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GROWTH RESPONSES OF *CATLA CATLA*, *LABEO ROHITA* AND *CIRRHINA MRIGALA* DURING CHRONIC EXPOSURE OF IRON

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The present research was conducted on different life stages (90, 120, 150 and 180-day age) of Indian major carps to check the growth responses under sub-lethal chronic toxicity of iron. Fish of each age group were stressed with iron for 30 days and growth parameters viz, average weight, fork and total length before and after exposure of iron were measured. Feed intake and feed conversion ratio of each fish species were also determined to evaluate the condition and health of fish during the stress period. Statistical analysis of data showed that age of 150 days for *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were the most sensitive in terms of fish growth. All the fish species of 150 days age showed significantly ($p<0.05$) negative growth in terms of weight, fork length and total length. Condition factor of control fish reveals that weight gain was maximum in relation to length of fish as compared to fish kept under chronic exposure of iron. The results also showed that feed intake increased during stress of iron but this feed does not take part in growth as shown by lower values of feed conversion ratio (FCR) of all fish species in iron stressed medium. This study concluded that among four age groups sub-lethal chronic exposure of iron disturbed the feed intake, FCR, growth, condition and fish health in 150-day fish.
Keywords: Sub-lethal, iron, growth, Indian major carps**.**

INTRODUCTION

Heavy metals are considered as major environmental pollutants and are regarded to be cytotoxic, mutagenic and carcinogenic (Muley *et al*., 2000; More *et al*., 2003). The environmental pollutants are those that tend to accumulate in organisms and are persistent because of their chemical stability (Wepener *et al*., 2001). Major carps could be appropriate monitoring organisms to observe the bioavailability of water-bound metals in freshwater habitats (Palaniappan and Karthikeyan, 2009). Heavy metals possess all of these characteristics and are one of the major contributors to the pollution of natural aquatic ecosystems (Mason, 1991). Iron is one of the essential elements for the entire aquatic organism especially for hemoglobin and myoglobin formation in fish. Unfortunately, increased industrial wastes polluted the natural ecosystem and this nutrient (iron) increased at significant contamination level (Williams, 1999).

Growth is a simple and straight forward index of metal's effect because it integrates all the effects within the fish (Javed, 2006). Many studies on effects of metals on *Catla catla, Labeo rohita* and *Cirrhina mrigala* have proved that the metals may fluctuate in their toxicity on living organisms depending on their concentrations, particular composition and duration of fish exposure (Kazlauskiene and Burba, 1997; Vosyliene *et al*., 2003; Javed *et al*., 2008). Heavy metals toxicity diminishes the food consumption in fish. Reduced food utilization and assimilation under such disturbance suggested that catabolic process exceed the anabolic process and hence resulted in reduced growth of fish (Vincent *et al*., 2002; Hussain *et al*., 2010).

Toxicity of iron showed variability in terms of accumulation in different organs of the fish. This variability of metal toxicity in fish is dependent on different organs, age, size of the fish, physiological state of the fish and feeding habits (Farkas *et al*., 2003). Sub-lethal effect is in general more subtle and quantitative; it is difficult to monitor these at the population or community level, due to the complexity of an ecosystem and the specificity of the induced effect. Therefore, for the lower toxic concentrations, laboratory studies at the level of organism are indispensable for the identification of relevant effects. This avoids the complexity of population dynamics and focuses on the study of more specific and mechanistic action (Javed, 2006). Interest in the essential metals, which are required for metabolic activities in organisms lies in between their essentiality and toxicity (Fatoki *et al*., 2002). Therefore, the main objective of this project was to determine the effect of sub-lethal chronic toxicity of iron on growth of Indian major carps of different life stages.

MATERIALS AND METHODS

Three fish species of different life stages (90, 120, 150 and 180-day age) were collected from fish hatchery, Faisalabad. 100 samples of each age group of *Catla catla, Labeo rohita and Cirrhina mrigala* were collected and kept under laboratory conditions in water tanks for two weeks before the start of experiment for acclimatization. The 90-day, 120-day, 150-day and 180-day fish of each species in good health and state were exposed to iron sub-lethal concentrations of 40.05, 23.33 and 40.08 mg L^{-1}; 41.21, 24.26 and 42.81 mg L^{-1}; 42.96, 26.18 and 44.91 mg L^{-1} and 44.06, 29.10 and 49.32 mg L^{-1} for *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*, respectively for 30 days as determined by Javed and Abdullah (2003) in glass aquaria (100 L). The stocking density of each species was kept 10. The exposure medium continuously replenish and partially exchanged to maintain the above mentioned sub lethal concentrations of iron for three species throughout experimental period of 30 days of each trial. During the trial exposed and controlled fish were fed to satiation daily at 9:00am and 17:00pm with the feed having digestible energy (DE) of 2.90 Kcal g^{-1} and 35% digestible protein (DP) with the following composition:

Table 1. Chemical composition (%) of feed ingredients

Ingredients	% age	Mineral and amino acids	% age
Fish meal	40	Lysine	1.9365
Corn gluten (30%)	39.27	Methionine	0.8205
Rice polish	7.51	Ca^{++}	2.2659
Wheat floor	5.00	PO_4^-	1.2023
Oil (Sunflower)	3.22	Na^+	0.3244
Vitamin and mineral mix	5.00		

Fish kept under sub-lethal concentrations of iron were reared for 30 days with three replications, while the control fish were reared in clean water for comparison. Before and after 30 days trial growth parameters including fish weight, fork length and total length of both fish reared in control and treated medium were measured. Condition factor and feed conversion ratio (FCR) were calculated by using following standard formulae (Carlender, 1970).

$$K = \frac{w \times 100}{(FL)^3}$$

Here W= Average weight (g)

$$FCR = \frac{\text{Feed intake}}{\text{Weight gain}}$$

FL = Fork length (cm)

The data on different variables, obtained from this experiment was analyzed statistically through Micro-computer to find out the statistically differences among variables under study, using analysis of variance (ANOVA) and Duncan's Multiple Range test (Steel *et al.*, 1996). Regression and correlation analyses were also performed to find-out relationships among various parameters under study.

RESULTS

At the age of 150 days all three fish species showed negative growth in terms of weight and length under sub-lethal concentration of iron. Similarly FCR for all three fish species (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) showed negative values by 150 days old fish. However, it was observed that controlled fish significantly ($p<0.05$) gains more weight, fork length and total length as compare to the stressed fish (Table 2).

Condition factor and FCR also showed significant variations ($p<0.05$) in different life stages and treatments during the whole trial. However, all fish species that were kept in controlled showed significantly ($p<0.05$) higher feed intake as compared to the treated fish. The results exhibited that values of condition factor increases as fish age and size increased in both controlled and treated fish it was observed that treated fish had better condition and health when compared to the treated fish.

FCR of Catla *catla* kept in controlled condition were ranged from 1.09 to 4.50 observed in 90 and 150 days old fish, respectively while the same was lowest and highest as -1.66 and 3.47 showed by 150 and 180 days old stressed *Catla catla.* However, *Labeo rohita* kept in treated (sub-lethal concentration of iron) exhibited that at the age of 180 days it showed maximum FCR value as 1.97 while fish of 90 days showed minimum value of 1.29. Similarly *Labeo rohita* at the age of 150 and 90 days showed maximum (3.42) and minimum (0.62) values of FCR. In controlled conditions *Cirrhina mrigala* of 120 days showed minimum as 0.86 while of 150 days showed maximum as 2.55 whereas, in stressed conditions *C. mrigala* exhibited highest and lowest values of FCR as 3.57 and -2.88 observed in the 120 and 180 days old fish.

The results of this study also showed that feed intake of treated fish were higher than controlled fish. It showed gradual increase in feed intake from 90 to 180 days. Its highest and lowest values were observed as 1.52 and 4.55 in treated *Catla catla* of having 90 and 180 days, respectively while in control *Catla catla* of 90 and 120 days the same was were remained minimum and maximum as 1.22 and 3.02, respectively. Other two species follow the same patterns for feed intake during the whole trial. Average fluctuation of condition factor in three fish species were presented in Table 2 and CF showed its minimum values as 1.60, 1.86, and 1.56 observed in 90, 120 and 90 days figure 3 (a-c). During the present experiment three fish species controlled showed higher values of condition factor (CF) when compared to stress fish under chronic exposure, *Catla catla, Labeo rohita* and *Cirrhina mrigala*, respectively. However, its maximum values were recorded as 2.84, 2.06

Table 2: Mean values of growth parameters under study

Fish age groups	*Catla catla*		*Labeo rohita*		*Cirrhina mrigala*		Mean ± SD	
	Treated	controlled	Treated	Controlled	Treated	controlled	Treated	controlled
Increase in weight (g)								
90 days	0.98 b	1.12 a	1.10 a	1.78 b	1.27 a	1.38 c	1.12 ± 0.15 a	1.43 ± 0.33 b
120 days	0.95 b	0.45 b	0.59 c	0.51 c	0.53 b	1.82 b	0.66 ± 0.25 b	0.93 ± 0.77 c
150 days	-2.01 c	0.42 b	-2.04d	0.52 c	-2.26 d	0.74 d	-2.10 ± 0.14 c	0.56 ± 0.16 c
180 days	1.31 a	1.42 b	1.02 b	2.18 a	-0.97 c	2.08 a	1.36 ± 0.18 a	1.89 ± 0.41 a
Increase in fork length (mm)								
90 days	0.60 a	0.20 b	-0.20 b	0.00 d	0.00 a	0.00 c	0.33 ± 0.30 a	0.06 ± 0.11 d
120 days	0.00 b	0.20 b	0.20 a	0.60 a	0.00 a	2.40 a	0.07 ± 0.11 b	1.06 ± 1.17 a
150 days	-0.40 c	0.20 b	-0.20 b	0.20 c	-0.20 b	0.20 b	-0.40 ± 0.20 d	0.20 ± 0.00 c
180 days	-0.40 c	1.20 a	0.20 a	0.40 b	0.00 a	0.00 c	-0.06 ± 0.30 c	0.53 ± 0.61 b
Increase in total length (mm)								
90 days	1.20 a	1.40 a	0.20 a	0.60 b	0.80 a	1.00 a	0.73 ± 0.50 a	0.53 ± 0.40 b
120 days	0.20 b	0.40 c	0.20 a	0.60 b	0.20 b	0.60 c	0.20 ± 0.00 b	0.53 ± 0.12 b
150 days	-0.20 d	0.20 d	-0.20 b	0.80 a	-0.10 c	0.80 b	-0.08 ± 0.12 d	0.60 ± 0.35 a
180 days	0.00 c	0.80 b	0.20 a	0.20 c	0.20 b	0.20 d	0.13 ± 0.02 c	0.40 ± 0.35 c
Condition factor (K)								
90 days	1.60 b	1.64 b	1.87 b	2.46 a	1.56 b	2.01 a	1.68 ± 0.17 b	2.04 ± 0.41 a
120 days	1.76 b	1.79 b	1.86 b	2.20 a	1.88 a	2.21 a	1.83 ± 0.06 b	2.07 ± 0.24 a
150 days	1.60 b	2.08 a	1.99 b	2.34 a	1.88 a	2.20 a	1.82 ± 0.20 b	2.21 ± 0.13 a
180 days	2.84 a	2.45 a	2.06 a	2.33 a	2.31 a	1.98 b	2.40 ±0.40 a	2.25 ± 0.24 a
Feed intake (g)								
90 days	1.52 a	1.22 a	1.42 a	1.11 a	1.58 a	1.39 a	1.51 ± 0.08 a	1.24 ±0.14 a
120 days	2.63 a	1.78 a	1.77 a	1.34 a	1.89 a	1.56 a	2.10 ± 0.47 a	1.56 ±0.22 a
150 days	3.33 a	2.99 a	1.99 a	1.78 a	2.19 a	1.89 a	2.50 ± 0.72 a	1.85 ±0.06 a
180 days	4.55 a	3.02 a	2.01 a	2.02 a	2.79 a	2.01 a	3.25 ± 1.14 a	2.35 ±0.58 a
Feed conversion ratio (FCR)								
90 days	1.55 c	1.09 d	1.29 c	0.62 c	1.24 b	1.01 b	1.36 ± 0.17 c	0.91 ± 0.25 d
120 days	2.78 b	3.96 b	3.61 a	2.63 b	3.57 a	0.86 c	3.32 ± 0.47 a	2.48 ± 1.56 b
150 days	-1.66 d	4.50 a	-0.98 d	3.42 a	-0.97 c	2.55 a	-1.20 ± 0.40 d	3.49 ± 0.98 a
180 days	3.47 a	2.13 c	1.97 b	0.93 c	-2.88 d	0.97 b	2.56 ± 533 b	1.34 ± 0.68 c

Means with similar letter in a single column are statistically similar at $p<0.05$.

and 2.31 in 180 days old *Catla catla, Labeo rohita* and *Cirrhina mrigala*.

(a)

(b)

(c)

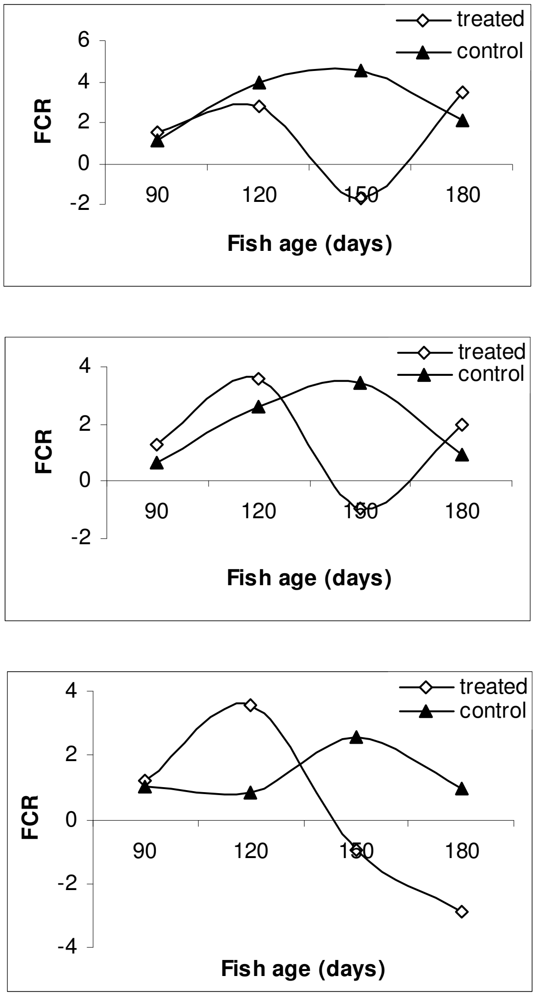


Figure 1. Comparison of FCR in controlled and stressed *Catla catla* (a) *Labeo rohita* (b) and *Cirrhina mrigala* (c).

DISCUSSIONS

Generally, iron is biologically non-biodegradable and its compounds have toxic potential to the fish. Its continuous exposure at low level may cause biological impacts of much greater intensity in fish (Karlsson-Norrgran and Runn, 1985). The present study showed negative growth performance of three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* under sub-lethal chronic exposure of iron for 30 days trial on fish four age groups viz. 90, 120, 150 and 180-days when compared to control fish. The effect of stress was more pronounced in 150-days old fish. Similar findings were reported by Javed (2005). He found that weight gain of *Catla catla, Labeo rohita* and *Cirrhina mrigala* reduced under chronic exposure of zinc. The results are also in line with the findings of Fracacio *et al*. (2003) and Hashemi *et al*. (2008). They observed growth reduction in Danio reiro (*Pisces ciprinidae*) and common carp (*Cyprinus carpio*) exposed to contaminated environment and sub-lethal doses of copper respectively.

(a)

(b)

(c)

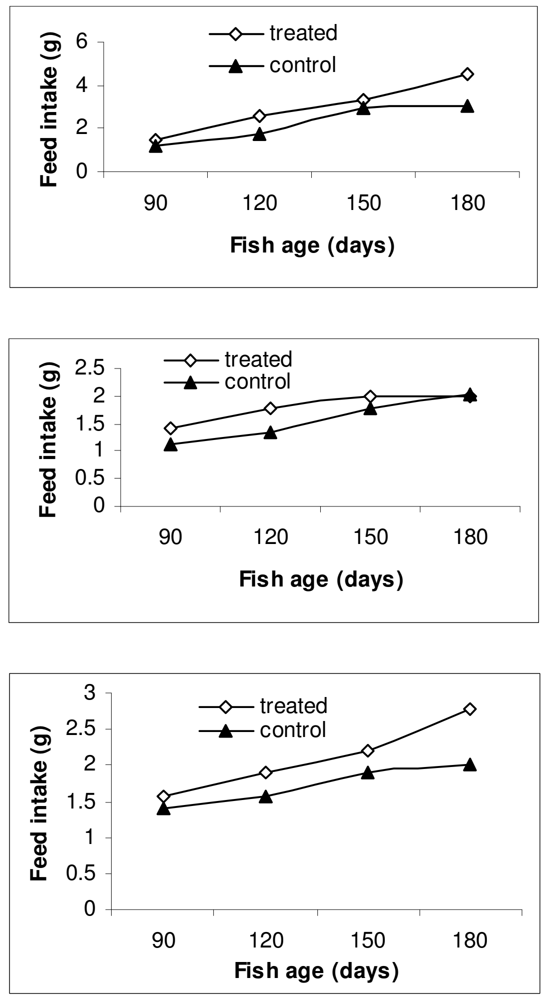
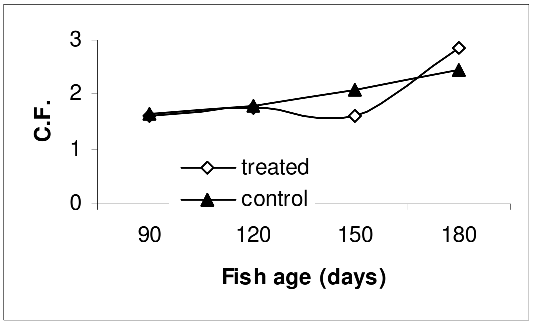


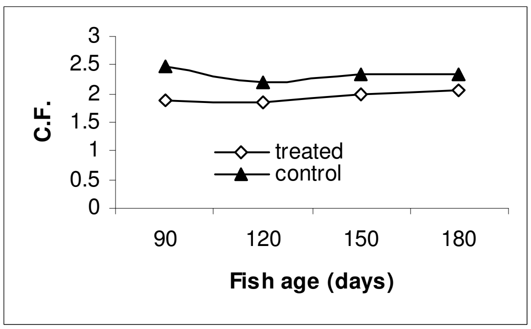
Figure 2. Comparison of feed intake in controlled and stressed *Catla catla* (a) *Labeo rohita* (b) and *Cirrhina mrigala* (c).

Average weight, fork length and total length of the fish showed significant variations (*p<0.05*) in different age groups. Feed conversion ratio (FCR) for all three fish species also showed negative values by 150 days old fish. However, it was also observed that controlled fish gain significantly (*p<0.05*) more weight, fork and total length as compared to the stressed fish (Giguere *et al*., 2004). *Cirrhina mrigala* showed increase in fork and total lengths in control pond (unstressed fish) as compared to metals stressed fish (Hussain *et al*., 2010).

(a)



(b)



(c)

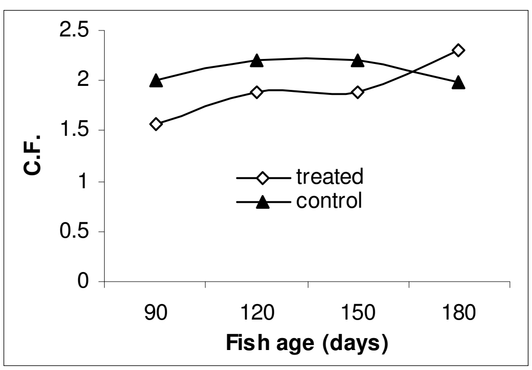


Figure 3. Comparison of condition factor (C.F.) in controlled and stressed *Catla catla* (a) *Labeo rohita* (b) and *Cirrhina mrigala* (c)

The result of this study showed that feed intake of treated fish was higher than controlled fish. Feed intake was gradually increased from 90 to 180 days. Javed (2006) reported that feed intake of major carps increased during stress of zinc. It can be concluded from the present study that exposure of iron to major carps did not only affects the feed intake but also make significant disturbance in FCR and condition of fish. Pelogram (1994) reported that sub-lethal concentration of Cd and Cu showed significant variation in age specific accumulation in different organs of fish. Persistence and accumulation of these heavy metals in tissues of fish upset the metabolic rate and hence increased the feed intake. Decrease of stress at higher stages of life is the result of decrease in daily feed intake of fish with age. Condition factor was estimation of weight-length relationship in fish. In the present study results showed that condition factor under sub-lethal concentration significantly decreased when it was compared to controlled fish of specific age. Farkas *et al*. (2003) studied the age and size specific patterns of heavy metals in the organs of freshwater *Abramis brama.* They reported the negative relationship between the heavy metal concentration of organs and the condition factor of fish suggests the relative dilution effect of the lipid content of tissues. Similarly, condition factor derived from length and weight measurements has also often indicated lower condition in polluted fish relative to reference fish (Eastwood and Counture, 2002; Lovesque *et al*., 2002) that may be due to the mixture of metals present in the natural contaminated environment.

CONCLUSION

This study concluded that overall iron stressed fish showed reduction in weight gain, fork and total lengths when compared with control fish. The feed intake in iron stressed fish was higher than control fish while FCR was better in control fish than iron stressed. Among four age groups sub-lethal chronic exposure of iron disturbed the feed intake, FCR, growth, condition and fish health in 150-day fish.

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