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EFFECT OF SYNTHETIC MALE HORMONE ON PHENOTYPIC SEXUAL DIFFERENTIATION OF NILE TILAPIA (OREOCHROMIS NILOTICUS)

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ABSTRACT: The effect of the synthetic male hormone 17 α-methyltestosterone (MT) on phenotypic sexual differentiation in Nile tilapia Oreochromis niloticus was studied. Fourty five hundreds sexually undifferentiated Nile tilapia fry were divided into four experimental groups and control, 60 ppm MT was added to the feed and fed to 7-day old fry for the 1st and 2nd groups, and for 14-day old fry for the 3rd and 4th groups. The hormonal treatments were terminated after application. Fry were fed at approx. rate of 20% of body weight twice daily. At 100 days of age fish were dissected, the gonads were classified on the basis of microscopic appearance. The dissected gonads were stained by aceto-carmine squash method. Ovarian tissue was positively identified by the presence of oocytes. Feeding Nile tilapia (Oreochromis niloticus) fry at 7days of age on diet added to it synthetic male hormone resulted in higher body weight, total body length, body weight gain, growth rate and survival rate then control. The hormone treatment resulted in male population ranged from 79.4 to 98.9% as compared to 53% for the control.

Key words: Monosex, sex differentiation, Nile tilapia, growth rate, survival rate.

INTRODUCTION

With ever-increasing need for cheap sources of protein to meet the world's overpopulation more and more attention is being focused on fish farming. In the developing countries where the problem is acute. tilapias culture is believed to offer one of the solutions especially in view of the depletion of existing fisheries.

The production of tilapias has long been hindered by over crowding of young that cause competition for the available hod and suppress growth of the entire population (Macintosh et al., 1985). In order to overcome the undesirable traits in titapias culture, hormonal sex reversal serves as a valuable tool to induce monosex populations. In Nile tilapia the first attempt to experimentally induce sex reversal was carried out by Jabber et al., (1974). This method prevents the unwanted reproduction and produce all male population which has reater growth potential and ensures better meat quality (Shelton et al, 1978). Steroids have been used for sex reversal in economically important species (Pandian and Sheela 1995). The efficiency of naturally or synthetic male hormone androgens on reversing the gonads of genetic female. can be arranged in the following orders: minolerone > 19 norehyl testosterone > 17-α-methyl testosterone > testosterone (Pandian and Sheela 995).

Several investigators reported that feeding tilapia on diet added to it 17 α-NT produced all male population (in Nile tilapia 30 ppm Jalabert et at, I974 In Sarotherodon niloticus 30 and 40 ppm Tayamen and Shelton, 1978; 60 and 120 ppm Chambers 1984 and Bocek et al., 1992).

In Nile tilapia (Oreochromis niloticus), sex occurred at 30 and 33 d post-hatch (when fry body length ranged from q to 12 mm (Alvendiq- .Casaway and Carìon 1988). While Nakamura and Nagahaiua (1989) observed that the intial testicular differentiation occurs at 23 to 26 d of age. More recently, Magouz et al. (1997) obtained all male population of Nile tilapia when they started the androgen treatment at 4 d of age.

Duration of treatment is one of several critical factors that are necessary for successful sex reversal in fish. It varies according to species. In tilapia Hunter and Donaldson (1983) reported that the proper duration for achieving 100% male ratwed from 15 to 60 d. Recently Bocek et al (1992); Phelps and Cerezo 1992 obtained 100% male when fry were fed diet containing male hormone for 28 d while Magouz et al 1997 obtained the same results by feeding male hormone for 2/- d.

Several investigators found that tilapia fry treated with hormone had higher survival rate than the untreated control (Shelton et al 1981 and Phelps et al, 1992). In contrast, to the aforementioned studies. Varadaraj and Pandian (1991) reported positive correlation between morality rate in tilapia and the dose of the MT hormone.

Some studies on the effect of hormonal treatment on growth of fish, Kuwaye et al., (1993) in tilapia reported an increase in rate of growth of hormonal treated tilapia fry. But not with Mc Andrew and Majundar (1989) who showed that androgens had no effect on growth .

The present study was conducted with the following objectives:

1- To study the effect of atiding synthetic anabolic male hormone 17 α - methyl testosterone on phenotypic sex differentiation of Nile tilapia (Oreochromis niloticus).

2- To further observe the effect of duration of treatment and fry age on the phenotypic sex differentiation in Nile tilapia

3- To demonstrate the effect of hormonal treatment on growth potential and survival rate of Nile tilapia.

MATERIALS AND METHODS

Broodstock maintainance:

Outdoor spawning concrete ponds were used in this study. Oreochromis niloticus brood fish were placed randomly with average weight 250 g for female and 350 g for male. Fry were fed on a pelleted commercial diet containing 25% crude protein, 4.1% crude fat, 5.5% crude fiber and 2044.5 kcal ME and fed at a rate of 3% of their biomass twice daily, 4500 fry were harvested, and transported to 60 liter aviated aquaria with municipal water which was processed against chlorine using 3 mg/L sodium thiosulphatc (Boyd, 1988)., (Table 1.).

Table 1: Water analysis of broodstock concrete ponds.

Water parameter	broodstock Concrete ponds	Manicipal water
Temperature: C	27 – 30: C	26 – 28: C
$_{P}^{H}$	8.0-8.5	7.75
Oxygen	3.3	4.0
Total dissolved solids (TDS)	0.45	0.1
Total alkalinity (as C_a CO_3)	365	150
Total hardiness (as C_a CO_3)	202	160
Ammonium (NH_4)	1.1	1.1
Ammonia (NH_3)	0,54	0 . 54
Nitrite (NO_2)	0.0	0.0
Nitrite (NO_3)	0.15	0.17

A stock solution contained 60 ppm 17 α -MT was prepared according to Guerrero (1974). Fry were fed on a formulated diet 2.7% crude fat 3.6% crude fiber 2505 kcal ME. One kilogram from the aforementioned finely grind feed was thoroughly mixed with one liter of hormone solution, then the hormone treated feed was kept on plastic sheet at room temperature 29° C for 24 h to dry.

Fourty five hundreds undifferentiated Nile tilapia fry which were counted by number were divided into four experimental groups and control, 900 fry in each. For the first and second groups, 7 days old fry were fed on 60 ppm MT treated feed for 21 and 28 days. respectively. For the third and fourth groups. 14 days old fry were fed on 60 ppm MT treated Feed for 21 and 28 days, respectively. Fry age was determined according to absorption of yolk sac. The fry of control group were divided into two subgroups, one was fed on diet without hormone and solvent and the other was fed on ethanol treated feed and was utilized as a vehicle control. Fry were fed at approximate rate of 20% of body weight twice daily.

The daily ration was calculated on basis Popma and Green (1990) that:

$$W = 0.02 X (L)^3$$

Where:

W Body weight per 1000 fry in grams.

L = Average total length of fry in mm.

Aquaria were partially exchanged it's water everyday by siphoning to eliminate the wastes. Five hundred liters fiber glass tank was filled by municipal water for manual exchanging water of aquaria.

Water temperature. was maintained at (27 °C ± 1°C) using a thermostatic control system provided with 14 h daylight. The green house ponds were used to place fry wintering. At 49 days of age, the fish of all treatments were placed at the same time in the green house as well as the controls.

Tilapia performance

Fry were weighed at weekly intervals and the total body weight gain was calculated and mortality was recorded.

Total body length of fry were determined using a petri dish and a ruler and the total weight gain was calculated and mortality was recorded.

Determination of the sex:

Fish were dissected at 100 days old as either testes or ovaries and the gonads were classified on the basis of microscopic appearance using magnification of 10 to 50 and stained by aceto-carmine squash method according to the technique described by Guerrero and Shelton (1914).

Statistical Analysis:

Data were subjected to one way analysis of variance with treatment effect using the General Linear Models (GLM) procedure of SAS User's Guide, (1994) Chi-square test was used to determine the difference in the data of sex ratio T-test was used to determine significance of difference ($P < 0.05$) between group means. The Data were analyzed by the model:

$$Y_{iik} = \mu + A_i + P_i + A^* P_{ii} + E_{iik}$$

where:

U = over all of mean,

A_i = effect of age.

P_i = effect of duration of hormonal treatment.

$A^* P_{ii}$ = effect of interaction

E_{iik} experimental error.

The total body weight gain was analyzed using PROC. REG, SAS, User's Guide, 1994 by the model: $Yij = \mu + T_i + E_{ii}$

where:

μ = overall of mean,

T_i=effect of treatment,

E_{ii} = experimental error.

Duncun's multiple range test was used to determine significance of difference in total body weight gain.

RESULTS AND DISCUSSION

Effect orandrogen on the phenotype of the sex:

Table (2) presents the percentage incidence of males and females of Oreochramis niloticus fry fed experimental diets. The male population for the treated fry ranged from 79.4 to 98.9% for the hormone treated groups compared with 53.0% male for the control groups. The difference in percentages of male between the hormonal treatments and control groups was significant (P<0 05) by Chi-square test.

It is readily seen from Table (2) and Figure (1), that the highest male percentage 98.9% was recorded for 7 days old fry that were fed on diet containing 60 ppm 17 α– MT for 28 days (T2) and the lowest percentage 79.4% for 14 days old fry that were treated for 21 days (T3). The difference between the two groups was insignificant.

There is a positive relationship between sex reversal and length of treatment irrespective of the age of the fry at the beginning of the treatment (initial age) and within the duration of treatment the same relationship between the age of the fry at the beginning of treatment and sex reversal was reported.

Either control group with ethanol (VC) or the control group with no ethanol (C) resulted in equal percentages of phenotypic male approximately 53.0%. The difference between (VC) and (C) groups was not significant (P<0.05) as presented in table (2), and Figure (1).

In the present study, it is evident that the duration of hormonal treatment was more effective in conrolling the phenotype of the sex than the age of the fry at the beginning of treatment (initial age) in Nile tilapia (Table 2).

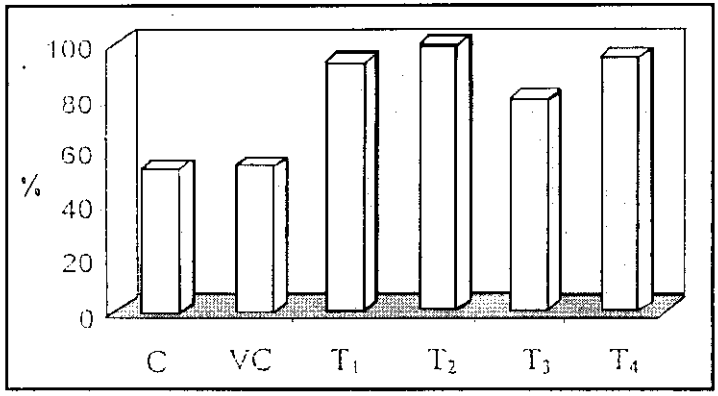


Fig. (1): Effect of hormonal treatment on producing all male *Oroechromis niloticus fry.*

Table 2. Incidence of males and females Oreochromis niloticus fry fed on diet supplemented with synthetic male hormone

X2	Treatment	percent incidence		No. sample
		Male	Female	
0.36	C	53.00	47.00	200
0.55	VC	53.45	46.55	174
74.46*	T1	92.31	7.69	206
87.04*	T2	98.90	1.10	182
33.49*	T3	79.38	20.62	194
80.40*	T4	94.18	5.82	206

*Significant ($p<0.05$)

This shows that the length of the hormonal treatment period could be the major important factor in producing the high percentage of male in tilapia species.

These findings agree closely with those of Hiott and Phelps (1993) who reported that the duration of treatment had a greater influence than initial age for producing phenotypic sex reversal in Nile tilapia. in contrast to the aforementioned findings, Magouz et al, (1997) showed that the percentage of phenotypic males was not significantly affected by the length of the hormonal treatment in Nile tilapia. Nakamura and Takajashi (1973) stated that sex reversal is assumed to be most effective if hormonal treatment coincides with the period of gonadal differentiation.

Therefore it appears that till now the results are equivocal; some studies reported that the duration of hormonal treatments is more effective in inducing phenatopic sex differentiation in the fry of Nile tilapia (Oreochromis niloticus) that the age at the beginning of treatment, whereas others found equal effects for age at the beginning of treatment (initial age) and the duration of treatment on sex ratio of treated fish.

Effect of hormonal treatment on Mortality:

Means ±SE survival rates of fry Oreochromis , Niloticus on experimented diet were 87.6% (C), 92.45% (VC). 91.47% (TI), 92.35% (T3), 93.92% (T2) and 93.21% (T4). Although the differences in survival rates between VC, TI, T2. T3 and T4 were insignificant, still these differences were statistically significant from the control without vehicle (C).

The long periods of treatments 28 d (T2 and T4) with relatively high level of 17 α MT (60 ppm) resulted in high survival values rates.

On the contrary, the results reported by Varadaraf and Pandian (1991) in Mozambique tilapia (Oreochromis mossambicus) who showed negative relationship between androgen (MT) concentration and the survival rate regardless of the duration of treatment . Also. Pandian and Sheela (1995) reported that treatment with synthetic steroid hormones resulted in decreased suivival rates in most tilapia species.

The difference between result of the present study and the above mentioned studies may be due to different experimental condition or different strains of tested tilapias.

Effect of hormonal treatment on tilapia growth performance:

Body weight: Averages body weigh of Nile tilapia (Oreochromis niloticus) fry fed on experimental diet are presented in table (3). The one way classification of analysis of variance shows significant differences ($P<0.05$) in body weights between treatments.

The average body weight of Oreochromis niloticus fry treated with 17 α MT experimental period were 748.2, 713.9 and 600 mg for T2, TI and T4, respectively.

However, the lowest mean body weight 500 rng, was for fry treated for only 21 days *T3 with male hormone but still was higher than those of C and 'IC groups, 308 and 286 respectively (Table3).

However, smaller body weights were recorded for fry that were fed on diet wfth male hormone at 14 days of age regardless of the duration of treatment.

The present results are in agreement with results of Panadin and Sheela (1995) who stated that inducing sex reversal was assured by 100% growth potential.

It is clearly noted that feeding tilapia fry on synthetic male hormone resulted in higher growth rate than the untreated ones.

Generally, it is evident that the fry fed on diet containing to it MT (60 ppm) from 7 days old had higher live body weights than others whether the duration of treatment was 21 or 28 days (figure 2).

The average weight of tilapia in the present study was positively correlated with the amount of hormone in the feed. Therefore it appears that the male hormone induced anabolic enhancement of growth when administered for long period. Furthermore, the male hormone might as an influence on somatic growth, increased appetite and food conversion of Nile tilapia fry throughout the experimental period.

Table 3. Means (X±SE) of live body weight (mg) of *Oreochromis niloticus* fry through the experimental period.

d	C	VC	T1	T2	T3	T4
0			11.324±0.5			
7	22.876±0.773^{c}	23.325±0.642^{a}	28.925±0.537^{a}	27.427±0.462^{ab}	26.76±0.625^{b}	28.219±0.598^{ab}
14	34.883±1.184^{d}	34.911±1.127^{d}	40.062±0.475^{b}	44.989±0.503^{a}	36.503±0.837^{cd}	37.343±0.752^{c}
21	40.403±1.419^{dc}	39.683±1.605^{c}	55.202±1.358^{b}	64.056±1.85^{a}	44.06±0.742^{cd}	46.337±1.264^{c}
28	46.805±1.848^{d}	44.612±1.774^{d}	107.713±2.415^{a}	98.445±1.381^{b}	90.373±2.208^{c}	94.953±1.097^{cd}
35	79.65±2.302^{e}	79.402±2.036^{e}	185.965±3.784^{a}	166.069±40578^{b}	137.779±2.894	153.632±3.377^{c}
42	117.951±2.716^{e}	117.51±3.243^{e}	356.878±6.857^{a}	313.552±5.777^{b}	246.964±3.359^{d}	280.598±3.962^{c}
56	307.849±6.478^{e}	286.128±8.921^{e}	713.929±11.256^{b}	748.237±15.404^{a}	500.087±10.442^{d}	608.839±10.618^{c}

Means in the same raw with the same leuers are not significantly different (P<0.05).

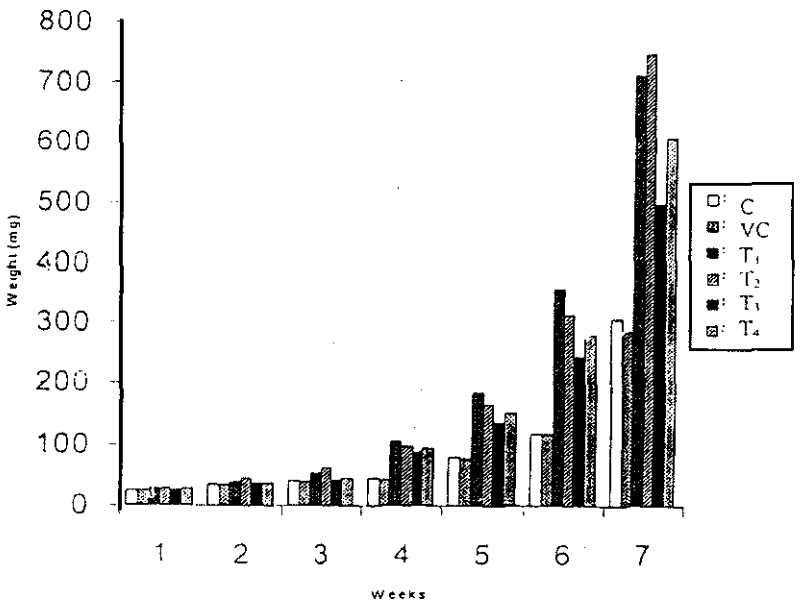


Fig. (2): Effect of hormonal treatment on live body weight of *Oreochromis niloticus* through the experimental period.

Table (4). Means of total body length (mm) (X±SE) of *Oreochromis niloticus* fry through the experimental period.

Wk	C	VC	T1	T2	T3	T4
0			**8.2±0.144**			
1	10.422±0.112^{e}	10.5±0.098^{e}	11.3±0.069^{a}	11.1±0.062^{ab}	11.0±0.086	11.2±0.079^{ab}
2	11.993±0.135^{d}	12.0±1.13^{d}	12.6±0.05^{b}	13.1±0.048^{a}	12.2±0.094^{cd}	12.3±0.083^{c}
3	12.59±0.15^{d}	12.5±0.168^{d}	14.0±0.116^{b}	14.7±1.144^{a}	13.0±0.073^{cd}	13.2±0.121^{c}
4	13.21±0.18^{d}	13.0±0.172^{d}	17.5±0.132^{a}	17.0±0.079^{b}	16.5±0.136^{c}	16.8±0.065^{bc}
5	15.807±0.156^{e}	15.8±0.137^{e}	21.0±0.143^{a}	20.2±0.186^{b}	19.0±0.135^{d}	19.7±0.145^{c}
6	18.04±0.138^{c}	18.00±0.167^{c}	26.1±0.168^{a}	25.0±0.156^{b}	23.1±0.104^{d}	24.087±0.106^{e}
7	24.84±0.174^{d}	24.2±0.252^{e}	32.9±0.172^{a}	32.4±0.239^{a}	29.2±0.206^{c}	31.19±0.182^{b}

Means in the same raw with the same letters are not significantly different (P<0.05).

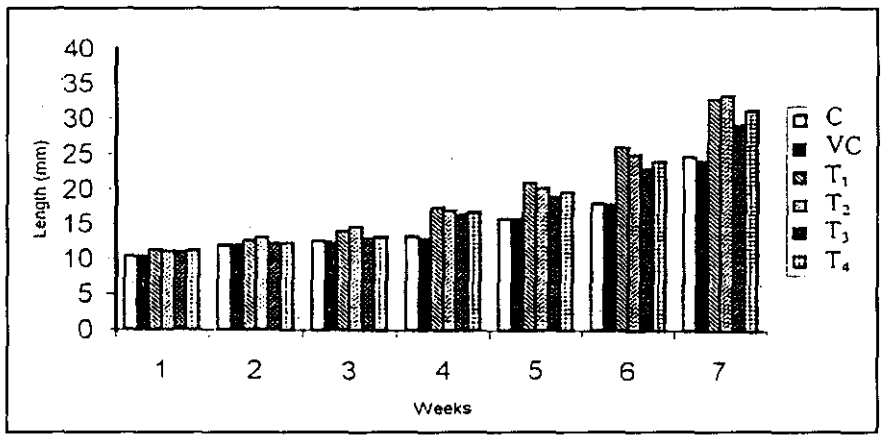


Fig. (3): Effect of hormonal treatment on total body length of Oreochromis niloticus fry through the experimental period.

Total body length: Table (5) shows that the averages total length (mm) of 7 days old Nile tilapia fry treated with MT (T2 andTl) were the highest, followed by T4, T3 and the controls respectively. The difference in total body length between the hormonal treatments was significant ($P<0.05$). It is readily seen from figure 3 that T2 and TI could he identified as one group with the longest total length.

Total body weight gain: Effect of 17 α– methyltestosterone on total body weight gain of Nile tilapia fry through the experimental period is presented in figure (4).

The difference in total body weight gain between groups were highly significant ($P<0.000$ I) (Figure 4).

These result clearly showed that the 7 days old fry fed on diet supplemented with MT at 60 ppm/kg feed, resulted in higher total body weight gain followed by fry fed on treated diet at 14 days of age compared with untreated controls (Figure 4). It is clearly noted that gain in weight of fry fed on hormonal treated diet for 28 days were significantly higher than those fed for 21 days.

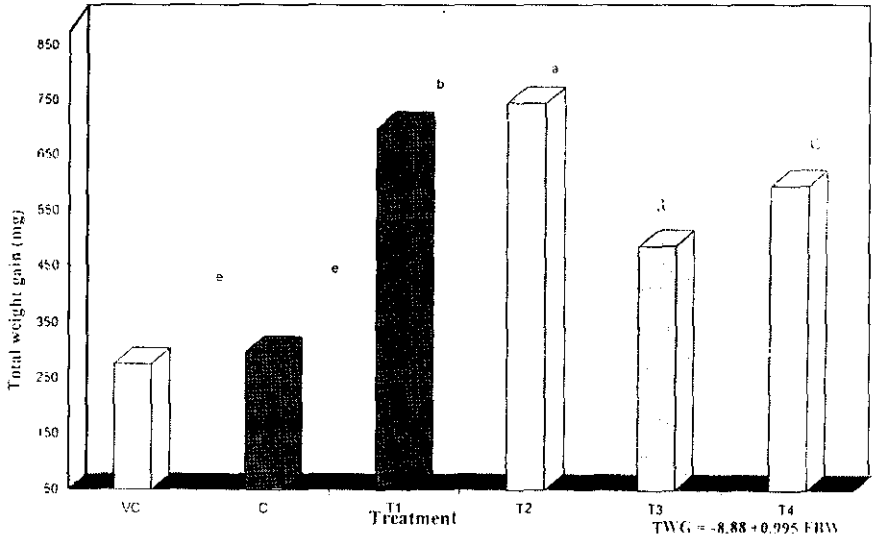
The present results are in agreement with the findings of McBride and Fagerlund (1973). They found significant weight gains in juvenile coho salmon fed on diet treated androgen hormone.

These results are in disagreement with Cannain and Lovell (199lb) who found that channel catfish fry weight gain fed on the control diet was higher than that of those fed on the treated diet. However, Perry and Wilson (1976) reported no differences in total body weight gain of treated and untreated channel catfish.

Shelton et al.(1978) stated that the benefits that would be gained by culture of all male tilapia include:- (I) reproductive control and therefore no reduction on growth because of crowding by the offspring. (2) increased production because males inherently grow faster than females.

Elfeet of Initial age X Duration of treatment on growth parameters:

The data show that the effect of initial age of fry during the experimental period is significant ($P<0.05$). The effect of duration of hormonal treatment is significant ($P<0.05$) in the first half of treatment period but it become insignificant in the second half in the last Wk of experimental period. The ellect of initial age X the duration of treatment is insignificant in the first three Wks. but ii is significant ($P<0.05$) in the last four Wks.



TWG = Total weight gain
FBW = Final body weight at 7 week of age.

Fig. (4): Effect of hormonal treatment on total body weight gain of Nile tilapia (Oreochromis niloticus) fry through the experimental period.

From these results, it can be noted that the initial age was more effective than duration of hormonal treatment on body weight of Nile tilapia during and after hormonal treatment, wherease there are interaction effect between initial age of fry and duration of hormonal treatment.

Table (5). Tow way classification of variance of body weight of *Oreochromis niloticus* fry through the experimental period.

	Bet . Ages		Bet. Periods		Age X period	
Wk	MS	F	MS	F	MS	F
1	14.714	1.34	0.0327	0.00	66.7819	6.1
2	941.6402	92.89*	249.3218	11.36*	125.2768	5.17
3	6247.613	104.36*	929.1881	15.56*	32494	5.43
4	3254.5834	31.99*	164.877	1.62	1438.1148	14.14*
5	27563.3079	85.94*	122.5737	0.38	9584.3963	29.88*
6	153083.62	245.82*	704.4146	1.13	44422.0816	71.33*
7	935844.0306	263.11*	153495.5117	43.16*	41563.2019	11.69*

*Significant (P<0.05)

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مجلة الأزهر للبحوث الزراعية
العدد(٣٥) يونيو ٢٠٠٢

تأثير هرمونات الذكورة الاستيرودية على التميز الجنسي الظاهري في اسماك البلطي النيلي (O.N)

احمد سف الدين صادق – اوسامة على – مي. فؤاد على

درست تأثيرات هرمونات الذكورة الاستيرودية ١٧ ميثيل تستسترون على التميز الجنسى الظاهري فى اسماك البلطى لنيلي حيث تم استخدام (٤٥٠٠) زريعه بلطي نيلي (O.N) غير مميزة جنسيا قسمت الى خمس مجموعات (أربعة تجربة ومجموعة مقارنة) .

تم تغذيه الزريعة على عليقه تحتوى على ٦٠ جزء فى المليون من الهرمون وذلك لمده ٧ أيام في المجموعة ٢,١ ولمده ١٤ يوم في المجموعة ٤,٣ . وبمعدل ٢٠٪ من وزن الجسم مرتين يوميا . وتم تقدير المعاملات الهر مونيه بعد أسبوعين.

عند عمر ١٠٠ يوم تم فحص وتصنيف الغدد التناسلية ميكروسكوبيا باستخدام طريقة الاسيتوكارمين.

نلاحظ أن الأنسجة المبيضة كانت إيجابية النمو بواسطة ملاحظة وجود الحويصلات (البويضات).

أعطت الزريعه التى تم تغذيتها لمده ٧ أيام على العليقه المهر منة وزن جسم وطول جسم كلى ونمو جسم ومعدل نمو ومعدل معيشة اعلي منه فى مجموعة المقارنة كما أعطت التغذية على العليقه المهر منه بسبه ذكور تراوحت من ٧٩,٤–٩٨,٩٪ بالمقارنة بمجموعة المقارنة والتى أعطت ٥٣٪ فقط.