

17-Alpha Methyltestosterone Clinical Field Trials - INAD 9332

Year 2004 Annual Summary Report on the Use of 17-Alpha Methyltestosterone in Field Efficacy Trials

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Summary

Various techniques have been developed for the control of sexual differentiation in a variety of fish species, including the use of 17-alpha Methyltestosterone. Specifically, this compound has been used effectively in the U. S. under compassionate INAD Exemption #11-236 for sex reversal in early life stage tilapia. In calendar year 2004 (CY04) the efficacy of 17-alpha Methyltestosterone (MET) was evaluated in 64 trials involving approximately 3.7 million early life stage tilapia for sex reversal. All trials were conducted at eight commercial tilapia culture facilities. The compassionate study protocol under which treatments were administered allowed the investigator to administer MET at a dosage of 9 mg MT/kg of fish biomass for 28 consecutive days. Overall, results from trials conducted in CY04 indicated that treatment appeared effective in approximately 64% of the trials, whereas treatments were characterized as inconclusive in the remaining 36% of the trials.

Introduction

Larval stages of many teleost species contain both ovarian and testicular tissue, and sexual differentiation commences shortly after hatching or after the initiation of feeding (Yamamoto, 1969; Donaldson and Hunter, 1982; Yamazaki, 1983). Various techniques have been developed for the control of sexual differentiation in a variety of fish species (Donaldson and Hunter, 1982). These techniques have typically involved the use of either androgen or estrogen treatment to override the endogenous mechanisms of sex determination in developing larval stages and direct sexual differentiation toward the production of either males or females. Treatment regimens have generally involved immersion of larval stages in water containing a steroid, incorporation of a steroid in the larval diet, or both. As numerous factors such as dosage, timing, duration, and environmental conditions often influence efficacy, results have been somewhat variable.

Although the gonadal tissue of tilapia remains undifferentiated at hatch, tilapia generally attain sexual maturity by three to six months of age, and begin to immediately reproduce. This somewhat precocious reproduction behavior is the number one impediment to the development of successful strategies for the commercial production of tilapia. In response to this problematic issue, strategies for monosex male culture have been evaluated including: 1) manual separation of the sexes by visual examination; 2) hybridization; and 3) sex reversal/direction via steroid administration.

1. Manual separation of the sexes via visual examination (or hand sexing) has been found to be a tedious and time consuming process, prone to significant human error, and less productive than other methods. Currently, hand sexing of tilapia is practiced by only a limited number of fish farmers in underdeveloped countries.
2. Hybridization involves the crossing of two different species of tilapia that can result in the production of 95-100% male offspring. The most frequently used crosses suitable for aquaculture production are *Tilapia nilotica* (F) x *T. hornorum* (M); *T. mossambica* (F) x *T. hornorum* (M); and *T. nilotica* (F) x *T. aurea* (M). However, there are limitations to hybridization that include behavioral incompatibilities between two species that lead to significantly decreased fingerling production; the high potential for the contamination of the broodfish populations; and the high costs associated with the sophisticated equipment and techniques necessary to ensure and confirm broodfish purity. Hybridization is currently practiced on only a limited basis worldwide.
3. Gonadal differentiation in tilapia typically occurs between 8 to 25 days post-hatch, dependent upon environmental conditions. It has been demonstrated that the oral administration of the synthetic androgen 17-alpha methyltestosterone (MET) to newly hatched tilapia fry (3-12 days old) for ~28 consecutive days results in populations comprised of greater

than 90% males (Green et al., 1997; Rani and Macintosh, 1997; and Teichert-Coddington et al., 2000). The excess androgen that is introduced into the early life stage fish overrides endogenous hormones and directs sexual differentiation towards the formation of testis. The use of orally administered MET has been shown to be an efficacious, cost-effective, and efficient methodology to produce populations of male tilapia.

Of the three above-described methods/strategies for the monosex culture of male tilapia, oral administration of MET is best suited for successful tilapia production in the United States.

Purpose of Report

The purpose of this report is to summarize the results of calendar year 2004 (CY04) MET field efficacy trials conducted under INAD #11-236. Furthermore, it is expected that data from these trials will be used to enhance the existing MET database that has been established from previous years studies for the purpose of developing an appropriate label claim for the use of MET in aquaculture.

Facilities, Materials, and Treatment Procedures

1. Facilities

Eight commercial fish culture facilities used MET for sex reversal of early life stage tilapia. Mean water temperature during all trials was 80.1 °F, and water temperature ranged from 75.0 - 83.0 °F during the test periods at the different testing facilities.

2. Test article used

The MET used during the reporting period was 17 beta-hydroxy-17-methylandrosta-4-ene-3-one. All MET medicated feed used in INAD trials was supplied by Rangen Inc, P.O. Box 706, Buhl, ID. All medicated feed was prepared with MET supplied by Hawkins, Inc. Pharmaceutical Group, 3000 East Hennepin Ave, Minneapolis, MN.

3. Treatment regimen

As described in the Study Protocol, Investigators were allowed to only use MET at a dosage of 9 mg MT/kg of fish biomass for 28 consecutive days (i.e., the standard dosage).

Fish Species Involved in CY 2004 Trials

1. Species of fish treated

Tilapia were the only fish species treated during CY04. Treated fish ranged in length from 5 - 10 mm. Tilapia strains that were treated included:

1. Nile tilapia *Oreochromis niloticus*
2. Tilapia aurea *O. aureus*
3. California mozambique *O. mossambica*
4. Tilapia nilotica x California mozambique *O. niloticus* x *O. mossambica*

Data Collected

1. Primary response variable (microscopic examination of gonadal tissue reports or gross examination of features characteristic of mature males/females)

The efficacy of MET medicated feed to produce tilapia populations comprised of greater than 90% male fish was the primary response variable of clinical field efficacy trials. The sex of individual fish was determined by evaluation of gonadal tissue according to procedures of the gonadal squash technique as described by Guerrero and Shelton, 1974. Investigators were required to send 60 fish to the U. S. Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership Program's research office in Bozeman, MT where gonads were examined and classified as testis, ovary, or ovotestis (gonads containing both ovarian and

testicular tissue). However, in CY2004, samples were sent from a limited number of Investigators, and subsequent microscopic examination of gonadal tissue reports were available for 2 of 64 (3%) of the trials. Investigators were also allowed to perform a gross visual examination of fish that had reached or were near reaching sexual maturity for external features characteristic of either male or female tilapia. Results from gross visual examinations were submitted with 39 of 64 (61%) of the trials. Overall, a report of sex determination was submitted for 41 of 64 (64%) of the trials.

2. Secondary response variables

Secondary parameters include general observations on fish behavior and response to routine culture activities. Secondary parameters of interest variables such as feeding activity, feed consumption, apparent level of stress, negative fish behavior, and mortality.

Discussion of Study Results:

- 1. General observations on the efficacy of MET to produce populations comprised of greater than 90% male fish** (Note: Table 1 provides a summary of all trials characterized as effective; Table 2 provides a summary of all trials characterized as inconclusive; Table 3 provides summary data for all trials; and Table 4 provides a summary of all trials conducted during CY04 under INAD #11-236).

A. Efficacy at 9 mg/kg bw/day for 28 days

Fish were treated with the standard dosage in all trials (Tables 1 & 2).

Treatments were determined to be effective by either microscopic examination of gonadal tissue or by the Investigators visually evaluating the treated fish a few months after the treatment period. Treatments were characterized as inconclusive due to situations in which Investigators were not able to evaluate fish because young fish were sold to a commercial grow out facility before they could be sexed. Results from all trials in which sex was determined using microscopic or gross visualization techniques showed that MET treatments was effective (i.e., such techniques were used to determine sex of fish in 41 of 64 trials). In the remaining trials, sex of fish was not determined before fish were sold or moved to a grow out facility. Consequently, results from these 23 (36%) trials were characterized as inconclusive.

2. Observed Toxicity

No toxicity or adverse effects relating to MET treatment were reported in any of the trials conducted in CY04.

Summary of Study Results

MET was administered at the standard dosage on four different species of tilapia and involved approximately 3.7 million fish. Treated fish ranged in length from 5 - 10 mm. Water temperature during treatment ranged from 75.0 - 83.0 °F, with a mean

treatment temperature of 80.1 °F. Overall results in all cases where microscopic or gross visual exams were performed to determine sex of fish showed that treatment was effective. However, in only 64% of trials were such techniques used. Consequently, treatment results in the remaining 36% of the trials were characterized as inconclusive. No evidence of toxicity or adverse effects related to MET treatment was reported in any of the trials. However, based on a absence of untreated control fish, replication, randomization, etc., it is understood that these data will only be considered as supportive or ancillary data. None-the-less, the data described above should provide useful corroborative data to support a label claim for MET for the sex reversal in tilapia. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #11-236. In future trials conducted under INAD #11-236, efforts will be directed towards the generation of higher quality data.

References

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Table 1. Summary of CY04 MET Treatment Results - Effective Trials

Hatchery	Number of Trials	Tilapia Species	Fish Size (mm)	Number of Fish	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Americulture	19	Nile Tilapia	9.00	621,200	28	9	82.0
Kent SeaTech Corp	1	California mozambique	10.00	10,000	28	9	78.0
Oceanrich Fisheries	7	California mozambique	9.00	70,000	28	9	83.0
Pacific Aquafarms	13	California mozambique	5.00	793,217	28	9	75.0 - 76.0
St. Croix Campus	1	Nile Tilapia	9.00	6,000	28	9	83.0

Table 2. Summary of CY04 OTF Treatment Results - Inconclusive Trials

Hatchery	Number of Trials	Tilapia Strain	Fish Size (mm)	Number of Fish	Number of Treatment Days	Dose (mg/kg)	Temp. (°F)
Aquasafra	12	Nile tilapia Tilapia aurea	5.0 - 6.0	1,825,000	28	9	80.0 - 83.0
Canyon Springs Fish Farm	1	Tilapia nilotica x California mozambique	6.00	40,000	28	9	80.0
Kent SeaTech Corp	8	California mozambique	10.00	229,779	28	9	78.0
Mill Creek Aqua Farms	1	Tilapia	?	100,000	28	9	?
St. Croix Campus	1	Nile Tilapia	9.00	2,000	28	9	80.0

Table 3. Summary Data Regarding Summary of CY 2004 MET Treatment Trials

Total Fish Treated:	<u>3,697,196</u>
Number of fish treated in efficacious trials	1,500,417
Number of fish treated in inconclusive trials	2,196,779
Total number of trials:	64
Efficacious trials	41 (64%)
Microscopic evaluation	2 of 41 trials
Gross visual examination	39 of 41 trials
Inconclusive trials	23 (36%)
No sex determination techniques used	
Treatment Regimen Used:	
9 mg/kg bw/day for 28 days	64 trials
Treatment Water Temperature (°F):	
Temperature Range	75 - 83
Mean Temperature	80.1
Size of Treated Fish (mm):	
Size Range	5 - 10
Tilapia Strains Treated:	
<u>Tilapia</u>	
Nile tilapia <i>Oreochromis niloticus</i>	
Tilapia aurea <i>O. aureus</i>	
California mozambique <i>O. mossambica</i>	
Tilapia nilotica x California mozambique <i>O. niloticus</i> x <i>O. mossambica</i>	

