Available online at www.sciencedirect.com
ScienceDirect
Aquaculture 270 (2007) 15–22
Aquaculture
www.elsevier.com/locate/aqua-online

Efficacy of bithionol as an oral treatment for amoebic gill disease in Atlantic salmon *Salmo salar* (L.)

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Received 30 October 2006; received in revised form 18 April 2007; accepted 28 April 2007

Abstract

This study examined the efficacy of bithionol as an oral treatment for Atlantic salmon *Salmo salar* affected by amoebic gill disease (AGD). The current commercial management strategy of AGD is a costly 3 h freshwater bath. It is labour intensive and the number of baths needed appears to be increasing; hence, there is an effort to identify alternative treatments. Efficacy was examined by feeding AGD-affected Atlantic salmon twice daily to satiation with bithionol, an antiprotozoal, at 25 mg kg^{-1} feed. Three seawater (35‰, 17 °C) re-circulation systems were used each consisting of three tanks containing 32 Atlantic salmon smolts with an average (±SEM) mass of 90.4 g (±5.2). Three feeds were examined in the trial including bithionol, plain commercial control and oil coated commercial control. Feeding commenced 2 weeks prior to exposure to *Neoparamoeba* spp. at 300 cells L^{-1} and continued for 28 days post-exposure. Efficacy was determined by examining gross gill score and identifying percent lesioned gill filaments twice weekly for 4 weeks post-exposure. Bithionol when fed as a two-week prophylactic treatment at 25 mg kg^{-1} feed delayed the onset of AGD pathology and reduced the percent lesioned gill filaments by 53% and halved the gill score from 2 to 1 when compared with both the plain and oil controls during an experimental challenge. There were no palatability problems observed with mean feed intake of bithionol over the trial duration with fish consuming higher levels of the bithionol diet compared to both the oil and plain controls. This study demonstrated that bithionol at 25 mg kg^{-1} feed, when fed as a two-week prophylactic treatment for *Neoparamoeba* spp. exposure, delayed and reduced the intensity of AGD pathology and warrants further investigation as an alternative to the current freshwater bath treatment for AGD-affected Atlantic salmon.

Keywords: *Salmo salar*; Amoebic gill disease (AGD); Bithionol; *Neoparamoeba* spp; Oral treatment; Protozoan parasite; Aquaculture

1. Introduction

Bithionol, a phenolic compound related structurally to hexachlorophene, has been used worldwide as a parasiticide for cattle, sheep, chickens, dogs and cats (Enzie and Colglazier, 1960). It is reported to have surfactant antimicrobial properties and thus is also effective against bacteria, moulds and yeast (Kim and Choi, 1998). It has been used as an alternative to praziquantel against human trematode and cestode infections, in particular paragonimiasis (Enzie and Colglazier, 1960; Takeuchi et al., 1984), and has been widely used in veterinary medicine, as it is active against flukes and cestodes (Mourot et al., 1987). Bithionol has

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doi:10.1016/j.aquaculture.2007.04.082

been found to stimulate lactic acid production, inhibit oxygen consumption and decrease glycolytic and oxidative metabolism in the human lung fluke, *Paragonimus westermani*, in vitro (Hamajima, 1973). Furthermore, it was postulated to be linked to a variety of processes including the inhibition of reduced nicotinamide adenine dinucleotide (NADH)-fumarate reductase and involved in protein phosphorylation (Reid et al., 2001). With regards to fish, bithionol has been shown to be effective against the fish ciliates *Philasterides dicentrarchi* in turbot *Scophthalmus maximus*, *Tetrahymena pyriformis* in vitro and *Trichodina jadranica* in eels *Anguilla anguilla*, acting upon the mitochondrial respiratory chain and uncoupling electron transport (Griffin, 1989; Iglesias et al., 2002; Madsen et al., 2000).

Bithionol has been recorded as efficacious *in vitro* and *in vivo* when used as a bath treatment for salmonid parasites, including *Gyrodactylus* sp., *Ichthyobodo necator* (Santamarina et al., 1991; Tojo et al., 1994) and *Neoparamoeba* spp. (Florent et al., 2007). Furthermore, due to bithionol being insoluble in water it was advantageous to use bithionol as an oral medication, providing easy administration and limited fish handling (Tojo and Santamarina, 1998c). Bithionol at 40 g kg^{-1} feed was offered for 10 days at 2% body weight (BW) per day to rainbow trout *Oncorhynchus mykiss* infected with *Hexamita salmonis* (*Spironucleus barkhanus*), *Gyrodactylus* sp. or *I. necator* and exhibited a reduction in parasitemia. Bithionol eliminated approximately 80% of *H. salmonis* from rainbow trout while both *Gyrodactylus* sp. and *I. necator* infections were reduced from a high to low intensity (Tojo and Santamarina, 1998a,b,c). Kim and Choi (1998) reported bithionol administered in-feed at 100–200 mg kg^{-1} BW significantly reduced the number of monogeneans *Microcotyle sebastis* on the gills of cultured rock fish *Sebastes schlegeli* with a 20-day feeding duration being most effective.

The protozoan parasite *Neoparamoeba* spp. is a free-living marine amphizoic amoeba and is believed to cause amoebic gill disease (AGD) (Adams and Nowak, 2004; Kent et al., 1988; Munday et al., 1990). It attaches itself to the gills particularly the secondary lamellae and is characterized macroscopically by the presence of raised, white mucoid patches with histological presentation of single or multi-focal epithelial hyperplasia leading to lamellar fusion (Adams and Nowak, 2001). It primarily affects salmonids and is a significant problem for the Atlantic salmon industry in Australia (Munday et al., 2001; Nowak et al., 2002). The current commercial treatment for AGD is freshwater bathing of affected fish, as it lowers gross gill lesions, mortalities and is environmentally friendly. However, it is labour and cost intensive as well as requiring fish handling, hence the need for effective oral medications (Munday and Zilberg, 2003; Parsons et al., 2001).

Several compounds have been examined for efficacy against AGD, including levamisole (Howard and Carson, 1995), chlorine dioxide, chloramine-T, hydrogen peroxide (Powell et al., 2003; Powell and Clark, 2003), amprolium, albendazole, toltrazuril and bithionol (Powell et al., 2003). These compounds have been examined either as a bath treatment or in *in vitro* toxicity testing in seawater, with levamisole reported as lethal to *Neoparamoeba pemaquidensis* at concentrations $\geq$10 ppm *in vitro* (Howard and Carson, 1994), while chloramine-T at a concentration of 25 and 50 ppm reduced amoeba numbers equivalent to those seen in deionized water after 2 h (Powell and Clark, 2003). Amprolium was found to be an amoebastat at 1 mg L^{-1} and bithionol amoebicidal at 1 and 10 mg L^{-1} *in vitro* (Powell and Clark, 2003). Compounds that have been examined as in-feed treatment for AGD include the mucolytic compound L-cysteine ethyl ester (LCEE), which was reported to delay progression of pathology associated with AGD as well as reduce percent lesioned gill filaments by approximately 50% relative to the control when fed at 52.7 mg kg^{-1} fish day^{-1} for 2 weeks (Roberts and Powell, 2005). The purpose of the present study was to determine if bithionol, when administered orally as a prophylactic and therapeutic treatment at 25 mg kg^{-1} feed to Atlantic salmon, could be used as an effective treatment against amoebic gill disease affecting Atlantic salmon *Salmo salar* L.

2. Materials and methods

2.1. Fish husbandry and maintenance

Atlantic salmon (AS) diploid mixed-sex spring smolts, with a mean (±SEM) mass of 90.4 g (±5.2) and a mean (±SEM) fork length of 21.1 cm (±0.3) (N=288) were obtained from SALTAS salmon hatchery (Tasmania, Australia). Fish were maintained at the University of Tasmania Aquaculture Centre for a minimum of 3 weeks prior to experimentation. Salmon were acclimated to seawater (30‰, 1 μm filtered, mean±SEM temperature of 16±0.8 °C) over 14 days in one 3000 L Rathburn tank with recirculated water and an individual biofilter system. The tank received constant aeration and oxygen levels were monitored daily using a Handy Gamma Oxy Guard (Birkerød, Denmark), with mean (±SEM) dissolved oxygen levels of 94% saturation (±0.2%). Fish