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Effect of starving and feeding on some haematological and physiological responses of the Nile catfish, *Clarias gariepinus* exposed to copper at extreme seasons

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Abstract The lethal concentration for 50% of fish for 96h (96h LC_{50}) of copper (Cu^{2+}) was estimated for the Nile catfish (*Clarias gariepinus*) in extreme seasons, winter and summer, 4.31 and 4.79 mg/l, respectively. The Nile catfish was exposed to 96h LC_{50} of copper for 7 days in extreme winter and summer. The body indices, haematological parameters as well as some plasma and liver enzyme activities and metabolite level were significantly differed in fish exposed to copper over than those of the control fish. Most of the tested parameters were not significantly different between the control fish of winter and summer (winter, water temperature 18 ± 2°C and summer, 27 ± 2°C). The effect of two ration sizes on copper toxicity in two different seasons on *C. gariepinus* was justified. It was found that the haematological parameters and the tested plasma activities of enzymes were significantly valid due to season differences. The blood parameters as well as plasma activities of enzymes were significantly differed in fishes fed elevated ration (3%) and exposed to copper challenge. On the other hand, the exploit of low feeding ration (0.5%) along with copper exposure during the examined seasons induced non-significant differences of the tested parameters, from those of the corresponding control. Therefore, the low feeding ration provides some tolerance against the possible water-borne copper exposure.

Keywords Food rations · *Clarias gariepinus* · Copper toxicity · Seasons

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Introduction

Copper (Cu) is one of the trace elements that is necessary for various biophysiological processes of all species (Uriu-Adams and Kleen 2005). It is required at extremely low level ($<10^{-18}$ mol/l) for normal cellular metabolism. At high levels, it becomes toxic due to interfering with several metabolic processes; so, different hazardous effects were reported (De Boeck et al. 2003). In aquatic habitat, Cu is a predominant pollutant as the copper-based compounds were used as herbicides, algicides and molluscicides (WHO 1993). Its levels ranging from 0.04 to 294 μg/l (An and Kampbell 2003) reaching 20 mg/l in critical situations (Goodyear and McNeill 1999). Also, the anthropogenic sources of environmental Cu include mining, smelting, municipal wastes, burning of coal for power generation and variety of Cu-based products used in electrical equipments and in other industries. Furthermore, Cu indirectly reaches the aquatic habitat as it is widely used as a base for many fertilizers and fungicides that is used in agriculture (Flemming and Trevors 1989;

WHO 1998). So, that in the globe, the copper environmentally realistic levels was demonstrated to pose a serious ecological risk to fish (Oliveira et al. 2008). This redox active metal has been documented as an oxidative stressor, promotion of highly reactive oxygen species (ROS), affecting lipid membranes and DNA integrity (Ahmad et al. 2005, 2007; Hashemi et al. 2007; Carvalho and Fernandes 2008; Oliveira et al. 2008) in different fish species. These effects consequently promote cell necrosis and apoptosis. In this regard, the enhanced caspase 3 and 7 activity was found as an early signs of apoptosis in trout hepatocytes after 2 h exposure to 10 μmol/l Cu (Nawaz et al. 2006). Although, Cu has shown significant deleterious effects on almost all physiological systems of fish (Kunwar et al. 2009), but different tolerance levels were recorded for different fish species.

Several studies have been reported that Cu induce deleterious effects on physiological, haematological and histological changes in different fish species (Sharaf-Eldeen and Abdel-Hameid 2002; Abdel-Tawwab et al. 2007; Carvalho and Fernandes 2008; Oliveira et al. 2008). The haematological variables were frequently used as fast indicators of fish response to environmental stressors (Webener et al. 1992; Shah 2006). Also, the hepatosomatic index (HSI) and condition factor (*K*) were reported as valuable environmental risk tool (van der Oost et al. 2003; Sanchez et al. 2008). The aspartate amion-transferase (AST) and alanine aminotransfearse (ALT) in addition to other serum metabolites were used as a diagnostic tool reflecting fish health status (Bernet et al. 2001; Barse et al. 2006).

The influence of different seasons has been found to induce seasonal variations in free radical metabolism of ectoderms (Ramos-Vasconcelos et al. 2005). Furthermore, studies have been reported that the acute changes of temperature induce disturbed oxidative stress biomarkers but only slightly affected the activities of antioxidant enzymes of diverse fish species (Lushchank and Bagnyukova 2006a, b; Bagnykova et al. 2007a, b).

It has been documented that chronic Cu exposure of brown trout inhibits ammonia excretory mechanism, most likely $Na^+/NH4^+$ exchange (Beaumont et al. 2003), and as a result enhance plasma ammonia level (Beaumont et al. 2000). The reduction in fish food ration was found to be helpful to avoid waste accumulations (Hashemi et al. 2008a, b). They reported that Cu exposure increases significantly the metalothionein (MT) level in the liver of fed fish (*Cyprinus carpio*), but no changes occurred in case of starved one. Carp were two fold more tolerant against acute Cu toxicity compared to fed fish (Hashemi et al. 2007, 2008b).

The African catfish (*Clarias gariepinus*) is one of the most important freshwater fish in the Egypt and many other countries and known for its ability to be cultured at high densities up to 500 kg/m^3 (Van de Nieuwegiessen et al. 2009). Therefore, the present study was highlighted to test the protective effect of food ration against the toxicity of Cu at extreme seasons (winter and summer). This may be supportive to evade the challenge of Cu toxicity during the propagation of *Clarias gariepinus*.

Materials and methods

Fish holding and experimental groups

Juvenile African *C. gariepinus* (Burchell 1822) were gifted from the central laboratory for aquaculture research (CLAR), Abassa, Abo-Hammad, Sharqia, Egypt. The fishes (45.50 ± 1.5 g) were apparently health, permit in cement aquaria (1 m^3) for 1 week to be acclimatised at laboratory conditions. The fish were fed with a commercial fish feed. The present study was conducted at a static system in well-aerated aquaria, at two different extreme seasons (winter and summer). The winter experiment was conducted in January where water temperature recording 18 ± 2.0°C, whereas the summer one was conducted in July (water temperature 27 ± 2.0°C). The $CuSO_4$ was used as a loyal source of Cu. At both experiment, the lethal concentrations for 50% of fishes for 96h (96h $LC_{50)}$ for Cu were determined in duplicate order by exposing five fishes to each of serial dilutions of Cu (1, 2, 4, 6 and 8 mg/l) for 96 h. The total died fishes at each concentration were used to estimate 96h LC_{50} of Cu according to Probit model (EPA 1993). The quantified 96h LC_{50} of Cu was 4.31 and 4.79 mg/l for winter and summer, respectively. In each season, the fishes were randomized into four duplicated groups in glass aquaria (40 cm height, 70 cm length and 30 cm width) each containing seven fish. The first and second groups rose in

ordinary dechlorinated tap water and fed 0.5 and 3% of body weight, respectively. Half LC_{50} of Cu was added to the water of the third and fourth groups. The fish of the third and fourth groups were provided with food at 0.5 and 3% of the body weight, respectively. Fish fed twice a day, in the morning (9 a.m.) and in the evening (15 p.m.), and the uneaten food particles deposited in the aquaria were removed by siphonation (after 2 h of feeding). Experimental aquaria are well aerated and the tank water was replaced twice a week (Cu level was renewed) to keep normal water quality. After 20 days from the beginning of the experiment, the fish were decapitated, anesthetized using 1 ml/l clove oil (40 ppt) (Ribas et al. 2007) and weighed.

Somatic indices

The liver and gonad weighed and also the fish without gut (gutted body weight). The somatic indices, hepatosomatic index (HSI), gonadosomatic index (GSI) and condition factor (K) were estimated using the following formulae:

$$\text{HSI} = 100 \times \text{liver weight}/\text{gutted body weight}$$
$$\text{GSI} = 100 \times \text{Gonad weight}/\text{gutted body weight}$$
$$K = 100 \times \text{total body weight}/L^3.$$

Haematological parameters

The haematological parameters were done following standard methods (Dacie and Lewis 1984). The red blood cells count (RBCs) was done by Nebauer haemocytometer slide using saline solution (0.8) as a diluting fluid. The haematocrit (Hct) value was determined using heparinized microhaematocrit capillary tubes after centrifugation in microhaematocrit centrifuge. The haemoglobin content was determined following cyanomethemoglobin method (Blaxhall and Daisely 1973). The calculated blood cell indices, mean cellular haemoglobin (MCH, ρg/cell), mean cellular haemoglobin concentration (MCHC, g 100/ml) and the mean corpuscular volume (MCV, μm^3) were computed using the following formulae were computed according to the equations proposed by Lee et al. (1998): MCH = (Hb/Hct) 10, MCHC = (Hb/Hct) × 100 and MCV = (Hct/RBCs) × 10. After that, the remained blood samples were centrifuged at 5,000 rpm for 5 min to isolate the plasma. The blood plasma was stored in deep freezer at −20°C till analysis.

Biochemical parameters

Small pieces of liver (approximately 0.5 g) were homogenised at ratio 1:2 in 0.8% sucrose solutions. The liver homogenates were centrifuged at 5,000 rpm for 5 min and the clear supernatants were separated and stored at −20°C till analysis. Fish blood was collected from the caudal artery using heparinized syringe. After that, the fish organs were excised for analysis. The AST and ALT were determined in plasma and liver following the method of Reitman and Frankel (1957). The determination of plasma and liver total proteins content and plasma albumin were done following the methods recommended by Henry (1964) and Dumas and Biggs (1972), respectively. The plasma globulin was estimated as the difference between the levels of total protein and albumin. The plasma glucose was manipulated based on the method of Trinder (1969). The liver glycogen content was determined by alkaline digestion of the tissue (Oser 1979) followed by enzymatic measurement glucose.

Statistical analysis

Data are presented as mean values ± standard deviation. Statistical analyses of the data were computed by SPSS (Version 10). For each of the studied items, significant differences between every pair-wise experimental group were done using two paired dependent Student's t test (Pipkin 1984).

Results

Somatic indices

The control fish fed low ration (LR, 0.5%) or high ration (HR, 3%) have non-significantly different somatic indices between the tested seasons (Table 1). In both seasons, Cu exposure induced significant reduction in the tested body indices of fish fed HR, compared with the relevant control, whereas those fed LR and exposed to Cu exhibited non-significant variations those of the control. The data showed non-significant variations between respective groups of the tested seasons.

Table 1 Effect of ration size on some somatic indices of Nile catfish (*C. gariepinus*) exposed to ½ 96 h LC_{50} of copper at winter and summer seasons

Parameters	Winter				Summer			
	Low ration		High ration		Low ration		High ration	
	Control	Cu	Control	Cu	Control	Cu	Control	Cu
HSI (%)	0.51 ± 0.09	0.58 ± 0.08	0.56 ± 0.08	0.69 ± 0.06[a,b]	0.54 ± 0.08	0.63 ± 0.11	0.55 ± 0.05	0.71 ± 0.07[a,b]
GSI (%)	0.36 ± 0.02	0.35 ± 0.03	0.37 ± 0.03	0.31 ± 0.03[a,b]	0.41 ± 0.03	0.39 ± 0.04	0.43 ± 0.04[c]	0.31 ± 0.03[a,b]
K (g/cm^3)	1.13 ± 0.07	1.14 ± 0.08	1.20 ± 0.15	0.92 ± 0.06[a,b]	1.35 ± 0.13	1.08 ± 0.09	1.28 ± 0.16	0.85 ± 0.07[a,b]

All data expressed as means ± SD, K = condition factor, *HSI* hepatosomatic index, *GSI* gonadosomatic index

[a] Is significantly different from respective control ($P < 0.05$)

[b] Is significantly different from low ration in the same season ($P < 0.05$)

[c] Is significantly different from the same group on the other season ($P < 0.05$)

Haematological parameters

In both seasons, Cu exposure and HR induced significant reduction in RBCs count, Hb content and Hct value, regarding those of respective control (Table 2). Non-significant changes of the same data were reported for fish treatment Cu and LR. In each season, the Cu and HR treatment induced significant reduction of these data from those of Cu and LR treatment. The MCH showed significant reduction at Cu and HR treatment, from those of the respective control at each season, whereas those of Cu and LR showed non-significant changes. Only in summer, the MCHC of fish subjected to Cu and HR showed significant rise either comparing with the respective control or with those of treatment Cu and LR of same season or with same group of winter. The MCV data only exhibited significant reduction for Cu and HR treatment in the summer compared with respective control.

Biochemical parameters

In each season, the AST and ALT enzymes levels were significantly elevated in plasma of fish exposed to Cu and HR, either compared with the respective control or with similar group fed LR (Table 3). The levels of these enzymes and glucose in plasma were significantly higher for fishes exposed to Cu and HR in winter than those of summer. In each season, the plasma total proteins, albumin and glucose levels recorded significantly higher values in fish exposed to Cu and fed HR over those of particular control. These data also were significantly higher than those of the Cu and LR group. The application of LR along with Cu exposure in both seasons induced non-significant fluctuations of all tested plasma biochemical parameters, compared with appropriate control. The data of estimated plasma globulin showed non-significant deviations in all tested experimental groups.

In each season, the AST and ALT enzyme activities in the liver of *C. gariepinus* exposed to Cu and fed HR were significantly enhanced compared with those of relevant control. In contrary, the total protein content and glycogen were depleted significantly in the liver of Cu and HR-treated fish (Table 4). Fish exposed to Cu and LR recorded non-significantly different enzyme activities and metabolite content from those of the corresponding control. The recorded data showed non-significant variations between the control fish of two seasons. Also, there are non-significant differences between the liver enzyme activities and metabolites for fish subjected to Cu and fed LR of the two seasons. In contrast, the liver enzyme activities and metabolites of fishes fed HR and exposed to Cu showed significant variations between the values recorded for winter and summer. According to the recorded results in Table 4, the effect of Cu was found to be more drastic in summer than in winter.

Discussion

Many of the research work pointed out that the cooper toxicity induces severe anomalies for fish (Carvalho and Fernandes 2006; Abdel-Tawwab et al. 2007; Hashemi et al. 2008a, b; Gravato et al. 2006;

Table 2 Effect of ration size on some haematological parameters of Nile catfish (*C. gariepinus*) exposed to ½ 96 h LC_{50} of copper at winter and summer seasons

Parameters	Winter				Summer			
	Low ration		High ration		Low ration		High ration	
	Control	Cu	Control	Cu	Control	Cu	Control	Cu
RBCs ($\times 10^6$/mm^3)	2.14 ± 0.07	2.24 ± 0.08	2.21 ± 0.05	1.55 ± 0.06[a,b]	2.04 ± 0.10	1.89 ± 0.12	2.17 ± 0.08	1.52 ± 0.13[a,b]
Hb (g/dl)	6.32 ± 0.14	6.29 ± 0.21	6.53 ± 0.24	5.80 ± 0.36[a,b]	6.06 ± 0.27	5.89 ± 0.32[c]	6.31 ± 0.11	5.13 ± 0.25[a,b,c]
Hct (%)	32.47 ± 1.24	30.62 ± 1.43	33.97 ± 0.97	23.94 ± 1.23[a,b]	31.21 ± 1.54	33.12 ± 2.03	32.17 ± 2.04	25.04 ± 2.13[a,b]
MCH (pg)	29.53 ± 1.23	28.13 ± 1.42	29.41 ± 2.02	33.68 ± 1.47[a,b]	29.70 ± 2.12	30.01 ± 1.24	29.77 ± 2.14	33.77 ± 2.04[a,b]
MCHC (%)	19.75 ± 0.74	20.83 ± 1.13	19.23 ± 1.65	19.44 ± 1.37	19.42 ± 1.72	19.93 ± 1.56	20.18 ± 1.81	22.60 ± 1.46[a,b,c]
MCV (μ^3)	151.73 ± 6.12	152.54 ± 4.67	153.71 ± 5.03	141.13 ± 5.09[a]	152.99 ± 6.01	157.49 ± 5.13	148.25 ± 4.14	144.72 ± 4.89

All data expressed as means ± SD, *RBCs* red blood cells, *Hb* haemoglobin, *Hct* haematocrit, *MCH* mean cellular haemoglobin, *MCHC* mean cellular haemoglobin concentration and *MCV* mean cellular volume

[a] Is significantly different from respective control ($P < 0.05$)

[b] Is significantly different from low ration in the same season ($P < 0.05$)

[c] Is significantly different from the same group on the other season ($P < 0.05$)

Table 3 Effect of ration size on some biochemical parameters in the plasma of Nile catfish (*C. gariepinus*) exposed to ½ 96 h LC_{50} of copper at winter and summer seasons

Parameters	Winter				Summer			
	Low ration		High ration		Low ration		High ration	
	Control	Cu	Control	Cu	Control	Cu	Control	Cu
AST (U/l)	31.24 ± 1.46	33.46 ± 1.76	31.98 ± 1.24	39.47 ± 1.79[a,b]	32.41 ± 2.07	33.97 ± 2.91	32.79 ± 2.04	43.66 ± 2.17[a,b,c]
ALT (U/l)	28.37 ± 1.39	30.14 ± 1.09	28.29 ± 1.91	35.13 ± 1.36[a,b]	29.07 ± 2.12	29.94 ± 1.96	29.26 ± 2.13	38.17 ± 2.21[a,b,c]
Total protein (g/dl)	7.82 ± 1.53	8.05 ± 0.74	7.91 ± 0.57	9.94 ± 0.76[a,b]	7.09 ± 1.43	7.51 ± 1.68	8.21 ± 0.57	10.18 ± 1.14[a,b]
Albumin (g/dl)	4.35 ± 0.96	5.07 ± 0.76	4.62 ± 0.84	6.18 ± 0.47[a,b]	4.62 ± 0.79	4.81 ± 0.83	4.92 ± 0.73	6.25 ± 0.96[a,b]
Globulin (g/dl)	3.51 ± 0.57	3.10 ± 0.87	3.05 ± 0.53	3.47 ± 0.46	2.66 ± 0.67	2.81 ± 0.54	3.02 ± 0.64	3.35 ± 0.56
Glucose (mg/dl)	28.53 ± 2.26	29.84 ± 1.71	28.71 ± 1.92	35.87 ± 1.83[a,b]	29.47 ± 2.15	30.42 ± 1.78	29.72 ± 1.46	39.84 ± 2.01[a,b,c]

All data expressed as means ± SD, *AST* aspartate aminotransferase and *ALT* alanine aminotransferase

[a] Is significantly different from respective control ($P < 0.05$)

[b] Is significantly different from low ration in the same season ($P < 0.05$)

[c] Is significantly different from the same group on the other season ($P < 0.05$)

Table 4 Effect of ration size on some biochemical parameters in the liver of Nile catfish (*C. gariepinus*) exposed to ½ 96 h LC_{50} of copper at winter and summer seasons

Parameters	Winter				Summer			
	Low ration		High ration		Low ration		High ration	
	Control	Cu	Control	Cu	Control	Cu	Control	Cu
AST (U/min/g fresh tissue)	49.63 ± 4.22	54.35 ± 4.14	50.24 ± 5.76	64.15 ± 3.26[a,b]	50.97 ± 4.28	55.72 ± 3.88	52.15 ± 5.24	70.78 ± 4.51[a,b,c]
ALT (U/min/g fresh tissue)	39.64 ± 3.68	44.26 ± 4.73	40.03 ± 3.87	50.38 ± 5.41[a,b]	40.14 ± 4.27	46.17 ± 4.19	41.37 ± 3.71	50.81 ± 5.15[a,b,c]
Total protein (mg/g fresh tissue)	93.52 ± 5.42	88.27 ± 4.96	95.14 ± 6.28	76.24 ± 5.19[a,b]	94.28 ± 4.22	87.32 ± 5.69	95.63 ± 5.15	64.63 ± 4.72[a,b,c]
Glycogen (mg/g fresh tissue)	1.53 ± 0.07	1.43 ± 0.07	1.59 ± 0.07	1.33 ± 0.05[a,b]	1.65 ± 0.06	1.57 ± 0.07	1.49 ± 0.08	1.07 ± 0.06[a,b,c]

All data expressed as means ± SD, *AST* aspartate aminotransferase and *ALT* alanine aminotransferase

[a] Is significantly different from respective control ($P < 0.05$)

[b] Is significantly different from low ration in the same season ($P < 0.05$)

[c] Is significantly different from the same group on the other season ($P < 0.05$)

Oliveira et al. 2008; Kunwar et al. 2009). In general, fish undergoing this command may utilise the energy and nutrients that could be used for reproduction (Foss et al. 2009). The body indices are still valuable ecotoxicological tool because of their low cost, ease and rapidly done (Van der Oost et al. 2003). In the present study, the rise HSI of fish exposed to Cu and HR may reflect hepatomegaly and accumulation of heavy metals in the liver, a phenomenon associated to metal contamination (Pait and Nelson 2003; Barse et al. 2006; Datta et al. 2007). In such circumstance, the fish liver is the second highest tissue accumulating metals following the skin (Yilmaz et al. 2010). The reduction in GSI of Cu and HR-treated groups reflects retarded growth of the gonad, which resulted from metabolic disturbances due to Cu intoxication (Papagiannis et al. 2004; Hoyle et al. 2007). Condition factor (*K*) and the other somatic indices provide a valuable information on fish physiological status. Condition factor (*K*) provides information of fish shape and energy reserves (Sanchez et al. 2008). In the present study, *K* values were reduced significantly for fishes exposed to Cu along with HR. This result agrees with those reported former for fish exposed to various pollutants (Abdel-Tawwab et al. 2007; Sanchez et al. 2007; Abdel-Hameid 2007). The reduction of *K* could be related to growth reduction (Laflamme et al. 2000). In the present study, no seasonal variation of tested body indices was found, either in the control or in Cu-exposed fish. This result matches those recorded by Sanchez et al. (2008).

Haematological analysis has been used frequently as a diagnostic tool for fish health under different experimental protocols (Pincus 1996; Cnaani et al. 2004; Řehulka et al. 2004). The reduction in RBCs, Hb and Hct reported for fish subjected to Cu along with HR may reflect disturbance of haemopoiesis or destruction of the haemopoiectic organ (kidney and spleen). This resulted anaemia may be due to osmotic changes resulting from hemodilution and hemoconcentarion (Tort et al. 1987), haemolysis and impairment in Hb concentration (Shah and Altinndag 2004; Shah 2006). Gill and Epple (1993) have postulated anaemia to impaired eryhtropoiesis due to metal direct action on kidney and spleen, accelerated erythroclacia due to altered membrane permeability and defective iron metabolism and/or impaired intestinal iron uptake due to mucosa lesions. The reduction in MCV and Hct recorded in the present

study due to Cu and HR may reflect the destruction of the hemopoietic organs with subsequent release of immature erythrocytes. This phenomenon recorded previously due actions of different metals on fish (Shah and Altinndag 2004; Carvalho and Fernandes 2006; Shah 2006). In the present undertaken, the tested blood parameters lack seasonal fluctuations. Only, the Hb and MCHC exhibited significant seasonal difference between fish exposed to Cu and fed HR.

The recorded data revealed elevated plasma AST, ALT, total proteins and albumin due to Cu and HR treatment. This may reflect hepatocelluar damage due to Cu toxicity (Levesque et al. 2002; Datta et al. 2007; Abdel-Tawwab et al. 2007). Eminent plasma albumin recorded for fish intoxicated with Cu and fed HR may be due to rise of their production in the liver. This result was in agreement with those reported by Ahmed et al. (2006) and Abdel-Tawwab et al. (2007). In a consequence to the rise of plasma, the plasma viscosity and osmolality will also be rise. It could also possibly due to that high energy demand requires for the synthesis of detoxifying enzymes (Begum and Vijayaraghavan 1995; Hori et al. 2006). The recorded hyperglycaemia besides reduced liver glycogen after Cu exposure along with HR may imitate energy mobilisation for the synthesis of detoxification enzymes and repair processes in fish exposed to Cu. This phenomenon is reported earlier for fish species encountering pollution (Begum and Vijayaraghavan 1995; Hori et al. 2006). During stress, energy mobilisation will lead to reduction in energy stores (Dangé 1986; Hori et al. 2006; Abdel-Hameid 2007).

The fish liver is one of the insightful organs in which various metabolic pathways happen. Consequently, the effects of a chemical intoxication usually appear primarily in the liver (Roy and Bhattacharya 2006). The enhancement of liver AST and ALT activities reported for Cu and HR group may be resulted from the stress caused by Cu. It is also may reflects tissue proteolysis and energy mobilisation due to pollutants intoxication. This phenomenon is earlier reported for phenol (Barse et al. 2006; Hori et al. 2006; Abdel-Hameid 2007) and for Cu (Abdel-Tawwab et al. 2007). This gets the support from the reduction in the liver glycogen recorded in the present study for fish exposed to Cu and HR. Similarly, Levesque et al. (2002) reported that chronic exposure of yellow perch (*Perca flavescent*) to heavy metals impairs growth and alters liver glycogen as well as the activities of metabolic enzymes.

In soft water, Cu is acutely toxic to freshwater fishes at concentrations 10–20 μg/l (McGeer et al. 2002). Ionic Cu (Cu^{2+}) and copper hydroxides are considered the most toxic species of aqueous copper, while the copper carbonates have proven much less toxic (Eisler 1998). It is well documented that Cu exposure induces ionoregulatory failure (Hoyle et al. 2007; Hashemi et al. 2008b). Furthermore, exposure to aqueous Cu is known to aggravate oxidative stress responses in fish (Ahmad et al. 2005). Copper toxicity can cause lipid peroxidation in fish (Hoyle et al. 2007) and hepatic fatty change (Handy et al. 1999) could be partly attributed to oxidative stress. Thiobarbituric acid (TBARS) assay measures the presence of lipid peroxides, and therefore, it is used as oxidative stress biomarker (Hoyle et al. 2007). Carriquiriborde et al. (2004) and Hoyle et al. (2007) reported progressive rise in TBARS in fish gills and intestine reflect some lipid peroxidation during Cu exposure. Also, its toxicity differs with ambient water temperature. Changes in membrane permeability and increased breathing frequency, which may accelerate copper absorption, are considered the main reason for increasing toxicity of copper at higher temperature (Carvalho and Fernandes 2006). Lushchank and Bagnyukova (2006a, b) found that acute temperature changes from an average summer (21°C) to extremely high temperature (35°C) led to oxidative stress of goldfish.

Compensatory growth in fish is commonly described as a period of rapid growth following a period of reduced feeding (Ali et al. 2003). The application of feeding and starvation succession was found to enhance the growth of Atlantic halibut fish (*Hippoglossus hippglossus* L.), hasten maturation and improve fish flesh quality. A pronounced rise in plasma ammonia level has been noticed during sub lethal exposure of brown trout to Cu (Beaumont et al. 2000). Most probably, Cu exposure enhance ammonia level by increased level of cortisol (due to stress) and inhibition of its excretory mechanism ($Na^+/NH4^+$) (Beaumont et al. 2003). Kunwar et al. (2009) found that common carp (*Cyprinus carpio*) exposed to Cu and fed high ration exhibited high plasma ammonia but those fed low ration remained unaffected. Fish are often fed minimal food rations to avoid accumulation

of wastes. However, previous studies clearly indicated that food ration affected the sensitivity of carp to copper exposure (Hashemi et al. 2008a, b; Kunwar et al. 2009). In the present study, fish exposed to Cu and fed LR exhibited non-significant differences of the tested items from those of the respective control at both examined seasons. This result recorded for *C. gariepinus* is in an agreement with reported by Hashemi et al. (2008a, b), Olsen et al. (2008) and Kunwar et al. (2009) for *C. carpio* and Atlantic cod (*Gadus morhua* L.). A potential reason why LR fish remained unaffected during Cu exposure could be due to the enhancement of metallothionein biosynthesis (low molecular weight metal binding protein) that bind more Cu and consequently lowers free concentration of the metal (Larsen et al. 1997; Kunwar et al. 2009). Hashemi et al. (2007) have suggested increased level of metallothionein in starved fish. Also, Hashemi et al. (2008b) reported increased metallothionein level in gills of LR and Cu-exposed fish. Thus, it could be concluded that relevance of low food ration (LR) during hostile contamination with Cu alleviates its toxicity. Also, the application of high ration (HR) during Cu exposure enhances its harmful effect. Moreover, the toxicity of Cu was amplified at summer than at winter.

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