Original Article

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Effect of Urea on Liver Protein of *Heteropneustes fossilis* (Bloch)

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Abstract

Heteropneustes fossilis (Bloch) is a common fresh water air breathing fish available in India. Due to continuous use in agriculture, the fish is constantly exposed to urea. The work was carried out to study the effect of this inorganic fertilizer on the liver protein of *H. fossilis*. The fish were exposed to 0.0078, 0.0156, 0.03125, 0.0625, 0.125 mg.l^{-1} concentration of urea for 7 and 14 days. Fish of both treated as well as control groups were killed by a severe blow on the head and liver was isolated for total protein estimation. Lowry *et al* (1951) was followed for protein(no particular protein was isolated, total amount protein which present in the extraction was estimated) estimation. The experiment was replicated five times. The study revealed that the amount of protein increased in most of the doses, indicating the resistance capacity of this fish to the sublethal doses of urea.

Keywords: *Heteropneustes fossilis;* Urea; Sublethal doses; Liver protein.

Introduction

Among air breathing catfishes, *Heteropneustes fossilis* is very popular and high priced fish in West Bengal, India. This fish is locally known as singi fish. It occurs in all types of pond and can survive for a very long time when kept in captivity in a small quantity of water for its massive paired sac like pharyngeal lungs as accessory respiratory organs (Das,1927).During the last few years,the natural abundance of this fish has been rapidly decreased due to indiscriminate use of fertilizers (Noor Khan *et al* 2003). The fertilizers which are liberated into the aquatic environment have harmful effect on fish and human. The excessive uses of urea can reduce dissolved oxygen of fish pond and cause fish death(Qin and Culver, 1992, Middleton and Reeder, 2003, Tew *et al* 2006). Fish production was found to decrease when urea was used as fertilizer in aquaculture practice (Jhingran, 1982). Many aquatic species are affected by the intermediate products of mineral fertilizers (Palinichamy *et al* 1985, Baskaran *et al* 1989).Use a very high urea which lead to algae problem that are affected to fish. Heath (1987) revealed that toxic chemicals when reach sufficiently high concentration in body cells will alter the physiological functions of aquatic organisms. Vijayavel *et al* (2006) has been reported the increase in aspartate transaminase significantly in serum and liver of *Oreochromis mossambicus* due to the application of urea.McDonald *et al* (2003) studied the elevated plasma urea concentration and its relationship with facilitated diffusion transport mechanism in *Opsanus beta*. Nanda *et al* (2010) suggested

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prevalence of hypoxic stress due to rotenone toxicity. Murad and Mustafa (1989) indicate direct relationship between urea and leucocytes count in H.*fossilis.* Exposure of live organisms to treated effluents deemed to have satisfied set standards before discharge have produced various types and degrees of physiological changes in fish and death in some cases (Lawani and Alawode, 1987, Kemdirim, 1999, Subhashini and Padmini, 2000). Arashisar *et al* (2004) revealed that urea caused disorder on branchial carbonic anhydrase enzyme and acid-base balance of Rainbow trout. Tripathi *et al* (2003) reported severe destructive effect of organophosphate on carbohydrate and protein metabolism in *Channa punctatus*. Again, Naveed *et al* (2010) revealed that exposure to sublethal doses of triazophosextract caused significant time and dose depended reduction in the levels of total protein and acetylcholinesterase. The present study examine the sublethal toxic effect of urea on protein levels in the liver of *Heteropneustes fossilis* (Bloch). This type of fertilizer and fish were selected for study because the former is used often in field and the latter is an important freshwater fish of West Bengal.

Materials and Methods

Fertilizer Used

Urea, a common fertilizer is widely used in West Bengal, India, a white crystalline solid granule containing 46% Nitrogen as main composition. Urea will provide the most Nitrogen at the lowest cost. Corn, wheat, mustard, sesame, sunflower, cotton, eggplant, pumpkin, melons, apples, grapes, tomatoes, strawberries and other heavy Nitrogen feeders, urea will supply immediate and powerful application of Nitrogen.

Experimental Design

Heteropneustes fossilis were collected in live condition from the market of Salt lake, Kolkata, West Bengal and were stored in glass aquaria containing tap water for 7 days for acclimatization under laboratory conditions. Water was changed after every 48 h. Commercial fish food was supplied to fish during acclimatization as well as treatment period. Dead fish (if any) were removed from the aquaria as soon as possible to avoid water fouling. Adult fish of nearly similar weight (49.64±0.27g), length (21.04±0.52 cm), and breadth (3.88±0.076 cm) were selected for experiments. Separate aquarium was set up for each concentration and each aquarium contained 5 fish in 40 l tap water. Water temperature was kept approximately 29^0C during whole experimental period. Qualities of experimental water (PH=7 approx.; Dissolved Oxygen=12.02mgl^{-1} approx; Free Carbon dioxide=62.64 mgl^{-1} approx; Alkalinity = 235 mgl^{-1} approx; Salinity=79.94mgl^{-1} approx; Nitrite= 0.5 mgl^{-1} approx; Nitrate = 5mgl^{-1} approx; Ammonium=0.5 mgl^{-1} approx) were measured. Control groups were kept in tap water without any treatment. Based on the result of 96h LC_{50}, *H.fossilis* were exposed to 0.0078, 0.0156, 0.03125, 0.0625, 0.125 mgl^{-1} concentration of urea for 7 and 14 days. Fish of both treated as well as control groups were killed by a severe blow on the head and Liver was isolated for total protein estimation. Lowry *et al* (1951) procedure was followed for protein (no particular protein was isolated, total amount protein which present in the extraction was estimated) estimation. The experiment was replicated five times.

Statistical Analysis

Correlation coefficient (r) and its significance level (t) were used to test for difference between different levels of treatments (Zar, 2009).

Results

The data collected on the liver protein of *H. fossilis* have been shown in Table 1. Fish exposed to urea exhibited a significant ($p<0.05$) dose dependent increase in liver protein in most cases than the control.

Table 1: Level of total protein (mean ± SE) in liver of fresh water fish *H. fossilis* after exposure to different concentrations of urea

Parameter	Tissue	Control	E P	Doses of urea mgl^{-1}				
				0.0078	0.0156	0.03125	0.0625	0.125
Protein (mg. g^{-1})	liver	33.54±0.25	7 days	45.8±0.19 r= 0.848 t=3.46*	29.5±0.25 r=0.692 t=2.71*	41.96±0.14 r=0.908 t=6.17*	38.75±0.14 r=0.8076 t=2.37*	42.69±0.29 r=0.754 t=3.25*
			14 days	27.8±0.20 r=0.663 t=2.51*	34.02±0.08 r=0.785 t=3.58*	48.04±0.30 r=0.626 t=2.305*	34.85±0.18 r=0.835 t=2.63*	39.13±0.27 r=0.873 t=5.07*

EP = exposure period, $*p<0.05$

Discussion

The study revealed that in 0.0156 $g.l^{-1}$ and 0.0078 $g.l^{-1}$ doses there was little decrease in the level of protein 29.5±0.20 $mg.g^{-1}$ and 27.8±0.20 $mg.g^{-1}$ during 7 days and 14 days of exposure respectively. But the amount of protein increase in most of the doses. Singh *et al* (1996) has been reported that due to breakdown of protein, free amino acids level was increased for energy requirement and impaired incorporation of amino acids in protein synthesis. Moreover in most cases stress induced elevation transamination pathway (Natarajan, 1985). Inhibition of DNA synthesis, was found to decrease both protein as well as aminoacid levels by decreasing the level of RNA requires for protein synthesis. This is in conformity with the effects of organophosphate pesticide on *Channa punctatus* (Tripathi *et al* 2003). Except these two cases, there was a trend of significant increase in protein level in all other doses. Such increase in the protein levels were probably due to check the effect of toxicant and try to recover from the stress of urea. Mukhopadhyay and Dehadrai (1987) reported the bioconversion of non-protein nitrogen (NPN) from urea to tissue protein took place in *Heteropneustes fossilis* when exposed to urea treated supplementary diet and no significant change in food value occurred when fish was fed on the NPN diet over a rather prolonged period. In the present investigation urea treated catfish showed elevation in protein level with increase of doses with both 7 and 14 days of exposure. Dabcowski and Wojno (1978) concluded that urea and ammonium citrate improved nitrogen balance and enhanced the rate of growth in *Cyprinus carpio*. Moreover, urea treated *H.fossilis* showed highly operative ornithine arginine urea cycle in liver as well as significant rise of urease activity might be the indication of utilization of the NPN compound (Mukhopadhyay and Dehadrai, 1987). Due to possessing such adaptive features in *H.fossilis,* this air breathing catfish was able to survive successfully in different sublethal doses of urea, which faces regularly in its natural habitat as use of this fertilizer viz. urea is increasing in agricultural practise all over India to yield more crop. This may ultimately affect human through food chain, as urea is already considered as important water and soil pollutant (Pandey *et al* 2005).

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