



Efficacy of AQUAFLO[®] (50% Florfenicol) to Control Mortality in Largemouth Bass Diagnosed with Systemic Columnaris Disease

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Nutrient-rich organic materials in the aquatic environment harbor bacteria that can cause disease in cultured fish (Plumb 1999). One such Gram-negative bacterium is *Flavobacterium columnare*, causative agent of columnaris disease.

Columnaris - also known as saddleback, cotton wool, or mouth fungus - is a chronic-to-acute, external-to-systemic disease often associated with stressful rearing conditions (Winton 2001). If left untreated, columnaris can cause substantial mortality in affected populations.

In the U.S., only two products are approved by the U.S. Food and Drug Administration (FDA) for use to control mortality in cultured fish populations diagnosed with systemic columnaris, and such use is restricted to a limited number of fish species. Terramycin[®] 200 for Fish (44% oxytetracycline dihydrate; Phibro Animal Health, Corp., Ridgefield Park, New Jersey USA) is approved for use on all freshwater-reared *Oncorhynchus mykiss*. AQUAFLO[®] CA-1 (50% florfenicol; Intervet/Schering-Plough Animal Health Corp., Roseland, NJ) is conditionally approved for use on catfish.

AQUAFLO[®] (50% florfenicol; Intervet/Schering-Plough Animal Health Corp., Roseland, NJ) is a sister product to AQUAFLO[®] CA-1. AQUAFLO[®] is approved for the control of mortality in all freshwater-reared salmonids due to furunculosis (causative agent, *Aeromonas salmonicida*) and coldwater (causative agent, *Flavobacterium psychrophilum*) diseases, and in catfish due to enteric septicemia (causative agent, *Edwardsiella ictaluri*). Florfenicol, the active ingredient in both products, is a broad-spectrum antibiotic with bacteriostatic and bactericidal properties and is active against a variety of Gram-positive and Gram-negative bacteria. Legally, both products must be used under veterinary prescription and administered at a dosage of 10 mg florfenicol per kg fish per d for 10 consecutive days.

Pursuant to future label expansions, AQUAFLO[®] is also being used experimentally to determine its efficacy against systemic columnaris in a variety of fish species. As part of the experimental-use program, we coordinated a field trial to evaluate the efficacy of AQUAFLO[®] to control mortality in

freshwater-reared largemouth bass *Micropterus salmoides* (LMB) diagnosed with systemic columnaris.

Methods

The trial was conducted May 20 through June 13, 2009 at the Florida Bass Conservation Center's, Richloam Fish Hatchery (RFH), Webster, Florida USA. Test fish were LMB fingerlings (mean weight, 3.3 g). AQUAFLO[®]-medicated feed was administered at a target dosage of 10 mg florfenicol per kg fish per d for 10 consecutive days.

Before the trial began, systemic columnaris had been presumptively diagnosed in a reference population of fingerling LMB. Reference population fish were then impartially collected by dipnetting, weighed, and randomly allocated among ten 382-L test tanks (5 treated and 5 control; mean, 473 fish/tank). Treatment conditions (AQUAFLO[®]-treated vs. nontreated control) were allocated among tanks systematically without bias. Tanks were supplied with first-pass water at flow and turnover rates suitable for rearing LMB.

The 25-d trial comprised 1-d acclimation, 10-d treatment, and 14-d posttreatment periods. During the treatment period, AQUAFLO[®]-medicated feed was administered to treated tanks and nonmedicated feed was administered to control tanks. During the posttreatment period, nonmedicated feed was administered to all tanks. During the treatment and posttreatment periods, feed was administered to tanks at 5.3% mean body weight/tank/d, and amounts were not adjusted for growth.

Mortality, general fish behavior, feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily. During treatment and posttreatment periods external and internal gross necropsies were performed on selected moribund fish. In addition, skin scrapes and kidney tissue from each fish necropsied were examined microscopically to presumptively identify cause of mortality. Bacterial isolates were cultured on Shieh's media, and confirmatory diagnosis was made by polymerase chain

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reaction. Florfenicol concentrations in medicated and nonmedicated feed samples were analytically verified by Eurofins Scientific Inc.; Portage, Michigan USA.

Results and Discussion

At the end the trial, mean cumulative mortality in treated tanks (5.7%; range, 3.9 - 8.0% per tank) was less than mean cumulative mortality in control tanks (12.0%; range, 9.3 - 14.0% per tank) (Figure 1). This result demonstrated a clinical reduction in mortality in treated fish compared to nontreated fish. Bacterial isolates recovered from moribund fish before the start of the trial positively confirmed *Flavobacterium columnare* as the cause of mortality. Necropsy results were also consistent with systemic columnaris, and indicated no concomitant pathogens present during the trial.

In both treatment groups, general fish behavior improved from 90% normal at the beginning to 100% normal at the end of the treatment period. The predominant abnormal behavior observed during this period was lethargy, with occasional hyperactivity. Hyperactivity was noted in only one treated tank early in the experiment but not observed otherwise during the treatment period. During the posttreatment period, fish in all tanks appeared to behave normally. Throughout the trial, feeding behavior was characterized as aggressive in all control tanks and in four of five treated tanks. Fish in one treated tank exhibited semiaggressive feeding during the first 7 d of treatment.

The mean analytically verified florfenicol dose administered to fish was 11.1 mg florfenicol per kg fish per d. No florfenicol was detected in control feed.

Mean water temperatures and dissolved oxygen concentration during the trial were 23.9°C (range, 22.6 - 24.9°C) and 13.7 mg per L (range, 12.0 - 14.6 mg per L) respectively. Mean water hardness (353 mg per L; as CaCO₃), alkalinity (380 mg per L; as CaCO₃), and pH (range, 7.7 - 7.8) were normal and within ranges suitable for rearing LMB at RFH.

In conclusion, AQUAFLO[®]-medicated feed administered at 10 mg florfenicol per kg fish per d for 10 consecutive days resulted in a clinical reduction in mortality in freshwater-reared LMB fingerlings diagnosed with systemic columnaris. This result was accepted by the FDA's Center for Veterinary Medicine as demonstrating the efficacy of florfenicol at the target dose, and, in combination with similar data from additional fish species, will be used to support expanding the approved use of AQUAFLO[®] in U.S. aquaculture to include treatment of systemic columnaris in all freshwater-reared, warmwater finfish.

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Figure 1. Mean (\pm SD) percent cumulative mortality (treated tanks vs. control tanks) of largemouth bass fingerlings diagnosed with systemic columnaris disease. Treatment period equals trial days 1 - 10.

