Effect of rearing temperature and hormone treatment on sex ratio, survival and body weight of *Oreochromis niloticus* fry

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ABSTRACT

The present experiment was carried out to investigate the effect of rearing water temperature in combination with or without hormone application on masculinization of newly hatched O. niloticus fry. Nile tilapia fry (0.025 g) were reared in three levels of temperature 25, 30 and 35°C and in each rearing temperature fry were fed diet with or without supplementation of 60 mg 17α-methyltestosterone (MT) for 7, 14, 21 and 28 days.

O. niloticus fry fed diet without MT and reared at different water temperature 25, 30 and 35°C significantly increased the male ratio from 48.67 to 48.67 and 65.33% after one week; 48.67 to 64.00 and 72.67% after two weeks; 48.67 to 68.67 and 77.33% after three weeks and 48.67 to 80.67 and 84.00% after four weeks, respectively. While fry fed diet supplemented with MT with increasing rearing water temperature from 25 to 30 or 35 °C significantly increased male percentage from 58.00 to 84.67 and 86.00% after one week; 67.66 to 93.33 and 96.67% after two weeks; 79.00 to 97.33 and 98.67% after three weeks and 85.67 to 98.00 and 99.33% after four weeks.

Mortality rate for *O. niloticus* fry reared at different water temperature 25, 30 and fed diet without MT significantly increased from 10.33 to 14.00 and 14.33% after one week; 10.33 to15.33 and 15.00% after two weeks; 10.33 to 15.33 and 15.17% after three weeks and 10.33 to 15.83 and 16.83% after four weeks. But, supplementation of *O. niloticus* feed diet supplemented with MT and increasing rearing water temperature from to 30 or 35°C significantly increased mortality rate from 14.67 and 14.00% after one week; 16.00 and 15.67% after two weeks; 15.33 and 15.67% after three weeks and 16.00 and 17.33% after four weeks of treatment.

The highest fry body weight was recorded for fry group administrated with MT and reared at water temperature of 35°C and the opposite trend was observed with fry group reared at the lower temperature (25°C) and fed diet without MT.

Keywords: Temperature, hormone treatment, sex ratio, *Oreochromis niloticus*

INTRODUCTION

Tilapias *(Oreochromis niloticus)* are a paradox in reproduction. The relative fecundity of *O. niloticus* species is low; 6,000-13,000 eggs/kg/spawn. However, this is compensated by high survival rate and its iteroparity nature. Ideally, a fish species used in aquaculture is not allowed to reproduce in the culture environment before reaching market size. This phenomenon presents a significant challenge to the fish culturist. Most tilapia species often reach maturity within 6-8 months of hatching at a size often less than 100 g. Under favourable conditions tilapia will start to reproduce

leading to intraspecific competition hence stunted growth and become unmarketable (Beaven and Muposhi 2012).

All male culture of tilapia is preferred because of their fast growth. Several techniques have been used to produce monosex tilapia to control; unwanted reproduction and among these include; manual sexing (Guerrero, 1982); genetic manipulation (Pandian and Varadaraj 1988); and sex reversal through sex oestrogenic hormone administration (Guerrero, 1982).

Hormone treatment does not alter the genotype of the fish but directs the expression of the phenotype. Production of all male population through administration of androgen (17α-MT) is considered to be the most effective and economically feasible method for obtaining all male tilapia populations (Guerrero and Guerrero 1988). Recently hatched tilapia fry do not have developed gonads such that it is possible to intervene at this early point in the life history and direct gonadal development to produce monosex populations. Exogenous steroids given during the gonadal development period can control the phenotype overriding the expression of the genotypically determined sex.

Some studies provided evidence that water temperature also governed the phenotypic sex of *Oreochromis* spp. A vast majority of experiments demonstrated that high temperatures favoured the production of (almost) monosex male progenies (*O. niloticus:* Baroiller *et al.,* 1995: 1996 a & b; *O. aureus:* Baras *et al.,* 2000). Except for some strains (Trewavas, 1983), 36-37°C is close to the upper incipient lethal temperature of Nile tilapia (Balarin and Hatton, 1979), and above the thermal optimum for its growth (Melard, 1986).

Therefore this study aimed to (i) measure the sex ratio and survival of progenies of Nile tilapia reared at different temperatures during their early life stages; (ii) measure the sex ratio and survival of progenies of Nile tilapia fed the diet supplemented or did not supplemented by 17α-MT (iii) measure the effect of interaction between thermal and hormonal treatments on sex ratio and survival of progenies of Nile tilapia.

MATERIALS AND METHODS

The present experiment was carried out at the hatchery unit of the experimental station of the World Fish Center, Abbassa, Abou-Hammad, Sharkia, Egypt to investigate the effect of rearing water temperature in combination with or without hormone application for different periods (1, 2, 3 and 4 weeks) on masculinization of newly hatched tilapia fry.

Broodstock and fry collection:

Oreochromis niloticus broodstock were brought from the same experimental station and stocked at a sex ratio of 3 female: 1 male in concrete tanks of 12 m^3 filled with filtered canal water from Ismailia canal to maintain a water depth of seventy (70) cm and supplied with compressed air through air diffusers to assure maintaining near optimum dissolved oxygen levels in the tank water.

Spawning tanks were monitored daily to collect any hatched fry using fine nets to undergo the experiment. Tanks were covered with plastic sheets (2 mm thickness) extended over a metallic frame of arched iron bars similar to those used in agricultural greenhouses.

Experimental design:

Three aquaria of 80 liters each were filled with water and used for each treatment. 300 hatched tilapia fry (0.025 g) were placed in each aquarium. All aquaria

were supplied with the specific diet (45% crude protein) with or without supplementation of 17α-methyl testosterone (MT) at 60 mg/kg diet. Feeding rate was 15% of the biomass of each aquarium divided into 5 meals per day. Tilapia fry were randomly distributed into 6 treatments as follows:

T1: C25H0 fry reared at 25°C and fed MT free diet.
T2: C25H1 fry reared at 25°C and fed MT supplemented diet.
T3: C30H0 fry reared at 30°C and fed MT free diet.
T4: C30H1 fry reared at 30°C and fed MT supplemented diet.
T5: C35H0 fry reared at 35°C and fed MT free diet.
T6: C35H1 fry reared at 35°C and fed MT supplemented diet

Diets preparation:

The hormone treated feed was prepared as described by (Killian and Kohler, 1991). The MT used in the present study was obtained from the Sigma Chemicals Ltd. A stock solution was made by dissolving 1 g of hormone in 1 L of 95% ethanol. Treatments were made by taking the accurate amount of the hormone from stock solution and brought up to 100 ml by addition 95% ethanol. This solution was evenly sprayed over 1 kg of the diet mixure (Table 1). The mixture was mixed again and this was repeated to ensure an equal distribution of the MT throughout the feed. Treated diets were fan dried in shade at 25ºC for 24 hours then kept in freezer till use.

Table 1: Formulation and composition of the artificial diet used for *O. niloticus* fry

Ingredients	%
Fish meal	38
Soy bean meal	30
Yellow corn	23
Bran	3.5
Vegetable oil	3
vitamins&minerals mixture[1]	2.2
Ascorbic acid	0.3
Sum	100
Crude protein	44.65
Metabolizable energy (Kcal/kg feed)	2895
P/E ratio (mg ptotein/kcal)	154.22

[1] Vitamin & mineral mixture/kg premix : Vitamin D_3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

Flow pattern:

All treatments aquaria were placed in a wet lab where a water recycling unit was used to supply the aquaria with temperature controlled water. In order to control water temperature in the lab, this experiment was conducted on three separate stages (25, 30 and 35°C). Each part the effluent water of the glass rearing tanks was passed through heater. After that, water passes through the filter unit before it was returned to the fish tank by pump. Daily partial water was added per day to reduce the accumulation of nitrate and substitute the water losses due to the evaporation. During the experimental period continuous monitoring and recording of the main water quality parameters took place and outlined in Table (2).

Table 2: Ammonia, dissolved oxygen and temperature after 1, 2, 3 and 4 weeks of different heat treatments 25, 30 and 35 ºC.

Temperature	Parameter	Experimental periods			
		1st week	2nd week	3rd week	4th week
25 ºC	Ammonia	0.2	0.4	0.3	0.5
	Dissolved oxygen	6.88	6.89	7.00	6.33
	Temperature	25.6	25.2	25.00	25.40
30 ºC	Ammonia	0.5	0	0.43	0.4
	Dissolved oxygen	7.1	6.88	6.17	7.8
	Temperature	30.1	30.55	30.2	30.4
35 ºC	Ammonia	0.4	0.2	0.5	0.3
	Dissolved oxygen	6.86	7.2	6.46	7.1
	Temperature	35	35.2	35.4	34.6

Means followed by different letters in each column for each treat for each trait significantly different ($P<0.05$).

Phenotypic sex determination (Squash Technique):

After each mentioned period for each treatment, a sample of 25 fry was collected from each aquarium to undergo sex ratio check using the squash technique.

Trinocular microscope with camera and monitor (Boeco Germany) were used to examine the gonads. Aceto-carmine solution was prepared according to Guerrero and Shelton (1974) where 0.5g of indigo carmine was added to 100 ml of acetic acid (45%) and boiled for 5 minutes. After cooling, the solution was filtered using filter paper and transferred to a dark bottle. Bouin's solution was prepared according to Clark (1981). A stock Alcoholic Bouin's Solution was prepared by mixing 750 ml ethanol alcohol 80% and 300 ml formaldehyde and 5 gm picric acid and this solution was well mixed and the working solution formed from 70 ml stock solution + 5 ml acetic acid. Acetic acid was added before use.

Fish samples were killed by cold shock (water temperature of 0ºC), weighed, measured, and dissected. For microscopic examination (Squash Technique) fish were cut ventrally, using a scalpel, from the genital papilla to the base of the pectoral fin. A window on the lateral side was opened and the viscera were removed, leaving gonads, swim bladder and kidneys in place. A few drops of Bouin's solution were applied topically to the gonads. This procedure hardened the gonadal tissue facilitating its removal. The anterior and posterior ligaments were cut, and both gonads were removed using a forceps and placed on a glass slide. Aceto-carmine solution was added and the tissue was covered with a cover slip. Both gonads were then examined over their entire length under a compound microscope using magnifications of 100 × and 400 ×.

Statistical analysis:

Statistical analysis of the obtained data was analyzed according to SAS (1996). Differences between means were tested for significance according to Duncan's multiple rang test as described by Duncan (1955).

RESULTS AND DISCUSSION

Sex ratio

The first fish group (C25H0) showed the lowest male percentage (48.67%) for the different rearing periods, 1, 2, 3 or 4 weeks (Table 3). After one week of treatment, the third fish group (C30H0) showed the same male population as control (C25H0) group (48.67%) while the successive treatment periods (2, 3 or 4 weeks of treatment) showed higher male percentages (64.00, 68.67 and 80.67%, respectively)

and the same trend was also observed for the C30H0 group indicating that the positive effect of rearing water temperature begin after two weeks at least. This also demonstrated that the temperatures used in treatments were sufficient to induce the sex reversal because there was change in the proportion of males in these groops.

Table 3: Sex ratio of *O. niloticus* fry reared in different water temperature and fed diet with or without 17α-MT for four weeks.

Treatments	Duration of treatments (week)			
	1	2	3	4
C25H0	48.67 d	48.67 d	48.67 d	48.67 d
C25H1	58.00 c	67.00 c	79.00 b	85.67 b
C30H0	48.67 d	64.00 c	68.67 c	80.67 c
C30H1	84.67 a	93.33 a	97.33 a	98.00 a
C35H0	65.33 b	72.67 b	77.33 b	84.00 bc
C35H1	86.00 a	96.67 a	98.67 a	99.33 a
SE	±1.97	±1.56	±1.47	±1.43

Means followed by different letters in each column for each treat for each trait significantly different ($P<0.05$).

After the first week of treatment, the 6th fish group (C35H1) showed the highest significant ($P<0.001$) male population (86.00%) compared the other fish groups. Male percentage increased to 96.67, 98.67 and 99.33% after 2, 3 and 4 weeks of treatment, respectively and relativelly similar results was also obtained for C30H1 group indicating the high efficiency of 17α-MT adminstration on sex reversal of *O. niloticus.*

The highest male percentages were obtained with the 6th fish group (C35H1) which did not significantly different from that recorded by the 4th fish group (C30H1) after 1, 2, 3 and 4 weeks from the treatment start.

Generally, the obtained results in the present study indicated that, supplementation of *O. niloticus* fry with 60 mg 17α-MT/kg feed with increasing rearing water temperature from 25 to 30 or 35°C significantly increased male percentage to 84.67 and 86.00% after one week; 93.33 and 96.67% after two weeks; 97.33 and 98.67% after three weeks and 98.00 and 99.33% after four weeks of treatment.

Producing a monosex population of *O. niloticus* for aquaculture is high priority since males have a higher growth rate as compared to females. In this study, we observed a relatively high male percentage compared to those not subjected to the hormone. These results in terms of male to female percentage are similar to other findings of Beaven and Muposhi (2012) who showed that *O. niloticus* fry fed to a diet treated with 17α-MT had a significantly high male population (90.06) as compared to those fed to a non hormone treated diet (50.63%).

The high male percentage in the different groups found in the present experiment cannot be attributed to differentially higher mortality of the females as suggested by Mair *et al.*, (1990), but to the hormone treatment given that high rates of survival were obtained and that there were no significant differences in survival among treated and control fry.

Hormonal treatments are very efficient, but they could pose environmental problems in the future, due to uncontrolled discharge of the resulting waste water (i.e. steroids and/or metabolites are not removed from the water before discharge).

We noticed that ambient water temperature during the period of sex differentiation of fry strongly influences sex ratio in Nile tilapia (O. *niloticus*), as shown by Baroiller *et al.*, (1995, 1996a and Tessema *et al.,* 2006) on *O. niloticus* (strain

Bouake), Baroiller *et al.,* (1996b) on Florida red tilapia, Baras *et al.,* (2001) on *O. niloticus* (strain Manzala), and Desprez and Melard (1998) on *O. aureus*, that high temperatures skewed the sex ratio in favor of males. On the other hand, we found that low rearing temperatures (25°C) did not affect the sex ratio of progenies C25H0, confirming results obtained for other strains and species of tilapia.

In *O. niloticus*, temperature influences on sex ratio have been detected that can (but not always) override the action of the sex determining genes (Abucay *et al.,* 1999; Baras *et al.,* 2001; Baroiller and D'Cotta, 2001). Temperature conditions are anticipated to have variable effects on sex differentiation depending on the height of temperature and the genetic background (Argue and Phelps, 1995). Desprez, and Melard (1998) reared tilapia fry *O. aureus* at 21°C (40 days), 27°C and 34°C (25 days) then at 27°C and sexed. They found that, high temperature regimes produced high male ratios (97.8%) while intermediate thermal regime gave balanced ratios (63.0%). Low temperature delayed the differentiation of gonads. Also, Varadaraj *et al.* 1994 and Abucay (1997) reported that, the temperature as high as 36°C during hormone treatment can increase the rate of sex reversal.

The results presented here provide evidence of a significant effect of temperature on sex ratio in *O. niloticus* (in the direction to male), confirming results obtained for this and other tilapia species in previous studies (Mair *et al.,* 1990; Mbahinzireki and Dabrowski, 1997 and Baras *et al.*, 2001). The observed sensitivity of the fish used in these studies to high temperature is likely to be related to effects during sexual differentiation. There are two possible developmental pathways whereby temperature can affect this process. First, an environmental shock such as high temperature might disrupt the normal development processes during sex differentiation causing the switch to males for the genetic females and switch to females for the genetically male progeny. Second, the high temperature might have an effect on the structure or action of a hormone or hormones acting during sex differentiation (Hunter and Donaldson, 1983). For example, Wibbels *et al.*, (1994) discuss the potential effects of temperatures on the action of aromatase the catalyst for the breakdown of androgens to estrogens.

All in all, the results obtained suggest that *O. niloticus* a thermo-sensitive specie and temperature can affect gonadal sex differentiation of the individuals during the early development stages.

The combined effect of rearing water temperature and 17α-MT treatment indicated a significant increase in male ratio compared to the effect of water temperature or 17α-MT alone and these results showed the possibility of reducing hormone treatment period from 4 to 2 weeks by increasing rearing water temperature to 30 or 35°C with obtaining an appropriate male population (93.33 and 96.67%, respectively) and this could be reduce hormone costs and its problems in producing sex reversal of *O. niloticus* fry. On the other hand, Drummond *et al.,* (2009) found no significant interaction between temperatore (26, 28, 30 and 30°C) and hormonal doses (0, 20, 40 and 60 mg17α-MTkg diet) for sex ratio of *O. niloticus* fry used for sex reversal.

Mortality rate:

The first fish group (C25H0) after one week showed the lowest mortality rate of *O. niloticus* fry (10.33%) which did not significantly different from 11.17% that obtained by the second fish group (C25H1) that reared at the same temperature (25°C) and received 17α-MT and the same trend was also observed during the other treatment period (2, 3 and 4 weeks). Indicating that MT treatment did not significantly affected mortality rate of *O. niloticus*. Varadaraj (1990) found no significant differences ($P>0.05$) in mortality rate between treated and untreated *O. mossambicus*

fry with 19-norethisterone acetate. Also, Cruz and Mair (1994), Goerrero III and Goerrero (1997) and Mainardes-Pinto *et al.*, (2000), found that 17α-MT has little or no effect on the growth and survival of Nile tilapia during the hormonal treatment of *O. niloticus fry*.

On the other hand, Pandian and Sheela (1995) decided that, treatment involving a synthetic steroids result in higher mortality in three natural populations of *O. niloticus*. Cruz & Mair (1994) mention that fry mortality observed during the hormonal treatment may be explained by the establishment of feed hierarchy among fish. Dominant individoals within the population may consume more food and grow faster leaving less food for submissive individuals who have less growth and become, consequently, vulnerable to cannibalism and death by starvation.

After one week from treatment the 3rd fish group (C30H0) showed high mortality rate (14.00%) which did not significantly different from 14.67, 14.33 and 14.00% that obtained for the other three fry groups C30H1, C35H0 and C35H1, respectively. Similar results were obtained after 2, 3 and 4 weeks from treatment statr and this results may be attributed to the effect of high rearing water temperature (30 or 35°C) compared to the normal rearing water temperature 25°C used in the first and second (C25H0 and C25H1) fish groups.

Supplementation of *O. niloticus* fry feed by 60 mg 17α-MT/kg diet with increasing rearing water temperature to 30 or 35°C (C30H1 and C35H1) significantly increased mortality rate to 14.67 and 14.00% after one week; 16.00 and 15.67% after two weeks; 15.33 and 15.67% after three weeks and 16.00 and 17.33% after four weeks of treatment. In the same trend, increasing rearing water temperature to 30 or 35°C without 17α-MT (C30H0 and C35H0) significantly increased mortality rate to 14.00 and 14.33% after one week; 15.33 and 15.00% after two weeks; 15.33 and 15.17% after three weeks and 15.83 and 16.83% after four weeks of treatment. These results are in agreement with those observed by Drummond *et al.,* (2009) who found that, the values of survival (%) of *O. niloticus* after 28 days of treatment there was significant interaction of temperatore (26, 28, 30 and 30°C) and hormonal doses (0, 20, 40 and 60 mg17α-MTkg diet).

The obtained results in the present study (Table 4) showed that the graded increase in water temperature significantly increased mortality rate of *O. niloticus* fry specially at 35°C and this effect was cleared observed after 4 weeks of water temperature treatment whereas mortality rate reached to 17.33% (C35H1) compared to 10.33% for control (C25H0) water temperature (25°C).

Table 4: Mortality rate of *O. niloticus* fry in different water temperature and fed diet with or without 17α-MT for four weeks

Treatments	Duration of treatments (week)			
	1	2	3	4
C25H0	10.33 c	10.33 c	10.33 c	10.33 b
C25H1	11.17 bc	12.50 b	12.83 bc	12.00 b
C30H0	14.00 ab	15.33 a	15.33 ab	15.83 a
C30H1	14.67 a	16.00 a	15.33 a	16.00 a
C35H0	14.33 a	15.00 a	15.17 ab	16.83 a
C35H1	14.00 ab	15.67 a	15.67 a	17.33 a
SE	±0.97	±0.97	±0.97	±0.97

Means followed by different letters in each column for each treat for each trait significantly different ($P<0.05$).

This suggests that the period of metamorphosis, including change in the style of energy-uptake from yolk to exogenous nutrition, is susceptible to damage resulting

from abrupt temperature changes. Similar results were also obtained by Baras *et al.,* (2001). In this respect, Azaza *et al.,* (2008) found that the best rate of masculizing (80%) was obtained after exposure to 36.83°C, but with lower survival rates during treatment (60%).Borges *et al.,* (2005) observed that with increasing temperatore survival rates of tilapia are directly related with the occurrence of cannibalism, significantly higher at 35°C.

Fry body weight (BW):

At all treatment periods (1, 2, 3 or 4 weeks) results indicated that, the highest BW averages were recorded for fry group administrated 60 mg 17α-MT/kg feed and reared at water temperature of 35°C C35H0 which did not significantly different from those recorded in C30H1 group and the opposite trend was observed with control fry group which did not received 17α-MT and reared at the lower water temperature (C25H0) and these results may be attributed to the high male percent related to the high rearing water temperature and 17α-MT (Table 5).

Table 5: Body weight of *O. niloticus* fry in different water temperature and fed diet with or without 17α-MT for four weeks

Treatments	Duration of treatments (week)			
	1	2	3	4
C25H0	0.12 d	0.23 c	0.56 d	1.02 f
C25H1	0.20 c	0.37 b	0.81 c	1.59 e
C30H0	0.25 b	0.45 ab	1.07 b	1.75 d
C30H1	0.29 ab	0.49 a	1.12 b	1.95 c
C35H0	0.28 ab	0.47 a	1.32 a	2.07 b
C35H1	0.31 a	0.51 a	1.34 a	2.27 a
SE	±0.012	±0.032	±0.051	±0.032

Means followed by different letters in each column for each treat for each trait significantly different ($P<0.05$).

Our ressults are in agreement with many previous studies. Drummond *et al.,* (2009) found a significant interaction between temperatore (26, 28, 30 and 30°C) and hormonal doses (0, 20, 40 and 60 mg17α-MTkg diet) for weight gain of *O. niloticus* fry used for sex reversal ($p <0.01$). Macintosh *et al.,* (1985) indicated that, there were significant differences between hormone treated and untreated tilapia fry. They obtained an average weight increase of 64% over control when feeding *O. mossambicus* fry with MT-30 mg for 60 days. Also, McAndrew and Majumdar (1989) obtained a 25.7% increase in BW over controls when *O. niloticus* fry were treated with MT-40 for 40 days. Varadaraj (1990) indicated that, the increase in BW of *O. mossambicus* fry treated with 19-norethisterone acetate than control for 15 days may be attributed to the anabolic effect of 19-NE on metabolism. Khater (1999) indicated that, *O. niloticus* fry treated with 17α-MT had significantly ($P<0.05$) higher BW as compared to the control group (received no hormone in the diet) after 14, 21 and 28 days of 17α-MT treatment. Also, Khalil *et al.,* (2011) and Beaven and Muposhi (2012) found that *O. niloticus* fry fed to a diet treated with MT had a significantly higher growth and body weight as compared to those fed to a non hormone treated diet.

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ARABIC SUMMARY

تأثير درجة الحرارة والمعاملة بالهرمون على النسبة الجنسية والحيوية ووزن جسم زريعة البلطى النيلى

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٣- المركز الدولى للأسماك بالعباسة ، أبوحماد شرقية ، مصر

أجريت هذه التجربة بهدف معرفة تأثير درجة حرارة الماء مع أو بدون هرمون الذكوره (١٧ ألفا ميثايل تستوستيرون) على إنقلاب الجنس فى أسماك وحيوية أسماك البلطى النيلى. ولذلك تم تقسيم زريعة أسماك البلطى النيلى إلى ثلاثة مجموعات وتم تحضين ورعاية المجموعات الثلاثة فى ثلاث درجات حرارة مختلفة ٢٥، ٣٠ ، ٣٥°م وفى كل مجموعة تم تقسيم الزريعة إلى قسمين الأول تغذت الزريعة على عليقة خلية من الهرمون أما المجموعة الأخرى فقد تغذت على العليقة التى أضيف إليها ٦٠ مجم هرمون/كجم عليقة. وكان من أهم النتائج المتحصل عليها مايلى:

وجد أن زيادة درجة حرارة الماء من ٢٥ إلى ٣٠ أو ٣٥°م بدون إضافة الهرمون إلى العليقة قد أدى إلى زيادة نسبة الذكور من ٤٨,٦٧ إلى ٤٨,٦٧ ، ٦٥,٣٣% بعد أسبوع من المعاملة، إلى ٦٤,٠٠، ٧٢,٦٧% بعد أسبوعين وإلى ٦٨,٧٨، ٧٧,٣٣% بعد ثلاثة أسابيع وإلى ٨٠,٦٧، ٨٤,٠٠% بعد أربعة أسابيع على التوالى كما أدت المعاملة بالهرمون مع زيادة درجة حرارة الماء من ٢٥ إلى ٣٠ أو ٣٥°م إلى زيادة نسبة الذكور من ٤٨,٦٧ إلى ٨٤,٦٧ ، ٨٠,٠٠% بعد أسبوع من المعاملة، إلى ٩٣,٣٣، ٩٦,٦٧% بعد أسبوعين وإلى ٩٧,٣٣، ٩٨,٦٧% بعد ثلاثة أسابيع وإلى ٩٨,٠٠، ٩٩,٣٣% بعد أربعة أسابيع على التوالى.

وجد أن زيادة درجة حرارة الماء من ٢٥ إلى ٣٠ أو ٣٥°م بدون إضافة الهرمون إلى العليقة قد أدى إلى زيادة نسبة نفوق الزريعة من ١٠,٣٣ إلى ١٤,٠٠، ١٤,٣٣% بعد أسبوع من المعاملة، إلى ١٥,٣٣، ١٥,٠٠% بعد أسبوعين وإلى ١٥,٣٣، ١٥,١٧% بعد ثلاثة أسابيع وإلى ١٥,٨٣، ١٦,٨٣% بعد أربعة أسابيع على التوالى، كما أدت المعاملة بالهرمون مع زيادة درجة حرارة الماء من ٢٥ إلى ٣٠ أو ٣٥°م إلى زيادة نسبة النفوق من ١١,١٧ إلى ١٤,٦٧، ١٤,٠٠% بعد أسبوع من المعاملة، إلى ١٦,٠٠، ١٥,٦٧% بعد أسبوعين وإلى ١٥,٣٣، ١٥,٦٧% بعد ثلاثة أسابيع وإلى ١٦,٠٠، ١٧,٣٣% بعد أربعة أسابيع من المعاملة على التوالى.

وجد أن أكبر متوسطات لوزن الجسم لزريعة البلطى قد تحقق مع المجموعة التى تغذت على العليقة المحتوية على الهرمون مع درجة حرارة ٣٥°م فى حين حققت المجموعة التى غذيت على العليقة الخالية من الهرمون مع درجة حرارة ٢٥°م أعطت أقل متوسط لوزن جسم الزريعة.