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Ammonia toxicity on biochemical constitutions in fingerlings of fresh water fishes *Catla catla* and *Labeo rohita*

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Abstract
Ammonia is present in the aquatic environment due to agriculture run-off and decomposition of biological waste. Ammonia is toxic to all vertebrates causing convulsions, coma and finally death. The focus is on this aspect and thus it was taken up in the present study. The effect of sub lethal concentration, namely 6 ppm of ammonia and 4.7 ppm of ammonia concentrations selected for fingerlings *Catla catla and Labeo rohita* respectively after evaluation of toxicity tests. The fingerlings were exposed to7days.Some metabolic components, namely ammonia, urea and glutamine estimated in the liver, muscle and brain tissues of *Catla catla* and *Labeo rohita.*

Keywords: Ammonia, *Catla*, *rohu*, ammonia, urea, glutamine

Introduction
Catabolism of proteins usually results in the production of unwanted nitrogenous products like ammonia, urea and uric acid. Ammonia is liberated in the metabolism of amino acids and other nitrogenous compounds. Some fishes have the capacity to convert ammonia to less toxic urea via the ornithine Urea cycle. Many fishes detoxify ammonia to glutamine, when exposed to environmental ammonia. Many fishes are ammonotelic but some species can detoxify ammonia to glutamine or urea. Certain fish species can accumulate high levels of ammonia in the brain or defend against ammonia toxicity by enhancing the effectiveness of ammonia excretion through active NH_4^+ transport, manipulation of ambient pH, or reduction in ammonia permeability through the branchial and cutaneous epithelia. In the case of ammonia neurotoxicity, the mitochondrial permeability transition could relate to the permeation of glutamine through the inner mitochondrial membrane and the production of ammonia through glutaminase in the mitochondrial matrix of astrocytes [1]. It has been established that several fish species can tolerate high levels of ammonia and/or glutamine in the brain [3, 4].
Ammonia can cause reductions in growth or even death [5]. It also addresses how certain fishes with high ammonia tolerance defend against ammonia toxicity through the regulation of the permeation of ammonia and related nitrogenous compounds through various types of membranes.
The present study is to concentrate on ammonia, urea, and glutamine levels in liver, brain and muscle tissues on exposure to ammonia. The work was carried out in two different fishes to examine the impact on different fishes.

Material and methods
Fingerlings of *Catla catla and Labeo rohita* were brought from fisheries Department, Tirupati, Kalyanidam, Chittoor district, Andhra Pradesh. Fingerlings of *Catla catla and Labeo rohita* weighing about 9±1 gr and 4±1 cm long selected for the study. They were maintained in tanks. A clear time of one week allowed for the fish to acclimatize themselves to the laboratory conditions before they used for the present study. The water in the tanks was changed every day. The fish fed with rice bran and groundnut oil cake in 1:1 ratio according to standard measurements. The temperature of aquaria maintained at 27 ± 4˚C and fishes were exposed to natural photoperiod. Toxicity test conducted using Ammonia Solution. LC_{50} was determined using Finney's method [6]. The sublethal concentration (LC_{50}) was found to18 mg/L, for *Catla catla* and 14.2 mg /L. for *Labeo rohita*1/3 of the LC50 concentration, namely 6 ppm was selected as sublethal concentration of *Catla catla*. 1/3 of LC_{50} concentration, namely 4.7 ppm was selected as a sub lethal concentration for *Labeo rohita.*

They were exposed to 7days to this concentration. Ammonia was estimated by the method of Bergmayer (1965) [7]. Urea content estimated by the diacetylmonoxime method as described by Natelson (1971) [8]. Glutamine was estimated by the acid hydrolysis method as described by Colowick and Kaplan (1967) [9]. The results were subjected to statistical treatment and mean, standard deviation and Analysis of variance (ANOVA) was carried out.

Result and Discussion

Ambient ammonia stress resulted in increment in ammonia levels in liver, muscle and brain tissues of fingerling of *Catla* and *Rohu*. The increment was in the order of Liver>Brain>Muscle in *Catla* and Muscle> Brain>Liver in *rohu*. It was more in rohu than *Catla* in brain and muscle tissues while it was in liver in *Catla* more than *rohu*.

The increased Urea levels were more in rohu than *Catla.* Among the tissues, greater increment in ammonia levels was observed in brain followed by muscle and liver in both *catla* and *rohu*. In *catla* and rohu, increase was higher in the brain compared to muscle. However, in case of *rohu*, the increase was more in the brain compared to muscle. Liver seems to show lowest increment though, is the center for ureogenesis. It has been observed that the last segment of urea cycle i.e. production of urea is operative in brain tissue.

Glutamine levels showed an increase in the all the selected tissues in the present study. Increased Glutamine levels were more in *Catla catla* than *Labio rohita*. (Table). The increment in the tissue of *Catla* was Brain>Muscle>Liver. In the case of *rohu* Muscle >Brain>liver.

Ammonia levels were increased in *Cyprinus carpio* by ammonia lethal exposure [10]. The increased levels of ammonia reported under the Chromium effect on protein metabolism in different tissues of fish, *Cyprinus carpio* [10]. The breakdown of protein leads to the elevation of amino acid level due to the increase of ammonia levels by the process of transamination and deamination. Under ammonia treated fish's significant increases ammonia content in *Cyprinus carpio* [11]. Ammonia levels were increased in freshwater fish *Catla catla* on Copper cyanide intoxication [12]. Under Chlorpyrifos toxicity increased ammonia levels observed in albino rats [13]. When ammonia concentration increased in the blood and other biological fluids, ammonia diffused into cells and across the blood / brain barrier, reported increased ammonia levels in the muscle of albino rat exposed to Cypermethrin [14].

Urea levels were increased under ammonia exposure as observed in *Cyprinus carpio* [10]. An increased urea level under the effect of Chromium on Protein Metabolism in Different tissues of fish, *Cyprinus carpio* might be due to activation of urea cycle for detoxification of ammonia [11]. Increased urea levels in the freshwater fish *Catla catla* under Copper cyanide intoxication [13]. An increased urea levels under Chlorpyrifos intoxication in albino rats [18]. Increased urea levels under cypermethrin exposed albino rats [15]. Increase urea levels observed in *Rana hexadactyly* exposed on Azadirachtin a bio pesticide [16].

Glutamine levels also gave increment under ammonia exposure as observed in *Cyprinus carpi*o [10]. The animal seems to remove ammonia through glutamine formation rather than urea formation. Similar increased Glutamine levels were observed in fry of fish *Cyprinus carpio* on exposure of ambient ammonia [17]. The observed increased glutamine levels under the study of toxicity of cadmium on certain aspects of protein metabolism of the freshwater mussel *Lamellidens marginalis* and fresh water fish *Labio rohita* (Hamilton, 1822) [18]. Increased glutamine levels in the freshwater fish *Catla catla* on Copper cyanide intoxication [13]. Increased glutamine levels on exposure to lihocin toxicity in freshwater, edible fish, *Channa punctatus* (Bloch) [19].

Table: Changes in the levels of Ammonia, Urea and Glutamine in Liver, Brain and Muscle tissues of fingerling of *Catla catla*and *Labeo rohita* exposed for 7 days to ammonia stress. (Values are expressed in mg/gram wet weight of the tissue).

Parameter	Tissue	*Catla*		*Rohu*	
		control	exposed	Control	Exposed
Ammonia	**Liver** Mean ±SD % Change over control	6.375 ±0.5064	8.301* ±0.3834 (30.23)	5.495 ±0.4073	6.868* ±0.316 (24.82)
	Muscle Mean ±SD % Change over control	4.150 ±0.3827	5.330* ±0.3782 (26.02)	4.150 ±0.4842	5.534* ±0.3278 (33.34)
	Brain Mean ±SD % Change over control	3.646 ±0.3411	4.544* ±0.5272 (30.0)	3.211 ±0.3165	4.200* 0±.3165 (30.84)
Urea	**Liver** Mean ±SD % Change over control	13.885 ±0.3983	15.022* ±0.4420 (8.18)	12.771 0.5062	14.429* ±0.4420 (12.98)
	Muscle Mean ±SD % Change over control	11.069 ±0.4419	12.502* ±0.398 (12.94)	10.624 ±0.3984	12.354* ±0.4419 (16.28)
	Brain Mean ±SD % Change over control	8.399 ±0.4427	9.537* ±0.3165 (13.67)	7.3133 ±0.4420	9.090* ±0.4432 (26.02)
Glutamine	**Liver** Mean	3.191 ±0.2398	3.721* ±0.1125	3.050 ±0.1547	3.702* ±0.1726

	±SD % Change over control		(16.57)		(21.4)
	Muscle Mean ±SD % Change over control	1.674 ±0.1127	2.221* ±0.1364 (32.59)	1.921 ±0.1424	2.538* ±0.1365 (32.1)
	Brain Mean ±SD % Change over control	1.428 ±0.1014	1.921* ±0.1875 (34.52)	1.604 ±0.2549	2.080* ±0.1576 (29.67)

All the values are mean and Standard deviation of six individual observations. *- Values are significant over control at $P<0.05$. SD-Standard Deviation

Conclusion

Ammonia exposure has led to increase ammonia, urea and glutamine levels. Suggesting utilization of metabolic components to withstand the effect of ammonia exposure and converted to non-toxic compounds of ammonia metabolism.

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