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Masculinization of Tilapia by Immersion in Trenbolone Acetate: Growth Performance of Trenbolone Acetate–Immersed Tilapia

Ninth Work Plan, Reproduction Control Research 5B (9RCR5B)
Final Report

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Abstract

Preliminary studies in our laboratory showed that the synthetic androgen trenbolone acetate (TA) is a good candidate for masculinizing Nile tilapia (*Oreochromis niloticus*) fry using short immersions. In this study we investigated the effects of TA treatment on the growth performance of Nile tilapia. We tested the potential anabolic effects of two treatments by growing treated and control fish for 81 and 114 days. Our results suggest that masculinizing treatments involving short-term immersions in TA and four-week feeding with 17α-methyltestosterone (MT) do not result in significant increases in fish growth. Despite significant masculinization (65 to 70% with TA and 100% with MT) in both treatments, we found no differences in final weight between treatments.

Introduction

Masculinization of tilapia continues to be an important tool for aquaculturists to prevent unwanted reproduction (which shunts energy away from growth towards gamete production) and to produce the sex with the larger growth potential (Green et al., 1997). Previous work in our laboratory has shown that short-term immersion in androgenic steroids can result in masculinization of Nile tilapia (*Oreochromis niloticus*) (Gale et al., 1995, 1999; Contreras-Sánchez et al., 1997). These studies showed that immersion in an androgen has the potential to be an alternative to dietary treatment with steroids for the masculinization of tilapia. A variety of androgens—especially synthetic androgens—are effective masculinizing agents (Hunter and Donaldson, 1983).

Tilapia have been effectively masculinized when fed trenbolone acetate (TA) (Galvez et al., 1996). 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. Such a treatment offers an alternative to the typical four-week feeding of tilapia with 17α-methyltestosterone (MT). However, in order to be a viable alternative, immersions in TA must be tested for its effects on fish performance. We decided to examine the potential androgenic effects of TA on Nile tilapia by analyzing fry and juvenile growth. To investigate these potential effects, we carried out an experiment to compare growth performance of fish under the following regimes: TA-immersed, EtOH-immersed, MT-treated food, and EtOH-treated food.

Methods and Materials

Breeding families of Nile tilapia were placed in 200-l aquaria (one male to three females). The temperature was maintained at 28 ± 1°C. Time of spawning was monitored every two hours. Spawning occurred between 1600 h and 1900 h. Once breeding occurred, the other fish were removed and the brooding female was left to incubate the progeny. At ten days post-fertilization (dpf; 280 CTU), fry were removed from the tank and randomly assigned to experimental groups. (Development of the fry was expressed in CTUs—mean water temperature in °C × the number of days since fertilization.) The fry used in the 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 ± 1°C under constant aeration.

Experimental Design

Immersion treatments consisted of two immersions, one at 11 dpf (308 CTU) and one at 13 dpf (354 CTU). The TA treatment was immersed in water containing 500 μg l^{-1} (EtOH used as vehicle), and the control group was immersed in water containing vehicle only. Fry were collected after each immersion, jars were thoroughly cleaned, and then fish were reallocated in fresh dechlorinated tap water. Feeding treatments consisted of MT-fed fish (60 mg kg^{-1} food) and control-fed fish

(EtOH-treated food). MT food was made by spraying crushed, flaked food with MT dissolved in EtOH; control food was made by spraying crushed, flaked food with EtOH. 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. This experiment was repeated with a different brood, and all treatments were triplicated each time.

To keep conditions similar for all treatments, fry to be used in the feeding treatments were counted and kept in jars until day 15 post-fertilization. On this day all fry from both immersion and feeding experiments were transferred to 50-l aquaria. Fry were fed the treatment diets for four weeks (from 15 to 43 dpf), commencing the day of the transfer to aquaria. Immersed fry received control food (no ethanol, no MT). Water temperature in the jars and aquaria was maintained at 28 ± 1°C. Temperature and pH were monitored daily, while ammonia, nitrites, and dissolved oxygen were checked weekly. Feeding rate was at 20% per calculated body weight for the first 23 days of treatment and then 10% per calculated body weight until the end of hormone treatment (day 28) (Popma and Green, 1990). Appropriate water quality was maintained using an activated charcoal filter (Whisper™ Filter I) and a 50% water exchange twice per week. At the conclusion of the 28 days of dietary treatment (on 40 dpf), fry from all treatments were transferred to the Oregon State University Warm Water Research Laboratory, Corvallis, Oregon, and reared in 75-l fiberglass tanks in a recirculating system. Fish from each replicate were placed into two grow-out tanks to allow faster growth. Water temperature in the grow-out system was maintained at 28 ± 1°C, and water quality parameters were also monitored.

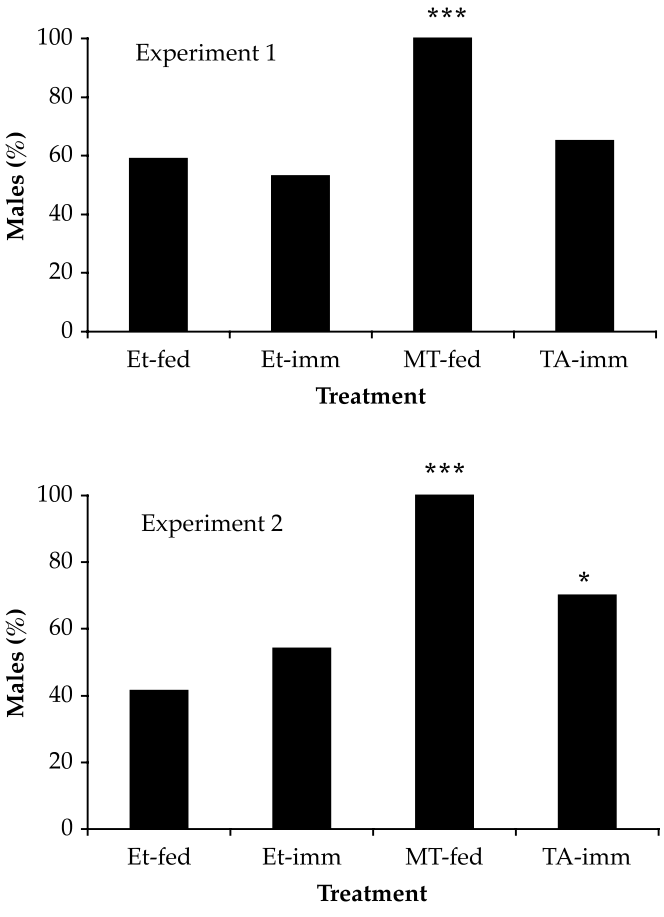

Figure 1. Masculinizing effects of MT-impregnated food (MT-fed) and TA immersion (TA-imm) on Nile tilapia fry. Control treatments were EtOH-impregnated food (ET-fed) and EtOH-immersion with regular food (ET-imm). Each treatment was triplicated. Significant differences between treatments and their respective control are denoted by asterisks (* $P < 0.05$; *** $P < 0.001$).

Growth Measurements and Sex Identification

Subsamples of fish (15 to 20) were measured to the closest 0.1 g at 15 and 51 dpf (experiment 1), and all fish were measured at 81 dpf. Fish from experiment 2 were measured similarly at 15, 80, and 114 dpf. Sex ratios were determined by examination of gonads using squash (10 and 40X) preparations after Wright staining (Humason, 1972) at days 81 (experiment 1) and 114 (experiment 2) post-fertilization.

Statistical Analysis

Sex Identification

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™.

Growth

The mean final weights of sampled fish were analyzed for differences between groups using one-way ANOVA, with density as a possible confounding variable. For all analyses,

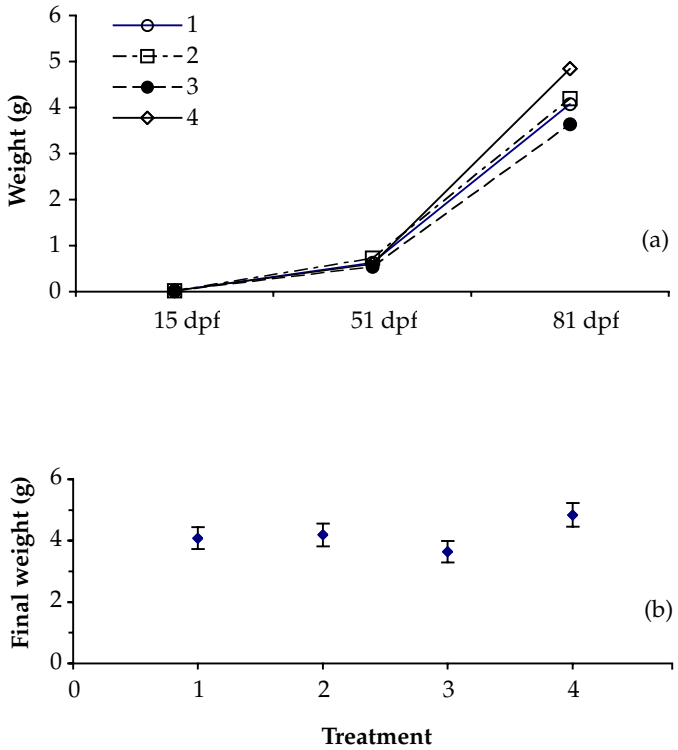

Figure 2. Growth in weight (a) and mean final weight (b) from experiment 1. Treatments were EtOH-impregnated food (1); EtOH-immersed with regular food (2); MT-impregnated food (3); and TA-immersed with regular food (4). Each experiment had three replicates.

differences were considered statistically significant when the p-value (P) was less than 0.05.

RESULTS

In both experiments, TA immersions resulted in significant masculinization; however, the efficacy of treatment was significantly lower than that of the MT treatment, which resulted in 100% males, compared with 59% males in the control-fed group in experiment 1 and 42% males in the control-fed group in experiment 2 (Figure 1). In experiment 1, TA treatment resulted in 65% males, compared with 53% males in the immersed-controls; in experiment 2 the TA treatment had 70% males, compared with 54.1% males in the immersed-controls.

We found no significant differences in weight at either 51 or 81 dpf for experiment 1 (Figure 2a). Due to differential mortalities in the grow-out tanks, some replicates showed larger growth in individuals than other replicates (e.g., TA-immersion); however, these differences were accounted for once density was used in the statistical analysis as a possible confounding variable (P = 0.17). Final mean weights (± SE) at 81 dpf for each treatment were: EtOH-fed = 4.1 g ± 0.3; EtOH-immersed = 4.2 g ± 0.4; MT-fed = 3.6 g ± 0.4; and TA-immersed = 4.8 g ± 0.4 (Figure 2b). Similar results were observed in experiment 2, where mean weight values were not significant at any sampling time (P = 0.29; Figure 3a). Final mean weights (± SE) at 114 dpf for each treatment were: EtOH-fed = 11.3 g ± 0.9; EtOH-immersed = 13.2 g ± 1.1; MT-fed = 12.61 g ± 1.0; and TA-immersed = 10.9 g ± 0.9 (Figure 3b).

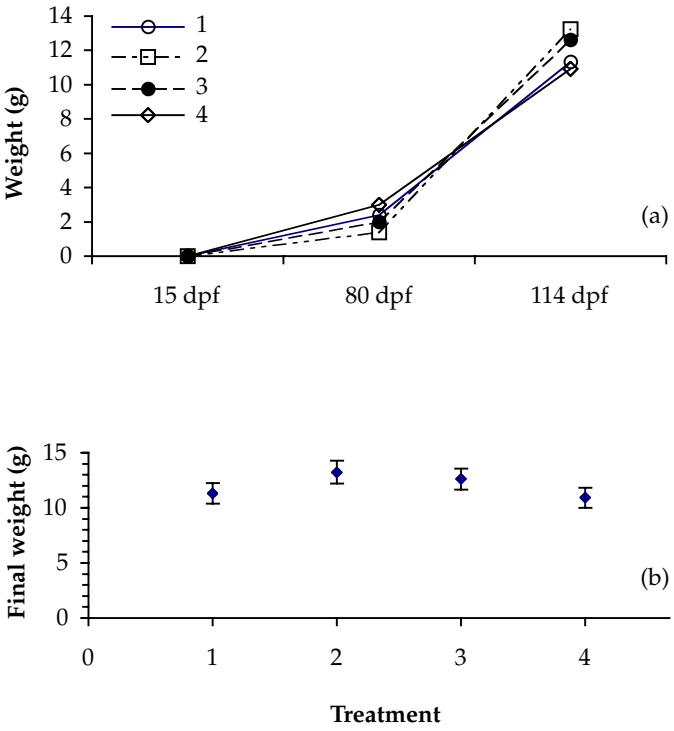

Figure 3. Growth in weight (a) and mean final weight (b) from experiment 2. Treatments were EtOH-impregnated food (1); EtOH-immersed with regular food (2); MT-impregnated food (3); and TA-immersed with regular food (4). Each experiment had three replicates.

DISCUSSION

Several authors have argued that a masculinizing treatment using steroids causes a significant increase in fish growth. However, few papers have tried to determine if these synthetic steroids are in fact potent growth enhancers or if the masculinizing effects of the hormone cause the observed differences in growth, resulting in a male-biased population of fish. Our experiments show that immersion of Nile tilapia on days 11 and 13 post-fertilization for three hours each caused significant masculinization without affecting growth. Similarly, feeding MT-impregnated food for 28 days—the time required for efficient masculinization—did not enhance growth performance of the fish despite the production of 100% males in this treatment. Green and Teichert-Coddington (1994) showed similar results in experiments conducted in nursery ponds, finding no differences between MT-fed and control-fed fish sampled after 94 days of growth. Furthermore, Green and Teichert-Coddington (1994) found similar results after growing the fish for 165 days in fertilized earthen grow-out ponds.

Kuwaye et al. (1993) and Ron et al. (1995) have shown that oral administration of MT can significantly increase growth of the euryhaline tilapia (*Oreochromis mossambicus)* if administered for very long periods of time. These authors used hormone-treated food for 60 days (one month over the masculinizing period), 180 days, and 210 days (Kuwaye et al., 1993), and 168 days (Ron et al., 1995). It is important to mention that the fish used in these studies were grown for periods of time longer than the ones used in our experiments; however, if comparable sampling days are analyzed (90 to 120 days), then the fish used in the cited papers do not show significant differences in mean weight (not tested by the authors, estimated from graphs).

Our results may indicate that some of the differences between treatments can occur after 120 days of growth, perhaps when the females present in the control groups are actively producing eggs, spawning, and incubating fry, thereby shunting energy into reproduction rather than growth.

ANTICIPATED BENEFITS

The use of anabolic steroids to produce 100% male populations of Nile tilapia showed no significant effects on mean final weight after 110 days of growth, despite the efficacy of both treatments on masculinizing fry. We have also shown in other experiments that the anabolic steroid 17α-methyltestosterone (MT) remains in the sediments of model ponds for up to three months after its use for masculinizing tilapia fry, representing a potential risk to hatchery workers and non-target species. Together, these results will be useful to those involved in aquaculture (e.g., farmers, researchers, and government workers) because they will discourage the unnecessary use of steroids for the purposes of enhancing growth.

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