ブロノポールを用いたアユ卵の水カビ病防除

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The Use of Bronopol to Control Fungal Infections in Ayu Eggs

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Abstract: We evaluated bronopol as a practical alternative anti-fungal agent to malachite green for use in hatcheries. Repeated daily exposure, from just after fertilization to the stage showing eye development, to 50 ppm and 100 ppm bronopol for 30 min showed an efficacy compared to the 0 ppm control for the inhibition of fungal infections in ayu *Plecoglossus altivelis* eggs. The 100 ppm bronopol treatment groups showed control of fungal infection up to the stage of eye development in eggs. Significant differences in terms of the number of hatched fish were seen between the 0 ppm control and treatments at 50 and 100 ppm bronopol.

Key words: Ayu eggs; Fungal control; Bronopol; Fungal infection

In Japan, fungal infections of coldwater fish and eggs have usually been controlled with malachite green. However, malachite green has never been permitted in fisheries medicine because of its teratogenic properties (Meyer and Jorgenson 1983) and the fact that the effects of its residues are still unknown (Meinertz et al. 1995). In 2005, the Pharmaceutical Affairs Law prohibited the use of malachite green in aquaculture (Miura et al. 2005). Thus, the fish culturing industry needs an alternative antifungal agent that is a safe and effective replacement for malachite green.

In Europe, bronopol has been used to control fungal infections in eggs of salmonids (Branson 2002). Bronopol (2-bromo-2-nitropropane-1, 3-diol) is water soluble, odorless and biocidal, and is widely used as a preservative in medical and pharmaceutical products such as cosmetics and shampoos (Bryce et al. 1978; Kumanova et al. 1989; Toler 1985). Bronopol is a thiol-containing dehydrogenase enzyme inhibitor that is thought to cause cell membranes to leak, consequently destroying cells (Branson 2002). This study examined the efficacy of bronopol in controlling fungal infections in ayu eggs.

Materials and Methods

Ayu eggs were taken from a single 1-year-old female and inseminated. A few minutes after insemination, approximately 100 fertilized ayu eggs were randomly attached onto 15 glass slides using a feather from a water bird. Glass slides with attached fertilized eggs were then held in metal baskets. This trial used a commercially available product, which contained 50% w/v bronopol with an inert carrier (Pyceze; Novartis Animal Vaccines Limited, Essex, United Kingdom). The eggs were treated with 50 ppm and 100 ppm bronopol for 30 min. Treatments were repeated everyday for 5 days, from fertilization until eye development was observed in the eggs. During treatment, the eggs were transferred to 2 *l* beakers with no aeration. Control groups, without treatment, were also prepared and transferred to beakers. After treatment, all groups were held in a 550 *l* tank in running water flowing at a rate of approximately 20 *ml*. No further treatment was performed after the eggs showed eye development. Numbers of eggs infected with fungi, dead eggs and eggs

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with eye development were counted 5 days after fertilization and numbers of eggs infected with fungi, dead eggs, hatched fish and deformities were counted 9 days after fertilization. This study was performed at the Kanagawa Prefectual Fisheries Technology Center Freshwater Fisheries Experiment Station. The source water for this hatchery was well water kept at a temperature of 20－22℃ throughout the test period.

Results

The number of dead eggs with fungal infection was 22 at 0 ppm, 12 at 50 ppm bronopol and 0 at 100 ppm bronopol. The number of dead eggs without fungal infection was three at 0 ppm, 11 at 50 ppm bronopol and 25 at 100 ppm bronopol. Repeated exposure from just after fertilization up to the stage of eye development in eggs with 50 and 100 ppm bronopol showed an efficacy compared to 0 ppm for the inhibition of fungal infection (Chi-square test, $p<0.05$). The 100 ppm bronopol displayed control of fungal infection up to the stage of eye development. The number of eggs with eyed in each group was almost same (Table 1).

Both the 50 and 100 ppm bronopol affected fungal infection from the stage of eye development in eggs until hatching. The number of dead eggs with fungal infection was 47 at 0 ppm, 18 at 50 ppm bronopol and 12 at 100 ppm bronopol. The number of dead eggs without fungal infection was one at 0 ppm and 50 ppm bronopol, and three at 100 ppm bronopol. The number of hatched fish was 227 at 0 ppm, 271 at 50 ppm bronopol and 296 at 100 ppm bronopol. Significant differences were observed in terms of the number of hatched fish between 0 ppm and the 50 and 100 ppm bronopol (Chi-square test, $p<0.05$; Table 2).

Discussion

The present study showed that treatment with 100 ppm bronopol for 30 min was effective in controlling fungal infections. Deformities were observed not only in groups treated with 50 ppm and 100 ppm bronopol, but also in those from the untreated group. It appeared that repeated exposure everyday, from just after fertilization to the stage of eye development in eggs, to 50 ppm and 100 ppm bronopol for 30 min, was effective in controlling fungal infections. This result was similar to the efficacy of bronopol to control fungal infections in rainbow trout *Oncorhynchus mykiss* eggs (Oono et al. 2007). The use of a fungicidal agent in hatcher-

Table 1. Control of fungal infection by bronopol from fertilization to the stage of eye development in ayu eggs [a)]

Concentration	Number of fertilized eggs	Dead eggs		Eggs with eyes	Unknown eggs
		With fungal infection	Without fungal infection		
0 ppm 1	58	6	0	50	2
0 ppm 2	66	3	0	63	0
0 ppm 3	65	5	0	57	3
0 ppm 4	74	3	1	69	1
0 ppm 5	70	5	2	59	4
50 ppm 1	79	0 [b)]	6	72	1
50 ppm 2	70	3 [b)]	1	65	1
50 ppm 3	58	3 [b)]	0	55	0
50 ppm 4	70	4 [b)]	3	62	1
50 ppm 5	59	2 [b)]	1	56	0
100 ppm 1	67	0 [b)]	5	60	2
100 ppm 2	87	0 [b)]	7	74	6
100 ppm 3	74	0 [b)]	3	70	1
100 ppm 4	74	0 [b)]	4	69	1
100 ppm 5	70	0 [b)]	6	64	0

Experiments were performed from September 22－27, 2004.
[a)] Treatments were performed for 5 days from fertilization to the stage of eye development in eggs.
[b)] Significantly different from the value for 0 ppm ($p<0.05$).

Table 2. Control of fungal infection by bronopol from the stage of eye development to hatching in ayu eggs [c]

Concentration	Number of eggs with eyes	Dead eggs		Hatched fish	Deformed fish	Unknown eggs
		With fungal infection	Without fungal infection			
0 ppm 1	50	13	0	32	0	5
0 ppm 2	63	10	1	48	0	4
0 ppm 3	57	5	0	49	1	2
0 ppm 4	69	15	0	50	0	4
0 ppm 5	59	4	0	48	0	7
50 ppm 1	72	5	0	60 [d]	0	7
50 ppm 2	65	3	0	58 [d]	0	4
50 ppm 3	55	3	1	49 [d]	0	2
50 ppm 4	62	3	0	56 [d]	1	2
50 ppm 5	56	4	0	48 [d]	0	4
100 ppm 1	60	5	0	48 [d]	0	7
100 ppm 2	74	0	0	70 [d]	1	3
100 ppm 3	70	4	0	60 [d]	0	6
100 ppm 4	69	3	0	61 [d]	0	5
100 ppm 5	64	0	3	57 [d]	0	4

Experiments were performed from September 27-October 1, 2004.
[c] No treatments were performed for the 4 days from the stage of eye development to hatching.
[d] Significantly different from the value for 0 ppm ($p<0.05$).

ies is usually stopped at the eye development stage of eggs. However, as it appears that fungal infections may continue from that stage to the hatched fish stage, the routine screening of dead eggs or the continuation of bronopol treatment after the eye development stage would be practical to increase hatching and control fungal infections.

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ブロノポールを用いたアユ卵の水カビ病防除

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サケ科魚類卵の水カビ病防除剤として日本で使用されてきたマラカイトグリーンは2005年で使用禁止となった。今回欧州でその代替薬として認可されたブロノポール50%含有製剤の水カビ病防除効果と毒性を検討した。試験はアユ *Plecoglossus altivelis* 受精卵を受精翌日から発眼までの5日間毎日，成分濃度で50，100 ppmに30分間浸漬した。対照区と比較した結果，薬浴区は水カビ病の発生を阻止した。また薬浴区で孵化尾数の有意差が認められた。