



Efficacy of 17 α -Methyltestosterone Administered in Feed to Produce Predominantly Male Populations of Tilapia

Dan Carty*, Jim Bowker, Molly Bowman, and Niccole Wandeleer

*U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road, Bozeman, Montana 59715, USA*

Tilapia (*Oreochromis* spp.) can reproduce at 3-6 months of age (Green et al. 1997), and such early reproduction is the primary impediment to their commercial production (Phelps 2006). In-feed administration of the synthetic androgen 17 α -methyltestosterone (17MT) to sexually undifferentiated Tilapia fry for ~28 consecutive days can produce populations composed almost entirely of phenotypic males (Green et al. 1997). Such “all-male” Tilapia populations are preferred for commercial production because reproduction is virtually eliminated and because males grow faster and more uniformly than females (Guerrero 1975).

In the United States, 17MT is a candidate for U.S. Food and Drug Administration (FDA) approval for use to produce predominantly (>80%) male populations of Tilapia when the drug is administered in feed to fish <14 d posthatch at a dosage of 9 mg 17MT/kg fish/d for 28 d. In 2006-2007, we conducted three field trials to evaluate the efficacy of this treatment regimen. In this bulletin, we summarize the results of those trials.

Methods

Trial sites, test fish, and test article.—Two trials (SeaPac1 and SeaPac2) were conducted at SeaPac of Idaho (Buhl, Idaho), and one trial (Simaron) was conducted at Simaron Fresh Water Fish (Hempstead, Texas). Tilapia hybrid (*Oreochromis niloticus* \times *O. aurea* \times *O. mossambique*) fry were used in the two SeaPac trials, and Nile Tilapia *O. niloticus* fry were used in the Simaron trial.

The test article was 17MT (100% active; Hawkins, Inc., Pharmaceutical Group, Minneapolis, Minnesota) incorporated into Rangen No. 0 Tilapia Starter Feed (Rangen, Inc., Buhl, Idaho) at the U.S. aquaculture industry standard concentration of 60 mg 17MT/kg feed. The control article was Rangen No. 0 Tilapia Starter Feed. Feed samples collected for dose verification were shipped to CanTest, Ltd. (Barnaby, British Columbia, Canada) to determine 17MT concentrations in treated and control feeds (Marwah et al. 2005).

Experimental designs.—In each trial, four replicates (tanks) of each treatment condition (17MT treated versus nontreated control) were allocated across eight tanks. In the SeaPac trials, completely randomized designs (CRD) were used to assign treatments to tanks. In the Simaron trial, a randomized

complete block design (RCBD; four blocks, with one treated tank and one control tank per block) was used to assign treatments to tanks. In all trials, CRDs were used to allocate fish to test tanks. Stocking densities were similar among trials, although the number of fish stocked per tank differed among trials.

Experimental procedures.—The in-life phase of each trial included a 1-d pretreatment period, a 28-d treatment period, and a posttreatment period (69 d in SeaPac1, 77 d in SeaPac2, and 110 d in the Simaron trial). Brine shrimp *Artemia franciscana* (Brine Shrimp Direct, Ogden, Utah) were fed to fish during the pretreatment period of each SeaPac trial, whereas control feed was fed to fish during the pretreatment period of the Simaron trial. During the treatment period, feeds were administered by hand four times daily to achieve 15% of the estimated mean body weight (BW)/d. Mean percent BW fed/d in each of the SeaPac trials was estimated from sample counts of test fish made periodically during the 28-d treatment period; however, no sample counts were made during the treatment period of the Simaron trial. During the posttreatment period, control feed was administered to all tanks, and fish were fed several times per day to satiation. Data collected and observations made during the in-life phase included fish mortality, general fish behavior, and fish feeding behavior; water temperature and dissolved oxygen (DO) concentration in tanks; and source water hardness, alkalinity, and pH.

At the end of the in-life phase of each trial, 40 fish were collected from each tank and euthanized in a solution of tricaine methanesulfonate (Tricaine-S, Western Chemical, Inc, Ferndale, Washington). For each fish collected, one or both gonads were removed and processed for histology. Histologically, each fish was characterized as male (100% testicular tissue in gonads), female (100% ovarian tissue in gonads), or intersex (both testicular and ovarian tissues present in at least one gonad). A fish characterized as a (a) male or an (b) intersex fish with $\geq 75\%$ testicular tissue in the gonads was classified as a treatment success (hereafter referred to as males). A fish characterized as a (c) female or an (d) intersex fish with $>25\%$ ovarian tissue in the gonads was classified as a treatment failure. Intersex fish were classified as either treatment successes or failures because fish with gonads composed of $\geq 75\%$ testicular tissue were likely

*Corresponding author: dan_carty@fws.gov

to exhibit male growth characteristics, whereas fish with gonads composed of >25% ovarian tissue were not (Bowker 2008). Results of the histological evaluations were used to calculate the percentage of males in each tank.

Data analysis.—To demonstrate efficacy within a trial, mean percent males in treated tanks had to be >80% and significantly different from mean percent males in control tanks. Consequently, each SeaPac trial (CRD) was evaluated with a *t*-test ($\alpha = 0.05$, two-sided; SYSTAT 2006), whereas the Simaron trial (RCBD) was evaluated with a General Linear Model ($\alpha = 0.05$, two-sided; SYSTAT 2007). Across trials, efficacy was evaluated with a Hierarchical Linear Mixed Model ($\alpha = 0.05$, two-sided), and potential treatment \times site interaction was evaluated with a General Linear Model ($\alpha = 0.25$, two-sided; SYSTAT 2007). In all statistical procedures, percentage data were arcsin square root transformed to radians before being analyzed (SYSTAT 2006).

Results

Treatment efficacy.—Within trials, mean percent males in treated tanks (range, 83-95%) was significantly ($P < 0.05$) different from mean percent males in control tanks (range, 40-64%; Table 1). Across trials, mean percent males in treated tanks (90%) was significantly ($P = 0.039$) different from mean percent males in control tanks (50%); however, a significant ($P = 0.022$) treatment \times site interaction was detected (Table 1). Combined, these results indicated that 17MT treatment was efficacious but that efficacy was influenced by differences among sites (i.e., differences among trials).

Fish behavior and mortality.—In SeaPac1, general fish behavior was characterized as normal and feeding behavior was characterized as aggressive in all tanks during the treatment period; however, no behavior data were collected during the posttreatment period. In SeaPac2, general fish behavior was characterized as normal and feeding behavior was characterized as aggressive in all tanks throughout the trial. No behavior data were collected in the Simaron trial; however, after the trial ended, onsite participants reported that fish in all tanks appeared to behave normally and feed semiaggressively or aggressively throughout the trial.

During treatment, mortality averaged <1% per treated tank and <1% per control tank in each SeaPac trial and <0.1% per treated tank and 0.1% per control tank in the Simaron trial. During posttreatment, little to no mortality occurred in all tanks in the SeaPac trials, and mortality was not recorded in the Simaron trial.

17MT doses administered.—Estimated 17MT doses administered to treated tanks were 5.1 mg/kg fish/d (57% of target) in SeaPac1, 9.4 mg/kg fish/d (104% of target) in

SeaPac2, and 9 mg/kg fish/d (100%) in the Simaron trial. In all trials, no 17MT was detected in control feed.

Water quality.—Water quality variables measured during the trials were suitable for rearing the test fish at their respective sites. The SeaPac trials were conducted at a mean water temperature of 31°C and mean DO concentration of 3-4 mg/L, whereas the Simaron trial was conducted at a mean water temperature of 24°C and DO concentration of 11 mg/L (oxygen supplementation). Mean source water hardness (range, 8-27 mg/L as CaCO₃) and alkalinity (range, 97-100 mg/L as CaCO₃) in the SeaPac trials were less than mean source water hardness (198 mg/L as CaCO₃) and alkalinity (248 mg/L as CaCO₃) in the Simaron trial; however, mean source water pH was similar among trials (range, 8.1-8.4).

Discussion and Conclusions

Within and across trials, 17MT treatment was efficacious for producing predominantly (>80%) male populations of Tilapia while not inducing any observable adverse behavior during the treatment and posttreatment periods. Efficacy results were significantly affected by differences among trials; however, this outcome was not unexpected because such differences included geographic location, season of year, Tilapia species (or hybrid) treated, age and size of fish at which treatment was initiated, number of fish stocked per tank, 17MT concentration in treated feed, estimated percent BW fed/d during treatment, estimated 17MT dose administered, and environmental culture conditions. One noteworthy difference occurred in SeaPac1, in which the 17MT concentration in the treated feed was only 39.5 mg/kg feed. Such a low concentration probably resulted from the facts that the feed used in this trial was manufactured nearly 7 weeks before the trial began and was stored at room temperature rather than at $\leq 4^\circ\text{C}$ as recommended by Barry et al. (2007). Regardless, the estimated 17MT dose administered in SeaPac1 (5.1 mg 17MT/kg fish/d) resulted in the highest percentage of males observed in all three trials.

Overall, our efficacy results were within the range of those reported by Phelps and Popma (2000), who reviewed 17MT trials conducted at many geographic locations on a variety of Tilapia species and tilapia hybrids treated at a variety of dosages and under a variety of environmental conditions. Results from our trials were accepted by FDA as demonstrating the efficacy of 17MT to produce predominantly (>80%) male populations of Tilapia when the drug is administered in feed to fish <14 d posthatch at a dosage of 9 mg 17MT/kg fish/d for 28 d.

Acknowledgments

Ray Gill (SeaPac1), Patty Sheen (SeaPac2), and Terry Washburn (Simaron) served as the onsite investigators. Miranda Dotson, FWS AADAP Program, prepared gonad

tissue samples for histological evaluation, and Beth MacConnell, Headwaters Fish Pathology LLC, histologically evaluated gonad tissue samples. Dave Erdahl, FWS AADAP Program, reviewed this bulletin.

References

- Barry, T. P., A. Marwah, and P. Marwah. 2007. Stability of 17α -methyltestosterone in fish feed. *Aquaculture* **271:523-529**.
- Bowker, J. D. 2008. Report to justify reclassifying 17α -methyltestosterone treatment successes (Report No. MT-EFF-Treatment Successes-01). U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, Bozeman, Montana USA.
- Green, B. W., K. L. Veverica, and M. S. Fitzpatrick. 1997. Fry and fingerling production. Pages 215-243 **In:** H. S. Egna and C. E. Boyd, editors. *Dynamics of Pond Aquaculture*. CRC Press, Boca Raton, Florida USA.
- Guerrero, R. D. 1975. Use of androgens for the production of all-male *Tilapia aurea* (Steindachner). *Transactions of the American Fisheries Society* **104:342-348**.
- Marwah, A., P. Marwah, and H. Lardy. 2005. Development and validation of a high performance liquid chromatography assay for 17α -methyltestosterone in fish feed. *Journal of Chromatography B* **824:107-115**.
- Phelps, R. P. 2006. Hormone manipulation of sex. Pages 211-252 **In:** C. Lim and C. D. Webster, editors. *Tilapia: Biology, Culture, and Nutrition*. Haworth Press, Binghamton, New York USA.
- Phelps, R. P., and T. J. Popma. 2000. Sex reversal of tilapia. Pages 34-59 **In:** B. A. Costa-Pierce and J. E. Rakocy, editors. *Tilapia Aquaculture in the Americas, Volume 2*. The World Aquaculture Society, Baton Rouge, Louisiana USA.
- SYSTAT. 2006. SigmaStat 3.5 statistical software. SYSTAT Software, Inc., Richmond, California USA.
- SYSTAT. 2007. SYSTAT 12.02 statistical software. SYSTAT Software, Inc., San Jose, California USA.

Table 1. Field trials conducted to evaluate the efficacy of 17α -methyltestosterone administered in feed to produce predominantly (>80%) male populations of Tilapia.

Trials		Test fish at start of trial						Mean (range) percent males at end of trial		
Sites	Dates	Tilapia species or hybrid	Age (d)	Mean length (mm)	Mean weight (g)	Number per tank	Density (g/L)	Treated tanks	Control tanks	P-value treated vs. control
SeaPac1	Jul 12 – Oct 17 (2006)	Hybrid	6-7	10	0.030	400	0.04	95 (90-100)	40 (28-50)	<0.001
SeaPac2	Dec 20 – Apr 4 (2006-2007)	Hybrid	12-13	11	0.034	400	0.05	83 (75-90)	46 (38-60)	<0.001
Simaron	Sep 6 – Jan 21 (2006-2007)	Nile tilapia	7	—	0.020	4,776	0.06	91 (83-98)	64 (50-78)	0.040
All trials	—	—	—	—	—	—	—	90 (75-100)	50 (28-78)	0.039