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Carotenoid accumulation against cadmium toxicity in fry of *Labeo rohita* and *Cyprinus carpio*

Surya Kumari Vadlamani, Charan Kumar Basuri and Prabhakara Rao Yallapragada

Abstract
Although many papers dealing with the cadmium toxicity in aquatic organisms, the expression of oxidative stress induced by heavy metals is still due in most common freshwater fishes. The present study deals with the toxicity of cadmium in fry of *Labeo rohita* and *Cyprinus carpio*. Preliminary toxicity tests results the sub-lethal concentration of cadmium for *Labeo rohita* and *Cyprinus carpio* were observed as 0.1998ppm and 4.938ppm respectively. It was noteworthy that 96h exposed fish fry recorded highest value of carotenoids such as 1.259±0.208mg/100 mg body weight and 1.291±0.079mg body weight in *Labeo rohita* and *Cyprinus carpio* respectively. The percent increase in exposed fry against to control was observed high at 10th day (61.65%) in *Labeo rohita* while in *Cyprinus carpio*, 32.29% at 24h.

Keywords: *Labeo rohita*, *Cyprinus carpio*, cadmium toxicity, carotenoid accumulation

1. Introduction

From the last few decades, the most traditional food promoting livelihoods in rural area farmers is aquaculture, which is based on scientifically evolved as traditional practice for fishery families. Aquaculture has become one of the valuable industrial sectors and contributes to national economy [1]. There should be awareness that environment friendly aquaculture makes good business sense. Presently, most industrial effluents are discharged into rivers untreated, leading to degradation of the aquatic environment. Therefore, the impacts of such pollution and the extent of damage need to be properly assessed and documented. The disposal of human wastes, the use of improper doses of pesticides and fertilizers are harmful to fish and other aquatic fauna. These discharges do not directly affect pond culture, but the industrial and agriculture wastes contain chemical pollutants of diverse nature [2]. Heavy metals are one of them, which are toxic to living organisms even in low concentrations. Two of the more harmful metals are mercury and cadmium which are biologically accumulative and they are non-bio-degradable. Hence, cadmium as a toxic element demands a detailed investigation to develop guidelines of safety for aquaculture development.

Recent investigations about metal induced oxidative stress causing cell damage, mainly as lipid per oxidation products, catalase, and glutathione in aquatic organisms like fish [3], crabs [4], Shrimps [5] and Mussels [6]. Chang *et al.,* (2009) [7] Studied Intra cellular calcium and DNA damage in haemocytes and hepato-pancreatic cells in *Litopenaeus vannamei*. Carotenoids are naturally occurring pigments, usually red, orange or yellow in colour belongs to the class of hydrocarbon carotenes and their oxygenated derivatives (Xanthophylls). They are important in human nutrition as a source of Vitamin – A (from beta-carotene) and a preventive agent for cancer, also heart diseases [8]. The antioxidant activity of carotenoids may shift into pro-oxidant activity (reduce oxidizing substances) depending on the redox potential of the carotenoid molecules as well as on the biological environment in which they act [9].

Susan and Damodaran (1997) [10] used carotenoid as a physiological index of cellular oxygen stress in Molluscs. Hardy (2002) [11] Investigated the Salmon pigments and found that they are the only group of fish to deposit carotenoids in muscle tissues. The bright pink color of Salmon flesh and the bright red of Salmon eggs are due to carotenoid pigmentation. Astaxanthin is the pigment that provides Salmon flesh with its characteristic rich pink red colour. Tunaxanthin (yellow) and Lutein (greenish yellow) are common pigments found in marine fishes.

Beta-carotene (orange), Lutein, Turaxanthin, astaxanthin(red), Tunaxanthin, α, β –doradexanthin (yellow) and Zeaxanthin (yellow-orange) are the carotenoid pigments occur in freshwater fishes. Carotenoids have multiple roles in aquatic animals especially on growth and survival [12]. Experimental evidences are available on improved brood stock performance in fish larvae by feeding with b-carotene diet [13]. Carotenoids can increase the strength of immune system of the body and also responsible for the various physiological functions such as light absorption, antioxidant properties [14, 15, 16].

Cadmium is a bio-persistent and once absorbed by an organism remains resident for many years, although it is eventually excreted for some extent. Cadmium has no known useful biological functions. It competes with Zinc for binding sites and can therefore interfere with some of the essential functions of Zinc. It may inhibit enzyme reactions and utilization of nutrients. Cadmium may be a catalyst to oxidation reactions, which can generate excessive free radical activity leading tissue damage. Sometimes cadmium pollution causes fatal diseases like itai-itai syndrome. Hence an attempt was made to study the effect of cadmium toxicity on fry of two freshwater marketable major carps *Labeo rohita* and *Cyprinus carpio* in relation to carotenoid concentration.

2. Materials and methods

2.1 Collection and Maintenance

Labeo rohita and *Cyprinus carpio* fry (Length of 0.8 to 1cm) were collected from fish breeding ponds of (16°55'12"N & 81°49'59"E) and Dwarapudi (16°55'56"N & 81°55'26"E). Precautions were taken in breeding ponds to avoid metal contamination. The fish fry were transported to the laboratory with well aeration and acclimated to laboratory conditions (pH-7, temp 29±1º C) for 48 h. They fed with the rice bran and groundnut oil cake powder mixture in 1:1 ratio. After acclimatization, healthy and active fry of *Labeo rohita* and *Cyprinus carpio* were isolated and divided into two groups, with the first group serving as control (without metal toxicant) and the other group as experimental or exposed group. The water was changed every day in both control and exposed groups without causing disturbance to the fry.

2.2 Experimental Design

Cadmium chloride (AR) was dissolved in distilled water to prepare 1% solution and used as the metal toxicant in these experiments. An appropriate amount of this solution was measured to reach the desired sub-lethal concentration of cadmium such as 0.1998ppm, 4.9381ppm for the fry of *Labeo rohita* and *Cyprinus carpio* respectively. These sub-lethal concentrations were calculated from earlier tolerance experiments conducted on these fry [17]. These fry were exposed to their respective sub-lethal concentrations for a period of 20 days; a parallel control was maintained without metal toxicant. Samples were collected from both the control and exposed groups at intervals of 24 h, 48 h, 96 h, 10 days and 20 days for carotenoid estimation.

2.3 Extraction of Carotenoids

The total carotenoid concentrations from the whole tissue of *Labeo rohita* and *Cyprinus carpio* fry were estimated by following the method of Susan and Damodaran (1997) [10]. Live fry were taken from control and exposed troughs and blotted in filter paper and then their wet weight was taken. The carotenoids from both fish fry tissues were measured with acetone in a glass homogenizer. The acetone extract was filtered under reduced pressure in a sintered glass funnel and the solid residue was returned to the glass homogenizer for further extraction. This step was repeated until the acetone extract was colourless. The volume of extract was measured and its optical density was recorded at 455nm by using a spectrophotometer (Chemito, 2000).

The concentration of carotenoid was calculated as follows:

mg/100gm of wet weight = (0.4 DV) / P where

D = optical density of the extract measured in the wave length of the carotenoid absorption maximum (455nm),

V = Total Volume of the acetone extract

P = Total wet weight of the tissue from which the carotenoid was extracted

2.4 Statistics

The mean values and standard deviation were calculated at each interval for both exposed and control fish fry. The percent (%) increase in exposed over their respective control was also calculated. Students't' test [18] was used to show the significant differences between control and exposed fry.

3. Results

The carotenoid accumulation was significantly higher in exposed fish fry of *Labeo rohita* and *Cyprinus carpio* than their respective control (Table 1; Fig.1 and 2). The levels of carotenoid of *L. Rohita* and *C. carpio* fry was presented in terms of percent increase in exposure against their control. Percent increase of carotenoid in exposure fry was recorded as 49.02% and 19.3% in *L. rohita* and *C. carpio* respectively at 24 h. In *L. rohita* the highest carotenoid concentration was observed at 96 h (1.2586 ± 0.2018 mg/100mg body weight) however, the percent exposure against control was noticed with low level (13.43%). While, in *C. carpio*, the exposed fish fry shows low difference between control, and exposed at 24 h (19.3). In both the fish fry (treated) the maximum amount of carotenoids (1.2586mg/100mg body weight and 1.2909 mg/100mg body weight in *L. rohita* and *C. carpio* respectively) was recorded at 96 h. On the other hand the maximum percent increase of carotenoid concentrations in treated fish fry when compared to their respective control was observed as 61.65%(10 d) in *L. rohita* and 32.29% in *C. carpio* (48 h). In *L. rohita* and *C. carpio*, the order of percent increase in exposure fish fry was identified as 96 h< 48 h<24 h<20 d<10 d and 24 h <20 d<96 h<10 d<48 h respectively (Table 1).

Table 1: Carotenoid concentrations in *Labeo rohita* and *Cyprinus carpio* fry on exposure to their respective sub-lethal concentrations of cadmium. Each value represents the mean ± standard deviation (n=5). The values in the parenthesis represent percent decrease over their respective controls. * Significantly different from their respective controls over at *P*<0.05

Period of Exposure	*Labeo rohita*		*Cyprinus carpio*	
	Carotenoid concentration (mg/100g body weight)			
	Control	Exposed	Control	Exposed
24 hrs	0.5505 ± 0.0616	0.8203 ± 0.0699 *	0.8988 ±0.06372	1.0723 ± 0.147 *
		49.02%		19.30%
48 hrs	0.6154 ± 0.0622	0.8735 ± 0.0959 *	0.9101 ± 0.084	1.2040 ± 0.1538 *
		41.94%		32.29%
96 hrs	1.1096 ± 0.1048	1.2586 ± 0.2018*	1.0095 ± 0.0861	1.2909 ± 0.0791 *
		13.43%		27.87%
10 days	0.6162 ± 0.0633	0.9961 ± 0.1590 *	0.9326 ± 0.1661	1.2133 ± 0.0510 *
		61.65%		30.09%
20 days	0.4726 ± 0.0269	0.7339 ± 0.0623 *	0.7950 ± 0.0883	0.9746 ± 0.2264 *
		55.29%		22.59%

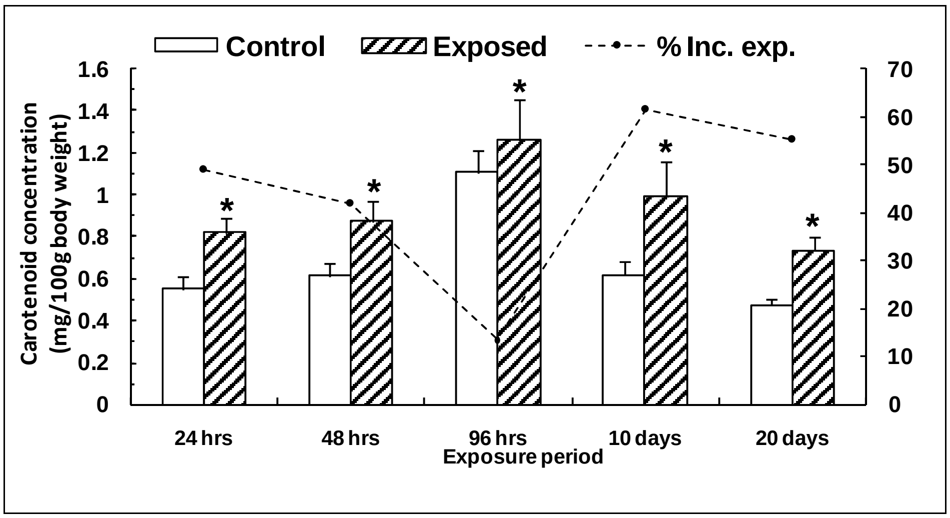

Fig 1: Carotenoid levels in fry of *Labeo rohita*; dotted line on secondary axis represents the percent increase of carotenoid concentration in exposure fry against control (*Significantly different from their respective controls at *P*<0.05)

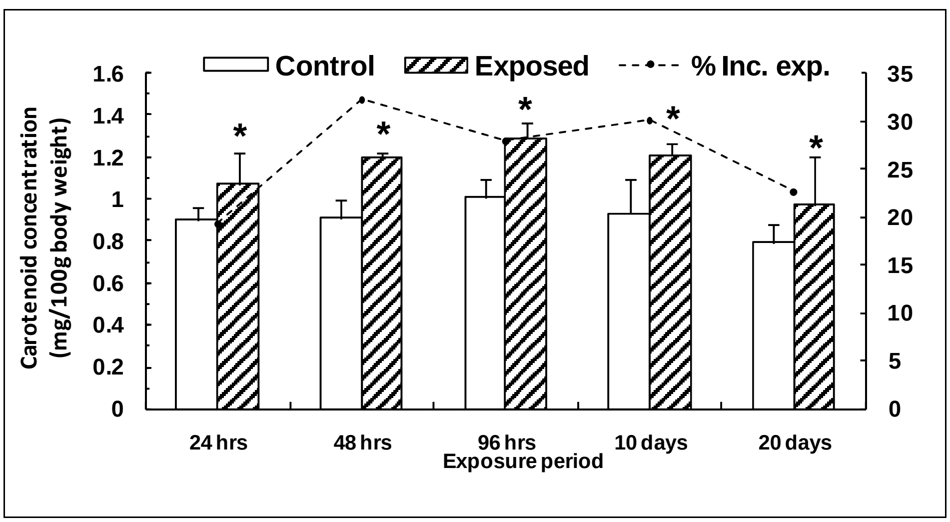

Fig 2: Carotenoid levels in fry of *Cyprinus carpio*; dotted line on secondary axis represents the percent increase of carotenoid concentration in exposure fry against control (*Significantly different from their respective controls at *P*<0.05)

4. Discussion

Since the heavy metal pollution has gained wide interest in the scientific community, the toxicity testing has been receiving much attention to know the facts and tools by assessing the effect and damage of toxicants in aquatic ecosystems. Fish can be taken as experimental animal because it acts as a bio-indicator in various studies [19, 20]. Cadmium present in various forms of organic and inorganic combinations in household and industrial effluents is the major contributors to aquatic pollution [21, 22, 23]. The deleterious effect of cadmium toxicity on fishes was studied in recent years, such as the gradual changes in physiological aspects of Rainbow trout such as biochemical and hematological changes of internal organs like liver, spleen, heart and brain tissues [20, 23, 24, 25]. However the present investigation was designed to find the effect of cadmium toxicity on fry of *Labeo rohita* and *Cyprinus carpio*, so that the amount of carotenoid concentration changes with respect to the exposure time.

The results of the present study clearly shows the carotenoid concentration in both fish fry, *Labeo rohita* and *Cyprinus carpio* increased in sub-lethal concentrations (0.1998ppm for *Labeo rohita* fry and 4.9381ppm for *Cyprinus carpio* fry respectively) of cadmium with increasing time. This fact indicates a time-dependent and concentration–dependent effect. An increase in carotenoid concentration could be attributed to compensate the metal-induced oxidative stress. In both the species of fish fry the carotenoid concentration increased with increasing exposure up to 96h exposure. From 10 days exposure on wards, the values show decreasing tendency. It shows that the fish fry of both species might have adopted a mechanism to tide over the metal toxicity. The study of comparative data of both fish fry indicate that the percentage increase of carotenoid concentration is high for *Labeo rohita* than *Cyprinus carpio* suggesting more sensitivity in the case of *Labeo rohita* fry on sublethal exposure to cadmium. In both the species yellow pigmented carotenoids are present perhaps tunaxanthin and dora-dexanthin.

The animal cell is supposed to get protection from oxidative stress [26, 27] and also from other mitochondrial inhibitor reactions by carotenoids. Czeczuga (1978) [28] Observed carotenoid concentrations in fertilized eggs, week old fry, month old fry, 4 month old fry, 1 year old fry of of *Cyprinus carpio* collected in muncipal water. Iyonouchi *et al*., 1991 [29] studied the role of carotenoid in immune defense mechanism. Astaxanthin being the major carotenoid would act as immune stimulant and useful in aquaculture [30]. Enhanced growth performance was observed in rainbow trout when fed with diet rich in carotenoids [31]. Jha *et al*., 2007 [32] observed no effect of immune stimulants in feed uptake by the Indian major carp, *Labeo rohita*.

5. Conclusion

Thus this investigation is aimed at carotenoid concentration in two major carps *Labeo rohita* and *Cyprinus carpio* fry as good bio-indicators of metal toxicity. It is proved that carotenoids give protection from oxidative stress due to metal toxicity by scavenging free radicals and quenching singlet oxygen produced. The increase in carotenoids, indicates the antioxidant status of fish fry, enhancing their resistance to heavy metal toxicity and it is clear that carotenoid pigment is an important bio-marker of biological superiority.

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