

17-Alpha Methyltestosterone Clinical Field Trials - INAD 11-236

Addendum to 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 (MET). Specifically, this compound has been used effectively in the U. S. under compassionate INAD Exemption #11-236 in early life stage tilapia to produce predominately male populations (i.e., sex reversal). In calendar year 2004 (CY04) the efficacy of MET was evaluated in two trials **not previously reported** involving 162,000 early life stage tilapia for sex reversal. Trials were conducted at one commercial tilapia culture facility. The compassionate study protocol under which treatments were administered allowed the investigator to administer MET at a dosage of 9 mg MT/kg body weight for 28 consecutive days. Overall, results **from these two additional** trials conducted in CY04 indicated that all treatments appeared effective.

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 **two additional trials conducted** in 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

A total of **two additional** MET field efficacy trials were conducted on early life stage tilapia at one commercial fish culture facility. Water temperature during trials was 75.2 °.

2. Test article used

The MET used during these trials was 17 beta-hydroxy-17-methylandrosterone-4-ene-3-one. All MET medicated feed used 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, the Investigator was allowed to only use MET at a dosage of 9 mg MT/kg body weight for 28 consecutive days (i.e., the standard dosage).

Fish Species Involved in CY04 Trials

1. Species of fish treated

Tilapia were the only fish species treated in **these two additional trials**. Treated fish were approximately 8 mm in total length. Tilapia species that were treated included:

1. Nile tilapia *Oreochromis niloticus*
2. California mozambique *O. mossambica*

Data Collected

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

The efficacy of MET medicated feed to produce populations of tilapia comprised of > 90% males was the primary response variable of clinical field efficacy trials.

The sex of individual fish was determined by evaluation of gonadal tissue prepared according to procedures 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

CY04, no samples were submitted **for these two additional trials**. Investigators

were also allowed to perform a gross visual examination of external features

characteristic of fish that had reached, or were very close to reaching, sexual

maturity. Results from gross visual examinations were submitted with both trials

submitted during this period.

2. Secondary response variables

Secondary parameters include general observations on fish behavior and

response to routine culture activities. Secondary parameters of interest included

evaluating 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 trials characterized as effective; Table 2 provides summary data for **both trials**, and Table 3 provides a list 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 (Table 1). Treatments were determined to be effective by the Investigators who visually evaluated the treated fish a few months after the treatment period. Results from both trials in which sex was determined using this technique indicated that MET treatment was effective.

2. Observed Toxicity

No toxicity or adverse effects relating to MET treatment were reported in either of **these two additional trials** conducted in CY04.

Summary of Study Results

MET was administered at the standard dosage **in two additional trials** conducted at one commercial fish culture facility on two different species of tilapia that involved treating a total of 162,000 fish. Treated fish were 8 mm in total length. Water temperature during the trials was 75.2 °F. Overall results in both cases where gross visual exams were performed to determine sex of fish showed that treatment appeared effective. No evidence of toxicity or adverse effects related to MET treatment was reported in either of the trials. However, based on an 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 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)
Simaron Fresh Water Fish Inc.	1	Nile Tilapia	8.00	72,000	28	9	75.2
	1	California mozambique	8.00	90,000	28	9	75.2

Table 2. Summary Data for Two Additional CY 2004 MET Treatment Trials

Total Fish Treated:	<u>162,000</u>
Number of fish treated in efficacious trials	162,000
Total number of trials:	2
Efficacious trials	2 (100%)
Gross visual examination	2 trials
Treatment Regimen Used:	
9 mg/kg bw/day for 28 days	2 trials
Treatment Water Temperature (°F):	
Temperature	75.2
Size of Treated Fish (mm):	
Size Range	8
Tilapia Strains Treated:	
<u>Tilapia</u>	
Nile tilapia <i>Oreochromis niloticus</i>	
California mozambique <i>O. mossambica</i>	