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Masculinization of Tilapia Fry by Immersion in Trenbolone Acetate (TBA) at a Production Level

Eighth Work Plan, Reproduction Control Research (RCR2C)
Progress Report

Ronald P. Phelps, J.T. Arndt, and R.L. Warrington
Department of Fisheries and Allied Aquacultures
Auburn University, Alabama, USA

Abstract

The precocious reproduction of tilapias (*Oreochromis* spp.) had been a serious impediment to successful commercial tilapia production until all-male cultures techniques were developed. Dietary treatment with 17α-methyltestosterone (MT) is an effective means of producing all-male tilapia populations; however, the treatment requires a minimum of several weeks exposure. Administration of steroids to the water containing sexually undifferentiated fish has also been effective in altering sex ratios and may provide aquaculturists with a safe and cost-effective alternative to treating fry with food that contains MT. Immersion requires substantially shorter exposure periods and the steroid is contained for controlled filtration or biodegradation. *Oreochromis niloticus* fry were stocked into aquaria and treated at a density of 33 fish l^{-1} with a stock solution of trenbolone acetate (TBA) dissolved in ethanol at 500 mg TBA l^{-1} for six hours on day 9, 11, 13, or 15 post-hatch. Fish were harvested and mean length, weight and survival were determined. Fish were restocked into outdoor 20-m^2 tanks and reared to 5 cm or larger. Sex ratios were determined by gonadal squashes. There was no treatment effect on sex ratio of Nile tilapia. The non-TBA treatment had a mean of 49.1% males while TBA treatments for the different age groups ranged from 43.7 to 54.3% males. Survival ranged from 64.0 to 82.4% with no observed correlation between age at treatment and survival. Average length and weight at 20 days of age was not correlated to treatment nor survival.

Introduction

The precocious reproduction of tilapias (*Oreochromis* spp.) had been a serious impediment to successful commercial tilapia production until all-male culture techniques were developed. In comparison with mixed populations, all-male populations of tilapia provide several important advantages for aquaculture, including superior growth (males grow faster and larger than females) and prevention of unwanted reproduction (which diverts energy away from somatic growth). Some techniques, including the manual separation of males and selective hybridization, are technically feasible but introduce labor and management constraints to commercial operations. The administration of androgens during early life stages, commonly called "sex reversal," effectively produces all-male populations of tilapia. The efficacy and operational simplicity of the technique have led to dramatic worldwide increases in commercial production of this group of fishes.

Dietary treatment with 17α-methyltestosterone (MT) is an effective means of producing all-male tilapia populations; however, the treatment requires a minimum of several weeks exposure. Administration of steroids to the water containing sexually undifferentiated fish has also been effective in altering sex ratios and may provide aquaculturists with a safe and cost-effective alternative to treating fry with food that contains MT, because immersion will require substantially shorter exposure periods and the steroid will be contained for controlled filtration or biodegradation. This technique is well-developed in salmonid aquaculture (Piferrer and Donaldson, 1989; Feist et al., 1995); however, it remains largely experimental in tilapia culture. For *O. aureus*, immersion of fry in mibolerone at 0.6 mg l^{-1} for five weeks resulted in populations that were 82% male (the remaining fish were intersexual), and a 0.3 mg l^{-1} mibolerone immersion for five weeks resulted in less than 1% functional females (Torrans et al., 1988). Immersion of *O. mossambicus* in 17α-methylandrostendiol at 5 µg l^{-1} for 11 days beginning at seven or ten days post-hatching caused 100% masculinization (Varadaraj and Pandian, 1987). Fitzpatrick et al. (1997) were able to produce greater than 90% male populations of *O. niloticus* when trenbolone acetate was administered as a 2-h bath on days 11 and 13 post-fertilization.

These studies suggest that immersion may be an alternative method for inducing masculinization in tilapia, but whether it is functional on a production scale is unknown. Immersions by Fitzpatrick et al. (1997) were successful because treatment time was initiated as a function of water temperature and time of egg fertilization. Individual females were monitored to determine when ovulation occurred. In practice this degree of monitoring is not feasible, but it is possible to determine time of hatch and swimup within a few hours. Tilapia reproduction systems have been developed where eggs are collected in commercial quantities and incubated artificially (Macintosh and Little, 1995). Using such techniques, it is possible to monitor time of hatch and swimup and apply a hormone bath to fry when age to the nearest day is known.

Methods and Materials

Brood Nile tilapia (*O. niloticus*) were stocked outdoors into 2-m^2 hapas at a rate of one male and three females per hapa. Brood fish were examined every ten days and eggs or sac fry were removed. Eggs and sac fry were held in flowing water McDonald jars at 28 to 32°C, and swimup fry were collected as soon as they were strong enough to swim out of the incubator. Swimup fry were held until 9, 11, 13, or 15 days post-hatch.

At the appropriate age, fish were stocked in aquaria at a density of 33 fish l^{-1} and treated with a stock solution of trenbolone acetate (TBA) dissolved in ethanol at 500 mg TBA l^{-1} for six hours. Three replicate aquaria were treated with TBA on

day 9, 11, 13, or 15 post-hatch. An additional three aquaria were stocked with nine-day post-hatch fry and treated six hours in an ethanol-only stock solution. Fish were held in aquaria until an age of 20 days and fed a non-hormone-treated feed four times per day. Fish were harvested and mean length, weight, and survival were determined. Fish were restocked into outdoor 20-m^2 tanks and reared to 5 cm or larger. Fish were harvested and preserved in 10% formalin for gonadal examination. Gonads from 100 fish per replicate were examined by the gonadal squash technique (Guerrero and Shelton, 1974) and classified as testes, ovaries, or intersex (if composed of both tissues).

Results and Discussion

There was no treatment effect on the sex ratio of Nile tilapia (Table 1). The non-TBA treatment (control) had a mean of 49.1% males while TBA-treated fish in the age groups tested ranged from 43.7 to 54.3% males. Survival ranged from 64.0 to 82.4% with no correlation observed between age at treatment and survival. Average length and weight at 20 days of age was not correlated to treatment or survival.

Table 1. Effectiveness of trenbolone acetate (TBA) to alter sex ratios of four age groups (days post-hatch) of Nile tilapia. Treatments were given a six-hour bath at 500 mg TBA l^{-1}.

Age Group	% Males	% Females	% Intersex	% Survival
9-d, control	49.1	50.9	0	82.4
9-d, TBA	54.3	45.7	0	69.6
11-d, TBA	54.3	45.0	0.7	72.0
13-d, TBA	43.7	56.3	0	64.0
15-d, TBA	49.7	50.3	0	75.4

The lack of response to TBA treatment is not clearly understood. Fitzpatrick et al. (1997) found that TBA is effective in altering the sex ratio of Nile tilapia under a variety of treatment protocols. Because of its effectiveness in these studies and its wider commercial availability, it was recommended that TBA be used for field testing in Auburn instead of 17α-methyldihydrotestosterone. The protocol followed in this study, in which nine-day-old fry were treated, is similar to successful protocols used by Fitzpatrick (personal communication).

Previous trials at Auburn University in which younger Nile tilapia fry were treated with bath applications of TBA to alter the sex ratio were also unsuccessful. The results of previous trials and this study show that fish with post-hatch ages of 3, 7, 9, 10, 11, 13, and 15 days do not have altered sex ratios when immersed in 500 mg l^{-1} TBA for two to six hours. In one study that used fry from only one or two spawns (Gale, 1995), mestanolone given as a bath resulted in highly skewed tilapia sex ratios. In another study, in which fry from ten or more different spawns were used, sex ratios were not as dramatically skewed (Phelps, unpublished data).

The lack of intersex fish in the current study might suggest why there was no treatment response. When tilapia fry are fed androgen-treated feed at an inappropriate dose rate or for an inappropriate treatment period, intersex fish are common. Whether such a response should be expected from a hormone bath treatment is unknown. When bluegill (*Lepomis macrochirus)* with an initial age of 34 days were given 5-h baths in 1000 mg l^{-1} TBA over a three-day period, 93% male populations with no intersex were produced, but when similar age bluegill were fed TBA for 30 days the result was a 100% intersex population (Al-Ablani, 1997).

Differences in water quality may be a factor influencing results. The efficacy of a variety of chemicals when applied to water is affected by water quality. Alkalinity, total hardness, and pH often alter the efficacy or toxicity of a chemical dissolved in water. The soft water conditions (alkalinity, 29 ppm; total hardness, 32 ppm) also may have affected the results of this study. Additional studies will be required to determine the effect of water chemistry on androgens when applied as a bath treatment.

Anticipated Benefits

Development of steroid immersion as an alternative treatment for masculinizing tilapia will minimize treatment time and potentially increase efficiency of exposure and safety in handling masculinizing steroids. This study has identified issues that must be addressed before the promotion of immersions as an alternative procedure for producing monosex tilapia.

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