MASCULINIZATION OF NILE TILAPIA FRY BY IMMERSION IN TRENBOLONE ACETATE: REUSE OF HORMONE SOLUTION AND EFFECTS OF TEMPERATURE

Ninth Work Plan, Reproduction Control Research 5D (9RCR5D)
Final Report

Wilfrido M. Contreras-Sánchez, Gabriel Márquez-Couturier, Thelma Ruth González Márquez, and Luis Arturo Dorantes Lopez
Laboratorio de Acuacultura
Universidad Juárez Autónoma de Tabasco
Villahermosa, Tabasco, Mexico

Carl B. Schreck
Oregon Cooperative Fishery Research Unit
Biological Resources Division—US Geological Survey
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon, USA

ABSTRACT

Preliminary studies in our laboratory showed that short immersions in the synthetic androgen trenbolone acetate (TA) constitute a good option for masculinizing Nile tilapia fry produced by a single female. This technique offers the potential to replace MT feeding for 28 days and avoid steroid accumulation in pond sediments. We investigated the effects of TA treatment on fry collected from a tank containing batches produced in multiple spawnings. Our results suggest that masculinization involving short-term immersions in TA results in significantly more males in the treated groups (55.9 and 61.6%) than in the controls (44.5 and 38.9%). However, the percentage of males produced is far below that recommended for aquacultural purposes. We further investigated the potential enhancing effects of elevated temperatures in combination with TA treatment during immersion time and found no significant effects of temperature on the proportion of males obtained.

INTRODUCTION

The production of single-sex populations of tilapia (*Oreochromis* spp.) is an important tool for aquaculturists to avoid unwanted reproduction and to produce the sex with the larger growth potential (Macintosh and Little, 1995; Green et al., 1997). Aquaculturists usually administer hormones to fish through the diet, but this method often requires long-term administration of the steroid and poses the risk of contamination by the uneaten or unmetabolized hormone that eventually reaches the pond environment. In previous studies we have demonstrated that a substantial amount of the methyltestosterone administered with the food quickly appears in the tank water and remains in the sediments for at least four months (Contreras-Sánchez, 2001). Therefore, the development of techniques that can offer significant masculinization of fry involving short-term treatments and presenting little or no risk to the environment or hatchery workers may be advantageous.

Recent studies in our laboratory have shown that short-term immersions can result in significant masculinization of Nile tilapia fry (Gale et al., 1995, 1999; Contreras-Sánchez, 2001). These studies showed that immersion in androgen has the potential to be an alternative to dietary treatment with steroids for the masculinization of tilapia. This technique has the advantage of using the steroid solution under controlled systems allowing for reuse and safe disposal. However, little is known regarding the efficacy of this technique in large-scale systems. A variety of androgens—especially synthetic androgens—are effective masculinizing agents (Hunter and Donaldson, 1983). Recently, the synthetic steroid trenbolone acetate (Galvez et al., 1996) has been used to masculinize tilapia using hormone-treated food. Trenbolone acetate (TA) has been widely used in the cattle industry for growth enhancement and is considered a potent androgenic and masculinizing agent (Galvez et al., 1996). We have previously shown that short-term immersion of tilapia fry in TA can result in significant masculinization (Contreras-Sánchez et al., 1997; Contreras-Sánchez, 2001). Such a treatment offers an alternative to the typical four weeks of feeding tilapia with MT. However, in order to be a viable alternative, short immersions in TA must be tested on fish.

Because recent studies have shown that elevated temperatures can induce masculinization to a certain degree, we decided to examine the potential enhancing effects that elevated temperatures may have when used in combination with short-term immersions in steroids. To investigate these potential effects, we carried out an experiment to compare masculinization rates between TA-immersed fry under normal temperatures (28°C), TA-immersed fry under elevated temperatures (32 and 36°C), and EtOH-immersed fry.

METHODS AND MATERIALS

Multiple breeding families of Nile tilapia, *Oreochromis niloticus*, were placed in reproduction tanks ($2 \times 4 \times 1$ m) at a ratio of one male to three females per square meter. Fry release was monitored every day, starting on day 10. Once breeding occurred, fry were removed from the tank. Fry were selected by grading with a 3-mm mesh (Popma and Green, 1990), counted, and randomly

assigned to experimental groups. All treatments were triplicated. The number of fry per replicate was determined by the amount of fry collected in a particular day. Each replicate was housed in a 10-l plastic container with dechlorinated tap water. The mean temperature at which fry were maintained was 29 ± 2°C. All containers were kept under constant aeration.

Experiment Ia: TA Immersions Using Multiple Broods

The objective of this experiment was to determine the masculinizing efficacy of TA in batches of fish produced by several females in fry production systems. Fry were immersed for three hours in 500 µg l^{-1} of TA or ethanol vehicle (both of which were mixed before addition of fry) at a density of 33 fish l^{-1}. All fish were immersed twice; one immersion was conducted at day 1 (day of fry harvest) and the other at day 3. Fry were collected after each immersion, containers were thoroughly cleaned, and then fish were reallocated in fresh dechlorinated tap water. At the conclusion of the immersions, fry from all treatments were transferred to 0.5-m^3 hapas located in a grow-out cement pond.

Steroids were obtained from Sigma Chemical Company (St. Louis, Missouri) and stored in stock solutions of ethanol (1 mg ml^{-1}) at 4 ± 1°C. Temperature and pH were monitored daily; dissolved oxygen was checked weekly.

Experiment Ib: Reuse of Steroid

The goal of this experiment was to determine the potential reuse of TA solutions after masculinizing tilapia fry. TA solutions were reused twice after the first immersion trial. Fry were immersed for three hours in 500 µg l^{-1} of TA or ethanol vehicle (both of which were mixed before addition of fry) at a density of 33 fish l^{-1}. All immersions were conducted as described in the previous experiment. Treatments were as follows:

1) Control (EtOH) first usage
2) Control (EtOH) first reuse
3) Control (EtOH) second reuse
4) TA first usage
5) TA first reuse
6) TA second reuse

Experiment II: Effect of Temperature

The goal of this experiment was to determine if elevated temperatures in combination with TA immersions could induce masculinization of Nile tilapia. Fry were collected and kept at either 24, 28, 32, or 36°C (± 1.5°C) from day 1 to day 4. Fry were immersed for three hours in 500 µg l^{-1} of TA or ethanol vehicle (both of which were mixed before addition of fry) at a density of 33 fish l^{-1}. All immersions were conducted as described in the previous experiment. Control groups were assigned to each temperature.

Growth Measurements and Sex Identification

Subsamples of fish (25 to 30) were measured to the closest 0.1 g after 65 days of growth. Sex ratios were determined by examination of gonads using squash (10 and 40X) preparations after Wright (Humason, 1972) staining.

Statistical Analysis

Data were pooled from replicate tanks because there was no evidence of tank effects within treatments (Chi-square test). Pairwise comparisons for 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™.

RESULTS

In Experiments Ia and Ib, TA immersions resulted in significant masculinization ($P < 0.05$); however, the efficacy of treatment was low. In Experiment Ia the control group had 44.5 ± 3.8% males while TA treatment resulted in 55.9 ± 4.1% males (Figure 1a). In Experiment Ib no significant differences were found between any of the control groups, and data were pooled (mean = 38.9 ± 4.3% males). TA treatment resulted in significantly more males than the control (mean = 61.6 ± 4.2%). Reuse of the hormone for the first and second time resulted in slightly more males (50 ± 4.8 and 51.7 ± 3.9%, respectively) than the control group; however, these results were not statistically different (Figure 1b).

In Experiment II we found no significant differences between control groups and any of the TA-treated groups. No significant effects on masculinization were observed when fry was kept at elevated temperatures (Figure 2). No significant differences in growth were found between any of the groups (weight = 1.39 ± 0.08 g; total length = 43.4 ± 0.7 mm).

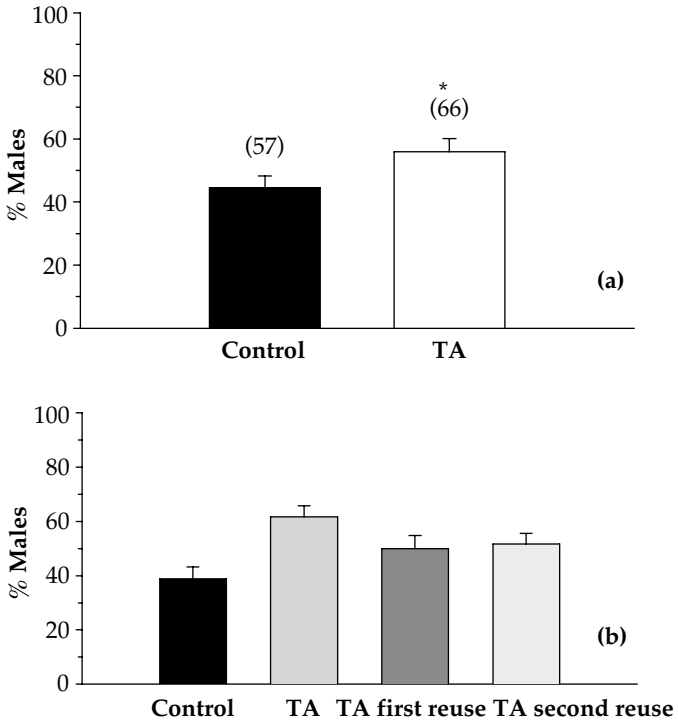

Figure 1. Effects of double immersions on masculinization of Nile tilapia fry. Graph shows mean percentage of males (± SE) obtained after immersions for 3 h in 500 µg l^{-1} of TA or EtOH vehicle (control; a). Effects of double immersions on fish immersed in TA and two cycles of reuse of the steroid solution (first and second reuse; b). The controls represent pooled data. Fish were immersed at days 1 and 3 after harvest. The numbers in parentheses indicate the numbers of fish sampled for each treatment. Asterisks indicate significant differences between control and treatment groups.

DISCUSSION

The results of this study indicate that short-term immersions of Nile tilapia fry (collected from spawning ponds) in masculinizing agents cause little or no masculinization of the fry. In a previous study Contreras-Sánchez (2001) reported that the period of gonadal sensitivity to exogenous steroids is limited to five or six days, with the gonad sensitivity to steroids resembling a normal distribution pattern. According to the cited paper, masculinization of tilapia fry kept at 28°C starts at 11 days post-fertilization (dpf), continues increasing through 12 dpf, and reaches a maximum at days 13 and 14. By 15 dpf, the response to steroid administration starts declining. The low masculinization rates found in our experiments may reflect the variability caused by multiple spawners, and it is possible that fry collected from spawning tanks have passed the labile period at which sex inversion can be accomplished. Several studies have been devoted to determining this valuable information in several species of salmonids (reviewed in Piferrer and Donaldson, 1993). However, most experiments have focused on attaining high numbers of individuals of one sex or the other using multiple-day immersion protocols, but few have approached the delimitation of the labile period by single immersion protocols.

The immersion technique poses disadvantages in terms of its feasibility for aquacultural purposes if more than 95% male populations are required. Early studies on immersion of tilapia fry in androgens reported 100% masculinization; however, these studies involved protocols that required one to five weeks of treatment (Varadaraj and Pandian, 1987; Torrans et al., 1988). Such a long-term protocol defeats the purpose of the immersion treatment (i.e., short-term usage of steroids, small amounts of hormone used, little manipulation of the fry).

It has been proposed that in Nile tilapia, female differentiation is inhibited by elevated temperatures during a sensitive period. Contreras-Sánchez (2001) suggested that this thermosensitive period may comprise the same days at which masculinization can be induced by the exposure of fry to synthetic steroids, and significant masculinization can be achieved by exposures to 34°C for as short as 3.5 d. In other studies tilapia fry were masculinized using long-term exposures (30 to 40 d) to elevated temperatures (Baroiller et al., 1995; D'Cotta et al., 1999; Wang and Tsai, 2000), but apparently such a long treatment may not be needed if conducted at the right time. Our results suggest that our treatments may have missed that window of sensitivity.

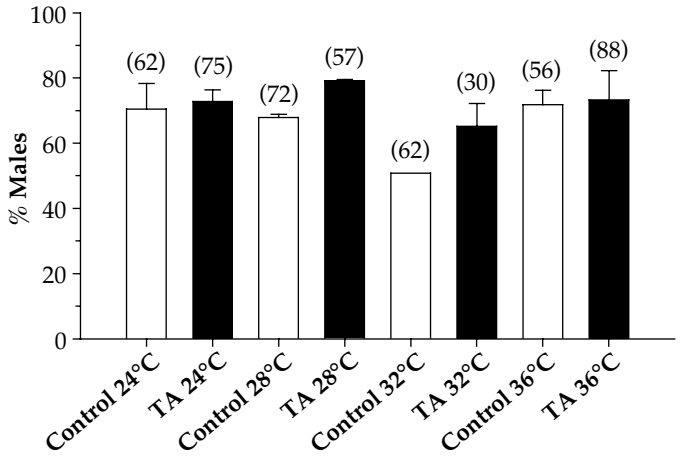

Figure 2. Effects of elevated temperatures and double immersions on masculinization of Nile tilapia fry. Graph shows mean percentage of males (± SE) obtained after immersions for 3 h in 500 μg l^{-1} of TA or EtOH vehicle (control). Fish were immersed at days 1 and 3 after harvest. The numbers in parentheses indicate the numbers of fish sampled for each treatment.

More research is needed to investigate if the immersion protocol can be improved by a combination of such factors and hormonal treatment. The development of this technology for masculinization of tilapia may enable farmers to masculinize tilapia with androgens while minimizing the risk of contamination of ponds with MT.

ANTICIPATED BENEFITS

The implementation of masculinizing trials using immersion will set up the base for the application of large-scale use of immersions under farm conditions. This will provide great opportunities for extending PD/A CRSP research and impacts to tilapia producers. In previous studies we have shown that short immersions in synthetic steroids can provide significant masculinization and that elevated temperatures may enhance the masculinizing effects of this treatment. However, our results indicate that more research is needed for large-scale trials.

The development of this technology for masculinization of tilapia fry may enable farmers to masculinize fish with androgens while minimizing the risk of contamination of ponds with MT. Furthermore, if steroid solutions can be reused for further masculinization trials, the benefits of the immersion technique may increase considerably.

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