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Ecotoxicological Assessment of Cobalt Used as Supplement in the Diet of Common Carp *Cyprinus carpio*

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Abstract Experiments were performed in the laboratory to determine if excess levels of Co used as dietary supplement (0.0, 0.05, 0.10 and 1.0%) to enhance growth of the fish *Cyprinus carpio* was safe for aquatic organisms. Lethal concentrations of Co for tadpole of toad *Bufo melanostictus* (96 h LC_{50}, 17.2 mg/L), oligochaet worm *Branchiura sowerbyi* (96 h LC_{50}, 179 mg/L) and crustacean zooplankton *Diaptomus forbesi* (96 h LC_{50}, 1.5 mg/L) were compared with the concentration of Co in the medium leached from the unused diets and faeces. The results indicated that the Co leached from diet containing 1.0% Co was ecotoxicologically unsafe for crustacean zooplankton.

Keywords Cobalt · Diet · Leaching · LC_{50}

Cobalt (Co) is an essential mineral micronutrient and freshwater fish require supplement of Co in their diet @ 0.5–5.0 mg/kg dry diet for optimum growth (Hasan 2001). However, Co is often used in the diet in excess of its requirement to enhance growth of fish (Anadu et al. 1990; Mahmoud 2009). Common carp (*Cyprinus carpio*) reared with a diet supplemented by 0.1–1.0% Co exhibited better growth as compared to fish reared with normal diet (Mukherjee and Kaviraj 2009). However, ecotoxicological assessments of the Co leached into water from the unused diets as well as from the faeces have not yet been evaluated.

Co is generally highly toxic to zooplanktonic species (Biesinger and Christensen 1972; Khangarot and Ray 1989; Das and Kaviraj 1994). Nagpal (2004) recommended that interim maximum total Co concentration in the freshwater environment should not exceed 110 μg/L to protect aquatic life from acute effects of Co and interim 30-day average total Co concentration should not exceed 4 μg/L to protect aquatic life from chronic effects of Co. Therefore, dietary requirement of Co for enhanced growth of fish should be correlated with the rate of leaching of Co into water and toxicity of Co to aquatic organisms.

The main objective of the present study was to determine if the excess level of Co used as dietary supplement for enhancing growth of *Cyprinus carpio* is safe for aquatic organisms.

Materials and Methods

Two experiments were designed in the laboratory. The first experiment was a feeding, accumulation and leaching experiment to determine concentration of Co in gut and faeces of common carp *Cyprinus carpio* fed Co supplemented diets for four days (96 h) and concentration of Co in water after 4 days of feeding. In the second experiment static bioassays were made for 96 h to determine lethal concentrations (LC) of Co for three aquatic organisms: a crustacean zooplankton *Diaptomus forbesi*, an oligochaete worm *Branchiura sowerbyi* and a one week old tadpole larva of toad *Bufo melanostictus*. Fingerlings of *Cyprinus carpio* were procured from a local hatchery and other organisms were captured from local ponds. Length and weight of the test organisms used in these two experiments have been given in Table 1. All organisms were acclimatized to test conditions for 96 h before use.

For the first experiment, diets were formulated with rice bran, wheat flour, mustard oil cake, fish meal, vitamin

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Table 1 Length and weight (mean ± SD) of the test organisms used

Test organisms	Length (mm)	Wet weight (mg)
Fish	81.43 ± 4.37	7410 ± 1010
Cyprinus carpio		
Amphibia	11.04 ± 0.14	20.00 ± 4.00
Bufo melanostictus (tadpole)		
Oligochaeta	30.52 ± 1.79	4.19 ± 0.08
Branchiura sowerbyi		
Crustacea	0.60 ± 0.02	0.05 ± 0.01
Diaptomus forbesi		

premix, mineral premix (without Co) and graded levels of $CoCl_2$, $6H_2O$ so as to prepare four experimental diets with an average crude protein level of 30.95% and any one of the four levels of Co (0, 0.05, 0.10 and 1.0%) supplemented in the diet. Details of ingredients used and proximate composition of the diet have been presented earlier (Mukherjee and Kaviraj 2009). The experiment was made in 10-L glass aquaria. Each aquarium contained four acclimatized fish. Altogether, twelve aquaria were arranged in randomized block design so that each of the four diets could be tested in three replicates. The experimental fish were fed a particular diet at 5% of their body weight at 10.00 and 16.00 h for all the 4 days of the experimental period. The uneaten diets were removed 4 h after each feeding. Faeces were collected every 3 h, soaked in blotting paper and stored at −20°C. During digestion the samples of faeces frozen over 96 h were brought to room temperature, thawed and pooled together to make a composite sample for each replicate and were digested in nitric acid and hydrochloric acid (Van Loon 1980). Water samples were collected before the start of feeding (initial) and after 96 h of feeding and were digested by nitric acid (APHA 1995). All fish were sampled after 96 h of feeding, soaked in blotting paper and dissected with a pair of clean, acid soaked scissors to collect gut tissue. The gut tissue was digested in nitric acid, sulphuric acid and perchloric acid (Churnoff 1975; Páez-Osuna and Tron-Mayen 1995). Levels of Co in the digested samples of faeces, water and gut tissue were determined in Flame Atomic Absorption Spectrophotometer (Varian Spectra AA 240). Detection limit of Co, determined as three times the mean standard deviation of absorbance of 10 replicate blank samples, was set at 0.01 mg/L. Variations in concentrations of Co in gut, faeces and water between treatments were tested by single factor ANOVA followed by Least significant difference (LSD) test (Gomez and Gomez 1984).

Acute toxicity bioassays for tadpoles were made in 3 L glass jar each containing 1 L water (temperature 34.0 ± 2.8°C; dissolved oxygen 7.37 ± 0.15 mg/L; pH 7.33 ± 0.15) and ten acclimatized tadpoles. On the other hand, acute toxicity bioassays for the oligochaet worm *Branchiura sowerbyi* and the crustacean *Diaptomus forbesi* were made in 300 mL glass beakers each containing 200 mL of the same water and ten acclimatized specimens. The glass jars and the beakers were arranged as per randomized block design so that there were three replicates for each of the concentrations of Co tested (Gomez and Gomez 1984). The test organisms were visually examined once every 3 h and the dead ones were removed in time. Number of organisms survived were recorded after 24, 48, 72 and 96 h. Response of the organisms to the concentrations of Co tested were normally distributed and the data were found fit to be analyzed by EPA probit analysis program (Version 1.5) for calculating LC (LC_5, LC_{50} and LC_{95}) of Co and the 95% confidence limit of each value.

Results and Discussion

Co could not be detected in the medium (<0.01 mg/L) in any diet group before the start of the experiment and in the control after 96 h of feeding. Co was detected in trace quantity in the medium after 96 h of feeding in the diet groups 0.05 and 0.1% Co (Table 2). There was no significant difference in the concentration of Co of water between these two diet groups. Concentration of Co significantly increased in the medium in the diet supplemented by 1.0% Co as compared to other two Co supplemented diet groups. The fish accumulated Co from the diet and the concentration of Co in gut increased gradually with the level of Co in the diet. Concentration of Co in gut showed a curvilinear relationship with the dietary Co ($Y = 1.367e^{0.951X}$; $r^2 = 0.98$; where Y = Co in gut and X = dietary level of Co). Concentration of Co in the faeces did not show any significant difference between the dietary levels of Co indicating that concentration of Co detected in the medium was leached mostly from the unused diet.

There was no mortality of the test organisms in the control. 96 h LC of Co at which 5, 50 and 95% mortality occurred (LC_5, LC_{50} and LC_{95}, respectively) and their 95% confidence limit (CL) are presented below (Table 3). The crustacean *D. forbesi* was most susceptible and the

Table 2 Concentration of Co in gut tissue, faeces and in test medium

Dietary Co (% of diet)	Co in gut (μg/g)	Co in faeces (μg/g)	Co in water (mg/L)
0.00	3.8 ± 0.8^a	5.4 ± 0.9^a	<0.01
0.05	9.4 ± 3.5^b	5.8 ± 0.5^a	0.05 ± 0.00^a
0.10	18.9 ± 3.9^c	6.2 ± 0.7^a	0.05 ± 0.00^a
1.00	71.6 ± 10.5^d	6.4 ± 0.4^a	0.28 ± 0.03^b

Different superscripts in a column indicate significant difference (LSD; $p < 0.05$) between two treatments

Table 3 96 h lethal concentrations (mg/L) of Co (LC_5, LC_{50}, LC_{95}) and their 95% confidence limit (CL) for different aquatic organisms

Test animals	LC_5 (95% CL)	LC_{50} (95% CL)	LC_{95} (95% CL)
Tadpole of *Bufo melanostictus*	6.8 (4.1–8.9)	17.2 (14.5–20.2)	43.0 (33.1–69.8)
Branchiura sowerbyi	47 (23–69)	179 (140–228)	690 (471–1375)
Diaptomus forbesi	0.6 (0.4–0.9)	1.5 (1.3–1.8)	3.7 (2.9–5.7)

oligochaet worm *B. Sowerbyi* was most tolerant to Co, The tadpole lava of *B. melanostictus* was moderately susceptible to Co.

96 h LC_{50} values of Co determined for the test organisms in the present investigation have similarities with the 96 h LC_{50} value of Co for *Rana hexadactyla* tadpole (Khangarot et al. 1985), the worm *B. sowerbyi* (Das and Kaviraj 1994), the crustacean *Daphnia magna* (Ewell et al. 1986) and 96 h EC_{50} values of Co for the worm *Tubifex tubifex* (Khangarot 1991). Comparing the LC_{50} values of Co with the concentration of Co detected in the medium in different diet groups it appeared that leaching of Co from the Co supplemented unused diets and the faecal matters might be deleterious to crustacean zoo-plankton when Co was supplemented at 1.0% level. Although concentration of Co detected at 96 h in the 1.0% Co supplemented diet group was much lower than the 96 h LC_{50} value of *D. forbesi*, the lower limit at which 5% of the animal was killed (LC_5) was close to the level of Co detected in the medium after 96 h of feeding. Moreover, Concentration of Co is likely to increase in water in long term culture of fish with regular feeding in closed water bodies like ponds and tanks. Mukherjee and Kaviraj (2009) observed a level of 0.4 ± 0.05 mg/L of Co in water after 60 days of rearing of *C. carpio* with 1.0% dietary Co.

Sensitivity of crustacean zooplanktons to Co was established from the results of the present study as well as from other studies mentioned above. 21-day LC_{50}/EC_{50} or NOEC of Co on reproduction and survival of water flea *D. magna* ranged from 0.01 to 0.02 mg/L (Biesinger and Christensen 1972; Nagpal 2004). There are many other aquatic organisms, which are even more sensitive to Co (Nagpal 2004). Therefore, the level of 1.0% Co used as dietary supplement for enhancing growth of *C. carpio* appeared ecotoxicologically unsafe for crustacean zooplankton population of the medium. However, this level of dietary Co was unlikely to cause any harmful effect to *B. sowerbyi* because this organism was found to be most tolerant to Co. But susceptibility of the tadpole of toad *B. melanostictus* to Co (lower limit of LC_5 being 4.1 mg/L) indicated that the water bodies used for long term culture of fish with 1.0% Co supplemented diet might not be safe for this organism also.

Therefore, it was concluded from this study that diets supplemented by 0.05–0.1% Co for enhancing growth of common carp *C. carpio* were eco-toxicologically safe for aquatic organisms, while leaching of Co from the unused diets containing 1.0% Co should not cause any lethal effects to these aquatic organisms or other aquatic organisms with similar sensitivity, but it might cause sub-lethal effects on reproduction and survival of crustacean zooplankton.

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