



Efficacy of AQUAFLO[®] (50% Florfenicol) to Control Mortality in Freshwater-Reared Sunshine Bass (female *Morone chrysops* × male *M. saxatilis*) Naturally Infected with *Streptococcus iniae*

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Intensification of freshwater and marine aquaculture has led to the emergence of several systemic bacterial fish diseases caused by Gram-negative or Gram-positive pathogens (Winton 2001). To combat such diseases, four antimicrobial compounds (sulfamerazine, oxytetracycline dihydrate, sulfadimethoxine / ormetoprim, and florfenicol) have been approved as medicated-feed articles by the U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine. However, none of these compounds has been approved by FDA for use to control mortality in freshwater-reared finfishes due to infections caused by the Gram-positive bacterium, *Streptococcus iniae*.

Streptococcus iniae infection in freshwater-reared sunshine bass (the most common of the five striped bass hybrids) was first diagnosed and experimentally treated by Stoffregen et al. (1996). However, relatively few controlled studies have since been conducted to evaluate the efficacy of antimicrobials to control such *S. iniae*-induced mortality (Darwish and Ismaiel 2003; Darwish 2007).

AQUAFLO[®] (Intervet/Schering Plough Animal Health Corporation, Summit, NJ) is a 50% florfenicol premix for inclusion into fish feed. Florfenicol is a broad-spectrum antibiotic with both bacteriostatic and bacteriocidal properties. Although the FDA has approved AQUAFLO[®] as a Veterinary Feed Directive (VFD) drug for a variety of claims, AQUAFLO[®] has not yet been approved for *S. iniae*. A VFD drug can only be administered on the order of a licensed veterinarian, and extra-label use is prohibited. Currently, AQUAFLO[®] can only be legally administered at 10 mg florfenicol/kg fish/d for 10 consecutive days.

In this bulletin, we summarize two field trials conducted to evaluate the efficacy of AQUAFLO[®] to control mortality in freshwater-reared sunshine bass naturally infected with *S. iniae*.

Methods

Trials were conducted under INAD No. 10-697 at the Kent

SeaTech Corporation aquaculture facility, Mecca, CA. Trial 1 (2001) was conducted on fingerlings (mean weight = 44 g). Trial 2 (2003) was conducted on subadults (mean weight = 377 g). In both trials, AQUAFLO[®]-medicated feed was administered at a target of 10 mg florfenicol/kg fish/d for 10 d.

In each trial, reference populations of fish were presumptively diagnosed with *S. iniae*. Impartially collected reference population fish and treatment conditions were randomly assigned to six 158-L test tanks (three treated and three control tanks). In Trial 1, there were 100 fish/tank. In Trial 2, there were 50 fish/tank. Each trial comprised ≤1-d acclimation, 10-d treatment, and 14-d posttreatment periods.

During treatment, AQUAFLO[®]-medicated feed was administered to treated tanks and nonmedicated feed was administered to control tanks. All tanks received the same base feed formulation, exclusive of drug. Mortality, general fish and feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily throughout each trial. Florfenicol concentrations in medicated and nonmedicated feed samples were analytically verified by the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC), La Crosse, WI (Trial 1) and Eurofins Scientific, Memphis, TN (Trial 2).

In each trial, SAS PROC GLIMMIX (logit link) was used to compare mean cumulative mortality in control tanks to that in treated tanks. Treatment conditions (treated vs. control) were considered significantly different if $P < 0.05$.

Results and Discussion

In Trial 1 (Figure 1), mean cumulative mortality in treated tanks was significantly less than that in control tanks from Trial Day 4 through Trial Day 17. However, during the posttreatment period, a *S. iniae* re-infection apparently occurred in one of the treated tanks. Consequently, at the end of Trial 1, mean (± SD) cumulative mortality in treated tanks (32.7 ± 7.4%) was not sig-

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nificantly different ($P = 0.389$) from that in control tanks ($37.3 \pm 3.8\%$). In Trial 2 (Figure 2), mean (\pm SD) cumulative mortality in treated tanks ($19.3 \pm 11.0\%$) was significantly less ($P = 0.040$) than that in control tanks ($52.0 \pm 13.1\%$) at the end of the trial. In both trials, test fish appeared to feed and otherwise behave normally.

In both trials, florfenicol doses administered were within FDA-acceptable limits (80 – 110% of target). In Trial 1, AQUAFLO[®]-medicated feed was administered at 9.8 mg florfenicol/kg fish/d (98% of target). In Trial 2, AQUAFLO[®]-medicated feed was administered at 8.3 mg florfenicol/kg fish/d (83% of target). In both trials, no florfenicol was detected in control feed.

Mean water temperatures and dissolved oxygen concentrations in Trial 1 (30.4°C and 12.6 mg/L) and Trial 2 (26.6°C and 13.3 mg/L) were normal for the time of year trials were conducted at the Kent SeaTech aquaculture facility and typical for the freshwater-rearing of sunshine.

The outcome of Trial 2 was clearly efficacious, but the outcome of Trial 1 was not. However, the combined results of these trials were accepted by FDA as demonstrating the efficacy of AQUAFLO[®] administered orally in feed at a target dose of 10 mg florfenicol/kg fish/d for 10 d to control mortality in sunshine bass associated with *S. iniae* infection. Consequently, we anticipate the results of both trials (online at www.fws.gov/fisheries/aadap/studiesFlorfenicol.htm) will contribute to a U.S. approval for this label claim.

Acknowledgments

Chue Vue, UMESC, analytically verified florfenicol concentrations in feed samples (Trial 1). Mark Gaikowski, UMESC, analyzed mortality data. Tom Bell and Dave Erdahl, AADAP, critically reviewed this bulletin.

References

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Figure 1. Trial 1: Mean percent cumulative mortality in treated and control tanks (error bar = ± 1 SD).

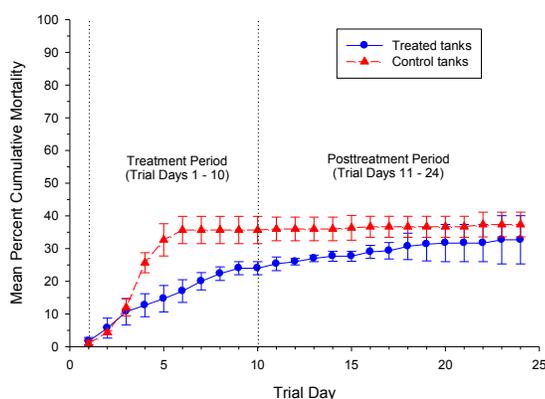


Figure 2. Trial 2: Mean percent cumulative mortality in treated and control tanks (error bar = ± 1 SD).

