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RECOVERY FROM AMMONIA STRESS IN FRY AND FINGERLING OF *CYPRINUS CARPIO* FOR TOTAL CARBOHYDRATES

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Recovery

Fry fingerling

Ammonia toxicity

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ABSTRACT

Ammonia toxicity in lakes and ponds results due to draining of excess toxicants and pollutants from agriculture and industries. The fish when exposed to these are a prey to ammonia stress. The potential of the younger stages of fish to combat this ammonia stress has been tested by taking fry and fingerling stages of fish *Cyprinus carpio*. Total carbohydrate content has been selected to understand the energy demands of the fish during ammonia stress (sub lethal concentration) and recovery periods in both fry and fingerling of *Cyprinus carpio*. These were exposed to ammonia solution for 14 days and later maintained in ammonia free water for 7 and 14 days to study their recovery capacities. The total carbohydrate content decreased in ammonia fry and fingerling of fish throughout the time course of the study against the normal fish. However during the recovery period of both fry and fingerling the total carbohydrates showed increment. The present investigation is carried out to fate of carbohydrates in recovery periods.

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INTRODUCTION

Considerable research effort has focused on the ammonia stress Neeraja and Santhi (1992). Ammonia in low concentration does not result in death of fish but results in metabolic changes (Santhi, 1989). To what extent these metabolic changes are permanent and whether the fish can recover back to normalcy, such studies are recovery studies. The recovery studies help to understand the basic biochemical and physiological mechanisms prevailing in the system. They also unravel the metabolic potentialities of the animals. Information regarding recovery response in fish after toxicant withdrawal is scanty (Holcombe *et al.,* 1976,). Chandravathy and Reddy (1994) reported a recovery trend in protein content, free amino acid level in fish *Anabas scandens* after transferring into lead nitrate free water for 15 days and reported that glycogen content, glycogen phosphorylase 'a' and 'ab' activities in Liver and Muscle slowly limped back to normalcy. The present investigation attempts to understand the impact of stress and recovery in fish with regard to carbohydrate metabolism.

MATERIALS AND METHODS

The young ones of fresh water carp *Cyprinus carpio* fry and fingerling were selected with the following parameters.

Fry – 25 days old, weight – 260 $\pm$ 5mg, length – 1.5 $\pm$ 0.3 cm, fingerling – 60 days old, weight – 550 $\pm$ 10mg, " – 4 $\pm$ 0.5 cm. They were acclimatized for 1 week and fed with rice bran and groundnut oil cake 1:1 ratio. The fry and fingerling were exposed to sublethal concentration of Ammonia stress 2.3 mg/lit (Doudoroff *et al.,*) 1951, Finney (1971) and 3.2 mg/lit. 7 and 14 days of stress were studied After that the same fishes were studied for recovery for 7 and 14 days *i.e* placing them in normal tap water. Total fishes were utilized, for estimation of carbohydrates. Total carbohydrates content was estimated by the method of Carrol *et al.,* (1956). The control and experimental animals were homogenized separately in 10% TCA (Trichloro acetic acid) to prepare 10% homogenate of whole animal fry and fingerling. The homogenates were centrifuged at 1000 rpm for 15 minutes. To 0.05mL of supernatant, 5mL of anthrone reagent was added and boiled for 15 minutes. The tubes were cooled and colour was read at 620nm in a spetrophotometer, using blank consiting of TCA and anthrone reagent in some proportion. The values were expressed as mg of glucose /gm wct weight of the tissue.

RESULTS

The percent decrease in total carbohydrates was progressive through out time course study in 7 and 14 days exposure and post exposure periods (Table 1a and 1b). The percent decrease in carbohydrate content of fry for 7 and 14 days exposure was 26.8, 25 respectively, while post exposure it was 26.3 and 28.7 respectively. The percent decrement in total carbohydrates in fingerling is 32.8, 36.9 and 35.6, 37.4 for both 7 and 14 days and post exposure period respectively. The percent increment in 7 and 14 days recovery fry and fingerling is (Table 1b) 2.04, 2.85, 5.88, 8.67 over 14 days of exposure fish.The carbohydrate content observed from the result of exposed period in three way ANOVA showed no significance between duration, age and experimental, while it is significant in recovery period at 1% level.

DISCUSSION

The levels of total carbohydrates were found to decrease in exposure and post exposure which suggests possible utilization of carbohydrates to meet the energy demands during stress conditions. (Nihira, 1982; Jabeen,1985; Santhi, 1989; Nadhamuni, 1992; Obula, 1994). Carbohydrates are converted to glycogen and are trapped extensively to meet the energy demands under ammonia stress, David and Schenkar (1981). Ammonia is known to produce anaerobiasis (Smith and Russo, 1975) in the animal during stress and substance like glycogen is rapidly utilized (Thillart and Kesbeke, 1978; Thillart *et al.,* 1980; Waarde *et al.*, 1982), which is resulting in decrease in carbohydrate levels. The decrease in carbohydrate level in the tissue of

Table 1a: Changes in levels of Carbohydrates in fry and fingerling of *Cyprinus carpio* on ammonia exposure for 7 and 14 days. (Values are expressed in mg/gram wet weight of the tissue).

	Control		Experimental	
	7	14	7	14
Fry	16	16.33	11.71	12.25
SD ±	0.589	0.276	0.961	0.796
% Change	-	-	(-26.8)	(-25)
Fingerling	20.69	21.55	13.9	13.6
SD ±	0.573	1.003	0.56	0.98
% Change	-	-	(-32.8)	(-36.9)

Each value is mean ±SD of Six individual observation.
Values in Parenthesis are percent increase (+) or decrease (-) over control.

ANOVA

Source	SS	df	MS	F
A	138.9922	1	138.9922	201.4266
B	394.7988	1	394.7988	572.1398
C	1.4639	1	1.46387	2.1214*
A x B	28.8604	1	28.86035	41.8242
A x C	0.0811	1	0.0105	0.1175*
B x C	0.7285	1	0.72852	1.0558*
A x B x C	1.4702	1	1.47021	2.1306*
Error	27.6016	40	0.69004	-
Total	593.9966	47	-	-

A-Age, B – Control x Experimental, C – Duration
* Non – Significant
All values are significant at 0.01 level

Table 1b:Changes in levels of Carbohydrates in fry and fingerling of *Cyprinus carpio* on post ammonia exposure for 7 and 14 days. (Values are expressed in mg/gram wet weight of the tissue).

	Control		Experimental	
	7	14	7	14
Fry	16.947	17.68	12.5	12.5
SD ±	0.747	0.367	0.646	0.435
% Change	-	-	(-26.2)	(-28.7)
Fingerling	22.35	23.61	14.4	14.78
SD ±	1.05	0.54	0.31	0.31
% Change	-	-	(-35.6)	(-37.4)

Each value is mean ±SD of Six individual observation.
Values in Parenthesis are percent increase (+) or decrease (-) over control.

ANOVA

Source	SS	df	MS	F
A	179.875	1	179.875	413.0097
B	521.9258	1	521.9258	1198.39
C	4.3896	1	4.38965	10.079
A x B	38.7393	1	38.73926	88.9489
A x C	0.585	1	0.58496	1.3431*
B x C	1.8916	1	1.8916	4.3433+
A x B x C	0.0195	1	0.01953	0.0448*
Error	17.4209	40	0.43552	-
Total	764.8467	47		-

A-Age, B – Control x Experimental, C – Duration
* Non – Significant
+ Significant at 0.05 level
All values are significant at 0.01 level

fish indicate the possibility of active glycogenolysis decrement in tissue of fish indicate the possibility of active glycogenolysis. Decrement in tissue carbohydrate level has been reported during ammonia stress (Shaffi, 1980; Jabeen Begum, 1985; Santhi, 1989; Nadamuni Chetty, 1992; Obula Reddy, 1994; Hariprasad,

1996). The decreased carbohydrate in ammonia exposed fish might also be due to stimulation of hormones that accelerate glycogen breakdown or inhibition of those which contribute to glycogen synthesis. It was reported that sub lethal concentration of ammonium salt can affect endocrine system (George *et al.,* 1981). Further the decreased glycogen was suppressed glycogen synthetase activity. A significant decreased in total carbohydrate levels in the fish to sublethal concentration of ammonia, observed in present study envisages, the possibility of their utilization under ammonia stress.

The decline in carbohydrate level was observed in recovery animal compared to 7 and 14 days stressed animal. This is in line with result obtained by Hariprasad (1996). A significant recovery was observed in fresh water field crab Barytelphusa Guerin, in carbohydrate levels on 15th day of post exposure period of lead nitrate (Venugopal *et al.,* 1990). In the present study 14 day exposure span followed by a 14 days post exposure the time course alternations in there constituent segment of the glycogen metabolism indicates an inconsistent depletion in metabolite levels and elevated enzyme activities during exposure. The levels of carbohydrates seem to be slowly coming back to normalcy in prolonged post exposure periods. In our present study carbohydrate levels are still showing stress effect. Slow elimination of ammonia coupled with the resynthesis of the enzymes may be the possible reason for progressive recovery.

Fingerling showed faster recovery (Table 1b) than fry, thus suggesting ammonia effect might be more in fry and so it naturally showed slow recovery. However, the total recovery in terms of carbohydrates levels was negligible, in present study as observed in ANOVA valves between age and duration. Thus carbohydrates may take more time to recover in contrast to protein parameter which seems to increase faster.

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