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Impact of copper on the oxidative metabolism of the fry of common carp, *Cyprinus carpio* (Linn.) at different pH

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Abstract: *In order to evaluate the impact of copper on the energetics of a fish, the levels of glucose, glycogen, pyruvate and lactate, the rate of tissue oxygen consumption and the activities of glycogen phosphorylase, isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) were estimated in the whole body of the fry of Cyprinus carpio immediately after 1, 7 , 15 and 30 days on exposure to a sublethal concentration of copper 0.08 mgl^{-1} at pH 7.5 (normal), 6.0 (weak acidic) and 9.0 (weak alkaline). A progressive increase in glucose level and glycogen phosphorylase activity with the corresponding decrease in glycogen level over the time of exposure at pH 7.5 indicated glycogenolysis. Increase in the rate of oxygen consumption, pyruvate level and ICDH and SDH activities at days 1 and 7 (day 1>7) followed by their decrease at days 15 and 30 (day 15<30) at pH 7.5 indicated an initial elevation in the energetics of the fish fry with a gradual suppression of it on prolonged exposure. During this period the animal might have relied more on energetically less efficient glycolysis as evident by the progressive increase in the level of lactate and LDH activity. The degree of glycogenolysis was relatively more at pH 6.5 than at pH 7.5. At that pH, a progressive decrease in glucose level with an increase in the pyruvate and lactate levels and in LDH activity and a decrease in the rate of oxygen consumption and ICDH and SDH activities revealed greater reliance of the fish on anaerobic glycolysis than on oxidative metabolism. At pH 9.0 also the fish fry initially exhibited glycogenolysis, but gradually it came to normal on day 30 (day 1>7>15>30). Decrease in the glucose level, increase in pyruvate level, rate of oxygen consumption, and ICDH and SDH activities at all the days of exposure suggested an elevation in oxidative metabolism, but it also came to normal on prolonged exposure. Even the lactate level and LDH activity initially increased but gradually reached to normal on day 30. These results indicated that copper suppresses the energetics of the fish fry at pH 6.0, elevates at pH 9.0 relative to the changes at pH 7.5 suggesting that the toxicity of copper is dependent on pH of the water.*

Key words: *Cyprinus carpio, Copper, Oxidative metabolism, pH*
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Introduction

There has been an excessive use of metals in industries in India (Lokhande and Kelker, 1999). They cause greatest threat to the health of Indian aquatic ecosystem (Joshi *et al*., 2002; Ohe *et al*., 2004; Satyaparmeshwar, 2006). Among metals, copper a group IB metal is used in industries like organic chemicals, fertilizers, iron and steel works, electrical works, antifouling paints, pulp and paper industries, pesticides, fungicides and automobile industries. Even though copper is an essential element in low concentrations, it is discharged into freshwater environments in large concentrations as an industrial effluent and severely affect the freshwater fauna, especially fishes (Venkataramana and Radhakrishnaiah, 2001; Lodhi *et al.,* 2006).

The solubility of copper can be considerably reduced with a pH increase; therefore formation of copper precipitate in alkaline condition followed by its removal is a generally acceptable means to reduce copper ion concentration in waste water (Lee *et al*., 2003). Water acidification has become one of the most important environmental factors affecting fish (Kossakowski Korwin, 1988). Dheer *et al*. (1987) reported mortality, loss of weight, increase in blood glucose and liver glycogen levels at low pH in *Channa punctatus*. Rask *et al.* (1990) reported delayed spawning in perch, *Perca fluvicetilis* in acidified lakes due to decreased gonadal maturation. Work on the impact of differential water pH with heavy metals on fishes is scanty. Hence, the present study is aimed at the determination of the changes in the energy levels of the fry of common carp, *Cyprinus carpio* in response to exposure to a sublethal concentration of copper at different pH, 7.5 (normal), 6.0 (weak acidic) and 9.0 (weak alkaline). As fry are important stage in the development of fishes and more prone to stress, either metal or otherwise, this size group has been selected for the study. As exposure period is also an important factor to study the toxicity of a metal, the observations were made at day 1, 7, 15 and 30.

Materials and Methods

Fry of *Cyprinus carpio* weighing 200 ± 10 mg were collected from the fisheries department of Anantapur, and were held in glass aquaria of 5'x 3'x 2' having the static waters for seven days under the conditions: (temperature 28 ± 0.5^{o} C, pH 7 ± 0.1, total hardness 100 ± 5 mgl^{-1} $CaCO_3$, chlorinity 0.08 ± 0.003% and dissolved oxygen 5.8 ± 0.4 mgl^{-1}) (Sivaramakrishna and Radhakrishnaiah, 1998). They were fed *ad libitum* with powdered commercial fish pellets (having around 40% protein content) once a day and water was renewed once every 24 hr to reduce the effects of metabolic wastes.

A stock solution of copper was prepared by dissolving 3.8 g of cupric nitrate [$Cu(NO_3)_2 3H_2O$] in one liter of distilled water that contains 1 g of copper. Appropriate amounts of stock solution were taken to obtain the desired concentrations of copper. The pH 6.0 ± 0.1 (weak acidic) and 9.0 ± 0.1 (weak alkaline) were maintained by adjusting the water with concentrated HCl and saturated NaOH respectively.

The LC_{50}/96 hr of copper was determined as 0.8 mgl^{-1} by using the method of Finney (1971) at pH 7.5. Twelve batches of fry containing 10 in each were exposed to a sublethal concentration 0.8 mgl^{-1} (1^{th} of LC_{50}/96 hr), of copper for 1, 7,15 and 30 days at pH 7.5, 6.0 and 9.0. Controls were also maintained alongside by the addition of 1 ml of nitric acid to 10 liters of water to nullify the nitrate effect. All the fry were fed *ad libitum* and feeding was stopped 24 hr before the animals were sacrificed for experimentation to avoid differential feeding effects, if any. Water of having the metal with pH 7.5, 6.0 and 9.0 respectively was changed everyday.

The following biochemical parameters were estimated in whole fry of the controls and experimentals *via* standard experimental procedures: Glucose (mg/g wet wt.) (Nelson and Somogyi, 1952), glycogen (mg g^{-1} wet wt.) (Caroll *et al.,* 1956), glycogen phosphorylase activity (GP) (µM Pi mg^{-1} protein hr^{-1}) (Cori *et al.,* 1955), the rate of tissue oxygen consumption (TOC) (µM 2/mg/5 min) gilson 5/6 oxygraph (Radhakrishnaiah *et al.,* 1993), isocitrate dehydrogenase (ICDH) (µM formozan mg^{-1} protein hr^{-1}) (Kerenberg and Pricer, 1951 as modified by Mastanaiah *et al.,* 1978), succinate dehydrogenase (SDH) (µM formozan mg^{-1} protein hr^{-1}) (Nachlas *et al.*, 1960), the levels of pyruvate (mg g^{-1} wet wt) (Friedman and Hangen, 1942), lactate (mg g^{-1} wet wt)(Barker and Summerson, 1941) and lactate dehydrogenase (LDH) (µM formozan mg^{-1} protein hr^{-1}) (Srikanthan and Krishnamoorthi, 1955).

Each experiment was done in a minimum of ten fish fry and the mean of it was taken for analysis. The results were analyzed statistically subjecting them to students 't' test. The significance was derived at 5% level among the exposure periods.

Results and Discussion

Relative to controls, at pH 7.5 glucose level and glycogen phosphorylase activity significantly increased with a corresponding decrease in the glycogen level at all the periods of study. Either the increase or decrease of them was in the order: day 1 < 7 < 15 <30. The rate of oxygen consumption and the activities of ICDH and SDH and the level of pyruvate increased initially at day 1 and 7 (1 > 7) followed by a decrease of them at day 15 and 30 (15 < 30). The increase of ICDH and SDH at day 7, was not significant compared to control. The level of lactate and LDH activity progressively increased from day 1 to 30 in the order: day 1 < 7 < 15 < 30 (Table 1 to 3).

These results indicate progressive glycogenolysis in fry exposed to copper at pH 7.5. Accumulation of glucose, however, revealed an imbalance in glucose homeostasis that may be due to its decreased utilization. Depletion in glycogen stores accompanied by an increase in blood glucose level in *Clarius batrachus* exposed to dimethoate was reported by Begum and Vijayaraghavan (1994). The breakdown of glycogen could also occur due to the induction of hormones like adreno cortico trophic harmone (Dalela *et al.,* 1981) or homeostasis induced by hypoxia under toxic stress (Sancho *et al.*, 1998). Increase in the rate of oxygen consumption, and the activities of ICDH and SDH at day 1 and 7 followed by their gradual decrease at day 15 and 30 suggests an initial elevation in the energetically more efficient oxidative metabolism to meet the energy demands in the toxic environment. But on prolonged exposure, the decrease in oxidative metabolism indicates a gradual susceptibility of the fry to copper stress. For its survival, however the fry might have relied more on the energetically less efficient anaerobic glycolysis than on oxidative metabolism as revealed by the progressive increase in the levels of pyruvate, lactate and LDH activity. Vijayaram *et al*. (1989), however, reported an adaptation of the air breathing teleost, *Anabas testudineus,* to a sublethal dose of copper after prolonged exposure.

At pH 6.0, a greater decrease was observed in glycogen level, relative to the decrease at pH 7.5, with a corresponding increase in glycogen phosphorylase activity at all the days of exposure. The degree of these changes was in the order: day 1<7<15<30. The glucose level, however, increased at day 1 but gradually decreased

Table - 1: The levels of glucose, glycogen and GP activity in the fry of *Cyprinus carpio* on different days of exposure to the sublethal concentration of copper at pH 7.5, 6.0 and 9.0. Each value is a mean of ten samples (n=10)

pH	Control	Exposure period in days			
		1	7	15	30
Glucose					
7.5	6.50 ± 0.64	7.35 ± 0.73 (+13.07)	8.46 ± 0.52 (+30.15)	10.20 ± 0.46 (+56.92)	11.40 ± 0.65 (+75.38)
6.0	6.85 ± 0.54	8.80 ± 0.65 (+28.46)	6.45 ± 0.42 (-5.83)	5.25 ± 0.50 (-23.35)	4.60 ± 0.71 (-32.84)
9.0	6.29 ± 0.53*	9.45 ± 0.61 (+50.23)	7.38 ± 0.49 (+17.32)	6.59 ± 0.73 (+4.76)	6.20 ± 0.62* (-1.43)
Glycogen					
7.5	3.58 ± 0.87	2.80 ± 0.72 (-21.78)	2.27 ± 0.61 (-36.59)	2.08 ± 0.50 (-41.89)	1.87 ± 0.42 (47.76)
6.0	3.89 ± 0.41	1.70 ± 0.73 (-56.29)	1.45 ± 0.62 (-66.72)	1.17 ± 0.51 (-69.92)	0.86 ± 0.55 (-77.89)
9.0	3.40 ± 0.63	1.90 ± 0.57 (-44.11)	2.25 ± 0.45 (-33.82)	2.65 ± 0.39 (-22.05)	3.22 ± 0.41 (-5.29)
GP					
7.5	0.380 ± 0.030	0.42 ± 0.0036 (+10.52)	0.475 ± 0.027 (+25.00)	0.510 ± 0.022 (+34.21)	0.569 ± 0.025 (+49.73)
6.0	0.325 ± 0.026	0.519 ± 0.022 (+59.69)	0.560 ± 0.029 (+72.30)	0.625 ± 0.031 (+92.30)	0.680 ± 0.037(+109.23)
9.0	0.360 ± 0.045	0.580 ± 0.062 (+61.11)	0.455 ± 0.069 (+26.38)	0.410 ± 0.033 (+13.88)	0.385 ± 0.054 (+6.94)

± SD = Standard deviation; Percent change over control is given in parentheses; * = Denotes insignificance between the exposure period and control

Table - 2: The TOC, ICDH and SDH activities in the fry of *Cyprinus carpio* on different days of exposure to the sublethal concentration of copper at pH 7.5, 6.0 and 9.0. Each value is a mean of ten samples (n=10)

pH	Control	Exposure period in days			
		1	7	15	30
TOC					
7.5	0.421 ± 0.044	0.645 ± 0.033 (+53.20)	0.526 ± 0.037 (+24.94)	0.327 ± 0.026 (+22.32)	0.238 ± 0.053 (-43.46)
6.0	0.480 ± 0.052	0.372 ± 0.076 (-22.5)	0.321 ± 0.080 (-33.12)	0.234 ± 0.062 (-51.25)	0.176 ± 0.068 (-63.33)
9.0	0.432 ± 0.039*	0.655 ± 0.031 (+51.62)	0.547 ± 0.050 (+26.62)	0.508 ± 0.057 (+17.59)	0.436 ± 0.042* (+0.92)
ICDH					
7.5	0.085 ± 0.002*	0.107 ± 0.004 (+25.88)	0.090 ± 0.004* (+5.88)	0.066 ± 0.003 (-22.35)	0.042 ± 0.006 (-50.56)
6.0	0.082 ± 0.003	0.063 ± 0.007 (-23.17)	0.05 ± 0.009* (-37.80)	0.042 ± 0.004* (-48.78)	0.030 ± 0.006 (-63.41)
9.0	0.081 ± 0.005*	0.100 ± 0.011 (+23.45)	0.091 ± 0.009 (+12.34)	0.083 ± 0.006* (+2.46)	0.076 ± 0.003* (-6.17)
SDH					
7.5	0.068 ± 0.009*	0.081 ± 0.005 (+19.11)	0.070 ± 0.003* (+2.94)	0.056 ± 0.008 (-17.64)	0.031 ± 0.005 (-54.41)
6.0	0.070 ± 0.006	0.052 ± 0.001 (-25.71)	0.041 ± 0.003* (-41.42)	0.033 ± 0.007* (-52.85)	0.020 ± 0.006 (-74.42)
9.0	0.064 ± 0.006*	0.085 ± 0.002 (+32.81)	0.074 ± 0.007* (+15.62)	0.066 ± 0.006* (+3.125)	0.060 ± 0.004* (-6.25)

± SD = Standard deviation; Percent change over control is given in parentheses; * = Denotes insignificance between the exposure period and control

Table - 3: The levels of pyruvate, lactate and LDH activity in the fry of *Cyprinus carpio* on different days of exposure to the sublethal concentration of copper at pH 7.5, 6.0 and 9.0. Each value is a mean of ten samples (n=10)

pH	Control	Exposure period in days			
		1	7	15	30
Pyruvate					
7.5	0.456 ± 0.052	0.620 ± 0.060 (+28.28)	0.585 ± 0.071 (+28.28)	0.325 ± 0.082 (-28.72)	0.218 ± 0.035 (-52.19)
6.0	0.430 ± 0.040	0.485 ± 0.032 (+12.79)	0.668 ± 0.035 (+55.34)	0.810 ± 0.042 (+88.37)	0.938 ± 0.039 (+118.13)
9.0	0.420 ± 0.065*	0.475 ± 0.047 (+13.01)	0.456 ± 0.073 (+8.57)	0.427 ± 0.071* (+1.66)	0.416 ± 0.050* (-0.95)
Lactate					
7.5	0.138 ± 0.027	0.167 ± 0.021 (+21.01)	0.198 ± 0.030 (+43.47)	0.220 ± 0.029 (+59.42)	0.256 ± 0.046 (+85.50)
6.0	0.148 ± 0.055	0.196 ± 0.070 (+32.43)	0.215 ± 0.065 (+45.27)	0.292 ± 0.052 (+97.29)	0.443 ± 0.049 (+199.32)
9.0	0.130 ± 0.071*	0.172 ± 0.062 (+32.30)	0.218 ± 0.049 (+67.69)	0.186 ± 0.050 (+43.07)	0.137 ± 0.045* (+5.38)
LDH					
7.5	0.044 ± 0.007	0.056 ± 0.006 (+27.27)	0.063 ± 0.005 (+43.18)	0.072 ± 0.004 (+63.63)	0.078 ± 0.002 (+77.27)
6.0	0.048 ± 0.008	0.068 ± 0.004 (+41.66)	0.073 ± 0.002* (+52.08)	0.079 ± 0.009* (+64.58)	0.084 ± 0.001* (+75.00)
9.0	0.043 ± 0.002*	0.065 ± 0.006* (+51.16)	0.061 ± 0.008* (+41.86)	0.056 ± 0.006* (+30.23)	0.045 ± 0.003* (+4.65)

± SD = Standard deviation; Percent change over control is given in parentheses; * = Denotes insignificance between the exposure period and control

from day 7 to 30 in the order: day 7<15<30. A significant decrease was observed in the rate of tissue oxygen consumption and the activities of ICDH and SDH. Whereas an increase was noticed in the levels of pyruvate and lactate and in the activity of LDH. These changes were progressive over the time of exposure in the order: 1<7<15<30 (Table 1 to 3).

Even though glycogenolysis was more in magnitude in the fry at pH 6.0, a progressive decrease in glucose level, the rate of oxygen consumption, ICDH and SDH activities with an increase in pyruvate and lactate levels and LDH activity warranted the fish fry to switch over from oxidative metabolism to anaerobic glycolysis even from the day 1 of exposure. This indicates a synergistic impact of the acidic pH and metal ions on the fry thereby the oxidative metabolism is greatly suppressed. The decrease in glucose level may indicate greater diversion to anaerobic glycolysis to derive necessary energy. The acidic pH with metal ions might have even damaged the respiratory epithelium of the gills thereby a decrease in oxidative metabolism is possible. Asztalos and Nemcsok (1985) correlated the magnitude of increase in LDH activity in carp tissues to the tissue damage and organ necrosis. Elevation in LDH was also reported in tissues of metal-intoxicated *Tilapia mossambica* (Usha Rani and Ramamurthi, 1987). The decrease in SDH activity and elevation in LDH activity leads to the accumulation of pyruvate and lactate in the tissues of the fish which may cause tissue acidosis (Gargiulo *et al*., 1996; Valarmathi and Azariah, 2003).

At pH 9.0 the glucose level significantly increased on day 1 but this increase gradually came down and reached to normal level at day 30. A decrease in glycogen level and increase in glycogen phosphorylase activity also followed the same trend and reached almost to normal at day 30. Even the rate of oxygen consumption, ICDH, SDH and LDH activities and pyruvate and lactate levels initially increased significantly at day 1 but the elevation gradually suppressed and reached to normal at day 30 (Table 1 to 3).

Thus glycogenolysis is the initial response of fry on exposure to copper (Emad *et al*., 2005). Increase in the rate of oxygen consumption and the activities of ICDH and SDH at all the days of exposure suggests an elevation in oxidative metabolism, thereby the animal could derive more energy through it to meet the stress caused by the metal as well as alkaline pH. At this pH the metal permeability through the cell membranes might be less, so the accumulation of metal in the cells might be tolerable. Gradual decrease in the degree of elevation in the levels of energetics with the increase in exposure period indicates the adaptation of the fry to the toxic stress on prolonged exposure; thereby all the metabolic activities came to normal levels. These changes thus indicate that the weak alkaline pH acts as an inhibitor to the toxicity of copper and might have prevented its binding with the active sites of the enzymes (Ghillebaert *et al*., 1995; Wu *et al*., 2006).

It can be concluded from the study that the carp fry can tolerate and develop resistance to sublethal concentrations of copper in alkaline habitats as the fish could derive the necessary energy through the elevation of oxidative metabolism. But, it appears that the fry can not survive in acidic environment due to the drastic decrease in their energy levels on prolonged exposure. The solubility of copper can be considerably reduced with a pH increase; therefore formation of copper precipitate in alkaline condition followed by its removal is a generally acceptable means to reduce copper ion concentration in waste water (Lee *et al*., 2003). So, the industries are advised to treat the effluents more alkaline before their discharges into the freshwater bodies, as majority of industrial effluents are acidic (Gilmour and Henry, 1991).

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