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# Stress Induced Status Of Blood Ammonia And Blood Urea With Reference To Hepatic Glutamate Dehydrogenase In Freshwater Fish, *Labeo rohita*.

**Shuvasish Roy Choudhury [a*], Rita Mahanta [b], Aparajita Borkotoki [c]**

[a*] *Research scholar, Department of Zoology, Gauhati University, Guwahati, Assam, India*
[b] *Retd. Associate Professor, Department of Zoology, Cotton College, Guwahati, Assam, India*
[c] *Retd. Professor, Department of Zoology, Gauhati University, Guwahati, Assam, India*

[a*]*Email: src_adonis@yahoo.co.in*
[b]*Email: ritamahanta@yahoo.co.in*
[c]*Email: a_borkotoki@yahoo.com*

**Abstract**

Ammonia, being highly toxic and readily soluble in water, is excreted by aquatic animals like fishes. However, scarcity of water in the surrounding medium, may force the fishes to convert ammonia into urea. In the present study, the possible role of ureogenesis to avoid accumulation of toxic ammonia under water-restricted condition was tested in *Labeo rohita*. Blood urea and ammonia were estimated in the blood of the fishes and glutamate dehydrogenase acitivity was measured in the hepatic tissue. From the present study, however, it is found that the relationship between blood ammonia and blood urea in *Labeo rohita* and the possible role of glutamate dehydrogenase is not so pronounced in the experimental fish species.

**Keywords:** ammonia; ureogenesis; glutamate dehydrogenase

## 1. Introduction

Many aquatic organisms, particularly those in freshwater, excrete ammonia in water. Ammonia is the end product of protein catabolism and is stored in the body of fish in high concentrations relative to basal excretion rates. Ammonia, if allowed to accumulate, is toxic and is converted to less toxic compounds or excreted. Ammonia is eliminated from the blood through the gills [1]. Organisms with less freshwater available, such as some marine organisms and all terrestrial organisms often invest some energy to convert the ammonia into urea. So, if conditions like exposure to exogenous ammonia, water limitations, or alkaline conditions hamper the release of ammonia, even some teleosts detoxify ammonia through synthesis of urea by urea cycle in liver [2].

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* Corresponding author. Tel.: +919864125414.
E-mail address: src_adonis@yahoo.co.in

The majority of teleost fishes are ammonotelic and excrete directly across the gills into the surrounding aqueous environment. But, a functional urea cycle and ureotelism have been documented in a few adult species as adaptations to unusual environmental circumstances as stress, air exposure, high pH, exposure to high concentration of ammonia. [3] reported an increased concentration of plasma and hepatic urea with salinity and suggested a direct correlation between hepatic productions of urea with osmoregulatory strategy of *Carcharhinus leucas*, a euryhaline elasmobranch. [4] reported urea synthesis in the African lungfish, *Protopterus dolloi*. [5] reported the excretion of nitrogen and expression of urea cycle enzymes in the Atlantic cod (*Gadus morhua l.*).

Some air-breathers when exposed to air, increase urea synthesis via the urea cycle. As reported by [6], the tilapia fish (*Oreochromis alcalicus grahami*), which can withstand the highly alkaline environment, excretes exclusively urea and has urea cycle enzymes in its liver.

[7] reported significant increase of both ammonia and urea levels in the plasma and other tissues of *Heteropneustes fossilis*. However, they also reported that the level of accumulation of urea was higher than ammonia in the *Heteropneustes fossilis*, indicating the activation of ureogenesis in a water-restricted condition. [8] also reported increased activity of the urea cycle enzymes in aestivating *Xenopus laevis*. In African lung fishes, *Protopterus annectens* and *Protopterus aethiopicus*, a greater part of waste nitrogen is converted to urea via the urea cycle, when they undergo aestivation [9].

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 [10,11,12]. [13] suggested that NADH- glutamate dehydrogenase was involved in the detoxification of high nitrogen levels while NAD- glutamate dehydrogenase was mainly responsible for the supply of energy to the cell during active assimilation.

## 2. Materials and Methods

Specimen: *Labeo rohita* were collected from a local pond and were kept in the aquarium for acclimatization.

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. Blood urea, blood ammonia and hepatic glutamate dehydrogenase activity were estimated in total ten fishes in ten consecutive days for both normal and experimental fishes.

### 2.1. Processing of the collected sample

The collected blood was centrifuged and the serum was collected for ammonia and urea analysis.

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

### 2.2. 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).

### 2.3 Estimation of glutamate dehydrogenase

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

## 3. TABLES

Table 1: Presenting the % deviation of blood ammonia from the mean values of normal control (mg/dl) in *Labeo rohita*.

| | DAYS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| % deviation | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th |
| Blood ammonia | 0.30 | 0.61 | 1.44 | 5.96 | 1.74 | 3.55 | 2.75 | 8.96 | 2.29 | 16.12 |
| Blood urea | 10.08 | 0.23 | 11.18 | 14.02 | 2.88 | 9.69 | 17.19 | 14.78 | 7.06 | 11.15 |

Table 2 : Presenting the % deviation of hepatic glutamate dehydrogenase activity from the mean values of normal control (U/mg) in *Labeo rohita*.

| | DAYS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| % deviation | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th |
| Hepatic Glutamate dehydrogenase | 42.50 | 17.50 | 37.80 | 11.25 | 8.97 | 2.50 | 15.47 | 20.00 | 6.41 | 20.98 |

## 4. DISCUSSION

In fish the general mode of nitrogen excretion 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 [14].

Whatever may be the environmental situation or metabolic signal to change over between ammonotelism and ureotelism as glutamate dehydrogenase is the enzyme initially releasing the nitrogen for excretion through both the system, it is a logical expectation that activity of this nitrogen releasing enzyme is influenced by the factors producing the transition from one type of excretion to the other. 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 circulating fluid.

In *Labeo rohita* there is fluctuation in blood ammonia concentration at alternate days without any significant differences in the alterations of blood ammonia during the period indicating absence of any marked variation in the blood ammonia level.

The correlation study between the blood ammonia and blood urea concentrations of each species as presented in Fig. 1 establishes presence of definite relationship between these two parameters.

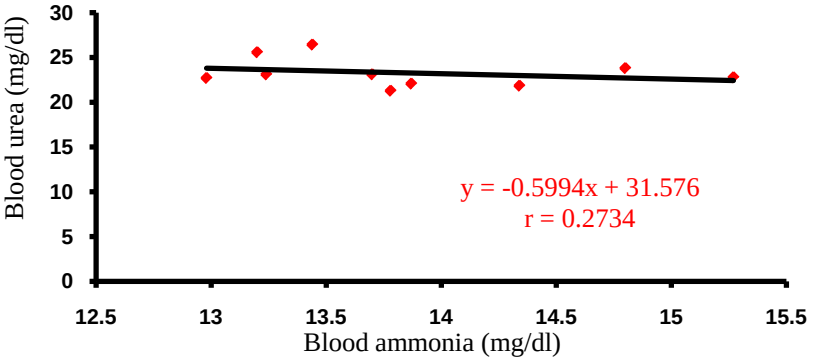


**Fig. 1 : Presenting the correlation between the mean values of blood ammonia (mg/dl) and blood urea (mg/dl) in *Labeo rohita*.**

The observed relationship between blood ammonia and blood urea suggests that under the present experimental set-up and in the selected fish species, a steady state of ureotelism is attained with concomitant conversion of ammonia to urea in response to increase in blood ammonia concentration.

The trend of changes in the activity of the hepatic glutamate dehydrogenase in *Labeo rohita* in the present study basically shows that there is daily fluctuation in the glutamate dehydrogenase activity. The resultant trend of the fluctuating glutamate dehydrogenase activity throughout the experimental period is a gradual decrease with increase in number of days in *Labeo rohita.*

However, on the simultaneous interpretation of the trends of glutamate dehydrogenase activity with trends of changing blood ammonia and urea under the same experimental set-up it is observed that there is no definite and appreciable relationship between the trends of these fluctuations (Fig. 2 and Fig. 3).

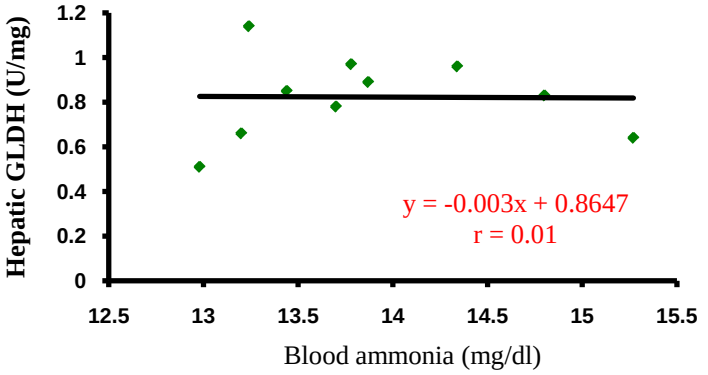


**Fig. 2 : Presenting the correlation between the mean values of blood ammonia (mg/dl) and hepatic glutamate dehydrogenase (U/mg) in *Labeo rohita*.**

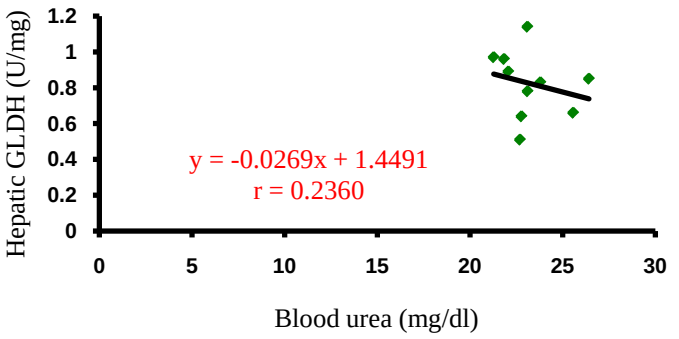


**Fig. 3 : Presenting the correlation between the mean values of blood urea (mg/dl) and hepatic glutamate dehydrogenase (U/mg) in *Labeo rohita*.**

The overall scenario of relationship between these parameters clearly shows that the relationship is lowest between blood ammonia and urea in *Labeo rohita*. (Fig. 1).

From the experimental outcome with determination of circulating nitrogen status in the form of blood ammonia and urea and their relationship with hepatic glutamate dehydrogenase (GLDH), it has been observed that the relationship is relatively not so pronounced in *Labeo rohita*.

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