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Feminization of Genotypically YY Nile Tilapia *Oreochromis niloticus* L.

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Abstract

The influence of duration (10-20 d) and concentration (250-1,000 mg•kg^{-1} diet) of diethylstilboestrol (DES) treatment on the production of phenotypically female populations of genotypically YY *Oreochromis niloticus* was evaluated. Sex ratio of all DES-treated populations deviated significantly ($P<0.05$) from the expected all male. Concentration of hormone was a significant factor in the production of phenotypic females, and the effective period for feminization treatment was 10-15 d following yolk-sac absorption. The critical factors influencing effective sex reversal treatment are discussed.

Introduction

The use of monosex populations in tilapia culture eliminates the problem of overcrowding resulting from the high reproductive capacity of the fish in mixed-sex populations. In monosex populations, males are preferred as they grow faster than females (Holden and Reed 1972). A more recent technique of producing all-male fingerlings in Nile tilapia (*Oreochromis niloticus* L.) is by breeding novel homogametic YY males with normal XX females. The development and largescale production of YY male broodfish involves stages of feminization of genotypically XY and YY fish during their sexually undifferentiated stage (Mair et al. 1993). Feminization of genotypically XY *O. niloticus* was optimized by a series of studies conducted by Mair and Santiago (1994) and Vera Cruz and Mair (in press). Exposure of sexually undifferentiated fry to diethylstilboestrol (DES) at 1 $g \cdot kg^{-1}$ of feed, given at 15-20% of the fish biomass per day, for 10 d in tank and 15 d in hapa-in-pond, resulted in populations composed of greater than 90% females. Preliminary trials on feminization of YY *O. niloticus* using the procedure developed for XY fish indicated that YY fish were more difficult to feminize than ordinary males. There was evidence for differential feminization of XY and YY genotypes in progeny from one XY x YY crosses (Abucay and Mair, in press) indicating that the success of steroid treatment was affected by the male genoytpe. Thus it was considered that previ-

ously developed protocols for feminization by oral administration of DES are required to be re-optimized for feminization of YY genotypes.

The experiment presented here examined the effect of duration of hormonal treatment (10, 15 and 20 d) and concentration of DES (250, 500 and 1,000 mg•kg^{-1} of feed) on the feminization of sexually undifferentiated YY *O. niloticus* fry. Hormone-treated feeds were given at higher feeding rates (30-40% of the biomass per day) than the previously optimized protocol. Feeding rate rather than DES concentration was increased because hierarchies in feeding among fry was observed in previous experiments as evidenced by wide variation in size (few large and numerous small) of harvested fish following treatment.

Materials and Methods

This study was conducted using pond facilities at the Freshwater Aquaculture Center (FAC), Central Luzon State University (CLSU), Philippines. The YY (from YY♀ x YY♂crosses) fry used were of the Egypt-Swansea strain of *O. niloticus* produced by the Genetic Manipulations for Improved Tilapia Project (Mair et al., in press). A 3x3 factorial design was employed for hormone treatment using 30 units of 0.25 m^2 hapas (50 x 50 cm, 1 m depth) immersed to a depth of 0.5 m in a 200 m^2 fertilized pond. The different doses of DES in the diet were 250, 500 and 1,000 mg•kg^{-1}, and each treatment was given for durations of 10, 15 or 20 d. A control treatment receiving non-DES treated feed was included to assess the sex ratio of untreated YY fish used in the study. All treatments were replicated three times.

Recently hatched, artificially incubated, sexually undifferentiated fry (<10 mm length) of the same age, were pooled and randomly divided into treatment groups. Each sex reversal unit was provided with a feeding ring and stocked with 250 fry. DES-treated feed was prepared using a method adapted from that of Guerrero (1975). DES was dissolved in 95% ethanol and the solution was mixed with powdered feed (30% fish meal and 70% rice bran) at 0.5 l solution per kilogram of feed. Non-DES treated control feed was prepared in exactly the same manner, with the exclusion of hormone.

Fry were fed four times a day at 40% of the biomass per day for the first week, and 35 and 30% during the second and third weeks, respectively. Ten percent fry samples were collected and weighed weekly for the adjustment of the diet ration. Water temperature, pH and dissolved oxygen were monitored twice a week at 600-700 and 1400-1500 h. Upon termination of the respective DES treatment duration (10, 15 and 20 d), all fry from each treatment-replicate were counted and bulk weighed for the calculation of survival rate and average weight. Fry were then nursed in 1-m^2 hapas suspended in an earthen pond up to a mean weight of 5 g, when the sex ratio was determined using the acetocarmine squash technique described by Guerrero and Shelton (1974).

Data were analyzed using analysis of variance for 3x3 factorial design with three durations of treatment and three concentrations of hormone. Comparisons among treatment means were made using Duncan's Multiple Range Test. Percentage data were arc sine transformed prior to analysis. A contingency test

was used to determine whether the observed sex ratio of the treated populations differed from the non-treated control.

Results

Table 1 shows the sex ratio (% female) of treated and control fish. All DES-treated groups had significantly ($P<0.05$) higher proportions of females than the untreated controls which were monosex males. Concentration of hormone had a significant effect ($P<0.05$) on production of phenotypic females at all treatment durations. Generally, higher concentrations of DES produced significantly higher proportions of phenotypic females. Little increase in the proportion of females was noted at the longer duration of treatment. The most effective DES concentration was the 1,000 mg•kg^{-1} diet which produced 64.1% females after 20 d of treatment.

Neither duration of treatment nor concentration of hormone significantly affected survival of fish (Table 1). Highest mean survival was observed in the control treatment (88.9%) and the lowest at 500 mg DES kg^{-1} diet given for 15 d (57.7%).

Fish attained mean length >16 mm and >20 mm after 10 and 15 d treatment, respectively (Table 2). Mean weight was ≥ 122 mg after 10 d and >145 mg after 15 d. Concentration of DES at specific durations had no significant effect on growth of fish.

Discussion

Sex reversal occurs when fish ingest the required critical minimum amount of hormone during their labile period of sexual differentiation. The critical minimum amount of hormone required by fish for sex reversal varies with the strain and species of fish (Rosenstein and Hulata 1994; Pandian and Sheela 1995). In this study, utilization of high concentration of DES (1,000 mg•kg^{-1}) did not achieve high rates (>90%) of feminization even though higher rates of feeding (30-40% of the BW•d^{-1}) were used as compared to the rates of feeding (15-20%) in the feminization protocol of XY *O. niloticus* (Vera Cruz and Mair, in press). This study supports the earlier findings that YY fry were harder to feminize than normal XY fry (Abucay and Mair, in press). The relatively low rate of sex reversal (≤64%) may be attributed to the male genotype (YY). Possibly this genotype may result in secretion of higher levels of testosterone during the period of sexual differentiation. The apparent increased maleness of the YY genotype is further evidenced by the absence of spontaneously occuring females in untreated crosses of YY♂ x YY♀ compared to the average 3-4% females found in YY♂ x YY♀ crosses. The precise physiological basis of sex differentiation in tilapia is not well understood but it seems likely that this differs between YY and normal XY males. Thus the critical minimum amount of DES required to override the action of these sex factors, for effective sex reversal of YY fish, will be greater than that required for XY fish. In this study, it is presumed that fish that were not sex reversed consumed less hormone than the required critical minimum amount, during their period of gonadal differentiation.

Table 1. Mean* percentage (±SD) phenotypic female *O. niloticus* and survival after the respective DES treatment in hapas at different hormone concentrations and durations.

Hormone dosage ($mg \cdot kg^{-1}$)	Duration of treatment (d) 10		15		20	
	% female	% survival	% female	% survival	% female	% survival
0	$0.0^{a,4**}$		$0.0^{a,4**}$		$0.0^{a,4}$	$88.9 \pm 7.3^{a,1}$
250	$7.5 \pm 5.6^{b,3}$	$80.0 \pm 1.6^{a,1}$	$36.2 \pm 17.9^{a,2}$	$74.1 \pm 27.8^{a,1}$	$15.7 \pm 6.6^{ab,3}$	$70.0 \pm 23.7^{a,1}$
500	$21.7 \pm 5.9^{a,2}$	$67.2 \pm 8.1^{a,1}$	$31.3 \pm 16.2^{a,3}$	$57.7 \pm 20.7^{a,1}$	$45.9 \pm 11.9^{a,2}$	$83.7 \pm 12.6^{a,1}$
1,000	$47.1 \pm 7.6^{a,1}$	$84.1 \pm 9.9^{a,1}$	$50.8 \pm 14.9^{a,1}$	$73.1 \pm 24.1^{a,1}$	$64.1 \pm 3.7^{a,1}$	$87.7 \pm 5.9^{a,1}$

* Mean of three replicates

** There was only one control treatment grown for 20 d, but since the sex ratio for this treatment was 100% male, it was intrapolated that the populations were composed of all-male fish at 10 and 15 d duration

Values within the same row, superscripted with different letters, are significantly different ($P<0.05$)

Values within the same column, superscripted with different numbers, are significantly different ($P<0.05$)

Table 2. Mean final length and weight (±SD) of the fish used in the study.

Hormone dosage ($mg \cdot kg^{-1}$)	Duration of treatment (d) 10		15		20	
	Mean length (mm)	Mean weight (mg)	Mean length (mm)	Mean weight (mg)	Mean length (mm)	Mean weight (mg)
0					21.0 (± 0.0)	148.7 (± 16.9)
250	16.8 (± 0.9)	122.0 (± 4.0)	21.1 (± 3.1)	163.3 (± 56.2)	22.5 (± 2.3)	176.7 (± 91.2)
500	16.8 (± 0.9)	138.0 (± 20.8)	21.9 (± 0.6)	165.7 (± 66.1)	22.4 (± 1.9)	163.0 (± 46.0)
1,000	17.0 (± 1.4)	124.7 (± 5.9)	20.8 (± 1.3)	149.0 (± 39.0)	21.9 (± 0.8)	145.7 (± 18.5)

The insignificant effect of duration of treatment on rate of feminization seems to indicate that sexual differentiation of most fish occurred early. According to Hiott and Phelps (1993), the species, age and size of fish, and temperature affected the period at which gonadal tissues differentiate. Popma and Green (1990) recommend 14 mm as the minimum harvest size for androgen sex reversal of *O. niloticus*, while Dunham (1990) recommends a smaller size of 12 mm for the same species. Dutta (1979) observed that in *O. aureus*, a higher temperature (31°C) resulted in earlier ovarian differentiation of gonadal tissue compared to a lower temperature (21°C; 14-15 and 24-27 d after hatching, respectively). In *O. niloticus*, gonadal differentiation took place 30-32 d post-hatch at 25-26°C where fry had body lengths of 9-12 mm (Alvendia Casauay and Carino 1988). In this study, the first feeding fry used had an initial length of less than 10 mm. The sufficient amount of food (both hormonal feed and natural food) available and the relatively high water temperature (25.5°C±0.9 at 600-700 h and 29.7°C±1.1 at 1400-1500 h) favored the fast growth of fish, attaining a length equal to or greater than 16 mm (Table 2), after just 10 d treatment. This length is greater than the minimum harvest size recommended by Popma and Green (1990) and Dunham (1990). It is possible that most sex-reversed fish had undergone sexual differentiation within the first 10 d treatment because further increase in treatment duration did not significantly increase the proportion of females. Similar results were obtained by Rosenstein and Hulata (1994), when DES was given *ad libitum* to *O. mossambicus*. These authors observed that the effective period of feminization was 10-15 d treatment starting immediately after yolk-sac absorption. However, in the feminization of XX and XY *O. niloticus* in hapa using a lower feeding rate (15-20% BW•d^{-1}), 10 d treatment had a significantly lower percentage of females than 15 and 20 d (Vera Cruz and Mair, in press).

The results of this study indicate that the age of fish at the start of hormone treatment, the concentration of hormone, rate of feeding and the period of gonadal differentiation are critical factors in sex reversal. The proportional increase in rate of feminization with increasing concentration of DES indicates that even higher levels of DES may further increase feminization rates.

The insignificant effect of hormone concentration on survival of treated fish indicates that the hormone dosages used were not toxic to the fish as has been indicated in our previous studies (Mair and Santiago 1994; Vera Cruz and Mair, in press), while the insignificant effect of duration of treatment may be attributed to the presence of sufficient food. In the study conducted by Vera Cruz and Mair (in press) using 1,000 mg DES kg^{-1} diet for 10-20 d, with XX and XY *O. niloticus* stocked in hapa at 1,000 fish m^{-2}, shorter duration treatment produced higher (but not significantly so) survival rates.

Conclusions

YY *O. niloticus* fry were harder to feminize than normal XY fry, indicating reduced lability of sex differentiation. Feminization rate was dosage-dependent rather than duration-dependent. Although feminization rate was relatively low

(64.1%±3.7) at a DES concentration of 1,000 mg•kg^{-1} diet for 20 d, the production of YY females is an important contribution to the YY-male technology. YY females, when crossed with YY males, will enable mass production of YY males. These males, when used as broodstock, crossed with normal females (XX) of the same strain, will yield all-male (XY) progeny for use in monosex culture of the fish. To obtain a higher feminization rate of YY *O. niloticus*, concentrations of DES higher than 1,000 mg•kg^{-1} diet should be tried in succeeding studies. Comparative studies of the physiology of sex differentiation including bioassays of critical hormones would be valuable in determining the physiological basis of the apparent increased "maleness" of the YY genotype.

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