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Steroid Immersion for Masculinization of Tilapia: Immersion of Tilapia Fry in MDHT

Eighth Work Plan, Reproduction Control Research 2A (RCR2A)
Final Report

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Abstract

The effects of a single immersion of fry in the androgen 17α-methyldihydrotestosterone (MDHT) on masculinization of Nile tilapia were investigated. Previous experiments had demonstrated that two immersions in 500 µg l^{-1} of this steroid for three hours each on days 10 and 13 after fertilization resulted in greater than 90% male populations. In the study described below, tilapia fry were immersed once in 500 µg l^{-1} of MDHT for two hours on days 10, 11, or 13 after fertilization. Significant masculinization occurred only in the group immersed on day 13 after fertilization, and the proportion of males produced (79.3%) was not significantly different from the proportion of males produced (82.9%) after two immersions on days 10 and 13 after fertilization.

Introduction

The production of single sex populations offers several advantages in tilapia aquaculture, including enhanced growth and prevention of unwanted reproduction. A number of androgens have been shown to masculinize various tilapia species, including 17α-methyltestosterone (MT; summarized by Pandian and Varadaraj (1990) for *Oreochromis mossambicus*); mibolerone (Torrans et al. (1988) with *O. aureus*); fluoxymesterone (Phelps et al. (1992) with *O. niloticus*); norethisterone acetate (Varadaraj (1990) with *O. mossambicus*); 17α-ethynyltestosterone (Shelton et al. (1981) with *O. aureus*); 17α-methylandrostendiol (Varadaraj and Pandian (1987) with *O. mossambicus*); and trenbolone acetate (Galvez et al. (1996) with *O. aureus*).

Aquaculturists usually administer hormones to fish through the diet, but this method is prone to inefficiencies such as uneven exposure to steroid due to the establishment of feeding hierarchies or the availability of supplemental feed from pond primary productivity. Immersion of tilapia fry in steroid solutions may be one way to achieve masculinization and avoid these inefficiencies. This technique is well-developed in salmonid aquaculture (Piferrer and Donaldson, 1989; Feist et al., 1995); however, it remains largely experimental in tilapia culture. Most of the reported studies immersed tilapia fry in androgens for periods of over one week to five weeks (Varadaraj and Pandian, 1987; Torrans et al., 1988). Recently, Gale et al. (1995) demonstrated that immersion for just three hours in 17α-methyldihydrotestosterone (MDHT) on two days resulted in masculinization of Nile tilapia. The study described below was undertaken to determine if these findings could be extended through examination of the effects of a single immersion in MDHT.

Methods and Materials

Breeding families of Nile tilapia, *Oreochromis niloticus*, obtained from Auburn University, were placed in 200-l aquaria (one male to three females). The temperature was maintained at 28 ± 2°C. Time of spawning was monitored every two hours. All spawning occurred between 4 and 7 pm. Once breeding occurred, the other fish were removed and the brooding female left to incubate the progeny. At 280 Celsius Temperature Units (CTU) post-fertilization, fry were removed from the tank and randomly assigned to experimental groups. A reference of 280 CTU was used because Gale et al. (1995) obtained 90-100% masculinization by immersing fry on 10 and 13 days post-fertilization (dpf) while maintaining the brooding females at a mean temperature of 28°C. In these experiments, a possible correlation between developmental stages and sensitivity to masculinization was sought, and CTU were estimated by multiplying the mean water temperature by the number of days, or estimated hourly when needed (e.g., day 10 at 28°C = 280 CTU). The fry used in this experiment came from an individual female. Each replicate was housed in a 3.8-l glass jar with dechlorinated tap water. The water in all treatments was maintained at 28 ± 2°C under constant aeration. Treatments consisted of immersions in either steroid or ethanol (EtOH), which were mixed 60 s before addition of fry. Steroids were obtained from Sigma Chemical Company (St. Louis, Missouri) and stored in stock solutions of ethanol (1 mg ml^{-1}).

Fry were immersed for two hours at 280, 310, or 364 (10, 11 or 13 dpf), or twice at 280 and 364 CTU in 500 µg l^{-1} of MDHT at a density of 33 fish l^{-1} in each replicate. Fish in the EtOH control group were immersed at 280 and 364 CTU. Each experimental group was triplicated. Fry were collected after each immersion,

jars were thoroughly cleaned, and then fish were reallocated in fresh dechlorinated tap water. After seven days, fry were transferred to the Oregon State University Warm Water Research Laboratory, Corvallis, Oregon, and reared in 75-l fiberglass tanks in a recirculating system. Temperature and pH were monitored daily; ammonia, nitrites, dissolved oxygen, alkalinity, and hardness were checked weekly. Water temperature in the grow-out system was maintained at 28 ± 2°C. At 60 to 70 dpf, sex ratios were determined by examination of gonads using squash (10 and 40X) preparations after aceto-iron hematoxylin (Wittman, 1962) staining. The weights of sampled fish were recorded at this time.

Data were pooled from replicate tanks, because there was no evidence of tank effects within treatments (Fisher's test or ANOVA). Sex ratio and mortality data were analyzed using Fisher's exact test with exact p-values (a more conservative test than the chi-square test for small sample sizes) estimated in GraphPad Prism™. The mean final weights of sampled fish were analyzed for differences between groups using one-way ANOVA including mortality as a possible confounding variable. For all analyses, differences were considered statistically significant when the p-value (P) was less than 0.05.

RESULTS

The sex ratios of tilapia treated with a single immersion in MDHT at 364 CTU (13 dpf) or two immersions at 280 and 364 CTU (10 and 13 dpf) were significantly skewed towards males in comparison to those of controls (56.6% males; $P < 0.001$). Single immersion in MDHT at 364 CTU resulted in 79.3% males, which was not significantly different from the two immersions at 280 and 364 CTU (82.9% males). No significant masculinization effects were observed in groups immersed once in MDHT at either 280 CTU (10 dpf) or 310 CTU (11 dpf).

Mortality and final weight data were not significantly different among treatment groups. Water quality in rearing tanks was maintained close to the optimal values for tilapia culture (data not shown).

DISCUSSION

We have demonstrated that short-term immersion in steroid can result in masculinization of Nile tilapia. Similar results were reported by Gale et al. (1995). A single immersion in MDHT at 364 CTU (13 dpf at 28°C) was as effective as two immersions at 280 and 364 CTU. The current experiments did not result in the level of masculinization (> 93%) that Gale et al. (1995) achieved; however, the latter study used two 3-hour immersions whereas two 2-hour immersions were used in this study.

The lack of significant masculinization in tilapia exposed to MDHT for two hours at 280 or 310 CTU suggests that the period of sensitivity to steroid-induced masculinization is several days after the onset of feeding, in contrast to salmonids, which must be immersed as yolk-sac fry (Piferrer and Donaldson, 1989; Feist et al., 1995).

ANTICIPATED BENEFITS

We have successfully developed a technique for masculinizing Nile tilapia with a single immersion in masculinizing steroid. This latest development will increase the ease of use of immersion as an alternative to dietary treatment with androgens for sex inversion.

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