2015 NCRAC ANNUAL PROGRESS REPORT

Project Title: Drug Approval Research on 17 α-Methyltestosterone (Official Transfer of 17 α- Methyl testosterone [MT] Analytical Method for Feed) [Termination Report]
Key Word(s): Aquaculture Drugs
Dates of Work: September 1, 2009-August 31, 2015.
Total Funds Committed: $54,615
Participants: Mark P. Gaikowski, USGS, Upper Midwest Environmental Sciences Center, Wisconsin; Nilmini Wijewickreme, Reference Laboratory, CANTEST Ltd., British Colombia
Extension Liaison: Kevin Fitzsimmons, University of Arizona
Industry Liaison: Mark Willows, Binford Eagle Fisheries, North Dakota
Reason for Termination: Project objectives completed and funds have been terminated.

Project Objectives

1. Develop study protocols to conduct the MT feed method transfer of the MT analytical feed method.
2. Submit method transfer study protocols to the Center for Veterinary Medicine (CVM) for concurrence.
3. Provide final study protocols to participating laboratories.
4. Prepare and ship medicated feed to participating laboratories.
5. Assay control and medicated feed samples according to the study protocols concurred with by CVM.
6. Complete report of analysis and submit along with raw data to the Upper Midwest Environmental Sciences Center (UMESC).
7. Compare and discuss the results of both the CANTEST, Ltd. (CANTEST) reference (expert) and transferred (naïve) analyses of the MT transfer study samples based on the MT analytical feed method developed by the University of Wisconsin-Madison (UW- Madison).
8. Determine whether any changes are needed to the MT analytical feed method developed UWMadison based on the results of the MT feed transfer study.
9. Validate that the naïve analyst at CANTEST can analyze the MT feed samples according to the analytical feed method developed by UW-Madison.
10. Compile Final Study Report (FSR), archive raw data, and submit FSR to CVM through the UMESC MT investigational new animal drug (INAD) exemption.
11. Respond to CVM comments.
12. Gain acceptance from CVM for the MT feed method transfer study.

Project Summary

The approval of 17α-methyltestosterone (MT) medicated feed for use in tilapia to produce male fish would be of significant benefit to the industry. Tilapia is now the fifth most consumed seafood in the United States. Male fish grow faster than do their female counterparts, and by using all male fish, reproduction can be minimized or eliminated in grow-out systems, further benefiting growers. Approval of MT will allow all tilapia producers to have legal access to MT without an investigational new animal drug permit and will provide them with a legal means to yield increased biomass, thus resulting in more revenue for those producers. The production of male populations of tilapia is

important to the U.S. tilapia industry if they are to remain competitive with foreign producers of tilapia. The remaining data requirements necessary for MT approval include a method transfer trial where naive and experienced laboratories process MT medicated feed. Data from the sources must match for the transfer trial to be successful, and the method to be accepted by CVM.

Technical Summary and Analysis
Objective #1: Protocol was developed.

Objective #2: Protocol was accepted by CVM.

Objective #3: Protocol submitted to participating laboratory.

Objective #4: Prepare and ship medicated feed to participating laboratories. Feed was shipped.

Objective #5: Feed samples were analyzed by the participating laboratory.

Objective #6: Reports from expert and naive analysts were received from the participating laboratory.

Objective #7 All data were reviewed.

Objective #8: The method was revised and a new standard operating procedure developed.

Objective #9: Study was conducted between December 2009 and June 2010.

Objective #10: Final report was compiled and the report and all associated data submitted to the UMESC archives and to CVM for review.

Objective #11: Respond to CVM comments. UMESC made a final response to CVM in July 2015 addressing all CVM comments.

Objective #12: All data were accepted by CVM.

Principal Accomplishments
The production of male tilapia populations is critical to the U.S. tilapia industry if producers are to remain competitive with foreign tilapia producers. Approval of 17-α methyltestosterone (MT)-medicated feed for use in tilapia to produce greater than 80% phenotypic male populations would be of significant benefit to U.S. producers because male tilapia generate more biomass with less effort and time making them more cost efficient to raise.

A data requirement needed for the approval of an original new animal drug application (NADA) for MT use in tilapia is a requirement to validate the method for determining MT concentrations

in fish feed containing MT. The validation requirement is termed a method transfer study where analysts naïve to the method procedures must fulfill method performance criteria when analyzing fish feed samples with the method.

The study was conducted in two phases, a familiarization phase and a method transfer trial phase. During the familiarization phase, analysts in the participating laboratory naïve to the method procedures successfully performed the method (met method performance criteria) when analyzing control feed and control feed fortified with MT at nominal concentrations of 30, 60, and 90 µg/g. During the method transfer phase, analysts in the reference laboratory experienced with the method procedures and analysts of the participating laboratory successfully performed the method when analyzing control feed, control feed fortified with MT at nominal concentrations of 30, 60, and 90 µg/g, and feed containing MT at a nominal concentration of 60 µg/g (Table 1).

The method for determining MT concentrations in fish feed marketed with the brand name Masculinizing Feed for Tilapia® was validated meaning the method fulfilled the method robustness criteria of a method transfer trial. Table 1. Summary of select results and performance criteria from reference and participating laboratory analysts processing control feed, control feed fortified with 17-α methyltestosterone (MT) at nominal concentrations of 30, 60, and 90 µg/g, and MT medicated feed. Parameter Reference laboratory results include Acceptance criteria Calibration curve linearity (R2) 0.99995 and 0.99991 0.99998 and 0.99999 ≥ 0.9950 MT concentration in control feed < 15 µg/g < 15 µg/g < 15 µg/g. Accuracy (mean % recovery) of MT from fortified feed 84.9% – 86.9% 96.9% – 105% 80 – 110% Precision (%RSD) from 2 days where five samples of MT medicated feed were analyzed each day 2.3% and 1.9% 1.7% and 8.4% < 10%.

Impacts

The industry now has a CVM accepted method to determine 17-MT concentrations in fish feed. The method will be used to verify 17-MT concentrations in fish feed when the medicated feed is produced for the fish farmer.

Recommended Follow-Up Activities

No follow up studies are projected

Publications, Manuscripts, Workshops, and Conferences

See the Appendix for a cumulative output for all NCRAC-Funded Aquaculture Drugs activities.

Table 1. Summary of select results and performance criteria from reference and participating laboratory analysts processing control feed, control feed fortified with 17-α methyltestosterone (MT) at nominal concentrations of 30, 60, and 90 µg/g, and MT medicated feed.

Parameter	Reference laboratory results	Participating laboratory results	Acceptance criteria
Calibration curve linearity (R^2)	0.99995 and 0.99991	0.99998 and 0.99999	≥ 0.9950
MT concentration in control feed	< 15 µg/g	< 15 µg/g	< 15 µg/g
Accuracy (mean % recovery) of MT from fortified feed	84.9% – 86.9%	96.9% – 105%	80 – 110%
Precision (%RSD) from 2 days where five samples of MT medicated feed were analyzed each day	2.3% and 1.9%	1.7% and 8.4%	< 10%