Masculinization of Tilapia through Immersion in 17α-Methyltestosterone or 17α-Methyldihydrotestosterone

Interim Work Plan, Africa Study 2

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Introduction

All-male populations are used in tilapia aquaculture because the culture of mixed-sex populations often results in precocious maturation and early reproduction (Mires, 1995). Early maturation shunts energy to gonadal rather than somatic growth. In addition, reproduction in ponds may lead to the harvest of many unmarketable fry. Individuals in mono-sex populations have increased somatic growth rate due to the avoidance of energy losses associated with gonadal development and reproduction. Furthermore, all-male tilapia populations are desirable because males achieve a larger final size than females (MacIntosh and Little, 1995).

One of the most common techniques for producing mono-sex populations is steroid-induced sex inversion (Hunter and Donalsdon, 1983). This involves administering synthetic androgens or estrogens to differentiating fry. The steroids act as sex-inversion agents by functionally masculinizing or feminizing individuals in the population. Several methods of steroid administration are possible, including injection, feeding of steroid, and immersion of fry in steroid solutions. Due to their non-invasive nature, the latter two are the most practical for application to aquaculture.

Use of steroid-treated feeds for the production of all-male populations is widespread in tilapia aquaculture (MacIntosh and Little, 1995).

Conversely, use of immersion techniques is not fully developed for practical usage. Torrans et al. (1988) successfully masculinized blue tilapia (*Oreochromis aureus*) using a long-term, continuous immersion in the synthetic androgen mibolerone (Mb). Optimum conditions for treatment were a five-week immersion period ([Mb]=600 mg/l H_2O) with steroid solutions replaced weekly. Pandian and Varadaraj (1987) masculinized Mozambique tilapia *(O. mossambicus)* by immersion in 17α-methyl-5-androsten-3β-17β-diol (5 or 10 mg/l). The immersion period lasted 10 days, beginning at 10 days post-fertilization. Although the authors reported 100% masculinization, detailed information regarding temperature, type of culture system used, fish density, and frequency of water exchange during the immersion period was not included.

A potential problem encountered when developing new methods for steroid-induced masculinization is paradoxical feminization, which results in the inadvertent production of feminized rather than masculinized populations. This phenomenon is caused by the aromatization of the synthetic androgen to a feminizing, estrogenic compound (Piferrer and Donaldson, 1991). Paradoxical feminization can be avoided by use of nonaromatizable androgens (Piferrer and Donaldson, 1991).

The objective of this research was to develop a short term immersion procedure for the masculinization of Nile tilapia (*O. niloticus*). Two synthetic androgens were tested, 17α-methyltestosterone (MT; 17α-methyl-4-androsten-3-one) and 17α-methyldihydrotestosterone (MDHT; 17α-methylandrostan-17β-ol-3-one). Methyldihydrotestosterone is a 17α-methylated nonaromatizable derivative of dihydrotestosterone. Methyltestosterone is one of the most commonly used sex-inverting agents but is susceptible to aromatization and has been associated with paradoxical feminization in chinook salmon (*Oncorhynchus tshawytscha*) (Piferrer and Donaldson, 1991).

Materials and Methods

Steroids were obtained from Sigma Chemical Company (St. Louis, MO) and stored in stock solutions of HPLC-grade methanol (10 mg/ml). Breeding families (one male to three females) were placed in 208-l aquaria. The temperature was maintained at 28-30°C. Breeding activity was monitored daily. Once breeding occurred between the male and one female, all fish were removed except for the brooding female, which was left to incubate the progeny. At 10 days post-fertilization (DPF), fry were removed from the female and randomly assigned to experimental groups (n = 100/group). Groups of fry were housed in 3.8-l glass jars with 3-l of fresh water. The water was maintained at 28 ± 2°C under constant aeration. Treatment consisted of a three-hour immersion on 10 and again on 13 DPF. After immersion, the fry were collected and placed in new jars that contained fresh water. For each immersion treatment, steroid was evaporated under N_2 (g) and delivered in 0.5 ml of ethanol. Steroid was allowed to mix by aeration for 30 min before addition of fry. Fry were immersed in MT or MDHT at 100 or 500 mg/l (MT-100, MT-500, MDHT-100, MDHT-500). Control groups included the following: immersion in water and ethanol vehicle (ethanol group), an immersion in water alone (control group), and water immersion followed by feeding of MT-treated diet (60 mg/kg) from 10 to 30 DPF. The MT-treated diet was made by dissolving steroid (30 mg) in 250 ml of 100% ethanol. The steroid solution was mixed with a commercial flake feed and allowed to dry before use. Other groups were fed commercial flake feed. Throughout the experiment, fry were fed to satiation 3-5 times daily.

The first experiment was repeated (experiment 2) with omission of the dietary MT control group. In experiment 1, the groups were held in the jars (3.8 l) until the end of the feeding treatment period (30 DPF). In experiment 2, fish were removed from the 3.8-l jars immediately following the 13 DPF immersion. Groups in both experiments were transferred to 20-l chambers for grow out in a recirculating system. Water temperature in the grow-out system was maintained at 28 ± 2°C. At 100 DPF, sex ratios were determined by examination of *in situ* (40X) and squash (100X) preparations after aceto-iron hematoxylin (Wittman, 1962) staining. Standard length and body weight of sampled fish was recorded in experiment 2.

Sex ratio data were analyzed using the chi-square test ($\alpha < 0.05$; Zar, 1984). The control and ethanol groups were not significantly different and were pooled for comparison to other groups. Mortality data were analyzed using the chi-square test ($\alpha < 0.05$; Zar, 1984). Length and weight data were

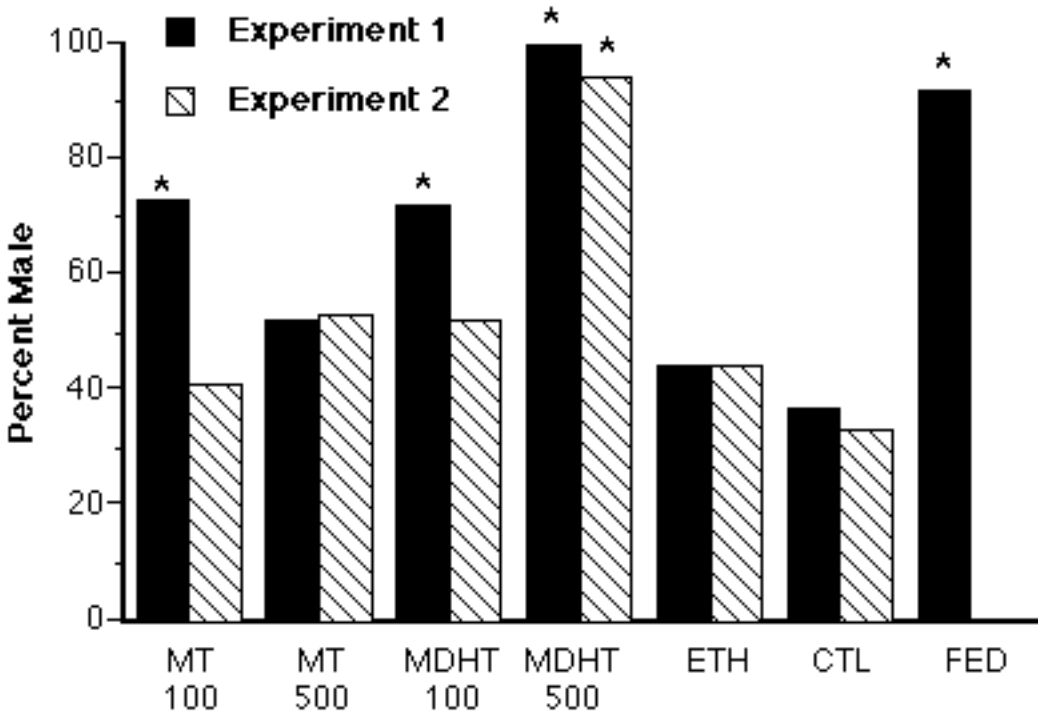

Figure 1. Percent males in each group for experiments 1 and 2. Group designations are as follows: immersion treatment in 100 or 500 mg 17α-methyltestosterone/l (MT 100, MT 500), immersion in 100 or 500 mg methyldihydrotestosterone/l (MDHT 100, MDHT 500), immersion in ethanol vehicle (ETH), immersion in water alone (CTL), and methyltestosterone feeding treatment (FED) from 10-30 DPF (60 mg/kg feed). Asterisks indicate significant (from chi square test; $\alpha \leq 0.05$) differences in proportion of males from the pooled control (ETH and CTL) group. Sample sizes ranged from 19 to 51 individuals.

not analyzed statistically, since these data were recorded for experiment 2 only.

Results

Immersion in MDHT at 500 mg/l resulted in 100 (experiment 1) and 94 (experiment 2) percent male populations (Figure 1). In experiment 1, MT and MDHT immersions at 100 mg/l resulted in significant skewing of the sex ratio toward males (73 and 72 percent male, respectively). However, in experiment 2, the proportion of males in these treatments was not significantly different from controls. Methyltestosterone at 500 mg/l had no masculinizing effect in either experiment. The MT feeding treatment resulted in 92 percent males.

Immersion treatment did not significantly affect mortality in either experiment (Table 1). High mortality was seen in the control group from experiment 1; this was associated with anoxic conditions caused by a clogged inlet during the grow-out period. The MT-500 group in experiment 2 suffered higher mortality due to cannibalization by an adult fish that jumped from an adjoining tank. Average final length and weight of fish were similar among treatments (Table 2).

Discussion

Immersion of Nile tilapia on 10 and 13 DPF with MDHT at a concentration of 500 mg/l caused masculinization. Conversely, MT at similar levels did not significantly alter the sex ratio. Lack of an effect in the MT treatment (500 mg/l) may be due to conversion of MT to a less active form or simply a higher rate of clearance from the body than MDHT. Another possible explanation for the differing effects of the two steroids is that MDHT is a more potent masculinizing agent than MT. Piferrer et al. (1993) found that MDHT was twice as potent as MT in masculinizing female chinook salmon. Furthermore,

Table 1. Mortality data for experiment 1 (EX 1) and 2 (EX2). Group abbreviations and sample sizes are the same as given in Figure 1.

Group	Mortality (%)	
	EX 1	*EX 2*
MT-100	58	26
MT-500	46	64
MDHT-100	53	33
MDHT-500	63	33
ETH	59	22
CTL	81	35
FED	62	--

MDHT can bind to androgen receptors in Nile tilapia gonads (Gale, 1996) and coho salmon (*O. kisutch*) ovaries (Fitzpatrick et al., 1995). These binding sites are specific for sex-inverting androgens, and are found in the gonadal cytosol.

Immersion treatment did not significantly affect mortality. Although mortality was not significantly different between treatments, fry in experiment 1 did suffer a higher mortality than did individuals in experiment 2. This discrepancy is likely due to improvements in culture conditions. Fish in experiment 1 were held at a density of 33 fish/l for 20 days (30 DPF) and then placed in grow-out tanks at a density of 5 fish/l. Fish in experiment 2 were held at the 33 fish/l density for only three days (13 DPF) and then transferred into grow-out tanks at a density of 5 fish/l.

Administration of steroid by incorporation in feed has a long history of use (see reviews by Schreck, 1974, and Hunter and Donaldson, 1983). Steroid is dissolved in a carrier (e.g., ethanol or acetone), uniformly mixed with feed, and allowed to dry before use. Fry are fed for several weeks, beginning between 10 and 14 DPF (Shelton et al., 1981; Nakamura and Iwahashi, 1982). Although this technique usually results in successful sex inversion, certain inefficiencies are cause for concern. MacIntosh and Little (1995) point out that any condition that adversely affects food consumption may decrease treatment efficacy. The dose received by an individual fish is variable—being dependent on body size, social status, and consumption of naturally-occurring food. This may result in an uneven distribution of steroid. The culturist must then accept partial or incomplete sex inversion or increase the treatment dose beyond the optimal requirement to achieve 100% sex inversion under laboratory conditions. Furthermore, the long period of treatment employed by typical feeding methods results in human handling of anabolic steroid three to five times daily for up to 35 days. This degree of handling presents an added risk to

Table 2. Mean weight and standard length (±SE) from sampled fish in experiment 2. Group designations and sample sizes are the same as given in Figure 1.

Group	Weight (g)	Length (mm)
MT-100	2.77 ± 0.21	41.7 ± 1.2
MT-500	3.45 ± 0.21	44.4 ± 1.0
MDHT-100	2.95 ± 0.30	41.7 ± 1.4
MDHT-500	3.29 ± 0.22	43.7 ± 1.1
ETH	2.97 ± 0.26	42.5 ± 1.3
CTL	3.17 ± 0.23	42.2 ± 1.0

the aquaculture worker, given the tumorigenic and teratogenic effects of anabolic androgenic steroids (Lewis and Sweet, 1993). This risk is easily mitigated by the establishment of proper handling procedures; however, these precautions are often improperly implemented. For instance, in developing countries, where much of the worldwide tilapia production occurs, disposable rubber gloves for the handling of treated feed may be either unavailable or too expensive to be practical. Furthermore, in developing countries workers generally have little or no protective clothing (e.g., rubber waders) for working in ponds containing dissolved steroid. Therefore, techniques that reduce worker exposure to anabolic steroid but are as (or more) effective as feeding treatments need to be established.

The technique described in our study consisting of immersion in MDHT decreases the treatment period, thereby reducing worker exposure while still achieving nearly complete masculinization. This technique is a promising alternative to the use of steroid-treated feed, but further evaluation is needed before application in large-scale aquaculture operations.

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