International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:1, 2014

Glutamate Dehydrogenase and the Changing Pattern of Excretory Ammonia and Urea in *Heteropneustes fossilis*

Shuvasish Roy Choudhury, Rita Mahanta, Aparajita Borkotoki

Abstract**—**Fishes, in general, follow ammonotelic mode of excretion. However, certain stress factors may provoke them to excrete urea. In the present study, the possible role of ureogenesis to avoid accumulation of toxic ammonia under water-restricted condition was tested in *Heteropneustes fossilis.* A total of hundred fishes were collected and sacrificed. Excretory urea and ammonia were estimated in the water of the aquarium and glutamate dehydrogenase acitivity was measured in the hepatic tissue. During the experimental period, excretory ammonia in *Heteropneustes fossilis* was found between 931% to 16% above the baseline ammonia and excretory urea was found between 112% to 898% 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 *Heteropneustes fossilis.* However, only a moderate degree of correlation is observed between the activity of glutamate dehydrogenase and excretory ammonia and urea.

Keywords**—**Ammonia, aquarium, glutamate dehydrogenase, urea, ureogenesis.

I. Introduction

EXCRETION of nitrogen is a necessary consequence of protein breakdown; when proteins are converted to carbohydrates to provide energy, the amino group is removed and must be dealt with. In the body, the amino group is quickly oxidized to form ammonia (or, at high body pH, the ammonium ion). Ammonia is highly toxic and highly soluble in water. Many freshwater organisms, having sufficient source of water, excrete ammonia in water.

In any event, ammonia must be dealt quickly because of its toxicity. Urea is commonly used as an excretory product in vertebrates, and is rarely used in invertebrates. Some organisms, such as sharks and snails, allow urea to accumulate in their blood to help with overall osmotic balance.

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 [12].

The majority of teleost fishes are ammonotelic, i.e., ammonia is simply excreted 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. Fishes are highly individualistic in the mechanisms they employ for adapting to varying environmental challenges, i.e., expression of the urea cycle is only one of the several possible strategies [1].

Reference [9] suggested postprandial increase in nitrogenous excretion and urea synthesis in the giant mudskipper, *Periophthalmodon schlosseri*. Reference [3] reported urea synthesis in the African lungfish, *Protopterus dolloi*. Reference [2] reported the excretion of nitrogen and expression of urea cycle enzymes in the Atlantic cod (*Gadus morhua* L.).

Reference [6] studied the sub cellular localization of different urea cycle enzymes in the liver and kidney of a freshwater air-breathing teleost *Heteropneustes fossilis.*

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. The endogenous ammonia production in different fishes has a significant role in glutamate catabolism [8], [11], [7].

Reference [4] suggested that NADH- glutamate dehydrogenase was involved in the detoxification of high nitrogen levels. Adaptation of nitrogen metabolism is one of the important prerequisites for any vertebrate species while migrating from aquatic to the terrestrial habitat [17]. 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 [10].

The presence of a functional urea cycle has recently been reported in some Indian air-breathing teleosts [13], [14]. It has been demonstrated that at least in two Indian air-breathing cat fishes (*Heteropneustes fossilis* and *Clarias batrachus*) ureogenesis is stimulated under hyper-ammonia stress [15], [16].

Hence, the present study was aimed to investigate the changing pattern of excretory ammonia and urea and the possible role of glutamate dehydrogenase in hepatic tissue of *Heteropneustes fossilis*.

Shuvasish Roy Choudhury is a research scholar in the Department of Zoology, Gauhati University, Guwahati, Assam, India (Corresponding author: phone: +919864125414, e-mail: src_adonis@yahoo.co.in).

Aparajita Borkotoki is a Retd. Professor in the Department of Zoology, Gauhati University, Guwahati, Assam, India (e-mail: a_borkotoki@yahoo.com).

Rita Mahanta is a Retd. Associate Professor in the Department of Zoology, Cotton College, Guwahati, Assam, India (e-mail: ritamahanta@yahoo.co.in).

II. Materials and Methods

A. Specimen

Heteropneustes fossilis were collected from a local pond and were kept in the aquarium for acclimatization.

B. Method

Total hundred fishes were collected. Those hundred fishes were divided in ten sets, each set comprising ten fishes. Every day, one fish from one aquarium was sacrificed for the experiment. The experiment was continued till tenth day. Enzyme activity was measured in the liver tissue of the freshly killed fishes of normal and experimental group.

C. Processing of the Collected Sample

The water of the aquarium was used for excretory 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.

D. Estimation of Ammonia and Urea

Ammonia was estimated by following the method of [18].

Urea was estimated by following Crest Biosystems Modified Berthelot method by [19].

E. Estimation of Glutamate Dehydrogenase

Glutamate dehydrogenase activity was determined by following the method [20].

III. Results

TABLE I
PRESENTING THE % DEVIATION OF EXCRETORY AMMONIA AND EXCRETORY UREA FROM THE MEAN VALUES OF NORMAL CONTROL (MG/DL) IN *HETEROPNEUSTES FOSSILIS*

	DAYS									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
% deviation of excretory ammonia	931.25	898.11	528.57	511.84	256.48	331.94	144.14	62.66	18.75	16.56
% deviation of excretory urea	112.50	126.82	288.17	470.58	586.41	878.31	823.07	834.56	848.23	898.80

TABLE II
PRESENTING THE % DEVIATION OF HEPATIC GLUTAMATE DEHYDROGENASE ACTIVITY FROM THE MEAN VALUES OF NORMAL CONTROL (U/MG) IN *HETEROPNEUSTES FOSSILIS*

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

IV. Discussion

The major nitrogenous waste product in fishes is ammonia. However, under some circumstances as reduced water level in the surrounding, fishes are reported to change their nitrogen excretion mechanism by excreting urea instead of ammonia [5]. In the present study, changes in the activity of glutamate dehydrogenase in *Heteropneustes fossilis* 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 medium.

In case of *Heteropneustes fossilis* there is sharp increase in the excretory ammonia with percent deviation of 930% above normal on the very first day of the experiment which gradually declines and finally touches the baseline level on the tenth day of experiment (Table I). The trend of decreasing excretory ammonia in this fish species is highly correlative with duration signifying a persistent and definite decrease in ammonia excretion with increase in duration of experiment.

In *Heteropneustes fossilis*, gradual and persistent increase in excretory urea with increasing duration of the experimental period with fairly high degree of correlation ranging from r = 0.936 (Fig. 1) is observed.

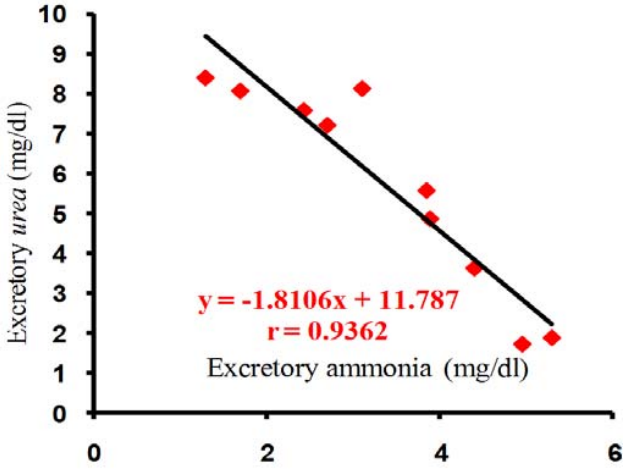

Fig. 1 The correlation between the mean values of excretory ammonia (mg/dl) and excretory urea (mg/dl) in *Heteropneustes fossilis*

The suggested acquisition of a ureotelic state is further supported in the present study by the observation that in the experimental species there is decrease in excretory ammonia associated with increase in excretory urea.

The trend of changes in the activity of the hepatic enzyme glutamate dehydrogenase catalyzing oxidative deamination of glutamate releasing ammonia in *Heteropneustes fossilis* in the

present study basically shows that there is daily fluctuation in the glutamate dehydrogenase activity (Table II). In *Heteropneustes fossilis* the fluctuating glutamate dehydrogenase activity results in a gradual increase in activity with increase in number of days of experiment followed by a decrease in the activity.

However, 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 this fluctuation (Figs. 2 and 3).

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=0.9362) and the relationship with glutamate dehydrogenase is quite pronounced in *Heteropneustes fossilis*. The findings of the present study may suggest ureotelism as the possible strategy of survival in *Heteropneustes fossilis*, living in a water-restricted condition.

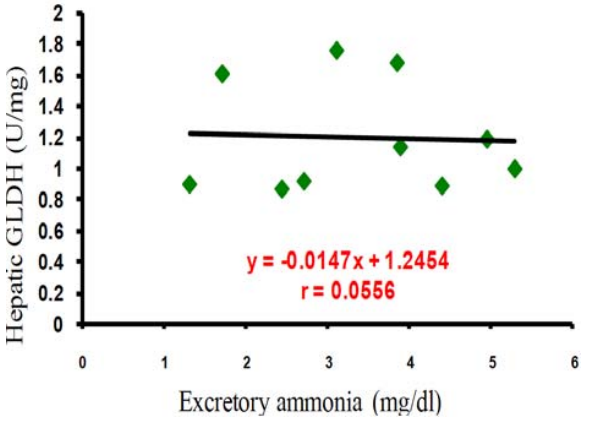

Fig. 2 The correlation between the mean values of excretory ammonia (mg/dl) and hepatic glutamate dehydrogenase (U/mg) in *Heteropneustes fossilis*

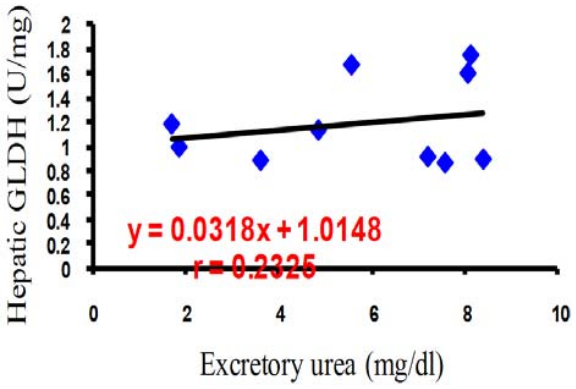

Fig. 3 The correlation between the mean values of excretory urea (mg/dl) and hepatic glutamate dehydrogenase (U/mg) in *Heteropneustes fossilis*

References

[1] Anderson, P. M., Urea and Glutamine Synthesis: Environmental Influences on Nitrogen Excretion. Fish Physiology, 20: 239-277, 2001.
[2] Chadwick, T., and Wright,P., Nitrogen Excretion and Expression of Urea Cycle Enzymes in the Atlantic Cod (*Gadus morhua*): A Comparison of Early Life Stages with Adults. Journal of Experimental Biology, 202: 2653-2662, 1999.
[3] Chew, S. F., Ong,T. F., Ho, L., Tam, W. L., Loong, A. M., Hiong, K. C.,Wong,W.P., and Ip,Y.K., Urea Synthesis in the African Lungfish *Protopterus dolloi* – Hepatic Carbamoyl Phosphate Synthetase III and Glutamine Synthetase Can Be Upregulated by Six Days of Aerial Exposure. Journal of Experimental Biology, 206: 3615-3624, 2003.
[4] Cammaerts, D., and Jacobs, M., A Study of the Role of Glutamate Dehydrogenase in the Nitrogen Metabolism of *Arabidopsis thaliana.* Planta, 163 (4): 517-526, 1984.
[5] Choudhury. R. S. and Mahanta. R (2013): "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" IJSER, 4 (5), pp. 1919-1323.
[6] Hirata, T., Kaneko, T., Ono, T., Nakazato, T., Furukawa, N., Sanae, H., Shigeo, W., Munekazu, S., Min-Hwang, C., Michael, R.F., and Shigehisa, H., Mechanism of Acid Adaptation of a Fish Living in a pH 3.5 lake. The American Physiological Society. APS Manuscript No. – R, 267-2, 2003.
[7] Ip, Y. K., Chew, S. F., Leong, I. W. A., Jin, Y., and Wu, R. S. S., The Sleeper *Bostrichthyes sinensis* (Teleost) Stores Glutamine and Reduces Ammonia Production during Aerial Exposure. Journal of Comparative Physiology, 171: 357-367, 2001.
[8] Ip, Y. K., Lim, C. K., Lee, S. L. M., Wong, W. P., and Chew, S. F., Postprandial Increases in Nitrogenous Excretion and Urea Synthesis in the Giant Mudskipper *Periophthalmodon schlosseri*. Journal of Experimental Biology, 207: 3015-3023, 2004.
[9] Janssens, P. A., The Metabolism of Aestivating African Lungfish. Comparative Biochemistry and Physiology, 11: 105-117, 1964.
[10] Lim, C. B., Anderson, P. M., Chew, S. F., and Ip, Y. K., Reduction in the Rates of Protein and Amino Acid Catabolism to Slow Down the Accumulation of Endogenous Ammonia: A Strategy Potentially Adopted by Mudskippers (*Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*) during Aerial Exposure in Constant Darkness. Journal of Experimental Biology, 204: 1605-1614, 2001.
[11] Mommsen, T.P., and Walsh, P. J., Biochemical and Environmental Perspectives on Nitrogen Metabolism in Fishes. Cellular and Molecular Life Sciences, 48 (6): 583-593, 2005.
[12] Saha, N. and Ratha, B. K., Active Ureogenesis in a Freshwater Air-Breathing teleost, *Heteropneustes fossilis*. Journal of Experimental Zoology, 241: 137-141, 1987.
[13] Saha, N. and Ratha, B. K., Comparative Study of Ureogenesis in Freshwater Air-Breathing Teleosts. Journal of Experimental Zoology, 252: 1-8, 1989.
[14] Saha, N. and Ratha, B. K., Alterations in the Excretion Pattern of Ammonia and Urea in a Freshwater Air-Breathing Teleost, *Heteropneustes fossilis* (Bloch) during Hyper-Ammonia Stress. Indian Jounal of Experimental Biology, 28: 597-599, 1990.
[15] Saha, N. and Ratha, B. K., Induction of Ornithine-Urea Cycle in a Freshwater Teleost, *Heteropneustes fossilis*, Exposed to High Concentrations of Ammonium Chloride. Comparative Biochemistry and Physiology, 108: 315-325, 1994.
[16] Saha, N., Datta, S., Biswas, K., and Kharbuli, Z. Y., Role of Ureogenesis in Tackling Problems of Ammonia Toxicity during Exposure to Higher Ambient Ammonia in the Air-Breathing Walking Catfish *Clarias batrachus*. Journal of Bioscience, 28(6): 733-742, 2003.
[17] Campbell, J. W., and Anderson, P. M., Evolution of mitochondrial enzyme system in fish: the mitochondrial synthesis of glutamine and citrulline. In: Molecular biology of fishes (eds: P.W.Hochachka and T.P.Mommsen) (Elsevier: Amsterdam) 1: 43-76, 1991.
[18] Anken H.C., Schiphorst M.E., Kinetic determination of ammonia in plasma. Clin. Chim. Acta. 1974;56:151–157. doi: 10.1016/0009-8981(74)90223-X. 56(2):151–157, 1974.
[19] Fawcett JK, Scott JE., A rapid and precise method for the determination of urea. *J Clin Patho,*; 13: 156-159, 1960.
[20] Dohertry, D., In Methods of Enzymol., 17, Part A. (eds. Tabor, H., and Tabor, C. W.) 850, 1970.