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Hepatic Glutamate dehydrogenase activity and the presence of ammonia and urea in the circulatory fluid of *Channa gachua* in water-restricted condition.

Shuvasish Roy Choudhury[1]*, Rita Mahanta[2]

[1]*Assistant Professor, Department of Zoology, Karimganj College, Assam, India*
[2]*Associate Professor, Department of Zoology, Cotton College, Guwahati, Assam, India*

ABSTRACT

Ammonia is the chief excretory product in fishes. However, non-availability of enough of water in the habitat, may lead to the formation of urea, in fishes. In the present study, the possible role of urea formation to avoid the toxicity of ammonia under water-restricted condition was tested in *Channa gachua*. Circulatory urea and ammonia were estimated in the blood of the fishes and glutamate dehydrogenase activity was measured in the hepatic tissue. From the present study, it is found that blood ammonia in *Channa gachua* showed a decreasing trend from 1^{st} to 10^{th} day and blood urea showed a steady increase during the experimental period. The correlation study between the blood ammonia and blood urea concentrations in *C. gachua* establishes the presence of definite relationship between these two parameters. However, hepatic glutamate dehydrogenase activity showed a fluctuating trend. Presence of high circulatory urea in the experimental fish indicates that ureogenesis may get activated, if the fishes face water-limitation.

Keywords: Ammonia, Blood, Ureogenesis, Glutamate dehydrogenase

INTRODUCTION

In fish the general mode of nitrogen excretion is in the form of ammonia diffusing directly to the environmental aquatic media. Ammonia is the end product of protein catabolism and is stored in the body of fish in high concentrations relative to basal excretion rates. It takes a lot of water to dissolve and flush ammonia. Each ammonia molecule carries only one nitrogen (Choudhury and Mahanta, 2013). Ammonia is eliminated from the blood upon passage through the gills (Randall and Wright, 2005). 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). The presence of a functional urea cycle has recently been reported in some Indian air-breathing teleosts (Saha and Ratha, 1987, 1989). Tay *et al.* (2006) reported the transportation of active ammonia and metabolism of excretory nitrogen in the climbing perch, *Anabas testudineus*, during four days of

Corresponding Author E-mail: src_adonis@yahoo.co.in

emersion or ten minutes of forced exercise on land. Choudhury and Mahanta, 2013 reported the presence of urea in the blood of *Heteropneustes fossilis*. Anderson *et al.* (2005) 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. Chew *et al.* (2001) reported decrease in ammonia and urea excretion, with aerial exposure, in *Misgurnus anguillicaudatus*, and suggested very high levels of accumulated ammonia in the muscle and liver.Glutamate dehydrogenase (GLDH) is an important enzyme, linking nitrogen elimination with utilization of amino acid carbons for energy metabolism. NAD-linked glutamate dehydrogenase catalyzes the major, but not sole, pathway for generation of ammonia from glutamate. In liver, excessive glutamate dehydrogenase activity results in increased ammonia production. Cammaerts and Jacobs (1984) suggested that NADH- glutamate dehydrogenase was involved in the detoxification of high nitrogen levels. The endogenous ammonia production in different fishes has a significant role in glutamate catabolism (Lim *et al.*, 2001; Hirata *et al.*, 2003). The activity of NADP-GLDH increases 2-4 times in the mitochondria and practically ten times in the cell cytoplasm of the fish muscle and liver. The established regularity is considered as the adaptation of hydrobionts to the changes of ecological conditions of dwelling (Hrubinko and Iavonenko, 1993). Hence the present study is aimed at finding the importance of ureogenesis in tackling the water shortage in the habitat of *Channa gachua.*

The findings of the present study suggested the possible involvement of urea cycle in *Channa gachua* to tide over water-shortage.

MATERIALS AND METHODS

Specimen

Channa gachua were kept in the aquarium for acclimatization.

Method: The hundred experimental fishes were divided in ten sets, each set comprising ten fishes to be sacrificed in ten consecutive days. 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.

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 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).

RESULTS

Table 1. Presenting the mean values of blood ammonia (mg/dl), blood urea (mg/dl) and hepatic glutamate dehydrogenase acitivity (U/mg) in different normal control and experimental *Channa gachua.*

	DAYS									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Blood ammonia	13.92	13.40	12.30	11.37	10.92	10.14	9.14	8.18	7.40	6.32
Blood urea	20.71	21.39	22.31	22.88	23.52	24.56	25.37	26.64	28.17	30.30
Hepatic GLDH	11.25	8.75	4.87	6.25	8.23	8.86	10.58	10.25	6.32	8.23

DISCUSSION

Fishes, though ammonotelic, are reported to change their nitrogen excretion mechanism by forming urea as the end product for nitrogen excretion, during water-restricted conditions (Saha *et al*., 2003). Activity of glutamate dehydrogenase is influenced by the factors producing the transition from one type of excretion to the other (Choudhury and Mahanta, 2013). In the present study, changes in the activity of hepatic glutamate dehydrogenase in *Channa gachua* is tried to probe with monitoring the circulatory urea and ammonia. The blood ammonia in *Channa gachua* showed a steady decrease with duration of experimental period (Fig.1)

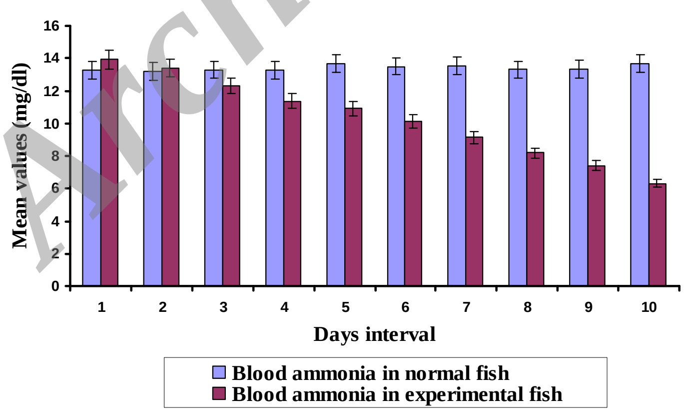

Fig. 1: Presenting the mean values of blood ammonia (mg/dl) in *Channa gachua* in different days of experiment.

suggesting significant decrease in ammonia concentration in blood which is dependent on duration of exposure to experimental condition. The blood urea level however showed a steady increase (Fig. 2).

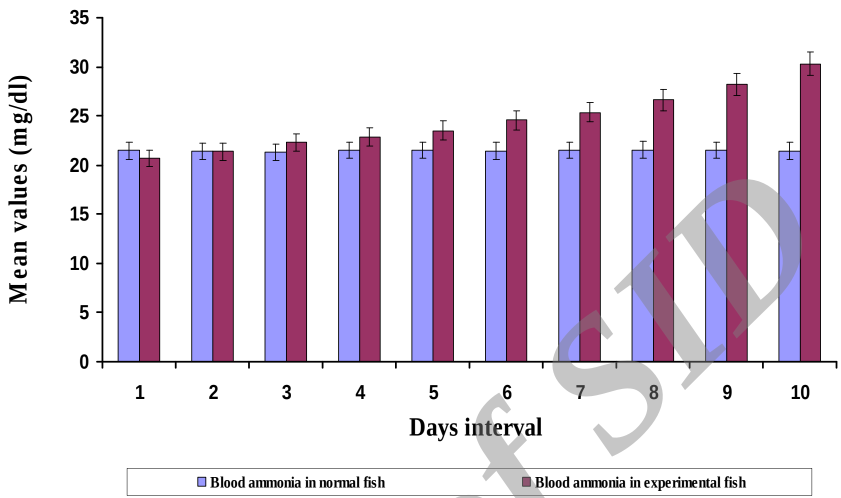

Fig. 2 : Presenting the mean values of blood urea (mg/dl) in *Channa gachua* in different days of experiment.

However, in the present study, daily fluctuation in the glutamate dehydrogenase activity was reported in the experimental fishes. In *Channa gachua* the fluctuating glutamate dehydrogenase activity results in a gradual increase in activity with increase in number of days of experiment. (Fig.3).

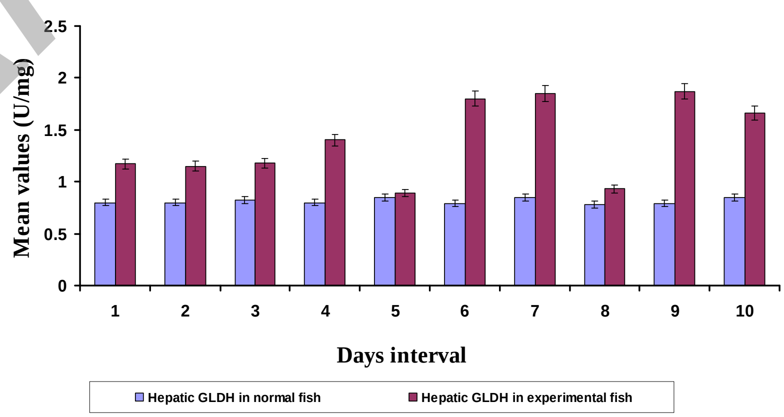

Fig. 3 : Presenting the mean values of hepatic glutamate dehydrogenase activity (U/mg) in *Channa gachua* in different days of experiment.

The correlation study between the blood ammonia and blood urea concentrations in *Channa gachua* as presented in Fig. 4 established presence of definite relationship between these two parameters.

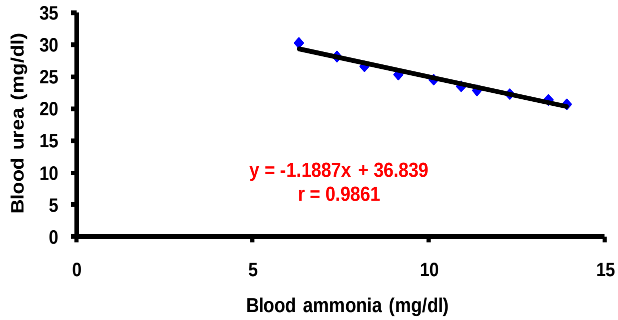

Fig. 4 : Presenting the correlation between the mean values of blood ammonia (mg/dl) and blood urea (mg/dl) in *Channa gachua*.

The Fig. 5 & Fig. 6 showed the relationship of GLDH with both blood ammonia and blood urea, which is not so prominent (r = 0.5004 and 0.5040 respectively).

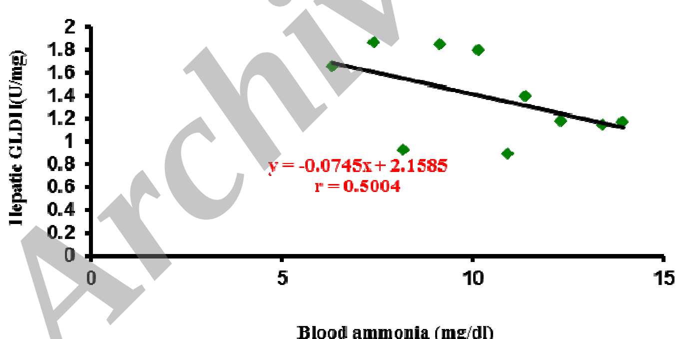

Fig. 5 : Presenting the correlation between the mean values of blood ammonia (mg/dl) and hepatic glutamate dehydrogenase (U/mg) in *Channa gachua*.

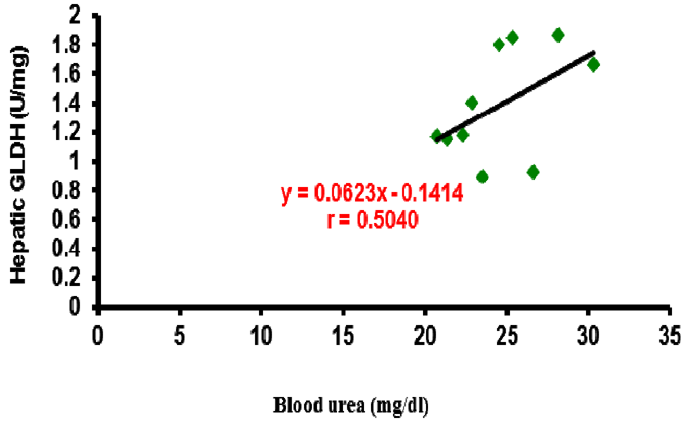

Fig. 6 : Presenting the correlation between the mean values of blood urea (mg/dl) and hepatic glutamate dehydrogenase (U/mg) in *Channa gachua*.

CONCLUSION

From the present study, the 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 blood ammonia and urea are interrelated with each other with certain degree of variation and the relationship is not so prominent in the experimental *C. gachua*. However, it is also observed with surprise that Ureogenesis is stimulated in *C. Gachua* when kept in the water-restricted condition.

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