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Research Article

The Effects of Cadmium on Levels of Glucose in Serum and Glycogen Reserves in the Liver and Muscle Tissues of *Cyprinus carpio* (L., 1758)

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Abstract: The common carp (*Cyprinus carpio* L., 1758) was exposed to sublethal concentrations of cadmium (0.05, 0.1, 0.5 and 1.0 mgl^{-1}) for 10 days. The levels of serum glucose and glycogen reserves in the liver and muscle tissues were measured both in fish exposed and not exposed to Cd. The levels of glycogen reserves in the liver and muscle tissues were significantly ($P < 0.05$) decreased in fish exposed to sublethal concentrations of Cd compared with the levels measured in the control groups. The decrease in glycogen levels in the liver and muscle tissues under the highest metal concentration (1.0 mgl^{-1}) were 24% and 29%, respectively. The blood serum glucose levels of fish exposed to Cd were significantly ($P < 0.05$) increased compared with the levels measured in the control groups. This increase was correlated with the increase in water Cd concentrations.

Key Words: *Cyprinus carpio*, cadmium, glucose, glycogen

Cyprinus carpio (L., 1758)'da Kadmiyum'un Serum Glukoz Düzeyi ile Karaciğer ve Kas Dokularındaki Glikojen Rezervleri Üzerine Etkileri

Özet: Sazan (*Cyprinus carpio* L., 1758), 10 gün süreyle kadmiyumun 0,05, 0,1, 0,5 ve 1,0 mgl^{-1}'lik subletal derişimlerinin etkisine bırakılmıştır. Cd etkisinde kalan balıklarla metal içermeyen ortamda bulunan kontrol balıklarının kas ve karaciğer dokularındaki glikojen düzeyleri ile serum glukoz düzeyleri ölçülmüştür. Kadmiyumun belirtilen ortam derişimlerinin etkisinde kas ve karaciğer dokularındaki glikojen düzeyi kontrole göre önemli ölçüde azalmıştır ($P < 0,05$). Bu azalma, en yüksek metal derişiminin (1,0 mgl^{-1}) etkisinde karaciğerde % 24, kas dokusunda ise % 29 düzeyinde olmuştur. Kadmiyum etkisinde kalan balıkların serum glukoz düzeyleri, metal etkisinde kalmayan balıklara göre önemli düzeyde artarken, bu artışın metalin ortam derişimindeki artışına paralel olduğu saptanmıştır.

Anahtar Sözcükler: *Cyprinus carpio*, kadmiyum, glikoz, glikojen

Introduction

Due to natural, geochemical and anthropogenic factors, the infiltration of toxic heavy metals into aquatic ecosystems is on the increase. These toxic heavy metals in aquatic ecosystems are carried via the food chain to the upper trophic levels and create important ecological problems.

Because cadmium is a non-essential heavy metal, it has a cumulative polluting effect, and could cause toxicity to aquatic organisms even in minute concentrations. The toxic effects of Cd on fish are numerous, for example, interrupting development and growth (1), preventing Ca^{+2} uptake through the gills (2), disturbing liver functions (3), skeletal deformations (4) and pathological changes in some tissues and organs (5).

Since the mechanisms of heavy metal excretion, deposition and detoxification in fish are not capable of handling heavy metals in short time frames, heavy metals tend to accumulate specifically in metabolically active tissues and organs (6). Metal accumulation rates in fish vary with species and among individuals in a population. They also depend upon the age, size, feeding status and sex of the organism (7).

It is known that physiological and biochemical parameters in fish blood and tissues could change when exposed to heavy metals and that these parameters are extremely sensitive to these elements (8). It has been found that Cd could change glycogen reserves and serum glucose levels in fish by affecting the activities of liver enzymes that have roles in the carbohydrate metabolism

such as gluconeogenesis and glycolysis (9). Thus, it was argued that several biochemical parameters in fish blood and tissues could be used as an indicator of heavy metal toxicity (10).

Because heavy metal contamination in an aquatic environment exerts an extra stress on fish, there must be several other changes in the fish metabolism when exposed to heavy metals (11). On the other hand, because glycogen reserves in the liver and muscle tissues of fish under stress are used as an emergency energy supply, changes in the glycogen levels in these tissues could indicate the health status of fish populations. It has been demonstrated that Cd might change glycogen reserves in fish via the endocrine system (12).

Because life span, condition factor, reproduction and health are all functions of metabolic events in fish exposed to heavy metals, this study aimed to demonstrate the effects of sublethal concentrations of Cd on the levels of glucose in the serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio,* which has economic value in Turkey.

Materials and Methods

The fish used in this experiment were transferred from farming ponds to a controlled laboratory environment and acclimatised for 4 weeks to laboratory conditions in aquaria measuring 40 cm in width x 120 cm in length and 40 cm in height. The initial mean weight and length of the fish were 55.23 ± 0.52 g and 12.38 ± 1.02 cm, respectively. There was no significant difference ($P > 0.05$) between the mean weights or lengths of the fish used in the experiments. Because metabolic activity changes with size and affects the parameters to be measured, individuals of similar size and length were used in the experiments (13). The room temperature and photoperiod during the experiments were 25 ± 1 °C and 12 L: 12 D, respectively. Five aquaria, 1 of which was designated as a control, were used to conduct the experiments. $CdCl_2.H_2O$ salt was utilised for the preparation of stock solutions.

Some of the chemical parameters of the Cd-free tap water used in the experiments were as follows:

pH 7.73 ± 0.5,

Total hardness 210 ± 0.8 mgl^{-1},

Total alkalinity 280 ± 3.15 mgl^{-1}.

Four aquaria were filled with 120 l of tap water and Cd stock solution was added to each aquarium to make the final concentrations 0.05, 0.1, 0.5 and 1.0 mgl^{-1} Cd. The fifth aquarium was used as a control. Six fish were added to each aquarium and the effects of Cd concentrations on glycogen reserves and serum glucose levels in liver and muscle tissues were investigated after 10 days. Fish were not fed during these 10 days. The aquaria were well aerated and dissolved oxygen levels were kept at around 7.5 ± 1.03 mgl^{-1} throughout the experiment. Every 2 days, the water in each aquarium was replenished to keep the metal concentrations constant. The fish were anaesthetised with MS 222 for tissue and blood sampling at the end of the experiments. The fish were washed with tap water and dried using drying paper before collecting the blood samples. Blood sampling was done by incising the caudal peduncle. The fish in each aquarium were grouped. Each group consisted of 2 fish and the blood samples collected from the 2 fish in each group were placed into the same anti-coagulant-free centrifuge tubes. These blood samples were then centrifuged at 3500 rpm for 10 min to obtain serum samples for glucose analysis. The glucose levels in the serum samples were analysed using the O-toluidine technique (14). In order to apply this technique, 50 µl serum samples were added to glass tubes and 3.5 ml of O-toluidine reagent was added to each tube and then all the tubes were kept in a hot water bath (100 °C) for 10 min. The glucose levels in cooled samples were measured spectrophotometrically.

The muscle and liver tissues to be analysed for glycogen levels were first wet weighed and then placed into centrifuge tubes containing 3 ml of KOH solution (30%). The centrifuge tubes were kept in a hot water bath for 20 min. Then 0.5 ml of saturated Na_2SO_4 and 3 ml of ethyl alcohol (95% pure) were added, followed by boiling for a further 15 min. After being cooled, all samples were centrifuged at 3500 rpm and the supernatants were discarded. The precipitations in the tubes were dissolved in 2 ml of distilled water followed by the addition of 2.5 ml of ethyl alcohol (95% pure). The tubes were then centrifuged at 3500 rpm for a further 10 min and the supernatants were discarded. The final precipitations in the tubes free of lipid and protein were then dissolved in 2 ml of HCl (5M) and neutralised with 0.5M NaOH followed by dilution to 50 ml with distilled water before analysis (14). The glycogen levels in

the samples were determined by the anthron method (15). All the data were treated with one-factor ANOVA followed by Student-Newman-Keul's (SNK) multiple comparison test.

Results

The effects of Cd on glycogen reserves and serum glucose levels in the muscle and liver tissues of *C. carpio* are shown in the Table and Figure, respectively. There was no mortality during the experiment.

The glycogen levels in the liver and muscle tissues of fish exposed to pre-determined concentrations of Cd were significantly ($P < 0.05$) lower compared with the levels found in the control fish. The highest concentration (1.0 mgl^{-1} Cd) tested in this study decreased the glycogen levels in the liver and muscle tissues by 23.72% and 29.05%, respectively. Although the decrease in the liver glycogen levels of *C. carpio* differed ($P < 0.05$) between all the Cd concentrations tested, there was no significant ($P > 0.05$) difference between those in the muscle tissues of fish exposed to 0.05 and 0.1 mgl^{-1} concentrations of Cd (Table). There was a significant difference ($P < 0.05$) for higher concentrations.

The serum glucose levels of fish increased in all Cd concentrations tested. However, this increment was significantly different in all Cd concentrations except for 0.05 and 0.1 mgl^{-1} ($P < 0.05$) (Figure).

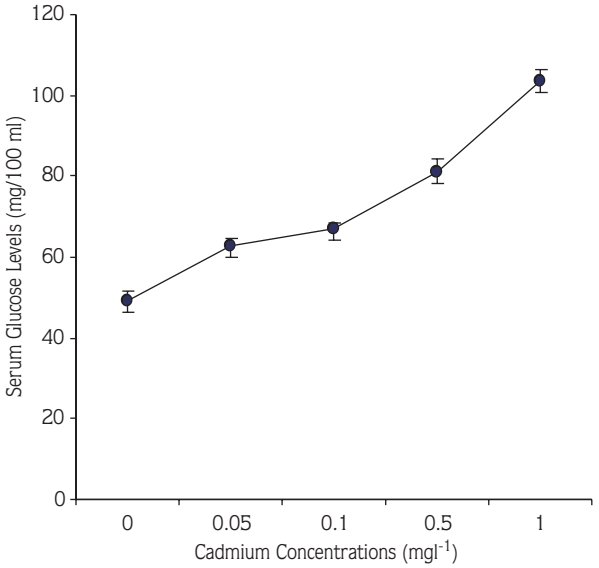

Figure. Serum glucose levels in *C. carpio* after 10 days of exposure to different concentrations of Cd.

Discussion

The results of the present study showed that pre-determined concentrations of Cd significantly altered the carbohydrate metabolism of *C. carpio* after 10-day exposure.

The glycogen reserves in the muscle and liver tissues of *C. carpio* exposed to Cd decreased significantly in this study compared with the glycogen reserves measured in the control groups. The decrease found in the muscle and liver glycogen reserves of *C. carpio* in this study might in fact be the result of Cd stimulating the activities of enzymes that work in glycogenolysis.

Carbohydrates are stored as glycogen in fish tissue and organs like the muscle and liver in order to supply the energy needs when there are hypoxic conditions, intensive stocking and a lack of food (16). It has been demonstrated that liver glycogen levels decreased in *Oncorhynchus mykiss* as a result of the activation of glycolytic enzymes via catecholamines under lack of food and hypoxic conditions (17). The carbohydrate

Table. The levels of glycogen reserves in the muscle and liver tissues of C. carpio after 10 days of exposure to different Cd concentrations.

Tissues	Cadmium Concentrations (mgl^{-1})				
	Control	0.05	0.1	0.5	1.0
Liver	43.12 ± 3.39^{a}	33.24 ± 1.42^{b}	26.18 ± 1.93^{c}	19.09 ± 1.98^{d}	10.23 ± 1.25^{e}
Muscle	4.13 ± 0.28^{a}	3.26 ± 0.15^{b}	2.88 ± 0.13^{b}	2.06 ± 0.20^{c}	1.20 ± 0.33^{d}

Values are mean ± se (n = 6) and different letters in the same row denote significant differences ($P < 0.05$).

metabolism of the fish used in the present experiment might also have been affected by the lack of food since they were not fed during the experiments. It was also found that heavy metals could create stress in fish (12) and that Cd could decrease glycogen reserves in the American eel (*Anguilla rostrata*) by increasing the production of catecholamines from the adrenomedulla (18).

Prolonged environmental stress in fish makes adaptation difficult and creates weakness in fish. Weakness is characterised by decreases in liver glycogen and serum cortisol levels, which subsequently create a series of alterations in the metabolism and shorten the life span of organisms (11).

Some investigations also showed that heavy metals could decrease the glycogen reserves in fish (9) and invertebrates (19) by affecting the activities of enzymes that play a role in the carbohydrate metabolism. Cd decreased the glycogen reserves in *Heteropneustes fossilis* by stimulating glycolytic enzymes like lactate dehydrogenase, pyruvate dehydrogenase and succinate dehydrogenase (20).

The decrease in glycogen reserves in the muscle and liver tissues of fish under heavy metal toxicity has been demonstrated to change with species (8,21). This change might stem from the metabolic differences between species and the environmental concentrations of heavy metals and durations which the fish are exposed to.

The feeding of experimental fish could also affect the fish glycogen levels in the muscle and liver tissues. When fish are exposed to heavy metals and fed, the amount of food given will supply extra energy to the fish, causing a miscalculation of the level of glycogen decrease which heavy metal toxicity alone could create. Furthermore, fish do not show the same feeding behaviour, resulting in differences in supplying their energy needs from food sources (22). Although starvation has an effect on the glycogen reserves of fish, to determine the effects of Cd on the carbohydrate metabolism, the fish were not fed throughout the present study, including the control fish.

Serum glucose levels of *C. carpio* exposed to sublethal concentrations of Cd for 10 days increased with increasing concentrations of Cd in the water. Serum glucose levels also increased with increasing concentrations of Cd in *Mugil cephalus* (23). It was shown that serum glucose levels in fish were affected by many stress factors, including heavy metals (24). The increase in serum glucose levels in both *O. mykiss* and *H. fossilis* exposed to Cd and *Channa punctatus* and *C. carpio* exposed to Hg were linked to the decrease in glycogen reserves in the muscle and liver tissues (8,12,25).

Energy gain or loss in fish is controlled not only by carbohydrates but also by other macronutrients like proteins (26). It was found that there was no change in glycogen reserves in *O. niloticus* exposed to Cd for 7 days, although total protein concentrations decreased and serum glucose levels increased (27). It was also determined that Cd increased the serum glucose levels and the activities of aspartate transaminase and alanine transaminase in *Notemigonus crysoleucas* (28), serum cortisol levels in the American eel (29) and the activities of enzymes that play a role in the amino acid catabolism like glutamate dehydrogenase, amino acid oxydase and the xanthine oxydase in *C. punctatus* (8). Thus, an increment in serum glucose levels without any decrease in glycogen reserves in fish exposed to heavy metals indicates the involvement of gluconeogenic events in this process (30).

In conclusion, this study showed that Cd altered the carbohydrate metabolism in *C. carpio* by affecting the levels of glucose in serum and the glycogen reserves in both muscle and liver tissues. Such changes in the glycogen reserves of muscle and liver tissues and serum glucose levels under the effect of Cd might result in impairments in energy requiring vital processes, and hence give an idea about the health status of the fish population.

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