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DRUG RESEARCH INFORMATION BULLETIN

Efficacy of AQUAFLOR® (50% Florfenicol) to Control Mortality in Bluegill Diagnosed with Systemic Columnaris Disease

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Flavobacterium columnare (causative agent of columnaris disease) is a Gram-negative bacterium that is often associated with high levels of mortality in a variety of freshwater fish species. Columnaris disease is a chronic-to-acute, external or systemic disease, which can infect virtually any wild or cultured fish (Plumb 1999). The worldwide distribution of columnaris makes it one of the most important diseases affecting aquaculture today (Morrison et al. 1981; Post 1987; Wagner et al. 2002; Thomas-Jinu and Goodwin 2004). As with most bacterial diseases, maintaining proper environmental conditions and practicing good management procedures can reduce the severity and occurrence of disease outbreaks (Post 1987; Jeney and Jeney 1995). Although columnaris is primarily an external disease, it may become systemic with or without advanced skin and gill necrosis (Noga 2000). Historically, systemic infections have been treated with oral or parenteral (e.g., intravenous or intramuscular injection) antibiotic administration (Tripathi et al. 2003).

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

AQUAFLOR® (50% florfenicol; Intervet/Schering-Plough Animal Health Corp., Roseland, New Jersey) is a sister product of AQUAFLOR® CA-1. AQUAFLOR® is approved for the control of mortality in all freshwater-reared salmonids due to furunculosis (causative agent, *Aeromonas salmonicida*) and coldwater disease (causative agent, *Flavobacterium psychrophilum*); 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/kg fish/d for 10 consecutive days.

In the interest of expanding the current label for AQUAFLOR®, additional potential uses in aquaculture are being tested experimentally. As such, this bulletin summarizes the results of a field trial conducted to evaluate the efficacy of AQUAFLOR® to control mortality in freshwater-reared bluegill Lepomis macrochirus (BLG) diagnosed with systemic columnaris.

Methods

The trial was conducted November 20 – December 14, 2009, at the Florida Bass Conservation Center, Richloam Fish Hatchery (RFH), Webster, Florida. Test fish were BLG fingerlings (mean weight, 25.2 g). AQUAFLOR®-medicated feed was administered at a target dosage of 10 mg florfenicol/kg fish/d for 10 consecutive days.

Before the trial began, fish health evaluations of moribund fish from a reference population of fingerling BLG indicated a presumptive diagnosis of systemic columnaris. Reference population fish were then impartially collected by dipnetting, weighed, and randomly allocated among eight 382-L test tanks (4 treated and 4 control; 100 fish/tank). Treatment conditions (AQUAFLOR®-treated vs. nontreated control) were allocated among tanks using a completely randomized design (CRD). Tanks were supplied with first-pass water at flow rates suitable for rearing BLG.

The 25-d trial comprised 1-d acclimation, 10-d treatment, and 14-d posttreatment periods. During the treatment period, AQUAFLOR®-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 2.0% of 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. Hardness, alkalinity, and pH of source water were measured twice during the trial.

During treatment and posttreatment periods, necropsies were performed on selected moribund fish. Kidney imprints from each fish necropsied were examined microscopically to presumptively identify *F. columnare*. Kidney tissue was inoculated on Shieh's medium and resulting bacterial colonies were assayed by polymerase chain reaction (PCR) to confirm *F. columnare*. Florfenicol concentrations in medicated and nonmedicated feed samples were analytically verified by Eurofins Scientific Inc., Portage, Michigan.

The SAS PROC GLIMMIX procedure was used to statistically compare mean cumulative mortality in control tanks to that in treated tanks on each day of the treatment and posttreatment periods. Treatment differences were judged significant if $P < 0.05$.

Results and Discussion

At the end of the trial, mean cumulative mortality in treated tanks (19%; range, 11 – 27% per tank) was significantly less ($P = 0.0098$) than mean cumulative mortality in control tanks (38%; range, 28 – 43% per tank; Figure 1). PCR assay of cultured bacterial isolates positively confirmed *F. columnare* as the cause of mortality.

In both treatment groups, feeding behavior ranged from nonaggressive to aggressive during the first 6 d of the treatment period, but fish in all tanks were feeding aggressively by 7 d. Throughout the trial, general fish behavior was characterized as normal.

The analytically verified florfenicol dose administered to fish was 10.0 mg florfenicol/kg fish/d. No florfenicol was detected in control feed.

Mean water temperatures and dissolved oxygen concentration during the trial were 22.3°C (range, 20.3 – 23.8°C) and 10.5 mg/L (range, 9.1 – 11.1 mg/L) respectively. Mean water hardness (365 mg/L $CaCO_3$), alkalinity (340 mg/L $CaCO_3$), and pH (range, 7.9 – 8.0) were normal and within ranges suitable for rearing BLG at RFH.

Based on results from this trial, we concluded that AQUAFLOR® -medicated feed was efficacious in controlling mortality when administered at 10 mg florfenicol/kg fish/d for 10 consecutive days to BLG fingerlings diagnosed with systemic columnaris. The results from this trial have been summarized in a final study report and submitted to FDA's Center for Veterinary Medicine in support of expanding the approval of AQUAFLOR® in U.S. aquaculture to include use to control mortality in all freshwaterreared, warmwater finfish due to systemic columnaris disease associated with *F. columnare*.

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Figure 1. Mean (± SD) percent cumulative mortality (treated tanks vs. control tanks) of bluegill fingerlings diagnosed with systemic columnaris disease. Treatment period equals trial days 1 – 10.