Abbassa International Journal for Aquaculture

Volume (4) Number (1), 2011

ISSN 1687-7638

Egyptian Society for Water, Aquaculture and Environment

Abbassa, Abou Hammad, Sharkia, EGYPT

ISSN 1687-7638

ABBASSA INTERNATIONAL JOURNAL FOR AQUACULTURE

Published by

Egyptian society for water, aquaculture and environment, Central Laboratory for Aquaculture Research (CLAR), Agricultural Research Center (ARC), Giza, Egypt

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GENERAL INFORMATION

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Abbassa Int. J. Aqua., V0l.4 No.(1) 2011 *201 - 220*

FEMINIZATION AND PROGENY TESTING OF CONVERTED MATERNAL (C-ZZ) GENOTYPE AFTER SEX REVERSE TO PRODUCE SUPER MALE (ZZ) IN *OREOCHROMIS AUREUS* EXPERIMENTALLY

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Received 15/ 10/ 2011 *Accepted 9/ 11/ 2011*

Abstract

The present study represents a first step in program for producing "supermales" of *Oreochromis aureus* in Egypt as a practical solution for the unwanted reproduction of tilapia in ponds and for increases the fish production. A trial of sex reversal for feminization of sexually undifferentiated progeny from *Oreochromis aureus* was conducted using 17-β ethynylestradiol as a feminizing stimulating agent. Oral administration of powdered feed containing 500 and 1000 mg/kg^{-1} were tested for 30 days.

Upon termination of the experiment, average weight and length on higher doses were generally higher (103±0.006 mg & 22.3±0.306 mm respectively) than those fed on lesser amounts (101±0.003mg & 22±0.153 mm respectively) indicating the effectiveness of the hormone for enhancement of fish growth. Through 2009 experiments hormone effect was highly detectable among fry that were orally fed on 1000 mg/kg hormone-treated feed for 30 days (mean percentage of phenotypic females (MPF) was 94.33 ± 2.08%). While, fry consumed the 500-mg/kg hormone-treated feeds for 30 days was 83.67 ± 4.04%. Fry was feeding with hormone 17-β ethynylestradiol treated-feed 500 mg/kg showed that male, inter sex and non differential percentages were 11.2±4.39, 3±1 & 2.33±0.58 % respectively, while, this percentage decreased in fry treated-feed 1000 mg/kg showed that male, inter sex and

non differential percentages were 3.33±1.53, 1.67±0.58 & 0.67±0.58 % respectively. From the 2010 experiments, the pseudofemales (C-zz) could be differentiated form the normal ones (wz) using pair-mating of normal genotypic males to the mixed females with normal males separately. Progeny testing of their offspring proved that the majority of females were originally genotypic females from the moment of fertilization and their offspring had a normal sex ratio of 1:1. Forty remaining females generated progeny including mean percentage of phenotypic males (MPM) was 70 ± 5.2% while female, inter sex and non differential percentages were 25 ± 4.3, 3 ± 1.9 & 2 ± 0.8 % respectively. Pseudofemales (C-zz) produce low number of male (70±5.2%) in first generation for estrogen-treated females. Also it is hypothetically supposed that one third of these males are super males of the "C-zz" genotype and two thirds should be normal males "N-zz". Further research by crossing these males after sexual maturation to normal females and testing their progeny will prove or disprove this hypothesis.

Key words: Sex reversal, *Oreochromis*, *aureus*, Feminization and Progeny testing.

INTRODUCTION

The most difficult problem associated with tilapia culture is the early sexual maturation and uncontrolled reproduction in ponds. A great deal of work has been done looking for solutions such as manual sexing, predator stocking, hybridization, gynogenesis and steroid-sex reversal to produce all male tilapia (super males) (Mair and Little, 1991). Wohlfarth (1994) evaluated these solutions, specially in the developing countries, and reported that all the traditional techniques have no longer been adopted widely in aquaculture. Manual sexing is laboreus and requires skill. The major disadvantages of this method are human error in sexing and the wastage of females. Interspecific and intergenic hybridization are known to produce all-male progeny. However, difficulty in maintaining

pure parental stocksthat consistently produces 100% male offspring, poor spawning success and incompatibility of breeders resulting in low fertility. Therefore, studies on the genetic basis of sex determination of *Oreochromis niloticus* and other *Oreochromis* species have been developed by Mair *et al*. (1991) and Trombka and Avtalion (1993) to provide an alternative and effectual monosex breeding program for producing all- male offspring (Desprez *et al.* (2003b).

Sex determination in fish is a very flexible process with respect to evolutionary patterns observed among genera and families, and within individuals is subject to modification by external factors. These influences can affect the fate of both somatic and germ cells within the primordial gonad, and include the action of genetic, environmental (e.g. temperature), behavioral and physiological factors. Both estradiol and the maturation hormone are produced by a two-step process involving different cell layers in the gonad, and have effects on the differentiation of gonadal and nongonadal tissues. Gonadal development and differentiation in some fish is also controlled by hormones from the pituitary gland (gonadotropins) that are regulated by release hormones and other neuroendocrine and gonadal factors. Genetic determination of sex in fish can involve monogenic or polygenic systems, with factors located on the autosomes or on sex chromosomes. In the latter case, both male (XY) and female (ZW) heterogametic systems have been described **(**Robert and Yoshitaka, 2002**)**. The use of oestrogens for sex control, either in the direct method of femenization or in the indirect method of masculinization. Special attention is given to the method of administration, including immersion and dietary treatment, and to the variables of the hormonal treatment itself. The importance of correct treatment timing in relation to the degree of gonadal development is emphasized and the outcome of the treatment evaluated in terms of survival, gonadal morphology and sex ratios, growth performance and deformities. The current methods to produce all-female or essentially all-

female stocks are presented for 35 different species, including eels, salmonids, cyprinids, poecilids, cichlids, gouramies and flatfishes. The overall goal is to emphasize the use of the indirect method, which means that fish that reach the marketplace have never been exposed to steroids. If this method is not feasible, as it happens in many species, an alternative is the use of the direct method, applied in an optimized protocol, to achieve maximum treatment efficiency with minimum exposure to steroids (Francesc, 2001).The monosex breeding program using YY-male broodstock might provide such a solution. The breeding program of generating YY-male comprises a number of distinct steps. The following genetic nomenclature for description of hormone - treated fish will be used in order to skim over the steps of this program: C-XY refers to converted genotypic male into functional phenotypic female. The breeding program starts with feminization of sexually undifferentiated progeny from normal crosses (Scott *et al*., 1989; Varadaraj 1989 and Rosenstein & Hulata 1994) in a number of *Oreochromis* species.

Desperz *et a*l. (2003a) carried out sex determination system on blue tilapia, *Oreochromis aureus* using pseudofemale populations Their data reported that sex reversal of fry with estradiol resulted in the production of some functional sex-reversed fish with a female phenotype and ZZ male genotype, known as pseudofemales. The first step which is feminization of sexually undifferentiated tilapia progeny from normal crosses has been achieved by many authors (Rosenstein & Hulata, 1994; Mair and Santiago, 1994 and Mohamed *et al*., 2004) in *Oreochromis niloticus* and in a number of other *Oreochromis* species. Sex reversal of tilapia either by feminization or masculinization must begin before the gonadal tissue of young genetic males or females has differentiated into testes or ovaries. Functional sex reversal is most easily achieved through oral application of estrogens or androgen incorporated into the feed and administered during the period of sex differentiation which is known to

be 30 days according to Alvendia-Casauay and Carino (1988) and 14 days according to Srisakultiew (1993). However, Popma and Green (1985) reported that fry can be effectively sex reversed in 20 days, but occasionally only 95% of the fry develop as phenotypic males.

The present study is a trial to carry out the first step in such program to feminize sexually undifferentiated progeny from normal crosses of *Oreochromis aureus* and the second step in such program, after feminization of sexually undifferentiated progeny from normal crosses of *Oreochromis aureu*s, to differentiate the converted tilapia after maturation through subsequent crossing with normal male. Progeny sex ratio approximating 3:1 is indicative of a maternal ZZ genotype.

The objective of this study was to produce supermale (ZZ) through the feminize sexually undifferentiated progeny of *Oreochromis aureu*s from normal crosses using 17-β ethynylestradiol for sex reversal as a feminizing stimulating agent as well as rcognizing the target converted maternal ZZ genotype.

MATERIALS AND METHODS

Fish and facilities:

Through the first season (Year 2009) a number of 120 broodstock females and 60 of active males of *Oreochromis aureus* were accommodated separately at a sex ratio of 2:1 in 6 hapas (net cages) installed in 2 concert pond each of which containing 20 females and 10 males. Dimension of hapa was 8 x 3 x 1 m. Average body weight of females was 100 ± 10 g. Fish were fed 2% of the total biomass daily with pilleted feed (25 % crude protein). Hapas were cleaned every week from uneaten food and feces. Females were checked regularly by opening their mouths gently. When females spawned, eggs were extruded from the buccal cavity of the female and incubated in Macdonald jars until hatching.

The first batch of hatched larvae were collected and stocked in 6 hapas (1*1*1 m), installed in the same pond, at a density of 1000 larvae /hapa., 6 hapas were randomly assigned into 2 groups representing 2 treatments with three replicates each. After the yolk sac absorption period, the so-called fry reached 7-12 mm total length and 10±3 mg body weight and started to search for exogenous food. The hormone of 17-β ethynylestradiol used for feminization of the fry, however fry of the 2 treatments were fed 6 days a week for 30 days on hormone- treated feed with 2 different dosages of hormone 500 and 1000 mg/kg feed, respectively.

Hormone-treated and feed preparation:

The hormone tested for feminization was 17-β ethynylestradiol. It is insoluble in water but readily dissolves in ethyl alcohol. 2 quantities of hormone impregnated feed weighing 1000 g each were prepared with varying dosages of hormone; 500 and 1000 mg/kg; for feeding the 2 treatments. The fish were fed an experimental diet 40% crude protein. It was finely ground and sieved to remove the particles that were too large to be ingested. An amount of 500 mg of 17-β ethynylestradiol were weighed and dissolved in 1000 ml ethyl alcohol and also, 1000 mg of 17-β ethynylestradiol were weighed and dissolved in 1000 ml ethyl alcohol 95% to prepare a two treatment of diet. The two volumes of alcohol with hormone mixed thoroughly to 2 quantities of 1000 g feed each with even distribution. The 2 mixtures were dried for the alcohol to be evaporated at room temperature with no direct sunlight by spreading out the mixture to a maximum thickness of 1 cm with light mixing by hand 2-3 times. The 2 mixtures were kept in 2 plastic bottles and used for feeding the fry of the 2 treatments.

Feeding frequency was two to four times daily during the daylight. The daily feed ration schedule of Popma and Green (1985). The daily ration was divided into approximately equal weights. Feeding

continued for 30 days after which it stop feed with hormone diet and fry of each hapa was feed normal diet without hormone in a hapa suspended in a concrete pond after taking the measurements of weight and length.

The water temperature ranged from 22 to 28 ^{o}C, dissolved oxygen varied from 5 - 6.5 ppm while pH ranged from 7 to 8.5.

Identification of the phenotypic sex:

After sex reversal, identification of the phenotypic sex of 100 fish from each hapa was determined by microscopic examination of the gonads when the fish reached 2 - 3 cm length. The thin gonad (thread-like structure lies along the dorsal side of the abdominal cavity) was extracted very carefully, placed on a glass slide and stained with a drop of aceto-carmine stain then it was lightly squashed with a glass cover slip and examined at 10 magnifications. The fish was a presumptive female if densely packed oocytes were found as reported in Guerrero and Shelton (1974). The remaining fry of the treatment that had the highest percentage of mixed females (converted and normal) from the two treatment were kept to the next season (Year 2010) in earthen pond (1000 m^2) reared to maturation. Throughout the rearing period, the water temperature ranged from 22 to 28 ^{o}C, dissolved oxygen varied from 5 - 6.5 ppm while pH ranged from 7 to 8.5.

The experiments were designed in 2010 to complement the results from the 2009 study. Through the second season (Year 2010) After maturation the males were gotten red of 100 females (100g) were distributed over 100 hapas (1*1*1 m) held in earthen pond (1000 m^2) one female crossed with one normal male (ZZ) per hapa. Fish were fed 2% of the total biomass daily with pilleted feed (25 % crude protein) for 30 days. The rest of females were stocked in an isolated hapa to substitute the mortality. The resulting siblings were isolated and reared separately in marked hapas. Fry were fed at 15% of body weight per day in three portions on diet 40% crude protein. The amount of diet was adjusted

weekly on the basis of the weight of a random sample of fish for 60 days and when reached 2-3 cm length, a representative sample of 100 fry was taken from each hapa and the gonads were examined microscopically using the squash method to estimate sex ratio. The actual converted females C-zz and the normal ones C-wz could be determined with normal male C-zz as the first should hypothetically generate progeny including 75% super males “zz” and the second (normal females wz) hypothetically generate progeny including 50% males “zz” and 50% females "wz". The remainder of the identified converted females and its fry in selected treatments were maintained separately alive in concrete pond for future progeny testing research.

RESULTS AND DISCUSSION

Sex reversal of tilapia must begin before the gonadal tissue of young genotypic males or females has differentiated into testes or ovaries. For producing sex reversed females; functional sex reversal is most easily achieved through oral application of estrogens incorporated into the feed and administered during the period of sex differentiation which is known to be 30 days according to Alvendia-Casauay and Carino (1988). Growth of genotypic males (XY) that were sex reversed to phenotypic females was slow as normal females. However, upon termination of the experiment, average length and average weight of fry of both species (*Oreochromis niloticus* and *aureus*) that were fed on high doses (80 and 100 mg/kg feed) of ethynylestradiol supplemented feed was generally higher than those fed on low doses (20, 40 & 60 mg/kg feed) for the same period, indicating the effectiveness of the hormone for enhancement of fish growth (Mohamed *et al*., 2004). From the present data, the initial weight and length of *Oreochromis aureus* post-hatching fry after the yolk sac absorption period weight were 10 mg/fry and length were 8 mm/fry, respectively. Average length and average weight per fry in the two treatments were demonstrated in table (1). It was noticed that, as the feeding dose increase the average weight and length increased in

dose 17-β ethynylestradiol hormone 1000 mg/kg (103±0.006 mg & 22.3±0.306 mm) than the dose 500 mg/kg (101±0.003mg & 22±0.153 mm respectively). In contrast, the survival rate was increased in treatment 500 mg/kg (67.67±1.528%) than treatment 1000 mg/kg (65.33±4.933 %). This result is in agreement with that of Farag *et al.* (2006) reported that growth of fry *Oreochromis niloticus* that were fed hormone treated-feed was affected by the dosages used. Upon termination of the experiment, average weight of fry that were fed on higher doses of 17-β ethynylestradiol supplemented feed was significantly higher than those fed on lesser amounts for the same period. Also, Mohamed *et al*. (2004) who assured the effectiveness of the hormone 17-β ethynylestradiol for enhancement of growth of fry reared in glass aquaria. On the other hand, Popma and Green (1985) reported that after sex reversal with androgens was demonstrated to be feasible, scientists began considering the use of estrogens to produce phenotypic female tilapia from genotypic males. The fattening of an all female population has little appeal to practical aquaculturists because females grow more slowly and several could spawn with single accidentally introduced male.

Table 1: Growth performances of first generation of *Oreochromis aureus* fry fed 17-β ethynylestradiol hormone–treated feed with two doses for 30 days.

	Initial weight (mg)	**Initial length (mm)**	**Final weight (mg)**	**Final length (mm)**	**Survival rate (%)**	**Condition factor**	**Specific growth rate**
500 mg/kg	10.00	8.00	101.00	22.00	67.67	1.01	0.18
± SD	0	0	0.003[a]	0.153[a]	1.528[a]	0.198[a]	0.002[a]
1000mg/kg	10.00	8.00	103.00	22.30	65.33	0.985	0.18
± SD	0	0	0.006[a]	0.306[a]	4.933[a]	0.390[a]	0.005[a]
F value			0.57	0.13	0.01	0.09	1.00
Probability			0.53	0.75	0.93	0.80	0.42
Significant			Ns	Ns	Ns	Ns	Ns

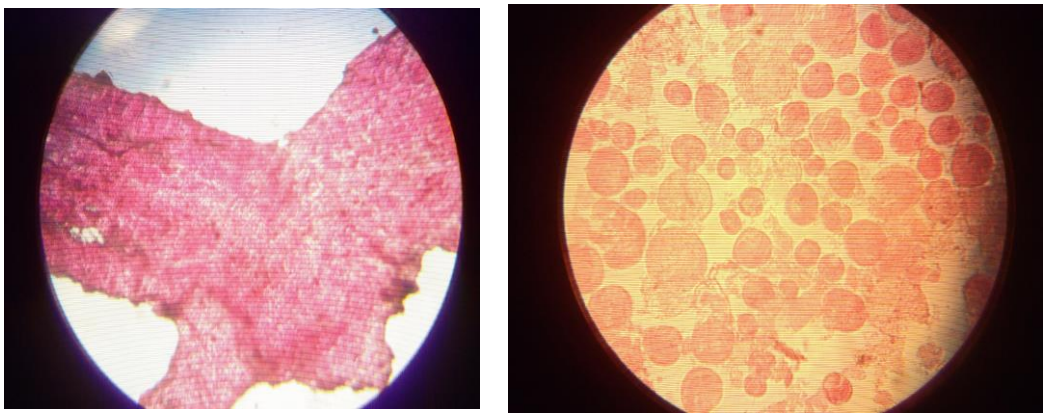
Ns = Non significant.

The possibility of mating a sex- reversed heterogametic female XY with a normal male of the same species to produce 75 % male offspring. The same authors excluded some fish species and reported that this would not be possible with *Oreochromis niloticus or O. mossambicus* but would theoretically be feasible with *Oreochromis aureus*. Researches have assured that *Oreochromis niloticus* could successfully be genetically manipulated with estrogen and the feasibility of producing "supermales YY" (Mair *et al*., 1997). From the present data, 17-β ethynylestradiol proved to be an effective feminizing stimulating agent for *Oreochromis aureus.* Its effect was highly detectable among fry that were orally fed on 1000-mg/kg hormone-treated feeds for 30 days mean percentage of phenotypic females (MPF) was 94.33 ± 2.08%. It was also obvious among fry that consumed the 500-mg/kg hormone-treated feeds for 30 days MPF was 83.67 ± 4.04%. MPF was decreased with decreasing the quantity of 17-β ethynylestradiol supplemented to the feed as indicated in table 2 and fig. 1&2. Also, the optimum treatment duration for optimizing feminization was agreement with Mair *et al*. (1997) who used diethylstilbestrol (1000 mg/kg) as a feminizing stimulating agent for 20 days as oral feeding *Oreochromis niloticus*. However, Popma and Green (1985) reported that fry can be effectively sex reversed in 20 days, but occasionally only 95% of the fry develop as phenotypic males using 17α-methyltestesterone for masculinization of *O. niloticus* fry. They also stated that sex reversal success is more consistent when the treatment duration is 25 to 28 days. It was also different from that reported by Srisakultiew (1993) for 14 days. Also, Mohamed *et al*. (2004) regarding *O. aureua*, mean percentage of phenotypic females were 64.37, 76.39, 60.75, 70.03 and 86.31%, respectively, when the fry were fed 20, 40, 60, 80 and 100 mg/kg ethynylestradiol hormone-treated feed, respectively for 30 days. While with 35 and 40 days feeding, mean percentage of phenotypic females were 46.03, 43.79, 55.33, 64.24 and 71.06 and 38.03, 39.54, 60.25, 84.22 and 71.07 % in the five treatments, respectively in glass aquarium.

Table 2: Phenotypic females (PF) of first generation among *Oreochromis aureus* fry fed 17-β ethynylestradiol hormone-treated feed for 30 day.

		Females %	Males %	Inter sex %	Non differential %
500 mg/kg	**Mean**	83.67	11.20	3.00	2.33
	SD	4.04 [a]	4.39[b]	1.00 [a]	0.58 [a]
1000mg/kg	**Mean**	94.33	3.33	1.67	0.67
	SD	2.08 [a]	1.53 [a]	0.58 [a]	0.58 [a]
F value		64.00	17.07	2.29	6.25
Probability		0.02	0.05	0.27	0.13
Significant		*	Ns	Ns	Ns

Ns = Non significant.



Tests Ovary

Fig. (1): Acetocarmine squash preparation from testis and ovary of fry *Oreochromis aureua* treated with 17-β ethynylestradiol.

As demonstrated in table 2 and figs. 1&2, after 30 days fry was feeding with hormone 17-β ethynylestradiol treated-feed 500 mg/kg showed that male, inter sex and non differential percentages were 11.2±4.39, 3±1 & 2.33±0.58 % respectively, while, this percentage decreased in fry treated-feed 1000 mg/kg showed that male, inter sex and non differential percentages were 3.33±1.53, 1.67±0.58 & 0.67±0.58 %

respectively. The present observation revealed that a few genetic males might consume so little hormone during sex reversal treatment that they develop into normal functional males. Another small fraction of genetic males could be sterile individuals with Ovo-Testis as a result of insufficient amounts of hormone. The majority of the sex-reversed fry that were fed sufficient feed with high estrogen dose develop into reproductively functional females. In normal mating of tilapia species, about half of the phenotypic females are originally genotypic females (WZ) from the moment of fertilization and a half of the offspring produced are normal males (ZZ) with a normal sex ratio of 1: 1. The same observation was reported by Mohamed *et al*., 2004 using the same hormone 17-β ethynylestradiol however with lower doses to produce nearly the low feminization percentage for *Oreochromis niloticus* and *aureus***.** While, they reported that the effect of feeding duration with the hormone-treated feed on sex reversal was obvious in case of *O. niloticus*, the shorter the feeding time the lower the mean percentage of phenotypic females produced. Sex reversal success was more consistent when the treatment duration was 35 to 40 days with the higher doses of estrogen. However, in case of *O. aureus*, there was no regular trend for the effect of treatment duration with different doses.

Table 3: Growth performances and phenotypic males % (PM) of second generation of fry *Oreochromis aureus.*

Growth performances	**Initial weight (mg)**	**Initial length (mm)**	**Final weig ht (mg)**	**Final length (mm)**	**Conditio n factor**	**Specific growth rate**
Mean	12	79	3.27	6.03	1.53	0.11
± SD	0.01	0.14	0.29	0.51	0.33	0.01
phenotypic males	**Females %**	**Males %**	**Inter sex %**	**Non differention %**	**Survival rate (%)**	
Mean	25	70		2	80	
± SD	4.3	5.2	1.9	0.8	0.8	

Condition factor (K) = (Weight/ (Length3))*100 Specific growth rate (SGR) = $(\ln W_2 - \ln W_1)\, T^{-1}$

Fig. (2): Phenotopic females (%) of first generation among *Orochromis aureus* fry fed 17-β ethynylestradiol hormone

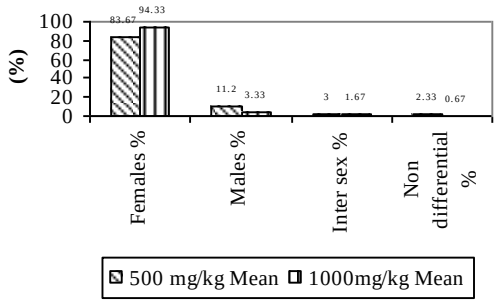
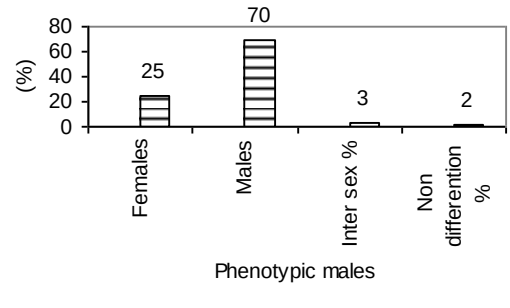


Fig. (3): Phenotopic males (%) of second generation among fry *Orochromis aureus* from progny.



Considering the possible role of methyltestesterone as inhibitory on gonadal differentiation, the development of pituitary during treatment would be important. Research on the pituitary gland of fish has been concerned mainly with the adult fish and its relation to adult's reproductive function; methyltestesterone is known to interfere with oogenesis and spermatogenesis (Dadzie and Hyder, 1976). Damien *et al.* (2008) studied the reproductive performance of two *O. aureus* pseudofemale groups in three successive breeding seasons to improve the spawning capacity in a line by studying the heritability of this reproductive trait in the successive generations of breeders. They reported that the reproductive potential in a group of *O. aureus* breeders depends on a few pseudofemales, those that have a high spawning capacity and a wide capacity of eggs production. From the present data in the 2010 experiments, when the sex reversed fry through 2009 experiments were reared to sexual maturity stage, the pseudofemales (C-zz) could be differentiated form the normal ones (wz) using pair-mating of normal genotypic males to the mixed females with normal males separately in 100 isolated hapas. Progeny testing of their offspring proved that the majority of females were originally genotypic females from the moment of fertilization and their offspring had a normal sex ratio of 1:1. Forty remaining females generated progeny including mean percentage of phenotypic males (MPM) was 70± 5.2% while female,

inter sex and non differential percentages were 25± 4.3, 3± 1.9 & 2± 0.8 % respectively as reported in table 3 and fig. 3 exceeding the normal ratio because, hypothetically, these brooders were converted maternal C_zz genotype and acted reproductively as phonotypical functional females. Pseudofemales (C-zz) produce low number of male (70±5.2%) in first generation for estrogen-treated females. Also it is hypothetically supposed that one third of these males are super males of the "C-zz" genotype and two thirds should be normal males "N-zz". Further research by crossing these males after sexual maturation to normal females and testing their progeny will prove or disprove this hypothesis. The same observation was reported by Damien *et al*. (2003) investigated a pseudofemale line in two populations of *O. aureus*, known as Egyptian Population (EP) and Israel Population (IP). In *O. aureus*, males are the homogametic sex (ZZ/ZW), and sex reversal of fry with estradiol results in the production of some functional sex-reversed fish with a female phenotype and ZZ male genotype, known as pseudofemales. Crosses between ZZ pseudofemales and ZZ males theoretically should provide monosex ZZ male progeny only. We have studied the sex ratios of progeny from 43 IP (F_2 to F_3 generations) and 51 EP (F_1 to F_5 generations), pair-matings between normal males and pseudofemales. In IP, the male percentage in progenies ranged between 83% to 100% in F_2 and 66% to 100% in F_3. In EP, male percentage was more constant, varying from 88% to 100% in F_1, from 96% to 100% in F_3 and from 97% to 100% in F_5. In EP, F_2 and F_4 pseudofemales produced only monosex male progeny. This apparent difference in sex ratio frequency distributions between the two *O. aureus* pseudofemale lines could be due to the selection of males. EP pseudofemales were mated with their siblings for F_2 and F_3 pseudofemales or with closely related males for F_4 and F_5 pseudofemales. The present study also shows that it is possible to fix the male sex determining factors (Z sex chromosome and genetic factors) in a line of pseudofemales, producing a high percentage of male

progeny in five successive generations. Also, Hopkins (1979) and Hopkins *et al*. (1979) reported that functional sex reversal of tilapia species by estrogen treatment of genetic males should produce all male offspring when the sex-reversed individuals are bred with normal males. This approach has been attempted with *Oreochromis aureus*; progress was evident in the successful sex reversal with estrogens but results have been inconsistent. While, Hackmann and Reinboth (1974) observed a feminizing effect from high levels of methyltestesterone and explained it by assuming that some of the exogenous hormone is metabolized to estrogen. This paradoxical effect probably occurs more readily at higher methyltestesterone concentrations. Also, Gerrero (1975) reported that increased effectiveness of ethynyltestosterone with increasing concentrations in *Oreochromis aureus*. The sex ratio in the control groups did not differ significantly from the expected 1:1 ($P>0.05$).

Conclusion: Results of gonadal examination of first generation for estrogen-treated *Oreochromis aureus* females suggested low numbers of males; this reduce the chance of identifying the altered individuals by progeny testing.

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تأنيث وأختبار الأجنة لذكور منقلبة جنسيا لأنتاج ذكور فائقة النمو (ZZ) فى سمكة البلطي الأزرق تجريبا

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المعمل المركزى لبحوث الثروة السمكية– مركز البحوث الزراعية – وزارة الزراعة – مصر

الملخـص العربـى

يعتبر هذا البحث خطوة أولي في برنامج يتم تنفيذه في مصر لإنتاج ذكور بلطي سوبر (ZZ) من البلطي الازرق وذلك كحل لمشكلة التكاثر الغير مرغوب فيه لسمكة البلطي في الأحواض وزيادة الإنتاج السمكي وذلك لانتاج ذكور البلطي في معدلات النمو عن الإناث. لذا أجريت التجربة لتأنيث أجنة بلطي أوريا غير مميزة جنسيا باستخدام هرمون ١٧–بيتاأثينايل أستراديول عن طريق التغذية بعلف يحتوي علي نسب مختلفة من الهرمون ٥٠٠و١٠٠٠ مج/كجم علف وقد أستخدمت لذلك هابات في حوض خراسانى ولمدة ٣٠ يوم.

أكدت النتائج أن زريعة البلطي الازرق التي تغذت علي علف يحتوي علي جرعة أكبر من الهرمون كانت متوسطات أوزانها وأطوالها (١٠٣±٠.٠٠٦ مج و ٢٢.٣±٠.٣٠٦ مم على التوالى) أعلي من تلك التي تغذت علي جرعه أقل (١٠١±٠.٠٠٣ مج و ٢٢±٠.١٥٣ مم على التوالى) بعد فترة التغذية مما يؤكد فعالية هرمون ١٧–بيتاأثينايل أستراديول كعامل محفز لمعدلات النمو. أما بالنسبة للإنقلاب الجنسي إلي إناث فقد تم عمل أختبار أجنة للزريعة بعد انتهاء التغذية وكان تأثير الهرمون ملحوظا بدرجة كبيرة في الزريعة التي تغذت علي جرعة أكبر من الهرمون، حيث وصل متوسط نسبة الإناث إلي ٩٤.٣٣± ٢.٠٨ %. ولقد أنخفضت هذه النسبة بانخفاض الجرعة في العليقة الى ٨٣.٦٧±٤.٠٤%.

أما بالنسبة لعدد الذكور وبين الجنسين والتى لاتميز إلي إناث أو ذكور فقد زادت فى الزريعة التي تغذت علي جرعة أقل من الهرمون وكانت١١.٢±٤.٣٩و ٣±١ و ٢.٣٣±٠.٥٨ % على التوالى ولقد أنخفضت هذه النسبة بزيادة الجرعة في العليقة الى ٣.٣٣±١.٥٣ و ١.٦٧±٠.٥٨ و٠.٦٧±٠.٥٨ % على التوالى.

بعد أن وصلت الزريعة إلي النضج الجنسي في الموسم التالي تم التخلص من الذكور ثم أجرى أختبار التزاوج بوضع الإناث المختلطة سواء المنقلبة أو الطبيعية في هابات كل أنثي في هابة مستقلة مع ذكرطبيعى للتزاوج. تم عمل أختبار أجنة مرة أخري للزريعة الجيل الثانى الناتجة علي حدة لتمييز الإناث التي هي في الأصل ذكور $C\text{-}_{ZZ}$ وتحولت ألي إناث من الأخري التي هي في الأصل إناث طبيعية WZ . وأظهر اختبار الأجنة أن ربع الأمهات من أسماك البلطي الاوريا من إجمالي عدد ١٠٠ سمكة هي في الأصل ذكور وتحولت إلي إناث $C\text{–}_{ZZ}$ حيث أن معدل التجنيس في إنتاجها من الزريعة كان ١ إناث : ٣ ذكور. أذ تراوحت نسبة الذكور في الزريعة من ٧٠ إلي ٧٥% تقريبا. تم الاحتفاظ بهذه الأمهات المنقلبة وكذلك إنتاجها من الزريعة لرعايتها حتي تصل إلي النضج الجنسي لاستكمال برنامج إنتاج الذكور السوبر ZZ منها.