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Aquatic Animal Drug Approval Partnership

DRUG RESEARCH INFORMATION BULLETIN

Efficacy of 35% PEROX-AID® (Hydrogen Peroxide) to Control Mortality in Fingerling Largemouth Bass Diagnosed with External Columnaris Disease

Molly P. Bowman, James D. Bowker, and Daniel Carty

*U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road, Bozeman, Montana, 59715, USA*

Columnaris (causative agent, *Flavobacterium columnare*) is an acute-to-chronic external or systemic bacterial disease affecting freshwater-reared finfishes worldwide. Severity of the disease increases with increasing water temperature, and mortality in affected populations is especially high when water temperature exceeds 15°C (Noga 2000). During external columnaris out-breaks, fish culturists can often minimize mortality by improving environmental rearing conditions or administering chemotherapeutic bath treatments or both.

Hydrogen peroxide (H₂O₂) is one of several chemicals that has been used historically and tested experimentally for the control of mortality in freshwater-reared finfish populations diagnosed with external columnaris. However, it was not until January 2007 that the commercial product 35% PEROX-AID® (35% active H₂O₂; Eka Chemicals, Inc., Marietta, GA) gained U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) approval for use in certain finfish species. Currently, 35% PEROX-AID® is approved for use to control mortality in all freshwater-reared coolwater finfish and channel catfish *Ictalurus punctatus* (a commonly reared warmwater finfish) due to external columnaris associated with *F. columnare*. The approved treatment regimen is as follows: Use at 50 – 75 mg/L H₂O₂ as a static bath for 60 min/d on three alternate days.

Public and private aquaculture would benefit from a broadening of the currently approved product label to include use in all fresh-water-reared warmwater finfish. Consequently, and in accordance with FDA/CVM requirements, we conducted a controlled field efficacy trial with 35% PEROX-AID® on another commonly reared warmwater finfish, largemouth bass (LMB) *Micropterus salmoides*. This bulletin summarizes the results of that trial.

Methods

The trial was conducted in July 2007 at the Florida Bass Conservation Center's Richloam Fish Hatchery (Richloam) in Webster, FL. The test article was 35% PEROX-AID®, which was administered at a target concentration of 150 mg/L active H₂O₂. This dose was tested in an effort to expand the current FDA-approved label to allow use at a higher therapeutic dose. Test fish were fingerling LMB (mean length, 10.8 cm). A single production tank of LMB fingerlings was used as the reference population after several moribund fish from the tank were presumptively diagnosed with external columnaris.

Completely randomized design procedures were used to assign test fish and treatment conditions (treated versus nontreated controls) to test tanks. Rectangular, fiberglass test tanks (rearing volume, 379 L) were stocked with fish impartially collected from the reference population. Each treatment condition was replicated four times (N = 8 test tanks; N = 800 fish/tank).

The trial comprised a 1-d acclimation period, 3-d treatment period, and 14-d posttreatment observation period. During the treatment period, 35% PEROX-AID® was administered to treated tanks (N = 4) in a static bath for 60 min/d on three consecutive days, while a sham treatment of pure hatchery water was administered to control tanks (N = 4). Mortality, general fish behavior, feeding behavior, water temperature, and dissolved oxygen concentration data were collected daily during the acclimation, treatment, and posttreatment periods. Water samples were collected from each test tank 30 – 45 min into each treatment to titrimetrically verify H₂O₂ concentrations (Jeffery et al. 1989).

The SAS PROC GLIMMIX (logit link) procedure was used to (a) compare mean cumulative mortality in control tanks to that in treated tanks on each day of the treatment and posttreatment periods and (b) generate mean daily odds ratios. Mean daily odds ratios > 1 indicated that odds of mortality in control tanks were greater than odds of mortality in treated tanks. Treatment levels were judged statistically significant if P < 0.05.

Results

On the last day of the trial, mean cumulative mortality in treated tanks (17.6%; range, 12.1 – 22.8% per tank) was less than mean cumulative mortality in control tanks (21.1%; range, 13.3 – 26.0% per tank; Figure 1); however, the difference was not significant ($P = 0.38$). Significant differences in mortality between control tanks and treated tanks were, however, detected on trial days 10 - 14. During the trial, mean daily odds ratios were > 1.0 from the last day of the treatment period through the end of the posttreatment period. These results indicate that the odds of mortality in control tanks during this period were greater than the odds of mortality in treated tanks during the same period. Odds ratio during the entire study ranged from 0.9 to 1.6.

Overall mean H₂O₂ concentration administered to treated tanks was 153.8 mg/L (range, 136.0 – 165.8 mg/L). Hydrogen peroxide was not detected in the control tanks.

Mean (\pm SD) water temperature ($24.8 \pm 0.40^\circ\text{C}$) and mean (\pm SD) dissolved oxygen concentration (15.2 ± 1.8 mg/L) during the trial were suitable for rearing healthy LMB. General fish behavior was considered normal and no substantial differences were seen between treated and control tanks. Except on the first treatment day, when fish were characterized as feeding semi-aggressively, all fish in all tanks appeared to feed aggressively.

Discussion

Results from this trial demonstrated that 35% PEROX-AID[®] administered at a target concentration of 150 mg/L active H₂O₂ for 60 min daily in a static bath on three consecutive days was effective in controlling mortality caused by external columnaris in LMB for a period up to 10 d posttreatment. We suspect a columnaris re-infection occurred in all treated test tanks near posttreatment day 11, which in turn altered cumulative mortality results at the end of the trial. The mode of action for non-selective oxidative disinfectants such as H₂O₂ make them effective microbicides but typically provide little therapeutic benefit to preventing re-infections following treatment. Had a re-infection of fish in the treated tanks not occurred, we suspect that a statistically significant difference would have been detected throughout the remainder of the trial.

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References

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Figure 1. Mean percent cumulative mortality in treated and control tanks and mean daily odds ratios (control tanks:treated tanks) during the trial (bar = ± 1 SD).

