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The chronic effects of dietary lead in freshwater juvenile rainbow trout (*Oncorhynchus mykiss*) fed elevated calcium diets

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Abstract

This study examined the impact of elevated dietary Ca^{2+} on the responses to chronic dietary Pb exposure in juvenile rainbow trout. Trout were fed reference (0.3 μg Pb/g, ~20 mg Ca^{2+}/g) and Pb-enriched diets (~50 or 500 μg Pb/g) in the presence of background Ca^{2+} (~20 mg Ca^{2+}/g) or (~60 mg Ca^{2+}/g) of added Ca^{2+} (as $CaCO_3$) for 42 days. The quantitative order of Pb accumulation in tissues reflected the exposure pathway of Pb via the diet (per tissue wet weight): gut > bone > kidney > liver > spleen > gill > carcass > brain > white muscle. The anterior intestine accumulated the most Pb per tissue wet weight, while the bone accumulated the most Pb per fish weight. Pb concentrations were much higher in the posterior kidney than the anterior kidney. Simultaneous addition of Ca^{2+} to the diet had an overall protective effect in all the tissues analysed in reducing Pb accumulation. The RBCs accumulated 100 times more Pb when compared to the plasma, while the whole blood δ-aminolevulinic acid dehydratase was inhibited in the high treatment group without added Ca^{2+}, by the end of the exposure. Neither plasma Cl^-, K^+, Mg^{2+} nor Na^+, K^+-ATPase activities in the gills, mid- and posterior intestine were affected. However, there were mild disruptions in plasma Na^+ and Ca^{2+} levels in the elevated Pb and Ca^{2+} treatment groups, and a significant up-regulation in Na^+, K^+-ATPase activity at the anterior intestine in fish fed the high Pb diets with background or added Ca^{2+}. By day 42, Pb levels in most tissues had either stabilized or started to decrease, indicating some capacity for regulation of accumulated loads. We conclude that elevated dietary Ca^{2+} levels will be protective in reducing Pb burdens in freshwater juvenile rainbow trout exposed to environments contaminated with waterborne Pb.

Keywords: Dietary; Lead (Pb); Calcium (Ca); Accumulation; Ionoregulation; δ-Aminolevulinic acid dehydratase (ALAD)

1. Introduction

In contaminated aquatic environments, fish can take up Pb through the water, diet and to a lesser extent the skin (e.g. Dallinger et al., 1987; Hodson et al., 1978; Köck et al., 1998; Rogers et al., 2003, 2005; Rogers and Wood, 2004). Waterborne Pb causes the disruption of Na^+, Cl^- and Ca^{2+} regulation during acute exposure, the induction of spinal deformities and black tails during chronic exposure, and disruption in hemoglobin synthesis during both types of exposure (Hodson et al., 1978; Rogers et al., 2003, 2005; Rogers and Wood, 2004). Dietary Pb is less well studied than waterborne Pb. However, physiological changes and morphological damage in the enterocytes at the mid- and posterior intestine have been observed in rainbow trout fed 10 μg Pb/g fish/day for 15–30 days (Crespo et al., 1986).

In contrast to the many acute waterborne Pb experiments (Hodson, 1976; Hodson et al., 1977, 1978; Varanasi and Gmur, 1978; Rogers et al., 2003, 2005; Rogers and Wood, 2004), only four studies have looked at the effects of dietary Pb exposure to rainbow trout. Hodson et al. (1978) found that Pb was not taken up via the diet, but in contrast, the studies of Alves et al. (2006), Mount et al. (1994), and Crespo et al. (1986) all found that dietary Pb accumulates in the whole body and in a number of internal tissues when present in the diet.

Alves et al. (2006) found mild physiological disturbances in juvenile rainbow trout fed diets containing up to 520 μg Pb/g for 21 days. These disturbances included transient decreases in plasma Mg^{2+} and Ca^{2+} levels, and increases in whole body waterborne Na^+ influx rates. Despite an absence of effects on growth and survival rates, rainbow trout accumulated significant Pb burdens in the intestine, kidney, gills, liver and carcass, with the RBCs accumulating 105 times more Pb when compared to

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the plasma by the end of the experiment. Pb accumulated to the greatest extent in the intestine, suggesting that the intestine may be a site of dietary Pb toxicity. Notably, there was some indication of stabilization or even depuration of tissue Pb burdens by day 21, but the exposure was not long enough to establish clear trends. Similarly, Mount et al. (1994) found that despite an absence of effects on survival and growth, rainbow trout accumulated whole body dietary Pb burdens when fed for 60 days with brine shrimp contaminated with Pb concentrations as high as 170 μg Pb/g dw. In addition, Crespo et al. (1986) reported morphological changes, decreased Na^+, K^+-ATPase activity, and decreased Na^+ and Cl^- absorption in the intestine of trout fed for 15–30 days with a comparable dietary load of Pb.

Generally, divalent metals such as Pb, Cd and Zn^{2+} are considered Ca^{2+} antagonists. In an early study (Varanasi and Gmur, 1978), coho salmon (*Oncorhynchus kisutch*) force-fed gelatin capsules containing 8.4 mg of calcium chloride ($CaCl_2$) and then exposed to 1300 μg/L of waterborne Pb for 168 h, had reduced Pb tissue burdens (Varanasi and Gmur, 1978). Similarly, several more recent studies (Zohouri et al., 2001; Baldisserotto et al., 2004a, 2004b, 2005; Franklin et al., 2005) have shown that dietary Ca^{2+} (as $CaCl_2$ or $CaCO_3$) is protective against the uptake of both waterborne and dietary Cd, as well as against the uptake of waterborne Zn^{2+} (Niyogi and Wood, 2006).

δ-Aminolevulininc acid dehydratase (ALAD), an enzyme that catalyses the formation of porphobilinogen (PGB) from the substrate aminolevulinic acid (ALA), has long been used as a biomarker for Pb toxicity in humans (Secchi et al., 1974) and fish (Hodson et al., 1977; Schmitt et al., 1984, 1993, 2002; Burden et al., 1998). Pb inhibits ALAD by binding to essential sulfhydryl (SH) groups and displacing the Zn^{2+} cofactors on ALAD (World Health Organization, 1977; Sassa, 1982). Hodson (1976) and Hodson et al. (1977, 1978) found that ALAD activity in rainbow trout was inhibited after exposing fish to waterborne Pb concentrations as low as 13 μg/L for 4 weeks, but the effects of dietary Pb exposure on ALAD activity in freshwater fish are not known.

Therefore, the objectives of the present investigation were: (a) to investigate the accumulation of Pb in various tissues; (b) to assess any possible physiological and toxicological effects on growth and survival rates, plasma Na^+, Cl^-, K^+, Ca^{2+}, and Mg^{2+} regulation, the enzyme ALAD and Na^+, K^+-ATPase activity in gills or intestine, in juvenile rainbow trout fed with two different levels of dietary Pb, in the presence of either background or elevated Ca^{2+} levels in the diet.

2. Materials and methods

2.1. Fish

Juvenile rainbow trout (*O. mykiss*) ($N \sim 400$) weighing 20–23 g were obtained from Humber Springs Trout Hatchery (Orangeville, Ont.). After arrival, fish were randomly selected and placed into eight, 200 L polypropylene flow-through, aerated tanks that were divided into 2×100 L sections (25 fish per section). The division was achieved using a 0.2 mm^2 mesh screen, which allowed free-mixing of water, but not the food or feces between the two sides. Each tank was supplied with 1 L/min of dechlorinated Hamilton water, pumped only once through the tanks and with the composition of $Na^+ = 0.65$ mM, $Cl^- = 0.8$ mM, $Ca^{2+} = 1.0$ mM, $Mg^{2+} = 0.4$ mM, $K^+ = 0.06$ mM and water hardness as $CaCO_3 = 140$ mg/L, total Pb = 1.3 ± 0.1 μg/L. The pH and temperature were kept at ambient conditions, 7.4–7.7 and 11–13 °C, respectively. Photoperiod was maintained at 12 h light and 12 h dark. Fish were acclimated three weeks prior to their use in the 42 day experiment. Prior to the start of the experiment juvenile rainbow trout had a mean weight of 25.8 ± 0.5 g.

Fish were fed commercial salmon fry pellets once daily (Silver Cup feed; Murray, UT, USA, see below for composition) at a ration of 1.5% body mass/day upon arrival and until the beginning of the experiment. At the start of the experiment, each tank section was assigned to one of six replicated nominal dietary Pb and/or Ca^{2+} treatments: 0 μg Pb/g + 20 mg Ca^{2+}/g dry weight (A); 0 μg Pb/g + 60 mg Ca^{2+}/g (B); 50 μg Pb/g + 20 mg Ca^{2+}/g (C); 50 μg Pb/g + 60 mg Ca^{2+}/g (D); 500 μg Pb/g + 20 mg Ca^{2+}/g (E), and 500 μg Pb/g + 60 mg Ca^{2+}/g (F) (Fig. 1). Additional replicated control tank sections (0 μg Pb/g + 20 mg Ca^{2+}/g and 0 μg Pb/g + 60 mg Ca^{2+}/g, Fig. 1) in tanks 1 and 5, in comparison to tanks 3 and 7, and 4 and 8, where the same treatments were paired with high dietary treatments, were used to control for possible waterborne Pb contamination as a result of Pb leaching across from the feces and/or food in the neighbouring tank section.

2.2. Diet

Pb-enhanced diets were made by adding Pb, in the form of lead nitrate, $Pb(NO_3)_2$ (Sigma–Aldrich) into 0.5 pt. commercial salmon fry food (Silver Cup feed, Murray, UT, USA).

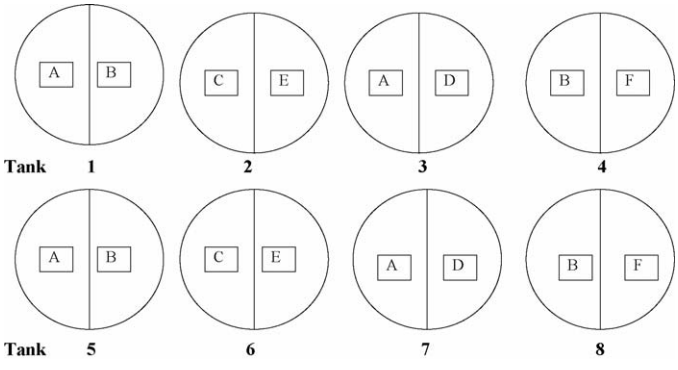

Fig. 1. Tank set-up. Tanks were set up by sectioning 200 L tanks into 2×100 L sections using a 0.2 mm^2 mesh screen. The letter in each section corresponds to the diet given in each section: (A) 0 μg Pb/g + 20 mg Ca^{2+}/g; (B) 0 μg Pb/g + 60 mg Ca^{2+}/g; (C) 50 μg Pb/g + 20 mg Ca^{2+}/g; (D) 500 μg Pb/g + 20 mg Ca^{2+}/g; (E) 50 μg Pb/g + 60 mg Ca^{2+}/g; (F) 500 μg Pb/g + 20 mg Ca^{2+}/g. Each of the above tanks were replicated. Tanks 1 and 5 were used as a control for possible waterborne Pb as a result of Pb leaching from the feces and food into neighbouring tank sections (i.e. treatment A was replicated in tanks 3 and 7, and treatment B was replicated in tanks 4 and 8, both of which had the highest dietary Pb levels on the opposite side). 20 mg Ca^{2+}/g represents background Ca concentrations in the salmon fry food used to prepare the diets.