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Journal of

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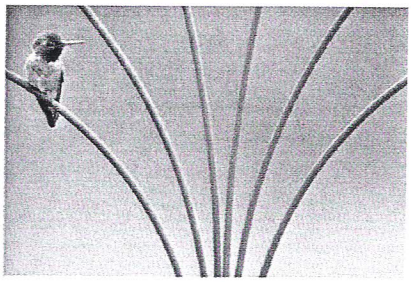
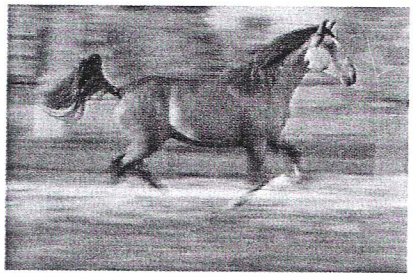
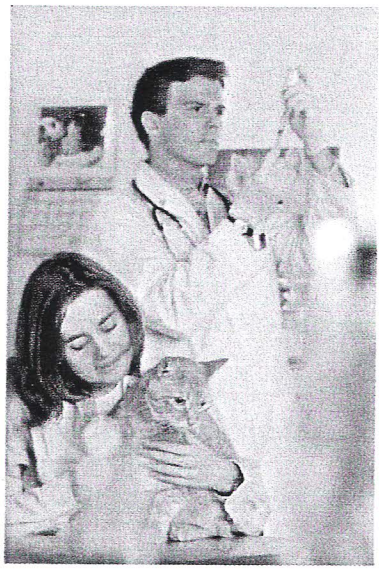
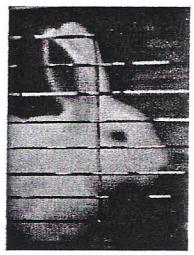
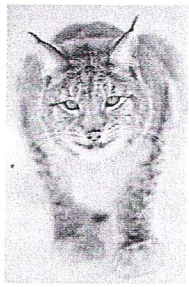

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Journal of Animal and Veterinary Advances 11 (1): 74-77, 2012
ISSN: 1680-5593

Accumulation and Effect of Chromium on Protein and Glycogen Levels of *Palaemonetes pugio*

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Abstract: Total metal accumulation and the levels of total protein and glycogen were measured in *Palaemonetes pugio* after exposing the animals to 0.05, 0.1 and 0.2 ppm concentrations of chromium over 1, 7 and 15 days. Metal accumulation in tisuues was measured using atomic absorbtion spectrophotometric techniques and the levels of total protein and glycogen were determined Lowry and Anthron methods, respectively. No mortality was observed under the effect of chromium at any concentrations and exposure periods tested. Total metal accumulation increased with increasing concentrations of chromium at given exposure period while total protein and glycogen levels showed a decrease on day 15th compared with day 1.

Key words: *Palaemonetes pugio*, chromium, accumulation, protein, glycogen, Turkey

INTRODUCTION

Heavy metal levels in natural habitats increased due to recent developments achieved in technology and usage of various chemicals in industry and agriculture as raw materials causing, a number of environmental and health problems.

Aquatic ecosystems constitute the main influx systems for pollutants originated from both natural and anthropogenic factors. The increase in metal pollution in an environment results in mass migrations or death resulting in environmental structure changes while accumulation, metabolic and physiologic changes might occur at sublethal concentrations (Biney *et al*., 1994).

Chromium is an essential element for organisms such as copper, zinc and iron and acts as an insulin cofactor in animals. It is used widely in various metallurgy and chemical industries such as metal and electrode plating, leather tanning, ferrochromium and pigment production. The increase in chromium concentration in aquatic environments results in accumulation in organisms which then is transferred to higher trophic levels through the food chain (Langard and Norseth, 1979; Abbas and Ali, 2007).

Studies carried out with various invertebrate animals in nature and under laboratory conditions revealed that beside accumulating in tissues chromium was shown to cause changes in biochemical parameters (Murti *et al*., 1983; Gopal *et al*., 1990).

Glycogen is the main storage form of fuel that supplies energy in animal organisms and its access is stored in muscle, liver in vertebrates and hepatopancreas in invertebrates. Compensation of increased energy need under metal stress results in exhaustion of carbohydrate reserves (Gopal *et al*., 1990).

The basic structural component of living organisms is protein which is also used as an energy source in aquatic organisms. The tissue levels of proteins were shown to decrease under metal stress in invertebrate and vertebrate animals at different levels of food web (Vutukuru, 2003).

Palaemonetes pugio having high protein content is an important food for a number of species in freshwaters. Hence, to study the accumulation and metabolic and physiologic effects of metals in this species reflect the state of metals at this food chain. The purpose of this study is find out total accumulation of chromium in tissues and its effect on total protein and glycogen levels in this species after exposing the animals to 0.05, 1.0 and 2.0 ppm of this metal over 1, 7 and 15 days.

MATERIALS AND METHODS

P. pugio was used as the experimental material. Experiments were carried out under controlled laboratory conditions at Mersin University. Animals were obtained from cultivation pools situated in a special protected area Silifke, Mersin. Animals were placed in stock glass aquaria, 40×100×40 cm in height and acclimated to laboratory conditions for 1 month. Individuals having a mean length of 2.24±0.07 cm and a mean weight of 3.03±0.51 g were exposed to 0.05, 0.1 and 0.2 ppm

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chromium over 1, 7 and 15 days. Hydrous solution of $K_2Cr_2O_7$, +6 valance of chromium were used in the experiments into which tri-sodium-citrate was added to prevent precipitation. Taking the exposure periods into account experiments were run in three series and 4 aquaria 40×100×40 cm in height were used in each series. The 1-3 aquaria were added with 100 L of selected chromium solutions while the 4th aquarium was filled with the same amount of tap water and used as control. Experiments were run in triplicate and 2 individuals were used in each replicate totaling to 72 individuals.

For possible variations in the concentration of experimental solutions due to adsorbtion, precipitation or evaporation, solutions were changed once in 2 days by a series of dilutions from the stock solution.

Accumulation and toxic effects of heavy metals depends on the physical and chemical properties of water. Some of the physical and chemical properties of the experimental media were as follows; water temperature: 20±1°C; pH: 8.1±0.03; dissolved oxygen: 7.56±0.72 mg L^{-1}; total hardness: 246.2±2.56 ppm $CaCO_3$; total alkalinity: 409±0.39 ppm $CaCO_3$. Shrimps were fed daily with phytoplankton organisms.

At the end of each experimental period 2 individuals from each replicate were pooled for accumulation, protein and glycogen analysis. Metal accumulation was carried out using Atomic Absorption Spectroscopy (AAS). Dry weights of the pooled tissues were determined after drying them at 150°C for 72 h. They were then transferred to glass tubes and nitric acid (65%, Baker)-perchloric acid (65%, Erba) mixture (2:1 v/v) was added and were digested at 120°C for 60 min. Digested tissues were transferred to polyethylene tubes and their total volumes were made up to 5 mL with distilled water.

For total protein analysis wet weights of the samples were determined. They were homogenized in 0.3 M Sucrose (Merck, extra pure) solution on an Ultra-Turrax T-25 homogenizer at 24,000 rpm for 5 min. Homogenates were then centrifuged at 2,000 rpm for 10 min for removing particles (Hettich; Universal-1200). Total protein levels in homogenates were determined using Lowry method (Wedemeyer and Yasutake, 1977).

Wet weight of the samples that are to be used for glycogen content were determined. They were transferred to centrifuge tubes for protein and lipid extraction and 3 mL KOH (30%) solution was added into each tube. They were then left in boiling water bath for 20 min. At the end of this period 0.5 mL saturated Na_2SO_4 and 3 mL ethyl alcohol were added to each sample and boiled for 15 min. Samples were then centrifuged at 3,500 rpm for 10 min and the supernatants discarded. The precipitates were dissolved in 2.0 mL distilled water and after adding 2.5 mL 95% ethyl alcohol and centrifuging the samples at 3,500 rpm for 10 min, the supernatant was discarded. The precipitates, cleared from protein and lipid were dissolved in 2 mL 5 M HCl, they were neutralized using 0.5 M NaOH and their total volumes were made up to 50 mL. (Wedemeyer and Yasutake, 1977). Glycogen levels of the samples were determined using Anthron method (Plummer, 1971).

Statitical analyses of the data were carried out using variance analysis and Student Newman Keul's procedure (SNK) (Rholf and Sokal, 1969).

RESULTS AND DISCUSSION

No mortality was observed in *P. pugio* exposed to 0.05, 0.1 and 0.2 ppm chromium solutions over 1, 7 and 15 days. Chromium accumulation increased with the concentrations tested and with increasing exposure periods ($p<0.05$) (Table 1).

Total protein levels decreased at the end of the experiments compared with day 1 at all the concentrations tested ($p<0.05$) (Table 2).

Total glycogen levels decreased with increasing exposure periods and concentrations of chromium ($p<0.05$) (Table 3).

Table 1: Total chromium accumulation at the concentrations and exposure periods tested in *P. pugio* (μg Cr g^{-1} d.w.)

	Exposure priod (Days)					
	1		7		15	
Concentration [Cr (VI) ppm]	$\bar{X}\pm s\bar{X}$	*	$\bar{X}\pm s\bar{X}$	*	$\bar{X}\pm s\bar{X}$	*
0.0	4.28±0.66	as	3.87±0.65	as	4.01±0.72	as
0.05	5.84±0.20	ast	8.32±0.17	bt	12.52±0.04	ct
0.1	7.51±0.36	at	11.95±0.52	bt	16.86±0.00	ct
0.2	14.04±0.66	ax	18.79±0.46	bx	21.40±0.30	cx

Table 2: Effects of chromium on total protein levels in *P. pugio* (mg g^{-1} w.w.)

	Exposure period (Days)					
	1		7		15	
Concentration [Cr (VI) ppm]	$\bar{X}\pm s\bar{X}$	*	$\bar{X}\pm s\bar{X}$	*	$\bar{X}\pm s\bar{X}$	*
0.0	42.06±1.03	as	42.70±1.06	as	42.35±2.22	as
0.05	30.52±2.34	at	35.60±1.16	at	28.87±0.72	at
0.1	30.09±0.75	at	33.49±0.43	bt	22.07±0.73	cx
0.2	35.50±0.71	at	30.47±0.20	bs	24.25±1.09	ctx

Table 3: Effects of chromium on total glycogen levels in *P. pugio* (mg g^{-1} w.w.)

	Exposure period (Days)					
	1		7		15	
Concentration [Cr (VI) ppm]	$\bar{X}\pm s\bar{X}$	*	$\bar{X}\pm s\bar{X}$	*	$\bar{X}\pm s\bar{X}$	*
0.0	22.01±0.59	as	21.89±1.11	as	21.54±1.35	as
0.05	14.53±0.34	at	9.88±0.50	bt	9.20±0.11	bt
0.1	7.85±0.75	ax	5.98±0.72	ax	4.30±0.00	ax
0.2	3.63±0.62	ay	2.19±0.10	ay	1.89±0.11	ax

*SNK: Letters a-c show differences among exposure periods and s, t and x among concentrations. Data shown with different letters are significantly different at the $p<0.05$ level; $\bar{X}\pm s\bar{X}$: Mean±SE

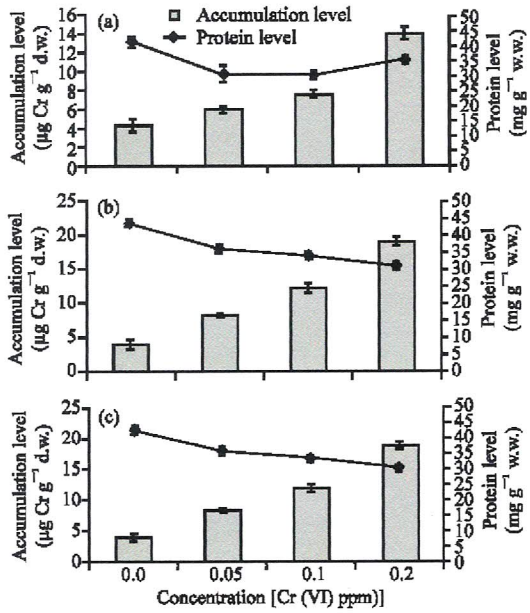

Fig. 1: Effects of chromium concentration on total metal accumulation (µg Cr g^{-1} d.w.) and protein levels (mg g^{-1} w.w). in *P. pugio*. (a-c refer to the exposure time 1, 7 and 15 days, respectively)

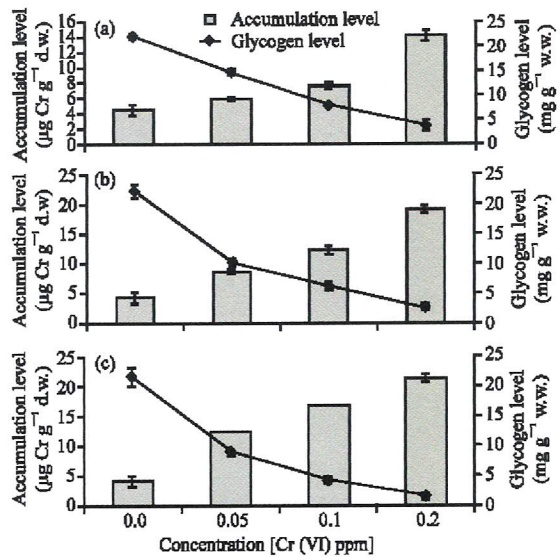

Fig. 2: Variations in total metal accumulation (µg Cr g^{-1} d.w.) and glycogen levels (mg g^{-1} w.w) in *P. pugio*. (a-c refer to the exposure time 1, 7 and 15 days, respectively)

Total protein levels increased with increasing concentration of chromium and exposure periods in *P. pugio* (Fig. 1). Total glycogen levels of *P. pugio* decreased with chromium accumulation at the concentrations and exposure periods tested (Fig. 2).

Effect of heavy metals on mortality in aquatic organisms is closely related to concentration of metal and exposure period. Mortality rates was shown to increase with increasing concentrations and exposure periods of Cr (III) in *Mysidopsis bahaia*, Ni in *Hyalella azteca* and Cd, Cu, Pb and Zn in *Chironomus tentan*a. Results of the present study revealed that the chromium concentrations (0.05, 1.0 and 2.0 ppm) and the exposure periods (1, 7 and 15 days) tested were below the mortality threshold for *P. pugio*.

Accumulation of metals in aquatic organisms not only depend on metal, concentration and exposure period but also on organization level, species and on the ecological needs of the species. It was shown that under the acute and chronical effect of Ni, accumulation was significantly higher in *Lamellidens marginalis* than in *Cyprinus carpio*. Chromium accumulation in *Perna viridis* increased with exposure period at a given concentration (Yap *et al*., 2004) whereas in *Mytilus galloprovincialis* accumulation increased with concentrations of the metal at a given period. The increase in concentration of chromium and exposure period also caused an increase in metal accumulation in *P. pugio*. In addition to accumulation heavy metals also cause metabolic, physiologic and biochemical changes in aquatic organisms. The excess amounts of glucose, the main high energy compound in animal organisms are stored as glycogen in hepatopancreas and muscle tissues of invertebrates. Glycogen is also used under other stress conditions such as hypoxia to compensate increasing energy needs. The excess energy need is supplied from proteins and lipids if carbohydrate reserves diminish. Exposure to sublethal concentrations of lead for 1, 2, 4, 10 and 30 days decreased the total protein, lipid and carbohydrate levels of post larval stages of *Penaus indicus* depending on the period.

Sublethal concentrations of chromium were shown to decrease the hemolymph glucose in *Macrobrachium lamarrei* (Murti *et al*., 1983) and in *Barytelphusa guerini* (Gopal *et al*., 1990). Chromium decreased total glycogen levels in *P. pugio* at the exposure periods and concentrations tested.

Various organic and inorganic pollutants decrease tissue protein levels in *Mytilus galloprovincialis* and *Haliotis rufescens* (Synder *et al*., 2001). Copper decreases hemolymph protein levels in *Carcinus maenas* (Rtal and Truchot, 1996). Antioxidative enzyme activity was inhibited by various metals in invertebrate animals (Conners and Ringwood, 2000; Jing *et al*., 2006).

About 96 h exposure to sublethal concentrations of cadmium, lead and arsenic increased tissue metal accumulation and decreased glycogen levels in *Biomphalaria glabrata* (Ansaldo *et al*., 2006). Exposure

to Cu, Cd and Pb increased metal accumulation and inhibited AST and ALT activities in *Ruditapes philipinnarum* (Blasco and Puppo, 1999). In present study accumulation of chromium increased with increasing concentrations and exposure periods whereas the total levels of glycogen and protein levels showed a decrease in *P. pugio*.

CONCLUSION

The decrease in total glycogen levels in *P. pugio* under the effect of chromium might be due to compensation of increased energy need and also use of glycogen in formation of glycoprotein and glycolipid and that of protein in formation of lipoprotein and mucoprotein.

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