



U.S. Fish & Wildlife Service

Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN**Efficacy of Terramycin® 200 for Fish (Oxytetracycline dehydrate) Feed to Control Mortality in Juvenile Channel Catfish Due to Columnaris**Niccole Wandelaar¹, Julie Schroeter¹, Shane Ramee¹, and Marilyn Blair¹¹*U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
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Columnaris (causative agent, *Flavobacterium columnare*) is a common bacterial disease that affects all freshwater finfish. It can rapidly infect a population and cause large scale mortality, if not promptly treated. Infections begin externally as the bacteria invades the epithelium commonly including the gill, buccal, opercular, dorsal, and caudal tissues. Clinical signs include the formation of erosive and necrotic epithelial lesions that begin as light colored patches with slight peripheral hemorrhaging and develop into large circular ulcers covered by yellowish mucoid material. Lesions commonly form at the base of the dorsal and/or caudal fin leading to the common names such as fin rot, caudal disease, and saddleback disease. Infections may become systemic, but only after significant epithelial damage has occurred. If left untreated, every member of the population may become infected. In the U.S. Channel Catfish industry, *F. columnare* is the second most prevalent disease and mortality causing bacterium, with yearly losses estimated at 30 million dollars (Delercq et al. 2013).

In the U.S., only two medicated feed products are approved by the U.S. Food and Drug Administration (FDA) to treat cultured fish diagnosed with systemic columnaris. Terramycin® 200 for Fish (TM200; 44% oxytetracycline dihydrate; OTC; Phibro Animal Health, Corp., Teaneck, NJ) is approved for use on all freshwater-reared Rainbow Trout *Oncorhynchus mykiss*.

AQUAFLO® (50% florfenicol; Merck Animal Health, Roseland, NJ) is approved for use on all freshwater-reared finfish.

Oxytetracycline is a broad-spectrum antibiotic with bacteriostatic properties that is active against a wide variety of gram-positive and gram-negative bacteria (Hochstein et al. 1953). As a member of the tetracycline family, it is used mainly in treating infections caused by gram-negative bacterial pathogens (Rigos et al., 2006). As a chemotherapeutant, it can be administered via enteral or parenteral routes with good tissue distribution (Stoffregen et al. 1996). Since its isolation and development in 1950, OTC has become one of the most commonly used antibiotics in aquaculture (Xu and Rogers 1994; Rigos et al. 2004).

In an effort to expand the current TM200 label, AADAP coordinated a field trial to evaluate the effectiveness of TM200 to control mortality in freshwater-reared Channel Catfish (CCF) *Ictalurus punctatus* caused by columnaris disease.

Methods

The study was conducted at the Florida Bass Conservation Center, Richloam State Fish Hatchery (Richloam SFH, Webster, FL) from September 24 – October 20, 2018. Test fish were CCF fingerlings (mean length, 64.3 mm; mean weight, 5.5 g). The 26-day study consisted of a 2 day acclimation period, a 10 day treatment period, and 14 day post-treatment period. During the treatment period, fish were fed either TM200 feed (Skretting Medicated Steelhead 2.5 mm), which was top-coated to deliver 3.75 g oxytetracycline/100lb of fish/ day, or a control diet (Skretting Extruded Floating Classic Fry 2.5 mm).

Ten days before the start of the study, the source population of test fish began to exhibit clinical signs and increased mortality indicative of columnaris disease. The day before the start of the study, 10 fish were sampled for a complete diagnostic work-up by the staff of the Richloam SFH. Of this sample, 8/10 fish were presumptively diagnosed with columnaris based on the

bacterial morphology seen on wet mounts of the skin and gills. The infections were confirmed to be systemic by culturing samples from the posterior kidney on plates of Shieh media. The resulting colonies demonstrated the characteristic yellow rhizoid growth, and were confirmed to be *F. columnare* via Polymerase Chain Reaction (PCR) at the USFWS Bozeman Fish Health Center (Bozeman, MT).

Before fish were stocked into the study tanks, the mean weight of the fingerling CCF was determined to average 5.5 g/fish. Approximately 1,900 CCF were randomly stocked by weight into each 387.2 L experimental tank, for a stocking density of 27 g/L. Four replicate tanks were randomly assigned to the TM200 and control treatments for a total of eight experimental tanks. Study participants that conducted the feeding and daily monitoring of the tanks were blinded from their respective treatments. Fish were fed 1.875% of their estimated body weight (212.6 g/tank/day) of the appropriate food, which was split into two feedings per day. Feed for each tank was weighed out ahead of time by non-blinded study participants. Cumulative mortality did not reach the *a priori* level (25%) that would have required adjustment of the feeding rates.

To verify the concentration and homogeneity of OTC in the feed, 6 samples were taken from the top, middle, and bottom of the feed bag and analyzed via high pressure-liquid chromatography (conducted by Phibro Animal Health Corporation, State College, PA). Two samples were also taken from the control feed to ensure there was no OTC in the control diet. Mortality, fish behavior, feeding appetite, temperature, and dissolved oxygen (DO) were recorded daily for each experimental tank by masked study participants. Moribund fish were sampled and submitted for *F. columnare* diagnosis, as above. Hardness, alkalinity, ammonia nitrogen, and pH were measured at least once during the study period. At the end of the treatment period, all fish were weighed out of each tank and held for the minimum investigational withdrawal period of 40 days.

The study focused on the proportional risk of death versus survival within each test tank with the feed treatment as the primary predictor variable. Probability of death was modeled with a mixed effects logistic model using SAS PROC GLIMMIX (logit link; SAS 2012; Wolfinger and O'Connell 1993). Cumulative mortality was compared between treatments on all treatment and post-treatment days. Significance was based on an α of 0.05.

Results and Discussion

Mean cumulative mortality at the end of the study in TM200 treated tanks (0.82%, range of 0.63-1.16%) was significantly less ($P = 0.0065$) than that of the control tanks (2.38%, range of 1.76-3.49%; Figure 1). Significant differences in cumulative mortality were detected on every day between day 3 and day 24. General behavior was normal during all observations except one, and all the food was consumed during all feedings except one.

During the study period, 33 moribund fish were sampled from all tanks for diagnostics, and all samples were confirmed positive for *F. columnare* via PCR. All of the 12 fish collected during the post-treatment period were presumptively diagnosed with *F. columnare* and subsequently confirmed positive with PCR.

The mean concentration of OTC in the samples of TM200 feed was 1.6 g/lb feed (range 1.5-1.7 g/lb). This means that the actual average dose administered to the tanks was 3.0 g OTC/100lbs fish/day, which was 80% of the target dosage. OTC was not detected in the control feed.

Water hardness, alkalinity, and pH of the source water were 209 mg/L (as CaCO_3), 288.5 mg/L (as CaCO_3), and 7.63, respectively. Overall mean water temperature in the test tanks was 23.8°C and mean DO concentration was 12.7 mg/L. All water quality variables measured were within ranges considered suitable for rearing healthy CCF (personal communication, Derek Piotrowicz).

In conclusion, TM200-medicated feed administered at a dose of 3.0g OTC/100 lbs fish/d for 10 consecutive days was effective at reducing mortality in freshwater-reared CCF fingerlings diagnosed with systemic columnaris. Results from this study were submitted to the FDA Center for Veterinary Medicine demonstrating the efficacy of OTC at the target dose to support expanding the use of TM200 in U.S. aquaculture to include treatment of systemic columnaris in all freshwater-reared finfish.

Acknowledgments

We thank Derek Piotrowicz, Richard Stout, Melissa Garnett, Katherine Childress and Arthur Agdeppa, of the Florida Bass Conservation Center for conducting the study, conducting fish health procedures, and shipping samples for PCR confirmation. We also thank Renee Martin and Rick Cordes of the U.S. Fish and Wildlife Service's Bozeman Fish Health Center, Bozeman, MT, for performing PCR to confirm the presence of the pathogen; and Melissa Whitsel of Phibro Animal Health Corporation, State College, PA, for performing dose verification and preparing the analytical report. We also Thank David Burbank for conducting an independent review of this publication.

References

- Delercq A. M., F. Haesebrouck, W. V. den Broeck, P. Bossier, and A. Decostere. 2013. Columnaris disease in fish: A review with emphasis on bacterium-host interactions. *Veterinary Research* 44:27.
- Hochstein, F. A., and eight coauthors. 1953. The structure of terramycin. *Journal of the American Chemical Society* 75:5455-5475.
- Rigos, G., I. Nengas, M. Alexis, and F. Athanassopoulou. 2004. Bioavailability of oxytetracycline in sea bass, *Dicentrarchus labrax* (L.). *Journal of Fish Diseases* 27: 119-122.
- Rigos, G., I. Nengas, and M. Alexis. 2006. Oxytetracycline (OTC) uptake following bath treatment in gilthead sea bream (*Sparus aurata*). *Aquaculture* 261:1151-1155.
- Stoffregen, D.A., P.R. Bowser, and J.G. Babish. 1996. Antibacterial chemotherapeutants for finfish aquaculture: a synopsis of laboratory and field efficacy and safety studies. *Journal of Aquatic Animal Health* 8:181-207.
- Wolfinger, R., and M. O'Connell. 1993. Generalized linear mixed models: a pseudolikelihood approach. *Journal of Statistical Computation and Simulation* 48:233-243.
- Xu, D., and W. A. Rogers. 1994. Oxytetracycline residue in striped bass muscle. *Journal of Aquatic Health* 6:349-354.

Figure 1: Mean percent cumulative mortality of CCF in treated (n = 4) and control (n = 4) tanks during the 24 day TM200-medicated feed study.

