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Effect of Dietary Selenium on Chosen Physiological and Biochemical Parameters in *Labeo rohita* (Hamilton, 1822) Reared in Copper Contaminated Water

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Abstract

Copper occurs naturally in the aquatic environment as a result of weathering of soils and rocks and land drainage. Copper is a micronutrient at low level and highly toxic to fish at high concentrations above 0.4 ppm can kill some species of fishes in few hours. The reduction of toxic elements in aquatic system by some acceptable methods is needed urgently. The present work was designed to study the effect of selenium on the reduction of copper toxicity in tissues and improvement of growth haematology and proximate composition in the freshwater fish, *Labeo rohita*.

Keywords: *Labeo rohita*; Water; Copper sulphate $CuSO_4$ 7 H_2O; Selenium (sodium selinite)

Introduction

Metals are introduced in aquatic systems as a result of the weathering of soils and rocks, from volcanic eruptions and from a variety of human activities involving mining, processing, or use of metals and/or substances that contain metal pollutants. Copper occurs naturally in the aquatic environment as a result of weathering and land drainage. But pesticides and fungicides and copper in water pipes and other human devices have introduced additional quantities of copper into the environment. Copper can be actually toxic to freshwater fish, the toxicity depending on species, age, size, water, temperature and ionic composition. The reduction of toxic elements in aquatic system by some acceptable methods is needed urgently. In the present study, selenium reduced the toxicity of copper and thereby enhanced the tested physiological and biochemical parameters. The present study concludes that, 100 mg sel kg^{-1} diet was required to reduce copper toxicity when copper exist in the environment.

Materials and Methods

An experimental animal (*Labeo rohita)* is the most important among the three Indian major carp species used in carp polyculture systems. its high growth potential, coupled with high consumer preference, have established *L. rohita* as the most important freshwater species cultured in India, *L. rohita* were collected from the Manimuthar Dam, Triunelveli district, and immediately transported to the laboratory in plastic buckets with the same Dam water. Fishes were acclimated to the laboratory conditions for a month. During acclimation, the animals fed with dried pellets of 35% protein diet. The water in the rearing tanks was changed daily. Well acclimated *L. rohita* (2.15± 0.75 g) were selected from the stock and divided into 6 groups of 10 individuals of each. Both male and female sexes were used. They were not fed for 24 h before commencement of the experiment.

Feed preparation

The ingredients of dried fish meal groundnut oil cake, cod liver oil, egg yolk, tapioca flour, vitamins and minerals mixture were used to prepare the 35% protein diet with appropriate proportions by square method [1]. Five diets were prepared with different levels of selenium (sodium selinite). The experimental diets (0, 25, 50, 100 and 200 mgselkg^{-1} diet) were prepared by adding the appropriate levels of selenium with chosen ingredients and an aliquot of boiled water, mixed well and steam cooked for 15-20 minutes. After moderate cooling pellets (2 mm) were prepared with a hand operated pelletizer and dried in sunlight. After drying, diets were separately stocked in refrigerator.

Test chemical preparation

A stock solution of copper (ppt) was prepared by dissolving exactly 3.93 g of copper sulphate $CuSO_4$ 7 H_2O in 1 lit of distilled water. From this stock solution, the desired concentration (ppm) was prepared by mixing appropriate quantity of fresh water. The static renewable bioassay method was adopted to estimate the LC_{50} [2]. The 96 hr LC_{50} value, its 95% confidence limit and slope function were determined following the method of Litchfied and Wilcoxon. 50% of LC50 was taken as the maximum sub lethal concentration for the present study.

Oxygen consumption was estimated by Winkler's idometric method described by Welsh and Smith [3]. A method derived by Yokayama [4] and later modified by Christensen et al. [5] was followed to estimate the RBC quantitatively using the Haemocytometer. The carbohydrate contents, proteins and lipids of the feed samples were estimated by Anthrone method and Lowry et al., and Bradgon [6,7] respectively.

Statistical analysis

The collected data were subjected in to the Standard deviation and Student's 't' test.

Results

Copper exposed animals fed on selenium free diet elicited the significant ($p<0.05$) reduction in the oxygen consumption (Figure 1) and

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specific growth rates and the parameters were improved significantly ($p<0.01$) in copper exposed fish fed on selenium supplemented diets (Table 1, Figures 1 and 2).

The red blood corpuscles of control fish was high and it significantly ($p<0.05$) declined in fish exposed to sub lethal level of copper and fed on selenium free diet. However RBC's count was improved in the copper exposed fish received the dietary supplementation of selenium diets (Table 2 and Figure 3).

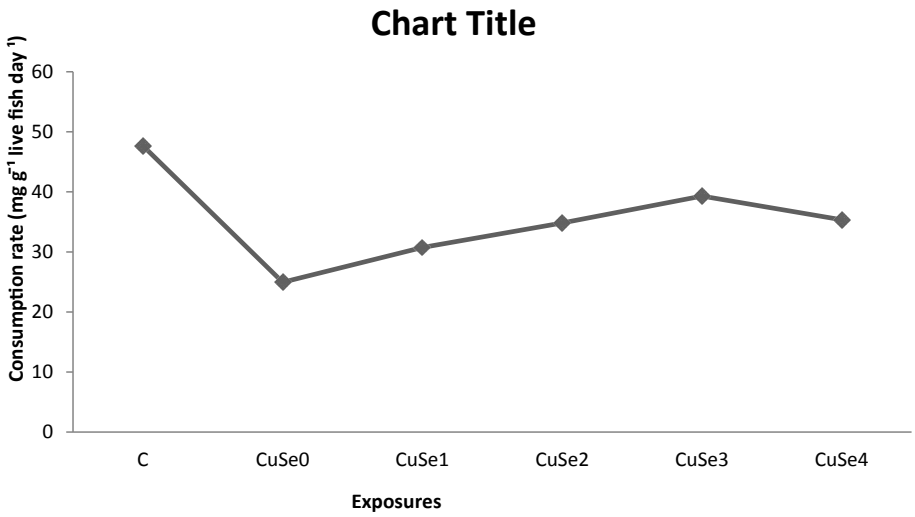

Figure 1: Effect of feeding different levels of dietary selenium on consumption rate in copper exposed *labeo rohita.*

Concentrations of copper(ppm)	Dead / tested	Percent mortality	Lethalconcentration (ppm)			95% confidence limit (ppm)		Slope function (S) ppm	Regression equation(R=a+bx)
			16	50	84	Upper	Lower		
0.100	0/6	0							
0.150	1/6	16.67							
0.200	3/6	50.00	0.09	0.23	0.56	1.82	-1.10	8.77	R=1.84 ± 8.77
0.300	4/6	66.67							
0.400	5/6	84.33							
0.500	6/6	100							

Table 1: Effect of copper levels on *Labeo rohita* exposed for 96 hr.

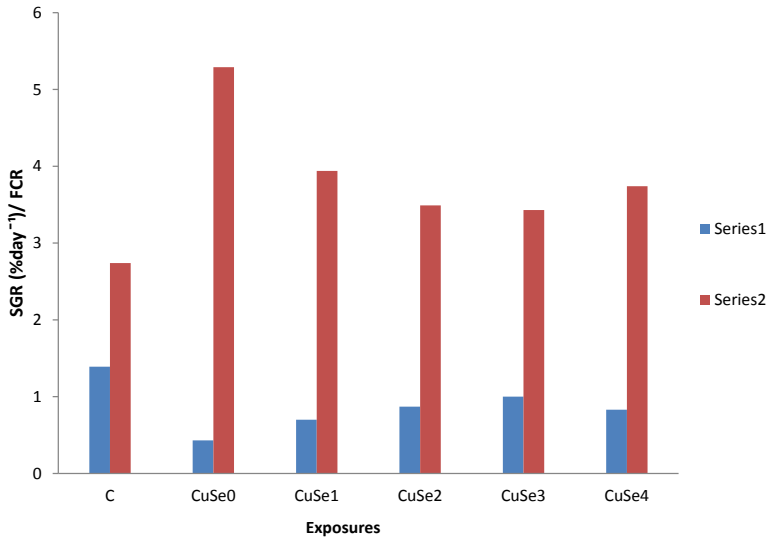

Figure 2: Effect of feeding different levels of selenium on specific growth rate (% day^{-1}) and feed conversion ratio in copper exposed *Labeo rohita.*

Parameters	Exposures					
	Control	CuSe0 0 mg	CuSe1 25 mg	CuSe2 50 mg	CuSe3 100 mg	CuSe4 200 mg
Initial wet weight (gm)	21.06 ± 1.45	21.90 ± 2.13	22.35 ± 2.30	21.15 ± 2.07	22.03 ± 1.77	20.61 ± 2.56
Initial mean wet weight (gm)	2.106 ± 0.20	2.190 ± 0.19	2.235 ± 0.17	2.115 ± 0.22	2.203 ± 0.20	2.061 ± 0.16
Final wet weight (gm)	32.06 ± 3.15	25.00 ± 1.96	27.58 ± 2.36	27.48 ± 2.60	29.61 ± 2.15	26.46 ± 2.19
Final mean wet weight (gm)	3.206 ± 0.27	2.500 ± 0.26	2.758 ± 0.26	2.748 ± 0.25	2.961 ± 0.28	2.646 ± 0.27
Total weight gain (gm)	11.00 ± 1.06	3.10 ± 0.35	5.23 ± 0.47	6.33 ± 0.38	7.58 ± 0.57	5.85 ± 0.49
Feed intake(g dry matter)	30.12 ± 3.86	16.4 ± 1.50	20.61 ± 2.18	22.10 ± 1.85	25.97 ± 2.61	21.86 ± 2.35
Consumption rate(mg live fish g^{-1} day^{-1})	47.67 ± 3.96	24.96 ± 1.57	30.74 ± 2.65	34.83 ± 2.94	39.30 ± 3.13	35.36 ± 3.70
Gain in weight(g wet weight)	11.00 ± 1.06	3.10 ± 0.35	5.23 ± 0.47	6.33 ± 0.38	7.58 ± 0.57	5.85 ± 0.49
Specific growth rate (%days)	1.39 ± 0.04	0.43 ± 0.02	0.70 ± 0.03	0.87 ± 0.06	1.00 ± 0.08	0.83 ± 0.07
Feed conversion rate	2.74 ± 0.18	5.29 ± 0.41	3.94 ± 0.27	3.49 ± 0.36	3.43 ± 0.45	3.74 ± 0.29
Red blood corpuscles count($\times 10^6 mm^{-3}$)	2.57 ± 0.22	1.557 ± 0.12	1.85 ± 0.11	2.02 ± 0.02	2.25 ± 0.05	2.12 ± 0.12
Heamoglobin	8.69 ± 0.45	5.10 ± 0.43	5.39 ± 0.51	5.89 ± 0.55	6.95 ± 0.62	6.04 ± 0.57
Oxygen carrying capacity of blood (mg O_2 g^{-1} Hb)	10.86 ± 0.94	6.38 ± 0.57	6.74 ± 0.66	7.36 ± 0.42	8.69 ± 0.67	7.55 ± 0.72
Rate of oxygen consumption (mg O_2 g^{-1} hr^{-1})	0.33 ± 0.01	0.56 ± 0.04	0.49 ± 0.05	0.44 ± 0.05	0.39 ± 0.04	0.43 ± 0.05

Table 2: Effect of sub lethal exposure of copper and supplementation of selenium (mg kg^{-1} diet) levels on weight gain, selected food utilization parameters, hematological parameters and oxygen consumption in labeo rohita. Each value is the mean ($\bar{x}$ ± SD) of three estimations.

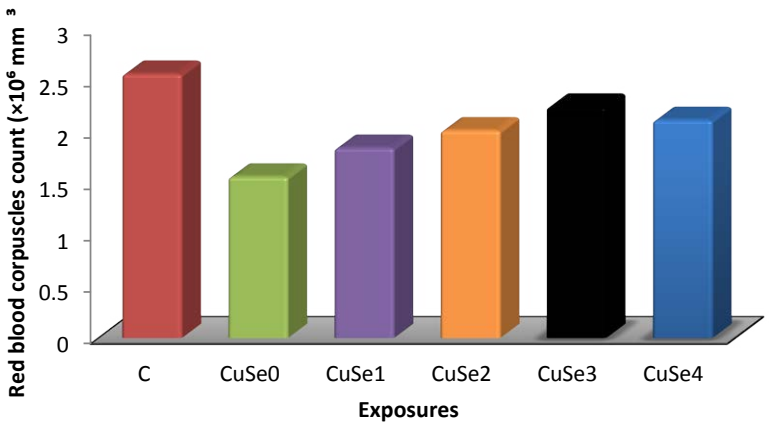

Figure 3: Effect of feeding levels of selenium on red blood corpuscles count in copper exposed *Labeo rohita*.

Haemoglobin content of copper exposed *Labeo rohita* ($CuSO_4$) at the end of the exposure period was 5.10 g% as against 8.69 g% in control. There was two times decline of haemoglobin content in copper exposed fish as compared to control. The exposures of Labeo rohita sublethal level of copper resulted the significant ($p<0.05$) decrease in the RBC count and haemoglobin content leading to anaemia. The anaemia condition may be due to inhibition of erythropoiesis and haemosynthesis and increase in the rate of erythrocyte destruction [8]. Oxygen carrying capacity of blood was also declined in metal exposed *Labeo rohita* may be due to the reduction of RBC count and Hb content. James and Sampath [9] found that the oxygen carrying capacity of blood of *Heteropneustes fossils* declined due to the reduction of RBC count and Hb content which reflected on tissue respiration. Ahmed et al., [10] found that the organic selenium supplementation to fry Nile tilapia enhanced the growth, feed utilization and reduced the susceptibility to *Aeromonas hydrophila* infection. The proximate compositions and energy contents were high in control fish. Copper exposed L. rohita fed on se free diet reduced the proximate compositions and energy and they were improved in copper exposed fish fed on se supplemented diets. Among these supplemented diets, fish fed on 100 mg Se k-1 dirt, significantly ($p<0.05$) improved the selected physiological and biochemical parameters then other levels. Selenium (Se) is an essential trace element required in the diet for normal growth and physiological function of fish [11,12]. Hence supplementation of 100 $mgSekg^{-1}$ diet is considered as optimum dose and selenium protects L. rohita from copper toxicity.

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