Original Article

L:ife Science

Excretory Pattern of Ammonia and Urea and the Activity of Glutamate Dehydrogenase in Freshwater Fish, *Labeo rohita*

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ABSTRACT [ENGLISH/ANGLAIS]

Fishes normally excrete ammonia as their main excretory product. However due to certain stress factors they may excrete urea. In the present study, the possible role of ureogenesis to avoid accumulation of toxic ammonia under water-restricted condition was tested in the major carp, *Labeo rohita*. A total of hundred fishes were collected and sacrificed. Excretory urea and ammonia were estimated in the water of the aquarium and glutamate dehydrogenase activity was measured in the hepatic tissue. During the experimental period, significantly high ($p<0.01$) excretory ammonia in *Labeo rohita* was found which was between 145% to 234% above the baseline ammonia and excretory urea was found between 142% to 638% above the baseline urea. A high degree of correlation with r (coefficient of correlation) above 0.9 is observed between excretory ammonia and urea in *Labeo rohita.* However, only a moderate degree of correlation is observed between the activity of glutamate dehydrogenase and excretory ammonia and urea.

Keywords: Ureogenesis, ammonia, urea, glutamate

RÉSUMÉ [FRANÇAIS/FRENCH]

Poissons normalement excréter de l'ammoniac comme leur produit d'excrétionprincipale. Cependant, en raison de certains facteurs de stress qu'ils peuvent excréterl'urée. Dans la présente étude, le rôle possible des ureogenesis pour éviter l'accumulation de l'ammoniaque toxique en vertu de l'eau restreint condition a été testéechez la carpe majeure, Labeo rohita. Un total de cent poissons ont été prélevés etsacrifiés. L'urée et l'ammoniac excréteur ont été estimées dans l'eau de l'aquarium et le glutamate déshydrogénase a été mesurée dans le tissu hépatique. Au cours de lapériode expérimentale, très élevé ($p <0,01$) l'ammoniac excréteur dans Labeo rohita a été trouvé qui était comprise entre 145% à 234% au-dessus de l'ammoniac et d'urée de base excréteur n'a été trouvée entre 142% à 638% au-dessus de l'urée de base. Un degré élevé de corrélation avec r (coefficient de corrélation) supérieur à 0,9 estobservée entre l'ammoniac et d'urée dans l'excrétion Labeo rohita. Toutefois, seul undegré modéré de corrélation est observée entre l'activité de la glutamate déshydrogénase et de l'ammoniac et l'urée excréteur.

Mots-clés: Ureogenesis, l'ammoniac, l'urée, le glutamate

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INTRODUCTION

Fishes, being aquatic, release ammonia as their chief excretory matter. It is assumed that urea cycle for urea production has been suppressed in all freshwater fishes. However, it is found that certain fishes release urea during stress as during less availability of water [1-5].

The tilapia fish, *Oreochromis alcalicus grahami,* can withstand the highly alkaline environment and excretes exclusively urea exhibiting urea cycle enzymes in its liver [6].

In *Piaractus mesopotamicus*, though ammonia is the main nitrogenous waste during embryonic development, the ureogenesis was also suggested [7].

The Batrachoidid fishes like toad fish can rapidly switch from ammonia to urea synthesis during a variety of stress condition [8]. The white edge freshwater whipray *Himantura signifer* though ammonotelic, but retains the capacities of urea synthesis, to survive in brackish water. *Himantura signifier* in freshwater was reported to be confronted with post prandial osmotic stress because of its capacity of conserving and increasing urea synthesis upon feeding [9].

In the experimental *Heteropneustes fossilis*, significantly increased amount of both ammonia and urea was reported in the plasma and other tissues. However, it was also reported that the level of accumulation of urea was

higher than ammonia in the mud-dwelled fish, indicating the activation of ureogenesis in a water-restricted condition [10].

In the Atlantic cod (*Gadus morhua l.*) the excretion of nitrogen and expression of urea cycle enzymes was reported [11].

Glutamate dehydrogenase (GLDH) is an important enzyme, linking nitrogen elimination with utilization of amino acid carbons for energy metabolism. The endogenous ammonia production in different fishes has a significant role in glutamate catabolism. [12-14].

NAD-linked glutamate dehydrogenase catalyzes the major, but not sole, pathway for generation of ammonia from glutamate [15].

In liver, excessive glutamate dehydrogenase activity results in increased ammonia production and depressed synthesis of N-acetylglutamate, a required allosteric activator of the first step in ureogenesis [16]. In view of the controversies regarding the interrelationship between glutamate dehydrogenase and excretory pattern, the present study was aimed to investigate

the excretory pattern of ammonia and urea with special reference to activity of glutamate dehydrogenase in hepatic tissue of freshwater fish, *Labeo rohita*.

MATERIALS AND METHODS

Specimen

Labeo rohita were collected from a local pond and were kept in the aquarium for acclimatization over a period of ten days.

Method

Total hundred fishes were collected. Those hundred fishes were divided in ten sets, each set comprising ten fishes to be sacrificed in ten consecutive days. Out of eleven aquariums used, one aquarium was kept only with water. It acted as "control water". In the other ten aquariums, fishes were kept as experimental specimen.

Every day, one fish from one aquarium was sacrificed for the experiment. The experiment was continued till tenth day. Urea, ammonia and glutamate dehydrogenase activity were estimated in case of total ten fishes in ten consecutive days for both normal and experimental fishes. Enzyme activity was measured in the liver tissue of the freshly killed fishes of normal and experimental group.

Processing of the Collected Sample

The water of the aquarium containing the fishes was collected for excretory ammonia and urea measurement.

The liver tissue from the normal and experimental fishes was weighed and homogenized using distilled water. The homogenized tissue was centrifuged and the supernatant was used for enzyme assay.

Estimation of ammonia and urea

Ammonia was estimated by following the method of Anken and Schiphorst (1974).

Urea was estimated by following Crest Biosystems Modified Berthelot method by Fawcett and Scott (1960).

Estimation of glutamate dehydrogenase

Glutamate dehydrogenase activity was determined by following the method of Dohertry (1970).

DISCUSSION

The general mode of nitrogen excretion in fish is in the form of ammonia. However, under some circumstances as stress or enhanced ammonia level in the surrounding, fishes are reported to change their nitrogen excretion mechanism by forming urea as the end product for nitrogen excretion (Saha *et al.*, 2003).

In the present study, changes in the activity of glutamate dehydrogenase in *Labeo rohita* in relation to ammonotelic and ureotelic nitrogen excretion is tried to probe with monitoring the excretory nitrogen forms as urea and ammonia in the rearing media.

A significantly increasing trend ($p<0.01$) was observed in both ammonia and urea excretion from first to tenth day of experimental period.

Significantly higher urea excretion, 142.50% to 638% (Table 2) against 145% to 234% ammonia excretion (Table 2) above normal control baseline was the important observation in the present study. However, hepatic glutamate dehydrogenase activity exhibited a fluctuating trend throughout the experimental period (Table 2). On the simultaneous interpretation of the trends of glutamate dehydrogenase activity with trends of changing excretory ammonia and urea under the same experimental set-up it is observed that there is no any definite and appreciable relationship between the trends of these fluctuation (Fig. 1).

Frequent fluctuation in glutamate dehydrogenase activity and absence of relationship with excretory ammonia and urea may be explained with the fact that the changing glutamate dehydrogenase activity is a resultant of overall production of ammonia and its conversion to urea under a changeover condition alternating between ammonotelism and ureotelism in the present study. However, it is also

observed with interest that whatever may be the state of glutamate dehydrogenase activity; the metabolically generated ammonia is efficiently disposed as either excretory ammonia or is converted to urea for excretion as supported by the observed relationship among the excretory ammonia and urea in the present study (Fig. 2). A very high degree of correlation with r above 0.9 is observed between excretory ammonia and urea in *Labeo rohita* (Fig. 3).

Table 1: Presenting the significance of difference in the mean values of excretory ammonia and excretory urea (mg/dl) between normal control and different experimental *Labeo rohita*.

			Day									
			1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Excretory ammonia	Between normal control and experimental *L. rohita*	t	2.32	2.94	2.16	1.98	3.01	4.78	4.90	5.74	6.21	7.02
		p	<0.05	<0.01	<0.05	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		df	18	18	18	18	18	18	18	18	18	18
Excretory urea	Between Normal control and experimental L. rohita	t	7.58	8.20	7.25	9.22	13.55	17.62	15.43	17.13	18.94	15.75
		p	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		df	18	18	18	18	18	18	18	18	18	18

Table 2: This table shows presenting the % deviation of excretory ammonia, excretory urea and hepatic glutamate dehydrogenase from the mean values of normal control (mg/dl) in *Labeo rohita*.

	Day									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
% deviation of ammonia from normal control	145.83	173.58	225.71	230.26	225.93	451.39	267.57	198.00	231.25	234.39
% deviation of urea from normal control	142.50	180.49	179.56	282.35	351.85	425.30	534.61	571.60	502.35	638.09
% deviation of glutamate dehydrogenase from normal control	42.50	17.50	37.80	11.25	8.97	2.50	15.47	20.00	5.41	20.98

Figure 1: This figure shows presenting the correlation between the mean values of excretory ammonia (mg/dl) and hepatic glutamate dehydrogenase (U/mg) in *Labeo rohita*

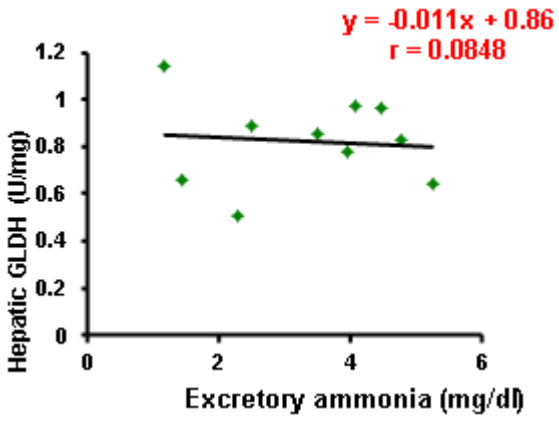


Figure 2: This figure shows presenting the correlation between the mean values of excretory urea (mg/dl) and hepatic glutamate dehydrogenase (U/mg) in *Labeo rohita*

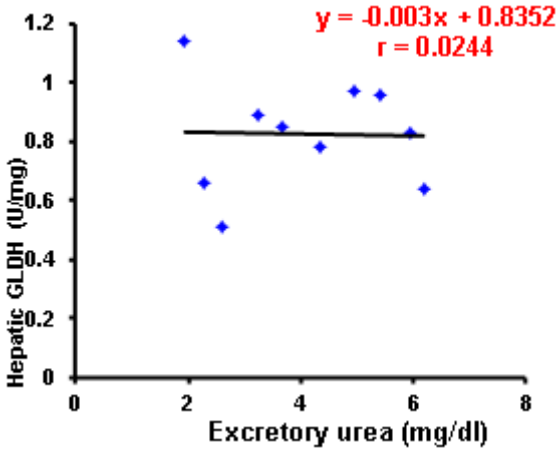
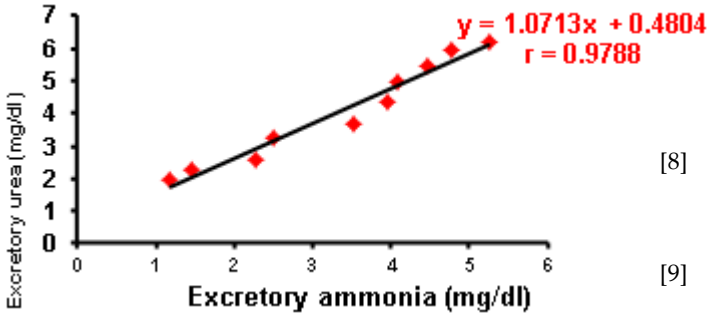


Figure 3: This figure shows presenting the corelation between the mean values of excretory ammonia (mg/dl) and excretory urea (mg/dl) in *Labeo rohita*



From the experimental outcome with determination of nitrogen excretion of ammonia and urea and their relationship with hepatic glutamate dehydrogenase (GLDH), it has been observed that excretory ammonia and urea are interrelated with each other (r=0.9788) but the relationship with glutamate dehydrogenase is not pronounced in *Labeo rohita.*The findings of the present study may suggest some amount of ureotelism in confined condition in this freshwater fish, *Labeo rohita* though ammonotelic excretion pattern cannot be ruled out.

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CONFLICT OF INTEREST

No conflict of interests was declared by authors

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