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Effects of Lead Nitrate ($PbNO_3$) on the Glucose and Cortisol Hormone Levels in Common Carp, *Cyprinus carpio*

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Abstract: The objective of this study was to evaluate the possible effects of $PbNO_3$ exposure on variations of glucose and cortisol levels in *Cyprinus carpio*. Fish were subjected to two sub-lethal concentrations of $PbNO_3$ for 14 days. Blood samples were isolated from the fish following the exposure, to measure the levels of cortisol and glucose compared to the control group. We found significant increases ($p<0.05$) in the levels of blood cortisol in two groups of fish after 14 days of exposure to two concentrations of $PbNO_3$ (1.3 and 2.6 mg L^{-1}) The results showed significant increases in the glucose levels of both fish groups exposed for 14 days In the later treatment, the rate of increase in group II (exposed to 2.6 mg L^{-1} $PbNO_3$) was higher than that of group I (exposed to 1.3 mg L^{-1} $PbNO_3$) (P = 0 compare to P = 0.007). Present findings attest that exposing to waterborne lead would affect the levels of both glucose and cortisol in *Cyprinus carpio*.

Key words: Lead nitrate, cortisol, *Cyprinus carpio*, glucose

INTRODUCTION

Anthropogenic activities during the past century lead to an increasing accumulation of toxic metals in soils and natural waters (Pinheiro *et al*., 1999). According to Markus and McBratney (2001), contamination of water by lead has occurred on a global scale with adverse effects to human and environment health. Lead is considered a major toxicant to several aquatic organisms and a non-specific poison affecting a wide range of physiological systems (Ma, 1996; Pain, 1997), but its uptake by fish is still a controversial issue, depending upon a variety of factors such as species and nutritional behavior, According to Zimmermmann *et al.* (1999), both water borne and trophic exposures can lead to a significant accumulation of this metal in distinct fish tissues with different consequences to physiological processes (Rabitto *et al*., 2005). It is known that physiological and biochemical parameters in fish blood could change when exposed to heavy metals and that these parameters are extremely sensitive to these elements (Cicik and Engin, 2003). Heavy metal stimulated the Hypothalamus-Pituitary-Interrenal (HPI) axis and causes an elevation of cortisol in blood of fish (Handy, 2003). Heavy metals such as cadmium and copper have been shown to cause a significant rise in cortisol levels in rainbow trout (Wu *et al*., 2002). Previous study reported that *Oncorhynchus mykiss* exposed to 10 g Cd L^{-1} for 30 days showed a significant increase in cortisol levels (Ricard *et al*., 1998). Shah *et al.* (2002), also reported the release of corticosteroid hormones in Sockeye salmon, *Oncorhynchus nerka*, when treated with copper. Cortisol affect the carbohydrate metabolism (Andersson *et al*., 1988) and increase the glucose 6-phosphatase activity in liver (Shahbazi and Maleknia, 1999), thus cause an elevation of glucose concentrations in blood of fish The depletion of liver glycogen (glycogenesis) and the rise in blood glucose levels were reported in carp (*Cyprinus carpio*) after exposure to several pollutants at sub-lethal concentration (Abdelmeguid *et al*., 2002). Increases in plasma glucose concentrations were previously described in *Salmo gairdneri* (Monteiro *et al*., 2005) and *Heteroclarias* (Kori-Siakpere *et al*., 2006) after exposed to copper and cadmium, respectively.

Fish are largely used in evaluation of aquatic systems quality and some of their physiologic changes can be considered as biologic markers of environmental pollution (Dautremepuits *et al*., 2004). It has a great potential to serve as sensitive indicators, signaling exposure and understanding the toxic mechanisms of stressors in aquatic ecosystems (Vutukuru *et al*., 2005). Common carp is a widely used species in aquaculture for food supply in Iran (Salehi, 2006). It provides a good model to study responses and possible adaptations of local fish populations exposed to diffuse pollution originated from various sources. In present study, we hypothesized that changes in the levels of blood cortisol and glucose of the carp exposed to sub-lethal concentrations of lead nitrate could be as bio-indices of pollution in a water source.

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MATERIALS AND METHODS

This study was carried out in the Laboratory of Fish Pathology of Iranian Artemia Research Center (Urmia, Iran), during the months July and August 2006. Common carp (200±50 g and 18.3±5 cm) were purchased from a local hatchery and were maintained in 200 L tanks containing aerated tap water. The other physicochemical elements of the water and experimental condition were kept quite instant during the course of study (Water temperature: 17±1°C; Ca^{2+}: 145.09 mg L^{-1}; Mg^{2+}: 398.7 mg L^{-1}; salinity: 1.7g L^{-1}; dissolved oxygen: 7.25±3 mg L^{-1}; photoperiod: 12L^{-1} 12D). Prior to the onset of treatment, the fish were acclimatized to the laboratory conditions for 14 days. They were fed once daily with commercial trout pellets (Chineh Co., Karaj, Iran), comprising the following ingredients: protein 36, lipid 14, ash 16, fiber 3.5, phosphorus 1, wet 11, carbohydrate 22.5 and fish meal 50%. Examined fish were divided in two groups exposed to two sub-lethal concentrations of lead (1.3 and 2.6 mg L^{-1} for group I and II, respectively). Lead stock solutions were made from lead nitrate ($PbNO_3$), added subsequently to aerate tap water in the tanks to obtain test concentrations. Control groups were the carps maintained in normal tap water. The water in each tank was replenished daily to keep the metal concentrations unchanged. From each exposed group, six fish were anesthetized by stroking on their head and their blood samples were taken through puncturing the caudal vessel.

Measurement of the levels of cortisol and glucose: Blood was taken from the tail vein of the fish with non-heparinized syringe, collected in plastic Eppendorf tubes. Serum was obtained by centrifugation of blood at 3000 rpm for 15 min and nonhaemolysed serum immediately frozen on liquid nitrogen and stored at -80°C until analyze. Serum glucose levels were measured by Spectrophotometer Enzymatic Methods using a commercial glucose kit (Enzymatic, GOD Trinder). Cortisol levels in the blood were determined by Radioimmunoassay (RIA), using LKB-Wallac apparatus and a cortisol kit made by Kavoshyar Co. (Iran).

Statistics: Data are expressed as mean±SEM. Difference among groups were tested by one-way ANOVA. The Tukey test, with 95% confidence limits, was applied to compare the means whenever there was a significant difference (using SPSS, version. 12). The level of significance was set at $p<0.05$.

RESULTS

The mean values (±SEM) of cortisol and glucose levels for exposed and control groups offish are shown in Table 1 and 2. Statistical analysis gives evidence of significant differences between exposed and control groups.

Table 1: The levels of cortisol (ng mL^{-1}) in blood serum of two groups of common carp exposed to two doses of $PbNO_3$ (1.3 and 2.6 mg L^{-1} for groups I and II, respectively) for 14 days

Group	Exposure time (14 days)
Control	193.6±10.2
I	354.3±13.2[a]
II	397.0±3.2[a]

a: $p<0.05$, Data shown as mean±SEM

Table 2: Concentrations of glucose (mg dL^{-1}) in blood serum of two groups of common carp exposed to two doses of $PbNO_3$ (1.3 and 2.6 mg L^{-1} for groups I and II, respectively) for 14 days

Groups	Exposure time (14 days)
Control	24±1.15
I	45±5.13[a]
II	72±1.15[a]

a: $p<0.05$, Data shown as mean ±SEM

Serum cortisol levels in common carp subjected to two sub-lethal concentrations of lead nitrate (1.3 and 2.6 mg L^{-1}) for 14 days had significant increases ($p<0.05$). Exposure to 1.3 mg L^{-1} of lead nitrate for a period of 14 days caused significant increase of about 85% in cortisol levels. Cortisol levels in group II (exposed to 2.6 mg Pb L^{-1}), elevated to 105% above the control levels (Table 1). Changes in concentrations of blood glucose of the treated fish are presented in Table 2. Glucose levels in lead-treated carps, increased to 87% and 200% above the control levels in group [2] (exposed to 1.3 mg Pb L^{-1}) and group II (exposed to 2.6 mg Pb L^{-1}), respectively ($p = 0.007$ against $p = 0$).

DISCUSSION

In fish lead is a non-essential heavy metal and in excess concentrations, have toxic effects, ranging from histological lesions (Forlin *et al*., 1986; Lévesque *et al*., 2003) to disruption of metabolic and endocrine functions (Lévesque *et al*., 2002, 2003; Campbell *et al*., 2003).Fish exposed to heavy metals activate several compensatory mechanisms, of which some are mediated by a non-specific stress response (Wendelaar Bonga, 1997). Cortisol is a non-specific stress response that release to the blood via stimulation of the Hypothalamus-Pituitary-Interrenal (HPI) axis by heavy metal exposure (Pelgrom *et al*., 1995; Dethloff *et al*., 1999). Cortisol is not stored in the interrenal tissue, but is synthesized on demand (Sumpter, 1997) and so, the elevation of circulating cortisol must be a function of *de novo* stimulation of the HPI axis

Cortisol affects carbohydrate metabolism and a rise in cortisol levels in frequently followed by hyperglycemia in fish (Wendelaar Bonga, 1997). Although the mechanisms involved remain unclear, the rapid rise in

plasma glucose concentration following an acute stressor has been associated with the activation of the Hypothalamus-Sympathetic-Chromaffin cell (HSC) axis (McDonald and Milligan, 1997), rather than with the cortisol rise mediated by the Hypothalamus-Pituitary-Interrenal (HPI) axis (Arends *et al*., 1999). Hyperglycemic response illustrated in this study is an indication of a disrupt carbohydrate metabolism, possibly due to enhanced the glucose 6-phosphatase activity in liver, elevated breakdown of liver glycogen (glycogenesis) (Shahbasi and Maleknia, 1999) and the synthesis of glucose from extra-hepatic tissue proteins and amino acids (Žikič *et al*., 2001). Accordingly, the high plasma levels of cortisol and glucose observed in the present study may be indicative of the stimulation activation of HPI and HSC axes by lead and induction of different compensatory responses.

In *Cyprinus carpio*, plasma cortisol and glucose levels significantly increased during the water lead exposure period. Increases in the cortisol and glucose levels were dose-dependent and previously described in *Prochilodus lineatus* (Martinez *et al*., 2004) and *Oreochromis niloticus* (Monteiro *et al*., 2005) in response to lead and copper, respectively. In *O. mykiss* (Dethloff *et al*., 1999) and *Cyprinus carpio* (De Boek *et al*., 2001), cortisol concentrations were significantly increased during the copper exposure period. Salmerón-Flores *et al*. (1990) reported increased glucose blood concentrations in *Sarotherodum aureus* in response to lead exposure. A strong hyperglycaemia was also observed in *Oncorhynchus mykiss* (Richards and Playel, 1999) and *Salmo gairdneri* (Haux and Larsson, 1984) after exposed to copper and cadmium, respectively.

To conclude, this study denotes that the response of the fish to lead nitrate was dose dependent and changes in cortisol and glucose levels were obvious after 14 days exposure.

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REFERENCES

Abdelmeguid, N., A.M. Kheirallah, Abou-Shaban, K. Adham and A. Abdel-Moneim, 2002. Histolochemical and biochemical changes in liver of *Tilapia zilli* G. as a consequence of water pollution. J. Biol. Sci., 2., 4: 224-229.

Andersson T., L. Förlin, J. Hardig and A. Larsson, 1988. Physiol. disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluent. Can. J. Fish. Aquat. Sci., 45: 1525-1536.

Arends, R.J., J.M. Mancera, J.L. Munoz, S.E. Wendelaar Bonga and G. Flik, 1999. The stress response of the gilthead sea bream (*Sparus aurata* L.) to air exposure and confinement. J. Endocrinol., 163: 149-157

Campbell, P.G.C., A. Hontela, J.B. Rasmussen, A. Giguere, A. Gravel and L. Kraemer, 2003. Differentiating between direct (physiological) and food-chain mediated (bioenergetic) effects on fish in metal-impacted lakes. Hum. Ecol. Risk Assess., 9: 847-866.

Cicik, B. and K. Engin, 2003. The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L.1785). Turk. J. Vet. Anim. Sci., 29: 113-117.

Dautremepuits, C., S. Betoulle, S. Paris-Palacios and G. Vernet, 2004. Immunol-related perturbations induced by copper and chiston in carp (*Cyprinus carpio*). Arch. Environ. Contam. Toxicol., 47: 370-378.

De Boeck, G., A. Vlaeminck, P.H. Balm, R.A. Lock, B. De Wachter and R. Blust, 2001. Morphol. and metabolic changes in common carp (*Cyprinus carpio*) during short-term copper exposure: Interactions between Cu^{2+} and plasma cortisol elevation. J. Environ. Toxicol. Chem., 20: 374-381.

Dethloff, G.M., D. Schlenk, S. Khan and H.C. Bailey, 1999. The effects of copper on blood and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol., 36: 415-423.

Forlin, L., C. Haux, L. karlsson-Norrgrehn, P. Runn, A. Larsson, 1986. Biotransformation enzyme activities and histopathol. in rainbow trout (*Salmo gaidneri*) treated with heavy metal. J. Aquat. Toxicol., 8: 51-59.

Handy, R.D., 2003 Chronic effect of copper exposure versus endocrine toxicity: Two sides of the toxicology process (review). J. Comp. Biochem. Physiol., 135: 25-38.

Haux, C. and A. Larsson, 1984. Long-term sub-lethal physiol. effects on rainbow trout, *Salmo gaidneri*, during exposure to cadmium and after subsequent recovery. J. Aquat. Toxicol., 5: 129-142.

Kori-Siakpere, O., J.E.G. Ake and U.M. Avworo, 2006. Sub-lethal effects of cadmium on some selected haematol. parameters of *Heteroclarias* (a hybrid of *Heterobranchus bidorasalis* and *Clarias gariepinus*). Int. J. Zool. Res., 1: 77-83.

Lévesque, H., T.W. Moon, P.G.C. Campbell and A. Hontela, 2002. Seasonal variation in carbohydrate and lipid metabolism of cortisol responses of yellow perch (*Prca flavescens*). J. Aquat. Toxicol., 60: 257-267.

Lévesque, H., J. Dorval, G. Van Der Kraak, P.G.C. Campbell and A. Hontela, 2003. Hormonal, morphological and physiological responses of yellow perch (*Prca flavescens*) to chronic environmental metal exposure J. Toxicol. Environ. Health, 66: 657-676.

Ma, W., 1996. Lead in Mammals. In: Environmental Contamination in Wildlife. Beyer, W.N., G.H. Heinz and A.W. Redmon-Norwood (Eds.), Lewis Publisher, Boca Raton. USA., pp: 281-296.

Markus, J. and A.B. McBratney, 2001. A review of the contamination of soil with lead II. Spatial distribution and risk assess/smelt of soil lead. Environ. Int., 27: 399-411.

Martinez, C.B.R., M.Y. Nagae, C.T.B.V. Zaia and D.A.M. Zaia, 2004. Acute morphological and physiological effects of lead in the neotropical fish, *Prochilodus lineatus*. Braz. J. Biol., 64: 797-807.

Mc Donald, G. and L. Milligan, 1997. Ionic, Osmotic and Acid-base Regulation in Stress. In: Fish stresss and Health in Aquaculture. Iwama, G.K., A.D. Pickering, J.P. Sumpter and C.B.S. Chreck (Eds.), pp: 119-144.

Monteiro, S.M., M.M. Juan, A. Fontinhas-Fernandes and M. Sousa, 2005. Copper induced alterations of biochem. parameters in the gill and plasma of *Orechromis niloticus*. J. Com. Bio. Physiol., 141: 375-383.

Pain, D.J., 1997. Lead in waterfowl. In: Environmental Contamination in Wildlife. Beyer, W.N., G.H. Heinz and A.W. Redmon-Norwood (Eds.), Lewis Publisher, Boca Raton. USA., pp: 251-280.

Pelgrom, S.M.G.J., R.A.C. Lock, P.H.M. Balm, S.E. Wendelaar Bonga, 1995. Effects of combined water-born Cd and Cu exposures on ionic composition and plasma cortisol in tilapia, *Oreochromis mossambicus*. J. Comp. Biochem. Physiol., 111: 227-235.

Pinheiro, J.P., A.M. Mota and M.F. Benedetti, 1999. Lead and calcium binding to folic acids: Salt effect and competition. J. Environ. Sci. Technol., 33: 3398-3440.

Rabitto, I.S., J.R.M. Alves Costa, H.C. Silva de Assis, É. Pelletier, F.M. Akaishi, A. Anjos, M.A.F. Randi and C.A. Oliveira, 2005. Effects of dietary Pb (II) and tributylin on neotropical fish (*Hoplias malabaricus*): Histopathological and biochemical findings. J. Ecol. Environ. Safe, 60: 147-156.

Ricard, A.C., C. Daniel, P. Anderson and A. Hontela, 1998. Effects of sub-chronic exposure to cadmium chloride on endocrine and metabolic functions in rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol., 34: 377-381.

Richards, J.G. and R.C. Playel, 1999. Protective effects of calcium against the physiological effects of exposure to a combination of cadmium and copper in rainbow trout (*Oncorhynchus mykiss*). Can. J. Zool., 77: 1035-1047.

Salehi, H., 2006. An analysis of the consumer market for carp products in Iran. Iranian J. Fish. Sci., 47: 370-378.

Salmerón-Flores, P., M.E. Melendez-Camargo and L. Martinez-Tabche, 1990. Efecto hepatotóxico y nefrotóxico del polmo sobre la tilapia (*Arotherodon aureus*) An. Esc. Nac. Cienc. Biol. Mex., 33: 147-156.

Shah, S., 2002. Behavioral abnormalities of *Cyprinus wastoni* on exposure to copper and zinc. Turk. J. Zool., 26: 137-140.

Shahbazi, P. and N. Maleknia, 1999. General biochem. Tehran Univ., pp: 465-488.

Sumpter, J.P., 1997. In: The Endocrinol. Stress. Iwama, G.K., A.D. Rickering, J.P. Sumpter and C.B. Schreck (Eds.), Cambridge Univ. Press, Cambridge., pp: 95-118.

Vutukuru, S.S., C. Suma, K. Radha Madhavi, Juveria, J. Smitha Pauleena, J. Venkateswara Rao and Y. Anjaneyulu, 2005. Studies on the development of potential biomarkers for rapid assessment of copper toxicity to freshwater fish using *Esomus danricus* as model. Int. J. Environ. Res. Public Health, 2: 63-73.

Wendelaar Bonga, S.E., 1997. The stress response in fish J. Physiol. Rev., 77: 591-625.

Wu, S.M., Y.Y. Chou and A.N. Deng, 2002. Effects o f exogenous cortisol and tolerance to waterborn cadmium in Tilapia (*Oreochromis mossambicus*). J. Zool. Study, 41: 11- 118.

Žikiě, R.A., A.Š. Štajn, S.Z. Pavilovic, B.J. Ognjanovic Z.S. Paviloviě, B.J. Organjanoviě and Z.S. SaiěIě, 2001. Activities of super oxid -dismutase and catalase in erythrocytes and plasma transaminases of gold fish (*Carassius auratus gibelio* Bloch.) Exposed to cadmium. J. Physiol. Res., 50: 105-111.

Zimmermmann, S., B. Sures and H. Taraschewski, 1999. Experimental studies on lead accumulation in the Eel-Specific endoparasites *Anguillicola crassus* (Nematoda) and *Paratemirsentis ambiguous* (Acanthocephala) as compared with their host, *Anguilla anguilla*. Arch. Environ. Contam. Toxicol., 37: 190-195.