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Effect of Cadmium on Blood Plasma Biochemistry in Carp (*Cyprinus carpio* L.)

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Trace amounts of cadmium occur naturally in water, for example, Svobodová et al. (1996) reported cadmium content in sediments of uncontaminated ponds in Southern and Western Bohemia (0.01-0.3 mg/kg dry matter). Wastewater from both industry and agriculture is the main source of pollution (Pitter 1999). Svobodová et al. (1996) report increased cadmium concentrations in bottom sediments of the Berounka river downstream from the town of Plzeň (14.0-15.0 mg/kg dry matter) and Leontovičová (2003) report increased cadmium concentrations in bottom sediments of the Svratka river downstream from the town of Židlochovice (12.8 mg/kg dry matter). Cadmium is a metal toxic to fish, with an important bioaccumulation capacity (Svobodová et al. 1987, Cibulka et al. 1991). Cadmium is released from bottom sediments into food chain in water environment. Among various effects, cadmium may alter biochemical parameters of fish blood plasma. Vaglio et al. (1999) observed a significant decrease in activities of liver cytoplasmic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) while a simultaneous increase of the serum activities of these same enzymes. Almeida et al. (2002) observed decreased activities of lactate dehydrogenase (LDH) and creatine kinase (CK), decreased glycogen content and glucose uptake in white muscle and increased CK and LDH activities and glucose uptake in red muscle. Soengas et al. (1996) observed changes in carbohydrate metabolism in the Atlantic salmon (*Salmo salar*) after only an 8 hr exposure to sublethal concentrations of cadmium. These changes included an activation of liver glycogenolysis and glycolysis as well as increased levels of plasma glucose and lactate.

The aim of this investigation was to assess intensity of stress, degree of damage to the parenchymatous organs and to calcium and phosphorus metabolism in carp following acute effect of cadmium.

MATERIALS AND METHODS

Blood plasma of one- to two-year-old common carp (*Cyprinus carpio* L.) was examined at the end of 96-hour acute toxicity test of cadmium chloride (ACS Reagent, f. Merck) at a concentration of 12.5 mg/L (7.67 mg/L of cadmium). The

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cadmium chloride concentration used was the LC50 of this substance for common carp of that age category. At the same time, biochemical examination was made on a control group of common carp. The test was carried out in a semi static way, the bath being exchanged every 24 hr. The basic physical and chemical indices of diluting water used in this acute toxicity test were as follows: pH 7.36, $ANC_{4.5}$ (alkalinity) 1.2 mmol/L, COD_{Mn} 1.6 mg/L, BOD_5 0.79 mg/L, NH_4^+ 0 mg/L, NO_3^- 11.51 mg/L, NO_2^- 0 mg/L, sum of Ca + Mg 14 mg/L. During the test, water temperature ranged from 18.9-20.5 °C, the oxygen saturation in the water ranged from 61.0-78.8%. The test was carried out in 2 control aquaria and 3 aquaria containing cadmium chloride at a concentration 12.5 mg/L.

Biochemical analyses of blood plasma involved 20 controls (C) (431.9±109.53 g body weight, bw) and 20 experimental carp (E) (515.7±113.17 g bw). Blood was sampled by means of cardiac puncture. Heparin in the amount of 50 IU sodium salt per 1 mL blood was used for stabilization. Individual blood samples of all investigated fish were centrifuged for 15 min at 400g. The activity of alanine aminotransferase (ALT) using the Bio-Vendor-test No. 10451, aspartate aminotransferase (AST) using the Bio-Vendor-test No. 10351, lactate dehydrogenase (LDH) using the Bio-Vendor-test No. 12352, creatine kinase (CK) using the Bio- Lachema-test No. 1303801, alkaline phosphatase (ALP) using the Bio-Vendor-test No. 10061, concentrations of total protein (TP) using the Bio-Vendor-test No. 12751, glucose (GLU) using the Bio-Vendor-test No. 11601, bilirubin using the Bio-Lachema-test No. 1105309 and electrolytes (Ca, P) using the Dialab-test No. 00363 were determined using the COBAS MIRA automatic analyser (Hoffmann, La Roche, Co. Switzerland). For the determination of CK activities, the plasma was diluted 5-10 times with a physiological solution. The cortisol concentration in blood plasma was assessed by radioimmunoassay by means of a kit (Immunotech Comp.).

Kidney, liver and muscle tissue were sampled for determination of cadmium residues. The samples of approximately 250 mg fresh tissue were treated in closed tubes with 0.5 mL of 7.2 M HNO_3 and subjected to acid attack for 12 hr at room temperature. The tubes were placed inside an ultrasonic water-bath at 50 ^{0}C, sonicated for 15 min and then placed into a heated block at 115 ^{0}C untill the content was clear (approx. 5 min). After solubilization, the tubes were cooled down, the dissolved samples were diluted to 10 mL by ethanol and analysed by AAS (Perkin–Elmer Z 5000) (Čelechovská, 2000). The proposed method was validated by CRM (BCR: No 278 - mussel tissue, NRC: TORT-2 lobster hepatopancreas). The AAS-Standard (f. Merck) was used for AAS analysis. The instrument was calibrated on four points (stock solution: $Cd(NO_3)_2$ in HNO_3 0.5 mol/L; solutions for calibration: 0.5, 1.0, 2.0, 4.0 µg/L). The limit of detection (12.5 µg/kg) was calculated as three times the standard deviation of replicates from the blank measurements.

QA/QC measures were consistently applied within all experiments. All the measurements described above were carried out according to validated standard operation procedures.

All statistics were counted by descriptive statistic basic and by one-factor analyses of variance (STATISTICA program 6.0 version). The signification of tests was calculated at the level $p<0.01$ and $p<0.05$. This program performed also categorized box and whiskers plots including box whiskers type mean, standard error of the mean (SE) and 1.96*SE.

Ethical Committee of the University of South Bohemia České Budějovice, Research Institute of Fish Culture and Hydrobiology Vodňany (approval No. 7/2002) approved the experiments on fish.

RESULTS AND DISCUSSION

The following clinical changes were recorded during blood sampling in the experimental common carp group after 96 hr treatment with cadmium chloride at a concentration of 12.5 mg/L: restlessness, jerky swimming movements and accelerated respiration. During blood sampling, the control group of carp showed no clinical changes.

Figure 1 shows a comparison of GLU concentration and AST, LDH, CK enzyme activities in the control group of common carp and in those exposed to acute action of cadmium. Cadmium caused a significant increase ($p<0.01$) in all these indices in the experimental group of carp compared to the controls.

Table 1 shows concentration of plasma cortisol, TP, bilirubin and ALP and ALT enzyme activities in the control and experimental groups. They are comparable, showing no significant differences between the two groups.

Stress is commonly defined as a state or condition in which the homeostasis of an individual is disturbed as a result of the action of external stimuli, termed 'stressors'. Stressors elicit changes in the animal physiological state, which is interpreted as the stress response (Leatherland and Woo 1998, Palíková and Svobodová 1995, Svoboda 2001). Primary response involves activation of the neuroendocrine system resulting in release of catecholamine and corticosteroid hormones. Secondary response includes physiological responses to these hormones (e.g. increased cardiac output, O_2 uptake and mobilization of energy resources). Tertiary response is identified at the level of the entire organism and it includes inhibition of growth, impaired reproductive success and impaired immune responses.

In our study, after 96 hr acute exposure to cadmium (in the form of cadmium chloride at a concentration of 12.5 mg/L), cortisol concentration in blood plasma was comparable in both the experimental and control groups. Mommsen et al. (1999) reported that under acute stress situations, plasma cortisol concentration tends to shoot up within a minute to hours time frame, followed by a gradual decrease to pretreatment levels within a day or so, depending upon subsequent maintenance conditions. A major drawback when using cortisol as the indicator of

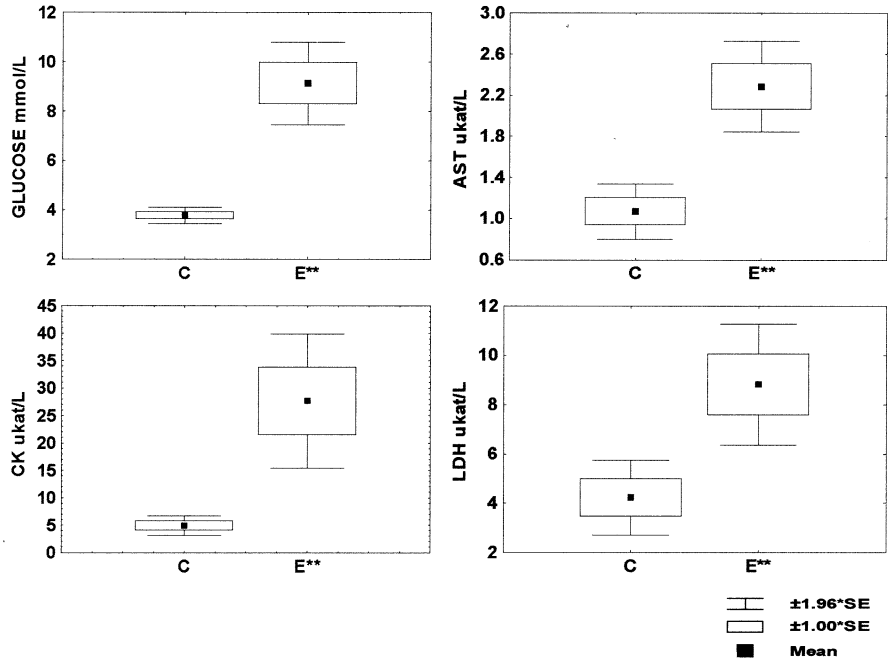

Figure 1. Effect of acute exposure to cadmium chloride (12.5 mg/L) on glucose concentration, AST, CK and LDH activities in the blood plasma of carp (N control = 20, N experiment = 20). C-control group, E- experimental group; significance **p<0.01.

stress levels is that chasing, capture and blood sampling procedures are also stressors and thus they may cause increases in blood levels of the hormone, as asserted by Leatherland and Woo (1998). They also declare that elevated plasma cortisol levels might not necessarily be indicative of exposure to a stressor.

The acute effect of cadmium on common carp caused a significant increase ($p<0.01$) of plasma glucose concentration compared to that in the control group. Post-stressor increases in blood glucose levels may be used as an indicator of the secondary phase of stress response (Leatherland and Woo 1998). Soenags et al. (1996) demonstrated the existence of changes in liver carbohydrate metabolism in Atlantic salmon (*Salmo salar*) after only an 8 hr exposure to sublethal concentrations of cadmium. These changes include an activation of liver glycogenolysis and glycolysis as well as increased levels of plasma glucose and lactate. Ghazaly (1992) observed increased plasma glucose levels in *Tilapia zillii* after a 48 hr exposure to sublethal concentration of cadmium (17.7 and 24.78 mg/L).

The activity of enzymes in blood may also be used as a stress indicator. Enzymes used for this purpose above all are LDH, CK and transaminases (ALT, AST). A

significant increase in concentration of these enzymes in blood plasma indicates tissue impairment caused by stress (Svoboda 2001). We observed a significant increase ($p<0.01$) of LDH, CK and AST activities in blood plasma of fish exposed to cadmium compared to the control group of fish. On the contrary, values of ALT and also of ALP were comparable in both the experimental and control groups. Vaglio et al. (1999) observed a significant decrease of activities of liver cytoplasmic AST and ALT while a simultaneous increase of the serum activities of the same enzymes after acute exposure to cadmium at a concentration of 2.5 mg/kg body weight. El-Demerdash and Elagamy (1999) observed an inhibition of activities of acetylcholinesterase, alkaline phosphatase and glutathione S-transferase. Changes in carbohydrate metabolism may result from metal-induced increase of locomotion activity involving high energy demand, accelerated glycogenolysis and increase of glucose oxidation. Disturbances of carbohydrate metabolism may also be caused by hypoxia due to metal-induced lesions in fish gills. The increase of LDH activity (accompanied by elevated levels of lactate and pyruvate) indicates greater reliance of poisoned fish on the energy less efficient anaerobic glycolysis (Jezierska and Witeska 2001).

Table 1. Effect of acute exposure to cadmium chloride (12.5 mg/L) on the concentration of plasma cortisol, TP, bilirubin and ALP and ALT enzyme activities in the control and experimental groups of carp.

Indices	Units	Groups	N	Mean	SD	SE	Probability
TP	g/L	control	20	30.71	4.15	0,93	0.085
		experiment	20	28.35	4.29	0,96	
Total bilirubin	μmol/L	control	20	5.04	3.42	0,76	0.570
		experiment	20	5.81	5.01	1,12	
ALP	μkat/L	control	19	0.43	0.36	0,08	0.333
		experiment	20	0.34	0.12	0,03	
ALT	μkat/L	control	20	0.02	0.02	0	0.332
		experiment	20	0.02	0.01	0	
Cortisol	mol/L	control	19	0.47	0.36	0,08	0.279
		experiment	20	0.59	0.30	0,07	

Total protein level is a frequently measured parameter of metal poisoning in fish. However, data available do not allow to assessment of the direction of these changes, since the same metal may cause both an increase and a decrease in total protein level (Jezierska and Witeska 2001). We observed no changes in plasma total protein level in the experimental and control groups of fish. Ghazaly (1992) observed hyperproteinemia in *Tilapia zillii* after a 72 hr exposure to sublethal concentration of cadmium (10.72, 17.7 and 24.78 mg/L). He explained it by changes in mobilization of serum protein or by the effect of metal binding to plasma proteins.

Concentration of electrolytes under study (Ca, P) in blood plasma of both the

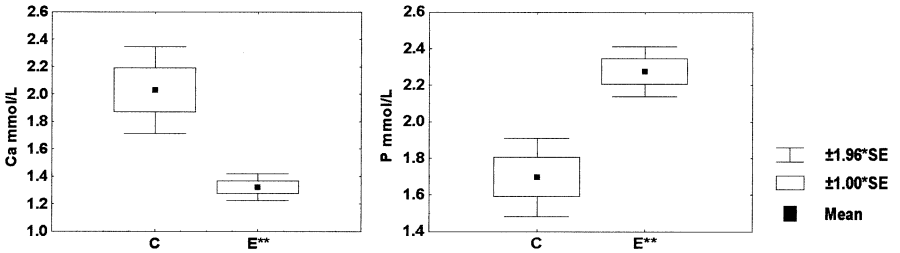

Figure 2. Effect of acute exposure to cadmium chloride (12.5 mg/L) on electrolyte concentration in the blood plasma of carp (N control=20, N experiment=20). C-control group, E- experimental group; significance **p<0.01.

control and experimental groups of common carp are compared in Figure 2. In the experimental group, the acute effect of cadmium caused significantly lower ($p<0.01$) concentration of calcium and significantly higher ($p<0.01$) concentration of phosphorus compared to the control group.

Internal fluids of freshwater fish are highly concentrated compared to the aquatic environment (hyperosmoregulators). Each species regulates the blood and body fluid salt concentration within certain limits and departure from this range is likely to be the result of stress (Leatherland and Woo 1998). Therefore, the significant decrease ($p<0.01$) in the calcium concentration and the significant increase ($p<0.01$) in the phosphorus concentration in the experimental fish fit in the diagnosis of the toxic effect of cadmium. Fish obtain most of their calcium from water, unlike terrestrial vertebrates for which food is the only source. In freshwater teleosts, the chloride cells in gills transport calcium (Evans 1993). Cadmium strongly affects calcium uptake. This is possible since cadmium competes with calcium for transport sites on the Ca-pumps in the gills (Leatherland and Woo 1998). That could also explain mutual competition resulting in reduced calcium uptake by cadmium and lower accumulation of cadmium at high calcium concentrations (Richards and Playle 1999, Jezierska and Witeska 2001). Apart from gills, skin, intestines, liver and gallbladder, kidney is an excretory organ for heavy metals in fish. Absorbed cadmium is redistributed to the kidney and liver following exposure (Leatherland and Woo 1998). Cadmium-induced pathological lesions and metabolic dysfunction may impair excretory processes in the kidney (Jezierska and Witeska 2001). This could also be a reason for plasma calcium and phosphorus concentrations in fish exposed to cadmium.

Figure 3 shows cadmium residues in kidney, liver and muscle in the control and experimental groups of common carp. Accumulation of cadmium was the highest in kidney followed by liver and muscle. The accumulation of cadmium in these organs was significantly ($p<0.01$) higher in fish exposed to cadmium than in the control group of fish.

Various metals show different affinity to various fish tissues. Cadmium is

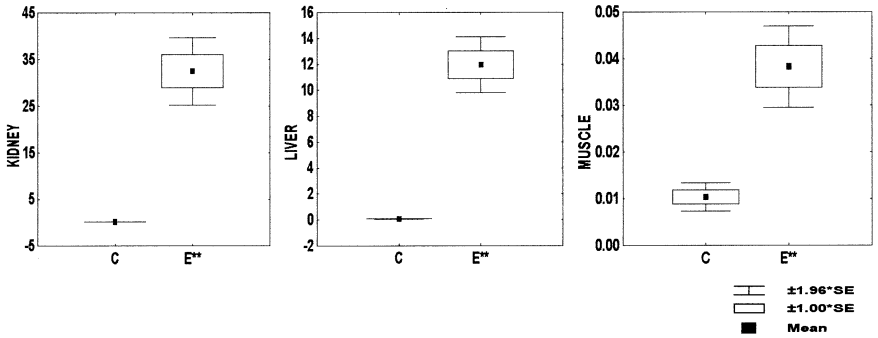

Figure 3. Effect of acute exposure to cadmium chloride (12.5 mg/l) on the concentration of cadmium in the tissues under study (mg/kg wet weight). (N control=20, N experiment=20). C-control group, E- experimental group; significance **p<0.01.

accumulated mainly in the kidney and then in spleen, liver and gills. Lower amounts of cadmium were found in the digestive tract, muscles and bones (Jezierska and Witeska 2001). We observed the highest accumulation of cadmium in the kidney followed by liver and muscle. The accumulation of cadmium in these organs was significantly ($p<0.01$) higher in fish exposed to cadmium than in the control group. De Smet and Blust (2001) show the cadmium accumulation in common carp tissues after chronic exposure in the following order: kidney > liver > gills. Woo et al. (1993) observed the highest cadmium accumulation in blue tilapia (*Oreochromis aureus*) after chronic exposure in the kidney, followed by liver, brain, gill filaments and muscles.

The increased concentration of glucose, CK and LDH in blood plasma indicates stress effect of cadmium in carp. Increased concentration of AST in blood plasma indicates impairment of parenchymatous organs (namely liver). Decreased calcium concentration in blood plasma indicates damage to gills. Increased phosphorus concentration in blood plasma indicates impairment of kidney.

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