Status of blood ammonia and urea with reference to hepatic glutamate dehydrogenase activity in freshwater airbreathing teleost, *Heteropneustes fossilis* kept in a water-restricted condition.

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Abstract— The airbreathing teleost, *Heteropneustes fossilis* was kept in an aquarium and the possible role of ureogenesis for their survival in a water-restricted condition was studied. The blood ammonia level showed a steady increase till 5th day of the experimental period. However, after that it maintained a more or less steady state, which supports the presence of glutamate dehydrogenase activity in the hepatic tissue. As considerably high blood urea level was found in the experimental fish, so it may be assumed that ureogenesis was used as a survival strategy by *H. fossilis* in a water-restricted condition.

Index Terms— aquarium, blood, glutamate dehydrogenase, hepatic tissue, survival strategy, teleost, ureogenesis

1 Introduction

Excretion of nitrogen is a necessary consequence of protein breakdown. In the body, the amino group is quickly oxidized to form ammonia. Most aquatic organisms, particularly those in freshwater, having sufficient source of water, excrete ammonia in water. Ammonia diffuses passively out of respiratory structures. It takes a lot of water to dissolve and flush ammonia. Each ammonia molecule carries only one nitrogen.

However, some marine organisms and all terrestrial organisms excrete urea as their chief excretory product. Sometimes conditions like exposure to exogenous ammonia, water limitations, or alkaline conditions hamper the release of ammonia. In such conditions, some teleosts detoxify ammonia through synthesis of urea by urea cycle in liver (Mommsen and Walsh, 2005).

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 emersion or ten minutes of forced exercise on land. Ip *et al.* (2004) suggested postprandial increase in nitrogenous excretion and urea synthesis in the giant mudskipper, *Periophthalmodon schlosseri*. Chew *et al.* (2003) reported urea synthesis in the African lungfish, *Protopterus dolloi*. Terjesen *et al.* (2002) demonstrated pathways for urea production during early life of an air-breathing teleost, the African catfish, *Clarias gariepinus* Burchell. Dkhar *et al.* (1991) studied the sub cellular localization of different urea cycle enzymes in the liver and kidney of a freshwater air-breathing teleost *Heteropneustes fossilis*.

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).

Hence, the present study is aimed at finding out the status of blood ammonia and urea with reference to hepatic glutamate dehydrogenase in *H. fossilis*.

2 Materials and Methods

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

2.2 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.

Everyday, 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.

Enzyme activity was measured in the liver tissue of the freshly killed fishes of normal and experimental group.

2.3 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.

2.4 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).

2.5 Estimation of glutamate dehydrogenase:

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

3 Results

Table 1 : Presenting the % deviation of blood ammonia and blood urea from the mean values of normal control (mg/dl) in *Heteropneustes fossilis*.

	DAYS									
% deviation	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Blood ammonia	4.59	20.72	32.09	32.73	47.91	47.61	39.68	45.21	24.29	29.10
Blood urea	15.88	7.88	0.87	2.45	18.45	16.46	30.30	22.44	19.17	17.30

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

	DAYS									
% deviation	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Hepatic Glutamate dehydrogenase	0.83	18.69	29.92	8.06	40.00	45.45	22.68	28.68	34.16	28.00

4 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. In the present study, changes in the activity of hepatic glutamate dehydrogenase in *Heteropneustes fossilis* is tried to probe with monitoring the excretory nitrogen forms as urea and ammonia in circulating fluid.

In *Heteropneustes fossilis* the blood ammonia level gradually increases with the duration of experimental period upto 6th day (Fig 1) which remains almost unaltered towards the end of the experiment on 10th day indicating a gradual increase followed by a sustained level as the general trend of the blood ammonia concentration in *Heteropneustes fossilis* under the experimental set up.

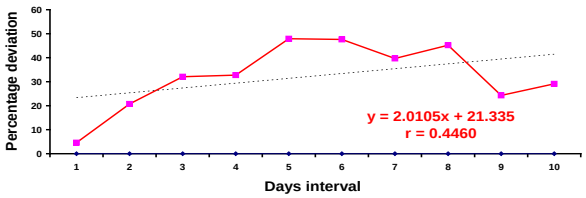

Fig. 1: Presenting the percentage deviation of blood ammonia from the normal mean values in *Heteropneustes fossilis* .

The blood urea level showed regular fluctuation which reached its pick on 7th day which gradually declines with the experimental period (Fig. 2).

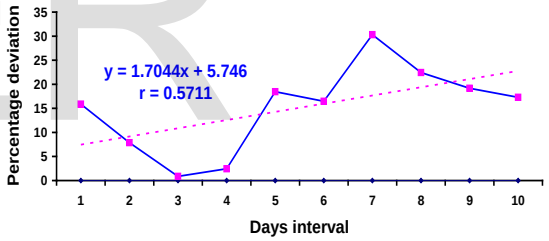

Fig. 2 : Presenting the percentage deviation of blood urea from the normal mean values in *Heteropneustes fossilis* .

The following figure (Fig. 3) clearly depicts the highly correlative relationship of r=0.7664 existing between blood ammonia and blood urea in *H. fossilis*.

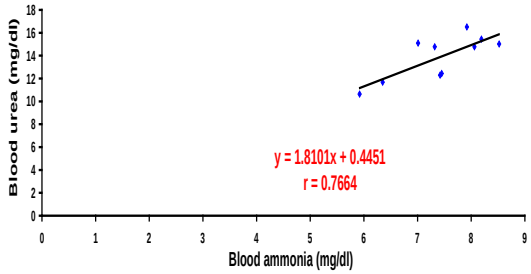

Fig. 3 : Presenting the correlation between mean values of blood ammonia (mg/dl) and blood urea (mg/dl) in *Heteropneustes fossilis* .

In *Heteropneustes fossilis* the fluctuating glutamate dehydrogenase activity results in a gradual increase in activity with increase in number of days of experiment (Fig. 4).

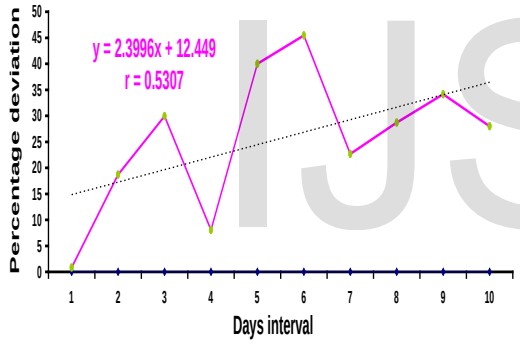

Fig. 4 : Presenting the percentage deviation of hepatic glutamate dehydrogenase activity from the mean values of normal control in *Heteropneustes fossilis* .

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 blood ammonia and urea are interrelated with each other with certain degree of variation and the relationship is quite prominent in the experimental *H. fossilis*.

5 References

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