It is apparent from Fig. (2) that SR in G_2 continued to decline for further 4 weeks until week 13, compared with G_1, which indicates a possible effect of hormonal treatment on SR.

Pandian and Sheela (1995) suggested that in most species of fish, using synthetic hormone for sex reversal resulted in a higher mortality. In contrast, the results obtained by Magouz *et al.* (1997) on Nile tilapia indicated that the relatively low levels of hormone (30 and 60 mg.Kg^{-1} 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^{-1} feed, on the SR of monosex Nile tilapia fry introduced in northern Vietnam. As illustrated in figure 2, SR was almost unchanged from the 13th week until the end of the experimental period.

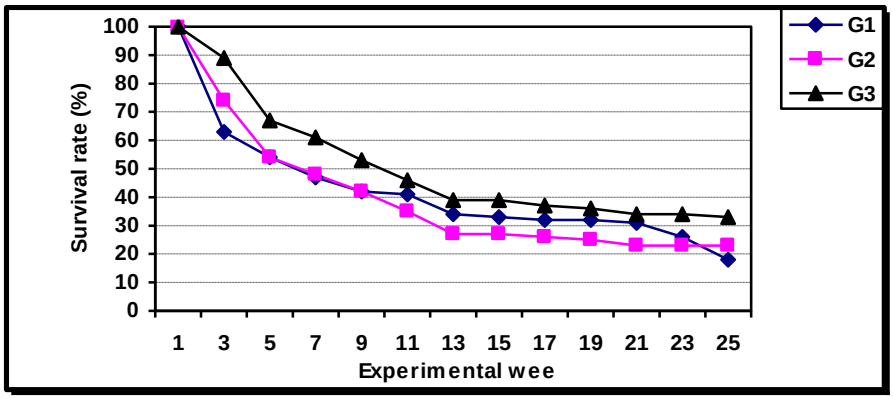

Fig. (2): Survival rate of fish at different experimental weeks as affected by hormonal treatment.

Histology of the testes

Regarding the histological examination, the spermatogenesis cells at different stages of development separated from the basement membrane of the semeniferous tubules and were noticed centrally in the lumen (Plate 1). Ruptured (stigma) of the basement membrane occurred in the semeniferous

tubules (Plate 2). In the interstitial tissue of the testes numerous of necrotic cells were observed (Plate 3), also atrophy of interstitial tissue was found within the testicular parenchyma (Plate 3) compared with the control group (Plate 4).

Generally, changes were found in the histological structure of the testes including degeneration in the semeniferous tubules and atrophy and necrotic interstitial tissue as a result of treatment with high dose of MT in Nile tilapia fish.

Plate 1. Section in the testes of Nile tilapia fry (treated group) showing the degenerated semeniferous tubules with clear intraluminal cellular depress. X100, H&E.

Plate 2. Section in the testes of Nile tilapia fry (treated group) showing interrupted basement membrane (arrow). X400, H&E.

Plate 3. Section in the testes of Nile tilapia fry (treated group) showing atrophy and necrotic cells of the interstitial tissue. X400, H&E.

Plate 4. Section in the testes of Nile tilapia fry (control group). Normal structure of the semeniferous tubules, lumen (a), and interstatial tissue (b) X400, H&E.

In conclusion, treating Nile tilapia fry with high dose of MT (500 mg.Kg^{-1} feed) produced 82% monosexed males with no significant effect on their growth performance, however, it resulted in lowering the survival rate and induced a marked negative changes in the histological structure of the tilapia fish gonads.

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إستخدام هرمون الإندروجين فى إنتاج ذكور أسماك من البلطى النيلى وحيدة الجنس

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أجرى هذا البحث بالتعاون مع معمل أبحاث السمك بكلية الزراعة بكفر الشيخ وذلك لدراسة تأثير استخدام مستوى عالى من هرمون الإندروجين على إنتاج أسماك من البلطى النيلى وحيدة الجنس (ذكور) وكذلك تأثيره على كل من النمو والحياتية والمظاهر التشريحية للغدد الجنسية ، تم إضافة هرمون 17- ألفا ميثيل تستستيرون المخلق صناعياً إلى عليقة إصبعيات البلطى عند عمر حوالى 6 أيام بجرعة قدرها 500 مجم/كجم عليقة لمدة 21 ، 56 يوماً كفترة معاملة فى كل من المجموعة الأولى والثانية على التوالى ، أما المجموعة الثالثة فكانت بدون معاملة (مجموعة مقارنة).

أظهرت النتائج أن المعاملة بالهرمون تحت الدراسة أدت إلى إنتاج حوالى 82% من ذكور أسماك البلطى النيلى فى كل من المجموعتين الأولى والثانية ، ولم يكن للمعاملة تأثير معنوى على نمو إصبعيات البلطى النيلى ، بينما أدت إلى انخفاض فى حياتية الإصبعيات فى كل من المجموعتين المعاملتين عن المجموعة غير المعاملة. كما أدى استخدام الجرعة العالية من الهرمون الذكرى المستخدم سواء لمدة 21 أو 56 يوماً إلى التأثير سلبياً على التركيب التشريحى للغدد الجنسية فى الأسماك المعاملة.

ويمكن القول بأن استخدام جرعة عالية من هرمون 17-ألفا ميثيل تستستيرون لمدة 21 أو 56 يوماً لقلب الجنس فى إصبعيات البلطى النيلى أدى إلى إنتاج 82% من الذكور دون التأثير على النمو ، ولكنه أدى إلى انخفاض فى حياتية الإصبعيات وحيدة الجنس من الذكور بالإضافة إلى ظهور تغيرات تشريحية سلبية فى تركيب الغدد الجنسية للذكور الناتجة.