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Response of Certain Tissues of *Cyprinus carpio* To *In-Vivo* and *Ex-Vivo* Exposure of Ammonia.

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ABSTRACT

Ammonium compounds are an important gamut of pollution and they may be defined as immutable and non biodegradable as far as aquatic pollution is concerned. The ammonia and its derivatives accumulate in soil and water consequently contaminating the aquatic ecosystem which may endanger the life of aquatic fauna particularly fish because of its high susceptibility to toxic ammonia. The present aim of the study was to understand the tissue potential for combating ammonia stress when it is directly exposed to ammonia(ex vivo) or indirectly exposed through the animal(in vivo). Hence, in vivo and ex vivo experiments were designed and the changes in liver, kidney and gill tissue were selected and study was done through estimations of protein, ammonia, urea and glutamine levels. Significant changes were observed in in vivo than ex vivo. But not much difference is there between the two exposure and animal seems to respond in a same way to ammonia detoxification.

Keywords: Cyprinus carpio , *in-vivo* , *ex-vivo*, ammonia exposure.

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INTRODUCTION

Environmental pollution is an unwanted side accelerated pace or industrialization during this century: However industrialization can't be stopped at this stage of our development since it has become indispensable for providing the basic necessities of life for our growing population, but every possible effort should be made for the control and abatement of environmental pollution. Ammonium compounds are an important gamut of pollution and they may be defined as immutable and non biodegradable as far as aquatic pollution is concerned. The ammonia and its derivatives accumulate in soil and water consequently contaminating the aquatic ecosystem which may endanger the life of aquatic fauna particularly fish because of its high susceptibility to toxic ammonia [1].

Invivo experimental research became widespread with the use of microorganisms and animals models in genetic manipulation experiments as well as the use of animal models to study drug toxicity in pharmacology. Ex vivo(Latin : out of the living) means that which takes places outside an organism . Ex *vivo* refers to experimentation or measurement done in or on tissues in an artificial environment outside the organism with the minimum alteration of natural conditions. Ex vivo conditions allow experimentation under more controlled conditions than possible in the intact organism, at the expense of altering the "natural" environment. Ammonia is known to result in metabolic changes in fish when they are exposed to ammonia concentrations.

The present aim of the study is compare the extent of tolerance in fish when fish are exposed to in vivo and ex vivo. Proteins form the basic nutrients in fish. Hence, the changes in these protein levels in these conditions have been studied.

MATERIAL AND METHOD

Healthy fishes, *Cyprinus carpio* were collected from the Fisheries Department in and around Tirupati with a mean weight of 120±10 gm. They were acclimated to laboratory conditions for one week prior to the experiment. They are fed with 1:1 ratio of ground oil cake and rice bran. The animals were starved for 24 hours before they were exposed to liquor ammonia. The temperature of aquaria was maintained at 27 ± 2 ºC and fishes were exposed to natural photoperiod. The fishes were starved for one day before being used for experiment. The toxicity evaluation of liquor ammonia was done by Probit Method of Finney [2] and LC50 value thus determined was 24.04 ppm. Sub lethal concentration of 1/5th LC50 i.e., 4.80 ppm was selected to study the effect of liquor ammonia as this concentration will result in hyperammonia state but does not cause mortality of fishes. The fish were exposed to this concentration and biochemical parameters were analysed in the animals after exposing the animals to their concentration for3 hours (*invivo)* and the tissues to this concentration for 3hours for *exvivo* study. Suitable controls without ammonia solution were also maintained. The tissues namely liver kidney and gill were selected for the study. The total protein content, Ammonia, Urea and Glutamine were estimated by the method of Lowry *et al.* [3], Bergmeyer [4], by the diacetylmonoxime method as described by Natelson [5] and by the acid hydrolysis method as described by Colowick and Kaplan [6] respectively.

RESULTS

Total protein:

Total protein content was estimated in liver, kidney and Gill tissues of both control and *ex vivo, invivo* experiments and presented in the Table 1.In control fish, highest amount of protein was observed in gill followed by kidney and liver. Total protein content of all tissues studied showed a decrement. Compared to *ex vivo, invivo* showed greater decrement.

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Table 1: Changes in the levels of total protein in different tissues of fish under Ex vivo and in vivo condition. (Unit: mg/gm Wet wt of tissue)

Name of the tissue	Control	Ex vivo	In vivo
Liver			
Mean	1.926	0.609	0.589
± SD	0.060	0.131	0.131
% change		-68.43	-69.41
Kidney			
Mean	1.51	0.683	0.668
± SD	0.262	0.127	0.120
%change		-54.76	-55.76
Gill			
Mean	1.078	0.770	0.756
± SD	0.078	0.112	0.114
%change		-28.57	-29.87

All the values are mean ± Standard Deviation (SD) of six individual observations and significant at (P<0.01).

Ammonia

The ammonia content was estimated in the liver, kidney and gill of control, *ex vivo and invivo* experiments, and presented in Table 2. In general, ammonia level was found to increase in all the tissues in both *ex vivo and invivo* experiments. Under *invivo* and *ex vivo* condition liver tissue contained more amount of ammonia when compared to kidney and gill

Table 2: Changes in the ammonia levels of different tissues of fish under Ex vivo and in vivo treatment of sub lethal liquor ammonia (unit: µmoles of ammonia/gm wet weight of the tissue)

Name of the tissue	Control	Exvivo	Invivo
Liver			
Mean	2.053	2.823	2.923
± SD	0.178	0.097	0.101
%change		37.50	42.37
Kidney			
Mean	2.359	3.209	3.223
± SD	0.357	0.408	0.402
%change		36.03	36.62
Gill			
Mean	1.464	1.836	1.915
± SD	0.247	0.487	0.390
%change		25.40	30.80

All the values are mean ± Standard Deviation(SD) of six individual observations and significant at (P<0.01)

Urea

Table 3: Changes in the urea levels of different tissue of fish Under Exvivo and invivo treatment of sublethal liquor ammonia(Unit: µ moles of urea/gm wet wt of tissue)

Name of the tissue	Control	Exvivo	Invivo
Liver			
Mean	6.444	7.746	7.768
± SD	0.391	0.166	0.133
%change		20.20	20.52
Kidney			
Mean	5.601	6.587	6.614
± SD	0.421	0.376	0.374
%change		17.60	18.08
Gill			
Mean	5.525	6.526	6.385
± SD	0.441	0.646	0.743
%change		18.11	15.56

All the values are mean ± Standard Deviation(SD) of six individual observations and significant at (P<0.01)

Urea content was estimated in liver, kidney and gill tissues of fish in control *ex vivo and, invivo* treatment and presented in the Table 3. In general, the urea levels were found to increase in all the tissues of *ex vivo and invivo* ammonia stress. In *invivo and ex vivo* liver tissue contained more amount of urea when compared to kidney and gill

Glutamine

The glutamine content was estimated in the liver, kidney and gill of both control, *ex vivo and invivo* treated fish and shown in the Table .4 .Glutamine level was found to increase in all the tissues of *invivo* and *ex vivo* experiments. In *invivo and ex vivo*, liver tissue contained more amount of glutamine than kidney and gill. The levels were more in in vivo than ex vivo.

Table 4: Changes in the glutamine levels of different tissues of fish under Exvivo and invivo treatment of sublethal liquor ammonia (Unit: µ moles of glutamine/gm wet weight of tissue)

Name of the tissue	Control	Exvivo	Invivo
Liver			
Mean	0.852	1.503	1.529
± SD	0.067	0.185	0.165
%change		76.40	79.46
Kidney			
Mean	1.274	1.703	1.746
± SD	0.052	0.148	0.133
%change		33.67	37.04
Gill			
Mean	1.288	1.345	1.361
± SD	0.370	0.164	0.165
%change		4.42	5.66

All the values are mean ± SD of six individual observations are significant at (P<0.01)± SD – Standard Deviation.

DISCUSSION

Total protein

Proteins are dynamic molecules whose functions almost invariably depend on interactions with other molecules, and these interactions are affected in physiologically important ways by sometimes subtle, sometimes striking changes in protein conformation [7]. Proteins being involved in the architecture and physiology of the cell seem to occupy a key role in cell metabolism [8]. Catabolism of proteins and amino acids make a major contribution to the total energy production. The depletion of total protein content observed in this investigation can be correlated to this fact. These results are in agreement with the earlier report of Ravider *et al.* [9] who demonstrated a similar situation in *Clarius batrachus* exposed to decis.

The decrement in the total protein content in both *invivo* and *ex vivo* exposure was found to be to the same extent in all the tissues. The decrement was slightly more under *in vivo* than *ex vivo* in all the tissues. Protein metabolism seems to be affected to the same extent in both types of exposure. Amongst the three tissues studied between in vivo and ex vivo, greater percent decrement was in the case of gill (2.13) followed by kidney (1.11) and liver. As the gill tissues are constantly exposed to changes in the environmental medium, the animal seems to respond to a greater extent under *invivo* exposure. Hence, the animal in general tries to remove the toxicant, through the gill, an increased decrement though slight seems to occur in the gill tissue.

Similarly kidney is the next centre for removal and the next decrement was observed in the kidney tissue. But as the liver is the main metabolic centre, it has responded least. The above result clearly suggests that the tissues under direct exposure (*ex vivo*) respond to the same extent and degradation of the protein seems to occur to the same extent in direct (ex vivo) and indirect (*invivo*) exposure. The above contention is supported by ANOVA test (Table.5). The comparison between control vs ex vivo and control vs *invivo was* significant at P<0.01, but comparison between ex vivo and *invivo* was non-significant.

Table 5: Results of one way Analysis of variance (ANOVA) conducted using Graph Pad InSat3

	Comparison	Liver	Kidney	Gill
PROTEIN	Control vs Exvivo	***p<0.001	***p<0.001	***p<0.001
	Control vs *invivo*	***p<0.001	***p<0.001	***p<0.001
	Exvivo vs *invivo*	Ns p>0.05	Ns p>0.05	Ns p>0.05
AMMONMIA	Control vs Exvivo	***p<0.001	**p<0.01	Ns p>0.05
	Control vs *invivo*	***p<0.001	**p<0.01	Ns p>0.05
	Exvivo vs *invivo*	Ns p>0.05	Ns p>0.05	Ns p>0.05
UREA	Control vs Exvivo	***p<0.001	**p<0.01	*p>0.05
	Control vs *invivo*	***p<0.001	**p<0.01	Ns p>0.05
	Exvivo vs *invivo*	Ns p>0.05	Ns p>0.05	Ns p>0.05
GLUTAMINE	Control vs Exvivo	***p<0.001	***p<0.001	Ns p>0.05
	Control vs *invivo*	***p<0.001	***p<0.001	Ns p>0.05
	Exvivo vs *invivo*	Ns p>0.05	Ns p>0.05	Ns p>0.05

Ammonia

Ammonia is produced endogenously in different tissues through deamination of amino acids via glutamate dehydrogenase and purine nucleotide cycle via AMP deaminase [10,11]. Removal of excess ammonia from the circulation is essential, since it is toxic to central nervous system and also interferes with peripheral metabolism [12-14]. In the present study under ex vivo and *in vivo* condition, liver tissue showed highest increment in ammonia content on exposure to ambient ammonia for 3hours (Table .2). Since liver is the main centre for detoxification, more amount of ammonia in liver when compared to kidney and gill is possible. The increment in ammonia content was more in *invivo* than ex vivo exposure. The order of difference of percent increment between in vivo and ex vivo was 5.8 in liver followed by 5.33 in gill and 0.64 in kidney tissue. There is least response for *invivo* over ex vivo treatment in kidney tissue while maximum type of response was shown by liver tissue. As the liver tissue is the centre for ammonia production and detoxification, the total animal on exposure (*invivo*) tries to metabolize the ammonia to the liver tissue and hence the above response in liver tissue is possible. As the ammonia is usually left out through through gill tissue, the present finding is supporting of this contention. The kidney tissue usually removes through urea production; there is little change in kidney tissue for *invivo* (indirect exposure) and ex vivo (direct exposure) treatment.

ANOVA was conducted for comparing control vs. *invitro* and *invivo* separately and between *invivo* and ex vivo (Table.5). The results were significant for liver and kidney tissue for control vs. *invivo* and ex vivo but non-significant for gill. Though there is increased difference of increment between ex vivo and *invivo*, the difference seems to be non significant statistically. Further the relation between *invivo* and ex vivo also gave non-significant value again suggesting that the animal responds by both methods to the same extent. The literature survey in support of the above findings is usually of *invivo* type of treatment. John Sushma *et al.* [15] reported increased ammonia content in tissues of mice exposed to aluminium acetate. Ammonia cannot be stored for longer period of time in the body as it leads to endogenous ammonotoxicity. The reduction in ammonia content suggests that the ammonia might have been converted into non-toxic compounds like glutamine and urea.

Urea

The purpose of urea cycle operation often has been defined as removal of excess ammonia from the organism. Conversion of ammonia to urea is one of the important mechanisms of ammonia detoxification in fresh water fishes [16]. In *invivo* experiments high urea content was found in liver followed by kidney and gill(Table 3), thus supporting the fact that hepatic tissue has a full complement of the urea cycle enzymes. This agrees with the earlier reports of Gregory [17] and Colombo and Bachman [18]. The increment in urea level in ex vivo type of exposure has also shown similar trend i.e liver followed by kidney and gill, but the amount of increment was less than *in vivo* exposure. The difference in percent of increment in *in vivo* compared to ex vivo is 0.53 in kidney, 0.45 in gill and 0.34 in liver tissue.

The above results support the fact that kidney is the centre for urea removal and this removal passes through gill tissue. Hence, the difference of increment was more in kidney followed by gill tissue. The difference of change in *in vivo* and ex vivo was least in urea levels compared to ammonia levels, suggesting that ammonia stress seems to act to same extent on urea levels for direct and indirect exposure.

The ANOVA value suggest that the changes in ex vivo and *in vivo* over control were significant in liver and kidney. The significant was only P<0.05 for control vs. ex vivo (Table .5), but not significant for *in vivo* for gill tissue. The comparison for ex vivo over *in vivo* was not significant similar to the protein and ammonia levels. The analysis of variance also supported that liver tissue showed greater response than kidney.

The liver is the primary organ for metabolism, biotransformation and detoxification of xenobiotics, excretion of harmful substances, etc. Since liver is the major functional and metabolic active centre to detoxify the ammonia, increased urea content in this tissue is quite reasonable. The increased level of urea in kidney tissue on ammonia exposure clearly suggests that the urea has been formed as a means of ammonia detoxification and requires further removal from the body through excretion. The gill tissue levels though not as significant as liver and kidney tissue still suggest an operation of arginase enzyme segment and hence the occurrence of urea level in the tissue.

Glutamine

The glutamine levels also showed an increasing trend in tissues of liver, kidney and gill in both *in vivo* and ex vivo experiments. The animal seems to remove ammonia through glutamine to the same extent as urea production. Comparing ex vivo and *invivo* treatment, the increment was more in *in vivo* than *ex vivo* treatment similar to ammonia and urea levels. The difference of percent increment in *in vivo* over ex vivo was 0.34 in liver and 0.53in kidney and 0.45 in gill tissue. The trend of greater response by kidney followed by gill and liver was similar to urea levels. As in addition to urea, another component of ammonia removal is the glutamine production. The reasons envisaged for urea production might be possible even for glutamine production.The relation between control and ex vivo and *invivo* and relation between *ex vivo* and *in vivo* was carried by testing using one way Analysis of variance (ANOVA) (Tabled.5) similar results of significance has been observed for glutamine levels also. The comparison between control, *in vivo* and control and ex vivo gave significant results for liver and kidney tissue while it was non-significant for gill tissue. The comparison between *in vivo* and ex vivo was non-significant for all the tissues.

As the increased ammonia availability leads to glutamine syntheses as reported by George king *et al*. [19] and James *et al*. [20], the present results are in agreement to the increased glutamine levels under ammonia stress (Table .4) .

CONCLUSION

Thus, ex vivo and *in vivo* study resulted in decrement in protein and increment in ammonia, urea and glutamine levels. The decrement or increment was more in *in vivo* than ex vivo study. It was to the same extent in liver, kidney and gill tissues with reference to urea and glutamine levels, while it was prominent with reference to ammonia and protein content. Liver and gill tissue showed greater response than kidney tissue in the case of ammonia levels while it was kidney and gill tissue is the case of decrement in protein content. These changes suggested that direct exposure of liquor ammonia to the tissues affects the metabolism to the same extent as direct ammonia exposure to the animal.

REFERENCES

[1] Shit FC. Fish Biol 2005;21;307-395
[2] Finney DJ 1971; Probit Analysis, Third Edition, Cambridge University Press, London.
[3] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. J Biol Chem 1951; 193:265-75.
[4] Bergmeyer HV 1965 In: Methods of enzymatic analysis. Ed. H.V. Bergmeyer, Academic Press, New York, 401.
[5] Natelson S 1971. In: Techniques of clinical chemistry 111 Edn. Charles. C. Thomas. Publishers, Spring field Illinois, U.S.A., pp. 263-268.
[6] Colowick SP and Kaplan NO. 1967 Methods of Enzymology. Academic Press, New York.
[7] Nelson DL and Cox MM. 2005. In: Lehninger Principles of Biochemistry, Fourth Edition, W.H.Freeman and Company, New York.
[8] Murray, R K., Daryl K G, Peter A M and Victor W R 2007. In: Harper's Illustrated Biochemistry. International 26th Edition, The McGraw-Hill Companies, Inc. pp 46, 47.
[9] Ravider VN, Suryanarayana and Narayana N. Indian J Comp Animal Physiol 1988; 6:5-12.

[10] Waarde A. Comp Biochem Physiol 1981;68(B): 407-413.
[11] Waarde, AV and. Kesbeke F. Comp Biochem Physiol 1982;70(B): 499-507.
[12] Mackie, BG and Terjung RL. J Appl Physiol Respirat Environ Excercise Physiol 1983; 55:1072-1078.
[13] Sabina RL, Swain JL, Olanow CW, Bradley WCh, Fishbein WN, Dimauro S and Holmes EW. J Clin Invest 1984; 73:720-730.
[14] Banister EW, Rajendra W and Mutch BJC. Sports Med 1988;2:34-36.
[15] John Sushma, N., Sivaiah U, Jacob Doss P and Jayantha Rao K. Indian J Comp Animal Physiol 2006;24(1):66-71.
[16] Florkin, M and Scheer BR. 1972; Chemical Zoology, Vol. 1, Academic Press, London.
[17] Gregory, RB. Comp Biochem Physiol 1977;57A : 33-36.
[18] Colombo TP. and Bachman C. 1978. Aminosaeuren, Ammonisk, Hepatis Chem Enzypholopathic. Int. Ammoniaksyrup. 3rd. 129-134.
[19] George king JC. J Environ Sci Health B 1979;25:653-663.
[20] James MJ, Cleland LG, Gibson RA and Hawekes JS. J Nutr 1991;121:631-637.