

Effect of Ammonia Stress on the Biochemical Constituents in Fingerlings of Common Carp *Cyprinus Carpio*, L.1758



Zoology

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ABSTRACT

*Fish is an indirect target to pollutants, xenobiotics pathogens substances present in these water bodies. These substances cause stress which in turn affects the metabolism and biochemical profiles of fish. Ammonia has received increasing attention over the past few years as potentially important aquatic pollutant. Fish when exposed to these aquatic pollutants are a prey to ammonia toxicity. In view of these, some of the important physiological and biochemical events occurring under ammonia stress is an interesting study. Cyprinus carpio is taken for the present study. Animal weighing 5 ± 0.5 and 4 ± 0.5 cm length and exposed to 3.2 ppm of ammonia for 0,3,7 and 14 days for the present investigation. Total protein, total carbohydrates, free amino acids were studied in the liver, kidney and gill tissues in order to understand the effect of ammonia stress on the detoxification aspect of fish (*Cyprinus carpio*).*

INTRODUCTION:

The aquatic environment is always subjected to different types of pollutants of industrial, domestic and agricultural wastes. Which severely affects the aquatic biota, including fish is receiving focus during the last few decades (Kalay and Canli, 2000). The toxicity of ammonia to fish and other aquatic organisms is primarily attributed to NH_3 and most biological membranes are permeable to NH_3 , but relatively impermeable to NH_4^+ (Hargreaves and Kucuk, 2001; Randall and Tsui, 2002). Ammonia, a chief constituent of fertilizers when present in high levels is quite toxic to most organisms and it must be either continuously eliminated or converted into less toxic compounds to prevent a build up to harmful concentration within the body (Randall and Tsui, 2002).

Ammonia detoxification takes place mainly through conversion of ammonia to glutamine by detoxifying enzymes. Ammonia excretion is common in aquatic animals, the ammonia and ammonium ion together stimulate growth but at the same time these can decrease tolerance to several stresses (Vander Eerden *et al.*, 1990). Stress has been linked as the primary contributing factor of fish disease and mortality in aquaculture (Petric *et al.*, 2006). However, under certain circumstances such as high ambient ammonia or aerial exposure, ammonia excretion is inhibited, and toxic (Wood, 1993). The capacity for accumulation of ammonia in catfish was found to be much higher than many teleosts and gobiid fishes (Jow *et al.*, 1999; Iwata *et al.*, 2000). The present study is to examine the role of proteins, carbohydrates, free amino acids of fish in liver, kidney and gills on exposure of ammonia

MATERIAL AND METHODS:

The young ones of freshwater carp *Cyprinus carpio* fingerlings were purchased from Government fish farm at Tirupati, Chittoor, Andhra Pradesh, They were acclimatized for 1 week and fed Commercial fish feed. Fingerling 120 days old, weighing 5.0 ± 0.5 g, 4 ± 0.5 cm. long are maintained at a constant room temperature ($30 \pm 2^\circ\text{C}$) with a 12:12-h light and dark photoperiod before using for experiments. The young ones of fresh water carp *Cyprinus carpio* fingerling were selected. Temperature and pH were maintained throughout experimentation. Toxicity test was conducted using ammonia solution. LC_{50} was determined using Finney's method. The fingerlings were exposed to sub lethal concentration of Ammonia of 3.2 mg/lit for 3, 7 and 14 days. Total Proteins content was estimated by the method of Lowry *et al.*, (1951). Total Carbohydrates are estimated by Carroll *et al.*, (1956) method. Free amino acids were estimated by Moore and Stein (1954). The results were subjected to statistical treatment and mean, standard deviation and analysis of variance (ANOVA) was carried out.

RESULTS:

In the present study there was a significant reduction in proteins, carbohydrates and an elevated levels of free amino acids in all the tissues of ammonia treated fish. The variations in the levels of proteins, carbohydrates and free amino acids in liver kidney and gills of common carp is presented in Table. Total protein content showed higher levels in liver (1.709 ± 0.166) when compared to those of kidney (1.367 ± 0.091) and gills (1.070 ± 0.049) in the control fish. Significant reduction of protein content was recorded in the tissues of experimental fish when compared to those of control. Maximum reduction in percentage of protein content was recorded in liver (-83.29%) which was followed by kidney (-82.29 %) and gills (-74.20%) of 14th day experimental fish when compared to that of 3rd and 7th day.

Significant reduction of carbohydrate content was observed in 14th day compared to 3rd and 7th day experiments in the order of Kidney (-37.68%), liver (-37.19%) and gills (-33.48%) respectively.

Free amino acid content showed a marked increase in all the tissues of experimental fish and showed highest free amino acid content in liver (+79.64%), kidney (+77.19%) and gills (+66.91%) in 14th day when compared to that of control fish.

DISCUSSION:

The variation in protein distribution suggests a gradual difference in metabolic calibers of various tissues and it's physiological strategy adopted by the animal to adjust itself to the changing metabolic systems and biochemical investigations besides mortality studies (Kawade and Khillare, 2012; Sreekala and Bellazutshi, 2012). Liver is the primary organ for the synthesis of various proteins and is the regulating centre of protein metabolism, biotransformation, and detoxification. The decrement in total protein levels suggests protein break down on prolonged exposure of fish to ambient ammonia (Hari and Neeraja 2012).

The levels of total carbohydrates were found to decrease in experimental fish which suggests possible utilization of carbohydrates to meet the energy demands during stress conditions (ObulaReddy, 1994). Carbohydrates are converted to glycogen and are trapped extensively to meet the energy demands under ammonia stress; Decrement in tissue carbohydrate level has been reported during ammonia stress in fish fingerlings by Vijayareddy and Neeraja, 2010.

Increased free amino acids are probably due to increased proteolytic activity under toxic stress (Singh *et al.*, 2010). This increase can also be attributed to the synthesis of amino acids in addition to their elevation by protein hydrolysis (Prashanth and

David, 2006). Increased free amino acids content in different animal models under chemical toxicity in fishes treated with carbofuran was reported by Begum, 2004.

Stress disrupts the respiration, excretion and osmo-regulatory functions are performed through gills in fish (Lease *et al.*, 2003; Raskovic *et al.*, 2010). Prolonged stress can lead to damage to gills at the cellular level and thus contributes significantly to autointoxication (Busova *et al.*, 2013). Ammonia, particularly NH₃, diffuses easily across the gill membranes, causing severe gill damage, gill necrosis that disrupts respiratory functions (Russo 1991; Bhakta 2006). Thus ammonia stress seems to affect the basic metabolic components in all the tissues in fish fingerlings.

Table: Changes in the Proteins, Carbohydrates and Free Amino acids levels in different tissues of Control and Experimental treated Fingerlings of *Cyprinus carpio*

Parameters	Tissues	Ammonia Stress in days			
		0Days (Control)	3 Day	7 Day	14 Day
Proteins (mg/gmwet wt of tissue)	Liver Mean ±SD % Change	1.709 ±0.166	0.866 ±0.055 -49.346	0.588 ±0.038 -65.579	0.286 ±0.0379 -83.298
	Kidney Mean ±SD % Change	1.367 ±0.091	0.697 ±0.0143 -49.012	0.501 ±0.048 -63.35	0.243 ±0.044 -82.29
	Gill Mean ±SD % Change	1.070 ±0.049	0.437 ±0.102 -59.16	0.327 ±0.031 -69.43	0.276 ±0.046 -74.208

Carbohydrates (mg/gm wet wt of tissue)	Liver Mean ±SD % Change	20.884 ±0.556	17.688 ±0.265 -15.308	15.733 ±0.194 -24.669	13.115 ±0.106 -37.199
	Kidney Mean ±SD % Change	22.974 ±0.435	18.359 ±0.268 -20.091	16.609 ±0.343 -27.709	14.315 ±0.266 -37.688
	Gill Mean ±SD % Change	19.322 ±0.231	17.380 ±0.251 -10.041	13.830 ±0.222 -28.41	12.850 ±0.223 -33.48
Free Amino Acids (μ moles of tyrosine/gm wet wt of tissue)	Liver Mean ±SD % Change	31.407 ±0.407	47.999 ±0.589 +52.800	53.048 ±0.588 +68.90	56.421 ±0.300 +79.64
	Kidney Mean ±SD % Change	28.28 ±0.803	36.89 ±0.518 +30.44	40.85 ±0.617 +58.59	50.65 ±0.617 +77.68
	Gill Mean ±SD % Change	24.605 ±0.767	31.79 ±0.394 +29.69	36.89 ±0.518 +49.95	41.06 ±0.719 +66.91

**All the values are mean of six individual observations
% - Percent change over control
SD – Standard Deviation
All the values are significant at P<0.05**

REFERENCE

- Begum, G., 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (Linn) and recovery response. *Aquatic Toxicology* 66 (2004) 83-92. | | 2. Bhakta J.N. 2006. Ammonia toxicity to four fresh water species: *Catla*, *Catla*, *Labeo*, *Cyprinus carpio*, *Oreochromis mossambica*. *Electronic Journal of Biology*, 2006, Vol. 2(3): 39-41. | 3. Busova et al 2013. Study of the adaptation process in common carp (*Cyprinus carpio* L.) After harvesting. *Journal of Microbiology, Biotechnology and Food sciences (special issue)* 1194-120 | | 4. Carroll, et al., Longley R.M. and Raw, J. H. 1956. Glycogen determination in liver and muscle by use of anthrone reagent. *J. Biol. Chem.*, 22:583. | | 5. Hari.P and Neeraja 2012. Ambient ammonia stress on certain metabolic aspects in liver tissue of fish, *Cyprinus carpio*. *The Bioscan* 7(1) : 111-113, 2012 | | 6. Iwata, K., Kajimura, M., Sakamoto, T., 2000. Function alreogenesis in the gobioid fish, *Mugilogobius abei*. *J. Exp. Biol.* 203, 3703-3715. | | 7. Jagadeesan, G., Jebanesan, A. and Mathivanan, A. 2001. In vivo recovery of organic constituents in gill tissue of *Labeo rohita* after exposure to sub lethal concentrations of mercury. *J. Exp. Indellieria*.3: 22-29. | | 8. Jow, L.Y., Chew, S.F., Lim, C.B., Anderson, P.M., Ip, Y.K., 1999. The marble goby *Oxyeleotris marmoratus* activates hepatic glutamine synthetase and detoxifies ammonia to glutamine during air-exposure. *J. Exp. Biol.* 202, 237245. | 9. Kalay, M. and Canli, M. 2000. Elimination of essential (Cu, Zn) and non-essential (Cd, Pb) metals from tissues of a fresh water fish *Tilapia zillii* following and uptake protocol. *Turk. J. Zool.* 24: 429-436. | | 10. Kawade S., J.Khallare Y.K. 2012 Toxicity of copper on the protein content of certain tissues of fresh water fish, *Channa Gachu* (Hum). *The Bioscan* 7 (1):53-56, 2012. | | 11. Lease, H. M., J. A. Hansen, H. L. Bergman and J. S. Meyerc, 2003. Structural changes in gills of Lost River suckers exposed to elevated pH and ammonia concentrations. *Comp. Biochem. Physiol. C*, 134: 491-500. | | 12. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin-Phenol reagent. *J. Biol. Chem.* 193, 265-275. | | 13. Moore, S., Stein, W.H., 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem.* 211, 907-913. | | 14. Obula, R. K. P. 1994. Certain metabolic modulations in carbohydrate metabolism of fry of *Cyprinus carpio* on ammonia stress. Ph.D thesis, S. V. University, Tirupati, India. | | 15. Petric D, Chris NG, Sonja Y, Kjersti K, Gro F, Rune WØ Marc H, Bemtsen G (2006). Sensitivity of Atlantic salmon (*Salmon Salta*r) to dietary endosulfan as assessed by hematology, blood biochemistry and growth parameters. *Aquat. Toxicol.* 80(3):207-216. | | 16. Prashanth, M. S. and David, M. 2006. Changes in nitrogen metabolism of the freshwater fish *Cirrhinus mrigala* following exposure to cypermethrin. *J. Basic Clin. Physiol. Pharmacol.* 17(1): 63-70. | | 17. Randall, D. J. and Tsui, T. K. N. 2002. Ammonia toxicity in fish. *Marine Pollution Bulletin.* 45(1): 17-23. | 18. Raskovic, B, Poleksic, V, Zivic, I, Spasic, M et al (2010). Histology of carp *Cyprinus Carpio*, L. Gills and pond water quality in semi intensive production. *Bulgarian Journal of Agricultural Science*, 16 (No 3) 2010, 253-262. *Agricultural Academy.* | | 19. Russo R.C., Thurston R.V. 1991. Toxicity of ammonia, nitrite and nitrate to fishes. In: *Aquaculture and Water Quality* (Brune E., Tomasso J.R.), World Aqua cultural Society Publication, pp. 58-89. | 20. Singh, K. S., Singh, S. K. S. and Yadav, R. P. 2010. Toxicological and biochemical alterations of cypermethrin (Synthetic pyrethroids) against freshwater teleost fish *Colisafasciatus* at different seasons. *World J. Zoology.* 5(1): 25-32. | | 21. Sreekala, G., Bela Zutshi., 2012 Alternations in bio chemical profiles and metabolic enzymes in tissues of *Labeo rohita*. *The bioscan*: 7(2): 359-364, 2012. | | 22. Vander Eerden C.J., Dueck, T.A., Elderson, J., Van Dobben, H.V., Berdowski, J. J., Lathuinhin, M. and Prins, A. H. (1990). Effect of ammonia and ammonium sulphate deposition on terrestrial semi natural vegetation on nutrient poor soils, report R 90/96 Research ammonia species, *Environ. Sci and Tehnol.* 15:837-84. | | 23. Vijaya Reddy and Neeraja. 2010. Recovery from ammonia stress in fry and fingerlings of *Cyprinus carpio* for total carbohydrates. *The Bioscan special issues*, Vol. 3: 659-664; 2010. | | 24. Wood, C. M. 1993. Ammonia and urea metabolism and excretion. In *Physiology of fish*. CRC Press, p. 379 - 425.