Sex Reversal of Florida Red Tilapia in Brackishwater Tanks Under Different Treatment Durations on 17 Alpha-Ethynyltestosterone Administered in Feed

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ABSTRACT

Florida red tilapia (5-22 days post-hatching) were reared in 530-l tanks (stocking density = 5.8 fish/L) supplied with recirculated brackish water (12 ppt) and fed a diet containing 17 alpha-ethynyltestosterone (ET) (60 mg/kg diet) for different durations: 0, 7-14, 14-21, 21-28, and 28-35 days. Fry were fed daily (0830-1630 h) to satiation (17-22% of biomass) by continuous automatic feeding. Each treatment was replicated three or four times.

All durations of androgen treatment produced nearly all=male populations (range = 94.3-98.1%), with no significant (P>0.05) differences observed among treatments. In contrast, non-treated fish were 61.7% males. No significant (P>0.05) effects of treatment duration on growth rate (avg=15.3% bw/d) or survival (avg=37.3%) was observed after 28 days.

Fry averaged 1.40 g after 28-35 days of androgen treatment, requiring 0.206 mg of ET per individual. In contrast, fry averaged 0.040 g after seven to fourteen days of androgen treatment, requiring only 0.003 mg of ET, or 1.5% of the 28-day total.

The results demonstrate the feasibility of reducing the duration of androgen treatment for sex reversal in Florida red tilapia to seven to fourteen days. This minimizes hormone requirements and size of sex-reversed fish, accelerates hatchery throughput, improves fry avaiability and transportability, and reduces the cost of sex-reversed seedstock to farmers.

KEY WORDS: Brackishwater, sex-reversal, Tilapia.

INTRODUCTION

Oral administration of androgenic hormones (e.g. methyltestosterone or ethynyltestosterone) is presently the method most widely used by commercial tilapia culturists to induce sex-reversal in juvenile females (Liao and Chen, 1983; Rothbard *et al.*, 1983; Guerrero and Guerrero, 1988; Pandian and Varadaraj, 1990; Hanley, 1991). For tilapias, proper timing and duration of androgen treatment have been contradictory (Shelton *et al.*, 1978; Shelton, 1989; Pandian and Varadaraj, 1990).

The objective of this study was to determine the minimum effective duration of androgen (ethynyltestosterone) treatment for sex reversal of Florida red tilapia in brackishwater tanks under commercial-scale hatchery conditions.

MATERIALS AND METHODS

The study was conducted at the Caribbean Marine Research Center's tilapia hatchery (Ernst, 1989) located on Lee Stocking Island (Exuma Cays, Bahamas) from July through November 1990.

Florida red tilapia fry (descendants of the hybrid *Oreochromis urolepis hornorum x O. mossambicus*) (Behrends *et al.*, 1982) were produced by year class 1 broodstock maintained in brackish water (12 ppt) tanks (34.2-m^3) at a ratio of 180 females to 60 males. On July 21 (experiment day 0), fry ranging in age from 5-22 days after hatching were stocked in fourteen, 530-l tanks at a density of 3100 fish/tank (5.8 fish/l).

To assess the effect of duration of androgen treatment on sex reversal, fry were fed Zeigler salmon starter (50% protein) treated with 17 alpha-ethynyltestosterone (60 mg/kg diet) (ET-60) for 7, 14, 21 or 28 days after stocking. Fry were fed untreated feed after the prescribed duration of androgen treatment. Because post-yolksac fry were treated with ET-60 upon collection from brood tanks, actual durations of androgen treatment in each group were 7-14, 14-21, 21-28, and 28-35 days respectively. The 7- and 28-day treatments were replicated three times and the 14-21-day treatments, four times.

Fish were fed daily to satiation with automatic belt feeders (Zeigler Bros., Inc., Pennsylvania, USA). The amount of food remaining on tank bottom each morning guided daily adjustment of rations.

Tanks were supplied with aeration recirculated brackish water (mean 12 ppt) produced by mixing ground water (4-6 ppt) with seawater (36 ppt). Ambient temperature and photoperiod prevailed throughout the experiment. Maximum (31.1 +/- 0.4°C) and minimum (30.0 +/- 0.3°C) water temperatures were recorded daily, while dissolved oxygen levels (5.4 +/- 0.7 mg/l) were measured on alternate days. Total NH_4-N (1.0 +/- 0.9 mg/l), NO_2-N (0.44 +/- 0.46 mg/l), NO_3-N (4.4 +/- 1.0 mg/l) pH (8.7 +/- 0.30) and alkalinity (273 +/- 16 mg $CaCO_3$/l) were monitored weekly. Dead fish were collected and tanks vacuumed daily.

After hormone treatment was completed for all groups (day 28), approximately 500 fish from each replicate were transferred to seven 3.9-m^3 outdoor tanks. Beginning 25 October (day 96) the sex of at least 200 individuals from each replicate was determined using gonadal squash method of Guerrero and Shelton (1974). Gonads containing both ovarian and testicular tissue were classified as intersex. The sex of 600 untreated fish was also determined as a control.

Treatment means for fish weights, lengths, growth rates, survival, feed conversion ratio, and percent males were compared by one-way analysis of variance (ANOVA). If the overall ANOVA was significant, differences between treatment means were analyzed by the Student-Newman-Keuls test (SNK).

Mean percentages of intersex fish were compared by the Kruskal-Wallis test. Level of significance in all tests was $P<0.05$.

RESULTS

Mean body weight and length, specific growth rate, survival, and feed conversion ratio did not differ significantly ($P>0.05$) among treatments (Table 1). All durations of treatment with ethynyltestosterone (ET) produced a high mean percentage of males (range=94.3-98.1%) with no significant ($P>0.05$) differences among treatments (Table 2). In contrast, non-treated fish consisted of only 61.7% males which was significantly ($P<0.05$) lower than observed in groups treated with ET (Table 2).

Table 1. Initial (day 0) and final (dat 28) body weights and lengths, specific growth rates (SGR), survival, and feed conversion ratios (FCR) of Florida red tilapia fry (5-22 days post-hatching) reared in 530-L tanks at 12 ppt and fed a diet treated with 17 alpha-ethynyltestosterone for different durations[1].

	Duration of Androgen Treatment (Days)			
	7-14	14-21	21-28	28-35
Initial				
Wt. (g)	0.0017+0.001	0.017+0.001	0.019+0.002	0.018+0.002
TL (mm)	10.1+0.4	10.4+0.2	10.4+0.4	10.3+0.5
Final				
Wt. (g)	1.16+0.02	1.17+0.09	1.31+0.07	1.40+0.08
TL (mm)	36.4+0.1	36.2+1.1	38.1+0.9	39.5+1.2
Survival (%)	38.9+1.1	40.5+3.8	36.1+1.7	33.8+1.3
SGR (% bw/d)[2]	15.1+0.1	15.2+0.3	15.2+0.2	15.5+0.3
FCR (dry/wet)[3]	2.31+0.08	2.20+0.14	2.42+0.17	2.48+0.08

[1] Data are presented as means + s. e. for three replicates (7-14 and 28-35 d) or four replicates (14-21 and 21-28 d). No significant ($P>0.05$, ANOVA) differences were observed among treatments for any parameter.

[2] SGR = specific growth rate = [(ln final wt. (g) - ln initial wt. (g)/time (days))] 100.

[3] FCR = feed conversion ratio = dry wt. fed/wet wt. gain.

Table 2. Sex composition of Florida red tilapia fry (5-22 day post-hatching) fed a diet treated with 17 alpha-ethynyltestosterone (60 mg/kg diet) for different durations.

Duration of Androgen Treatment (d)	% Males[1] (P<0.001)[2]	% Females[1] (P<0.001)	% Intersex[1]	No. of fish sexed
0	61.7±2.9a[3]	38.8±2.6a	0.2±0.2a	600
7-14	95.8±1.3b	3.6±1.2b	0.6±0.3a	712
14-21	97.2±0.4b	1.8±0.5b	1.0±0.3a	1,025
21-28	94.3±1.5b	4.4±1.8b	1.3±0.7a	708
28-35	98.1±0.7b	1.9±0.7b	0.0±0.0a	480

[1] Data are presented as means ± s. e. Means are based on 3-4 replicates (≥ 200 fish/replicate) except for the 28-35 day treatment, which consisted of three replicates. One replicate was lost from each of the 21- and 28-day treatments due to a damaged hapa which allowed mixing of fish between these groups.
[2] Probability level when overall ANOVA was significant.
[3] Means in a column followed by the same letter are not significantly different (P>0.05, SNK).

Fry averaged 1.4 g after 28-35 days of androgen treatment, requiring 0.206 mg of ET per individual. In contrast, fry averaged 0.040 g after 7-14 days of androgen treatment, requiring only 0.003 mg of ET per individual, or 1.5% of the 28-day total.

DISCUSSION

Treatment of sexually undifferentiated fry with oral administration of the androgenic hormones ethynyltestosterone or methyltestosterone for 21-28 days is considered necessary for producing nearly all-male (*i.e.* 95%) populations in most tilapia species, including *O. aureus* (Guerrero, 1975; Shelton *et al.*, 1981; McGeachin *et al.*, 1987), *O. hornorum* (Obi and Shelton, 1983), *O. niloticus* (Tayamen and Shelton, 1978; Owusu-Frimpong and Nijjhar, 1981), and hybrid *O. mossambicus x O. niloticus* (Rothbard *et al.*, 1983) and *O. niloticus x O. aureus* (Buddle, 1984). For *O. mossambicus* androgen treatment for 21-28 days also was effective (Guerrero, 1975), but other studies indicated that longer durations (42-69 days) are necessary (Clemens and Inslee, 1968; Macintosh *et al.*, 1985; Das *et al.*, 1987).

The results of the present study demonstrated that treatment of Florida red tilapia with ethynyltestosterone for only 7-14 days was sufficient to obtain a high rate of sex reversal (e.g. 95.8% males). Extending androgen treatment to

28-35 days did not ensure 100% success, indicating that a small percentage of females must be accepted.

Reducing the effective duration of androgenic treatment for sex reversal in Florida red tilapia to 7-14 days can increase hatchery productivity by accelerating throughput and allowing transport of fry to growout facilities at much smaller sizes. This should lower the cost and increase the availability of sex-reversed seedstock to farmers.

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