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COMMUNICATIONS

Hydrogen Peroxide Treatment during Egg Incubation Improves Channel Catfish Hatching Success

BRIAN C. SMALL* AND WILLIAM R. WOLTERS

U.S. Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, Thad Cochran National Warmwater Aquaculture Center, Post Office Box 38, Stoneville, Mississippi 38776, USA

Abstract.—Three trials were conducted to evaluate the effect of hydrogen peroxide (H_2O_2) treatment on the hatching success of channel catfish *Ictalurus punctatus* when administered during egg incubation as a 15-min bath or as a flow-through treatment. In the first trial, initial treatment with 100 mg povidone iodine/L followed by daily 15-min baths of 250 mg H_2O_2/L yielded a 26% increase ($P < 0.05$) in hatching success above controls. In the second trial, daily 15-min baths with H_2O_2 (250 mg/L) yielded a 30% increase ($P < 0.05$) in hatching success compared with povidone-iodine-treated controls and significantly improved hatching success compared with formalin-treated (1,600 mg/L) eggs. In the third trial, hydrogen peroxide was administered in flow-through hatching troughs. Egg masses treated with 70 mg H_2O_2/L had significantly improved ($P < 0.05$) hatching success compared with untreated controls (68.3% and 24.2%, respectively). The results of this research show that significant improvements in channel catfish hatching success can be obtained through the use of hydrogen peroxide as a cost-effective alternative to formalin.

Commercial channel catfish farmers typically report average hatch rates of 60–80%; however, fungal and bacterial egg infections can pose significant problems for hatcheries (Brunson 1992), resulting in highly variable hatching success. Potential infections are often controlled with chemical therapeutics such as povidone iodine or formalin (Brunson 1992; Walser and Phelps 1993). Povidone iodine is designated by the U.S. Food and Drug Administration (FDA) as a low regulatory priority (LRP) aquaculture drug when used as an egg surface disinfectant, and formalin is approved by the FDA for the control of fungi of the family *Saprolegniaceae* on the eggs of all finfish. Although formalin is an effective therapeutic, concerns of safety exist among users due to its suspected carcinogenicity and odoriferous nature. Hydrogen peroxide (H_2O_2) has been investigated as an alternative treatment to control egg diseases in a number of fish species (Rach et al. 1998; Ardt et al. 2001), and is currently designated by the FDA as an LRP aquaculture drug when administered at 250–500 mg/L. For the research presented here, three trials were conducted to evaluate the effect of hydrogen peroxide treatment on channel catfish hatching success when administered as a 15-min bath or as a flow-through treatment representative of a commercial hatchery situation.

Methods

The channel catfish *Ictalurus punctatus* eggs used in all three trials were collected from U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Catfish Genetics Research Unit (CGRU) ponds in Stoneville, Mississippi, during the months of May and June 2001 and 2002, and were from USDA103 strain channel catfish. The spawning containers were checked in the morning every 2 d for egg masses and microscopically staged to determine embryo development (Silverstein and Small, in press). Only fertilized eggs less than 24 h old were used at the start of these studies. No visible fungal growth was observed by gross observation in any of the egg masses prior to starting each trial. In the first trial, catfish eggs from three spawns of approximately 300–400 g each were subdivided into 12 equal masses and randomly assigned to thirty-six 76-L glass aquaria equipped with a wire hatching basket and an air stone. The number of eggs per mass was calculated after determining eggs per gram. Each aquaria was supplied with flowing well water (temperature = 26°C; pH = 8.6; total hardness = 120 mg/L; alkalinity = 410 mg/L; total ammonia nitrogen = 1.5 mg/L; nitrite nitrogen = 0 mg/L) at a rate of 7.6 L/min. Following collection and removal from the pond, all eggs were initially treated by immersion in a povidone iodine solution (Aquadine, Aquacenter, Leland, Mississippi) at a dose of 100 mg/L for 10 min. Hydrogen peroxide (Natchez Animal Supply, Natchez, Mississippi)

* Corresponding author: bsmall@ars.usda.gov

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treatments (0, 100, 250, and 500 mg/L) were randomly assigned to three aquaria per spawn and administered every afternoon until embryonic eye pigmentation became apparent. During treatment, the flow of well water to the aquaria was stopped for 15 min and then restored. The egg masses were allowed to hatch to completion within individual aquaria. When hatching was complete, the fry were siphoned into a graduated cylinder and the volume of fry was recorded. The total number of fry was calculated (number of fry in 1 mL × total volume of fry collected), and hatching success was determined as the percentage of eggs hatched.

In the second trial, a comparison was made between the optimal hydrogen peroxide treatment from the first trial and treatment with formalin (formalin-F, Natchez Animal Supply, Natchez, Mississippi) at a concentration of 1,600 mg/L and a povidone iodine-treated control. Catfish eggs from three spawns (300–400 g) were initially treated with a povidone iodine solution (100 mg/L for 10 min), then subdivided into nine equal masses and randomly assigned to twenty-seven 76-L glass aquaria. The environmental conditions were similar to the first trial. The treatments (control, 250 mg H_2O_2/L, and 1600 mg formalin/L) were randomly assigned to three aquaria per spawn and administered every afternoon until embryonic eye pigmentation became apparent. During treatment, the flow of well water to the aquaria was stopped for 15 min in all but the control aquaria and then restored. Hatching success was calculated as previously described.

In the third trial, the treatment of channel catfish eggs with H_2O_2 in a hatching trough with continuously flowing well water was evaluated. Initially catfish eggs from four spawns (300–400 g) were collected, subdivided into equal masses, and assigned to four 380-L spawning troughs. Each spawning trough received well water at 7.6 L/min, and contained three large air stones and four standard hatching baskets lined with 1.6-mm mesh. Hydrogen peroxide treatments (0, 100, 200, and 300 mg/L) were randomly assigned to the four troughs. The treatments were again administered every afternoon until embryonic eye pigmentation became apparent. Treatment consisted of adding the appropriate amount of hydrogen peroxide to the trough at the water inlet with a continuous flow of well water. Egg masses were allowed to hatch to completion within the hatching baskets. Hatching success was then calculated as previously described. This trial was repeated with four new egg masses and hydrogen peroxide treatments of 0, 35, 70, and 100 mg/L.

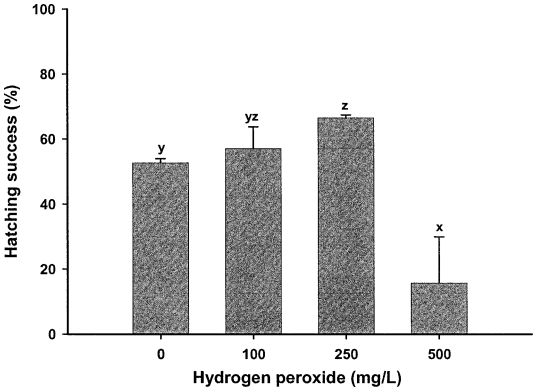

FIGURE 1.—Mean (+SE) hatching success for channel catfish eggs treated once daily until the eyed stage with increasing concentrations of hydrogen peroxide for 15 min. Significant ($P < 0.05$) differences are indicated by different letters.

The statistical comparisons for each trial were conducted using the SAS software system (SAS Institute 1996). The assumptions for homogeneity of variance and normality of the data were tested by examining the correlation between absolute residuals and predicted values, and the Shapiro–Wilkes *W* test for normality. Hatching success data, expressed on a percentage basis, were arcsine-transformed prior to an analysis of variance (ANOVA) using mixed-model procedures. Pairwise contrasts were used to identify significant differences at the 5% level among treatments at different time points.

Results and Discussion

In the first trial, the hatching success of channel catfish eggs was significantly improved ($P < 0.05$) with H_2O_2 treatment for 15 min as a bath solution of 250 mg/L (Figure 1). The control treatment (treatment with povidone iodine only) resulted in an average hatching success of 52.7%, compared with 66.5% for the 250 mg/L H_2O_2 treatment—a 26.2% increase. The highest H_2O_2 treatment (500 mg/L) resulted in a significant decrease in hatching success due to premature hatching. Rach et al. (1998) suggested that a reduction in hatching success during the treatment of various warmwater and coolwater fish eggs with high levels of hydrogen peroxide was the result of toxicity. In that study, Rach et al. (1998) report their best H_2O_2 treatment for channel catfish eggs as 1,000 mg/L, with even 3,000 mg/L being an acceptable con-

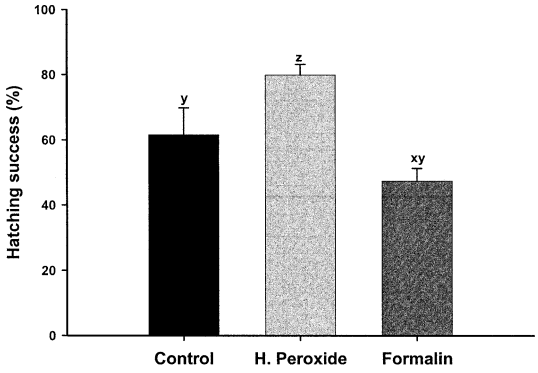

FIGURE 2.—Mean (+SE) hatching success for channel catfish eggs treated once daily with hydrogen peroxide (250 mg/L) or formalin (1,600 mg/L), compared with that of controls treated once with povidone iodine. Significant ($P < 0.05$) differences are indicated by different letters.

centration. There is no discussion of the premature hatching in their study; however, at an H_2O_2 concentration of only 500 mg/L in the present study, we observed chorionic deterioration causing the premature release of the embryos into the water column after just three treatments, resulting in high mortality. Dead ''fry'' were not included in our calculation of hatching success.

Formalin is approved by the FDA for the control of *Saprolegniaceae* on channel catfish eggs and is widely used by hatchery managers for treating fungal infections. The formalin concentrations used in hatchery treatments vary greatly depending on the species being cultured, the culture water temperature, and other factors. The most widely used concentration is 1,667 mg/L, administered as a daily 15-min bath (Piper et al. 1982; Rach et al. 1997). Rach et al. (1997) demonstrated adequate safety margins for treating channel catfish eggs with 1,500 mg/L for 15 min. For the present study, we selected a concentration of 1,600 mg/L as a standard treatment for comparison with H_2O_2 in the second trial.

In the second trial, the treatment of catfish eggs with H_2O_2 at a concentration of 250 mg/L was compared with treatment with formalin (1,600 mg/L) and the povidone iodine control. As in the first trial, eggs representative of distinct spawns received each treatment in triplicate. Hatching success was significantly ($P < 0.05$) higher for eggs treated with hydrogen peroxide (79.9%) compared with the formalin (47.4%) and control (61.6%) treatments (Figure 2). The 29.7% increase in the hatching success of H_2O_2 treated eggs above the controls is consistent with the improvement observed in the first trial. Although formalin has been shown to be an effective bath treatment in controlling *Saprolegnia* infections on catfish eggs (Rach et al. 1997), treatment with formalin in this study had no impact on the hatching success relative to the controls ($P > 0.05$). The research reported by Rach et al. (1997) was conducted at 22°C and did not include a pretreatment of the eggs with povidone iodine. Both the higher temperature (26°C) and the use of povidone iodine may have reduced the effectiveness of formalin in the present study.

Incubation troughs in commercial catfish hatcheries commonly contain 380 L, with water entering the trough at one end at a rate that will allow one complete water exchange in 45–60 min (Brunson 1992). In large hatcheries, turning off the water for a 15-min therapeutic treatment every day can be a substantial risk, as millions of eggs would be lost if the water flow is not restored. As an alternative, we sought to identify a concentration of hydrogen peroxide that would provide a beneficial treatment in a continuous-flow situation. The third trial presented here involved two phases. To minimize costs, the control group in this trial received no treatment. In both phases, four egg masses were each divided among four troughs such that eggs representative of each spawn received every treatment. In the first phase, eggs were treated with 0, 100, 200, and 300 mg H_2O_2/L with the 100 mg/L treatment yielding the highest hatching success (Figure 3). Premature hatching was observed in both the 200- and 300-mg/L treatments. In the second phase, eggs were treated with 0, 35, 70, and 100 mg H_2O_2/L, and 70 mg/L yielded a significant ($P < 0.05$) improvement in hatching success. The results of phases one and two of this trial were standardized for comparison based on the common 100 mg/L treatment. The shape of the curve shown in Figure 3 is similar to that reported by Rach et al. (1998) of the probability of catfish hatch at various concentrations during 15-min H_2O_2 bath treatments.

The FDA has approved the use of hydrogen peroxide treatment to control infections on fish eggs at levels up to 500 mg/L through a low regulatory ruling. The results presented here indicate that 500 mg/L is too concentrated for treating channel catfish eggs at 26°C, resulting in premature hatching, mortality, and poor hatching success. In two separate trials, treatment with hydrogen peroxide at a concentration of 250 mg/L for 15 min was shown to significantly increase hatching success over

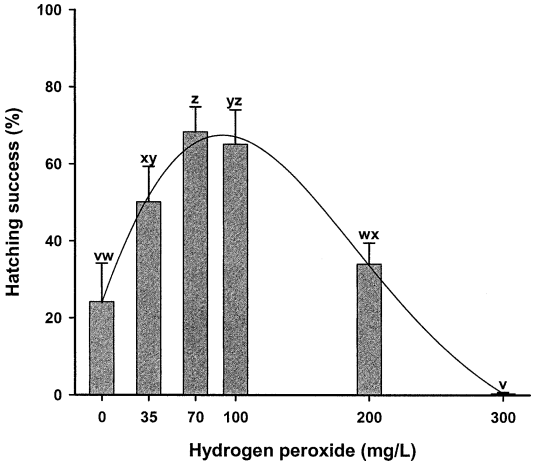

FIGURE 3.—Mean (+SE) hatching success for channel catfish eggs treated once daily with increasing concentrations of hydrogen peroxide in a 380-L hatching trough with continuous water flow (7.6 L/min). Significant ($P < 0.05$) differences are indicated by different letters.

povidone iodine-treated controls and formalin-treated eggs by approximately 30%. Considering that catfish fry and fingerling production in 2002 was approximately 1.6 billion (NASS 2002), this could mean an increase of 480 million fry and fingerlings for the commercial catfish industry. Additionally, this level of treatment currently falls within the FDA's low-regulatory ruling for hydrogen peroxide use.

Hydrogen peroxide treatments were also found to be effective in flow-through hatching troughs. Once-daily addition of 70 mg/L hydrogen peroxide at the water inlet end of the trough yielded a hatching success of 68.3%, compared with 24.2% for untreated eggs. Hydrogen peroxide clearly provides an effective alternative for the treatment of catfish eggs, and is both odor-free and noncarcinogenic. The cost of treating one 380-L hatchery trough with 70 mg/L H_2O_2 is about US$0.10/d (based on 2003 prices of ~$280/55 gal 35% technical grade H_2O_2). Additional tests to determine the treatment effect on eyed eggs may still be necessary.

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