Blood Metabolite Changes Under Exposure Of Ammonium chloride In Freshwater Fish *Clarias batrachus* (Linn.)

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ABSTRACT

This study includes the blood biochemical alterations induced by chronic (20 days) exposure of the fish Clarias batrachus to a sublethal concentrations (20.9 ppm) of Ammonium chloride. Significant changes were observed in the blood biochemical properties in the form of hyperglycemia, hyperproteinemia and hyperchlosterolemia. The present study therefore points towards a severe metabolitic dysfunction in response to Ammonium chloride toxicity in the fish Clarias batrachus (Linn.). So, it is suggested that more suitable to culture at water fertilizer, ammonium chloride concentration of < 20 mg/l for optimum growth performance and survival rate than other water conditions.

Key words: ammonium chloride, *Clarias batrachus*, hyperglycemia, hyperproteinemia.

INTRODUCTION:

The aquatic organisms are sensitive to environmental changes. Sub-lethal concentrations of fertilizers may cause ecological imbalance of these organisms after sufficiently long time of exposure probably as a result of cumulative impact of impaired metabolic functions (Abedi *et al.*, 2013).

Exposure to ammonia in an aquatic environment produces many physiological changes in fish, including changes in their metabolism (Cavero *et al.*, 2004, Barbieri *et al*, 2018). Un ionized farm of ammonia released from ammonium chloride. Two forms of ammonia occur in water, un-ionized (NH_3) and ionized (NH_4^+) ammonia, and the relative proportion of each form is dependent on pH, temperature and salinity (Bower and Bidwell 1978). The un-ionized form of ammonia (NH_3) is highly toxic to fish, while the ammonium ion (NH_4^+) is much less so. Un-ionized ammonia mainly enters fish via the gills, as it can readily pass through the gill epithelium (Hampson, 1976), which, however, is rather impermeable to ionized ammonia (Sheehan and Lewis 1986). Exposure of fish to high levels of ammonia therefore results in a rapid increase in plasma levels of the compound (Person-Le Ruyet *et al.* 2003), and it might result in net accumulation of ammonia at toxic levels in the fish (Rasmussen and Korsgaard 1998).

Hence, in this paper efforts have been made to illustrate the blood biochemical alterations induced by inorganic fertilizer, ammonium chloride exposure on air breathing teleost *Clarias batrachus.*

MATERIALS AND METHODS :

The air-breathing teleost *Clarias batrachus* procured live from the local fish market, Darbhanga were washed with 0.1% $KMnO_4$ solution to remove dermal infection if any. Healthy fish of average length (15–18cm) and weight (30–34 g) were acclimated for 15 days to laboratory conditions. Commercial diet containing 26.58% crude protein was used through the experiment period with daily ration rate 3% of fish weight in the in morning (10.00 AM). Running tap water was used in all the experiments and the fish were adjusted to natural photoperiod and ambient temperature. No aeration was done.

Static acute bioassays were performed to determine LC_{50} values of ammonium chloride, the mortality was recorded after 24, 48, 72 and 96 h, and were calculated by the Finney method (1978). The LC_{50} values for these periods were 275 ppm, 240 ppm, 221 ppm and 209 ppm respectively. 1/10th value of the LC_{50} value for 96 hr was taken as the sublethal concentration (Sprague, 1971). Twenty acclimated fish were exposed to a sub-lethal concentration (20.9 ppm) of ammonium chloride for 20 days. Side by side same number of fish as that of experimental one was maintained as the control group. At the end of exposure period the fish were anaesthetized with 1:4000 MS 222 (tricane, methane, sulfonate, sandoz) for two minutes. On 20^{th} day blood samples was extracted from the caudal dorsal of the test fish and were then processed for quantitative estimation of blood glucose (Sinha, 1990), serum protein ('Biuret method' of Varley *et. al.*,1980) and serum cholesterol (Kabara's method,1966).

RESULT :

BLOOD BIOCHEMISTRY STUDIES

The carbohydrates, proteins and lipids are the three principal constituents of the cell. These are also known as metabolites and play a major role as energy precursors for fishes exposed to stress conditions. The optimum concentrations of these biomolecules are required for the proper and normal functioning of a cell. Any alteration in their organization or concentration may therefore, disturb the normal physiology of the cell. Therefore, the ultimate search for any disruptive process brought about by the chemicals must be made within the cell. One of the probable and logical ways which may eventually lead to an understanding of the events that follows fertilizers treatment could be an examination at biochemical level.

BLOOD METABOLITE LEVELS:

It was thought worthwhile to identify the metabolic dysfunctions in the non-target species, the (*C. batrachus*) in the present case challenged with a chronic sub-lethal concentration (20.9 mg/l) of ammonium chloride. The variables monitored at blood metabolites levels are blood glucose, serum protein and serum cholesterol of experimental fish *C. batrachus.* On exposure to a sub-lethal concentration (20.9 mg/l) of ammonium chloride the fish reveals following changes in the biochemical parameters.

BLOOD GLUCOSE:

The blood glucose level in the control fish group is assessed to be 68.66 ± 0.33 mg/100ml of blood. The experimental fish become hyperglyceric as evident by a highly significant ($p < 0.001$) elevated level of glucose in the blood (88.82 ± 1.41 mg/100ml) which counts to an increase of 28.53% (Table-1) the present values of the present observations are expressed as meant S.E. of 5 fish in each group.

SERUM PROTEIN:

Contrary to the elevated blood glucose level the experimental fish group show highly significant ($P< 0.001$) depletion in the level of serum protein in control group estimated to be 6.01 ± 0.37 g/100 ml as against 3.73 ± 0.15 g/100 ml in the treated group (Table-1).

SERUM CHOLESTROL:

The serum cholesterol level in the fish of control group has been analysed 202±2.08 mg/100 ml. The treated fish show hypercholestrolemic response as evident by significant ($p < 0.05$) increase (228.8± 1.96 mg/100 ml) in its level (Table-1) the serum cholesterol has been found to increase by 13.03% in the present case.

Tables:-1

Changes in the blood / serum metabolite levels in *Clarias batrachus* exposed to ammonium chloride (20 mg/l) for 20 days. Values are mean ± SE of 5 observations.

Parameters	**Control**	**Ammonium chloride exposed**
Blood glucose (mg/100 ml)	68.66±0.83	88.82± 1.41(+28.53)
Serum protein (g/100ml)	6.01 ± 0.37	3.73 ± 0.15 (- 37.90)
Serum cholesterol (mg/100 ml)	202±2.08	228.8±1.96 (+13.07)

Values indicate percent increase (+)

Or decrease (-) over control values significant at

* *P<0.05*

*** *p<0.001*

DISCUSSION :

BLOOD GLUCOSE:

Blood glucose level in serum of *C. batrachus* after treated with different concentration of NH_4Cl showed highly significant increases ($P<0.001$) (88.82±1.41 mg/100ml), in all groups compared to the control fish group (68.66±0.33 mg/100ml). Ammonium chloride an effect is known to increase the levels of activating glycogenolysis and glyconeogenesis with a net result of increasing plasma glucose levels. These results confirm the corticosteroid response to high ammonia observed by Tomasso *et al.* (1981). Blood glucose increased due to stimulation of glucocorticoids in stressed catfish. Davis *et al.* (2003) reported that High NH_3-N concentration caused a significant increase in plasma glucose concentrations in both PC and isoeugenol treated catfish. Recently Jha (2009) and Poonam *et al.* (2010), Singh and Chaudhary (2011), Koley and Kumar (2012) has observed similar result under the exposure of Nuvan, fenvalerate and Eklaux in various fresh water fishes.

However, the hyperglycaemic response in ammonium chloride treated fish *C. batrachus* indicated an imbalance of glucose homeostasis which might be due to decreased utilization of glucose (glycolysis) or increased glucose formation (gluco-neogenesis). Blood glucose concentration of fish can be decreased by starvation or increased in response to acute and chronic stressors. Changes in blood glucose concentration is the most widely recognized and consistent response to stressor. An increase in the O:N ratio represents an increase in the catabolism of lipids and carbohydrates (Zhang et al., 2017).

The present study also suggests the involvement of adrenal pituitary glucocortcoied axis and the depletion of energy stores and resultant debility under chronic ammonium chloride exposure. Again the tissue acidosis might have favoured glycogenolysis by changing the pH of the blood thus, disturbing the buffering system of organs. It is further assumed that the activation of glycogen phosphorylase and depression of glycogen transferase might have been caused by the toxic stress as the former is known to accelerate glyconesis and the latter limits the glycogen storage.

PLASMA PROTEIN:

In the present investigation decreased levels of plasma proteins was observed. Serum proteins appear to be very sensitive to ammonium chloride exposure as is evident by the apparent fall (36%) in its content. The decrease in serum protein as observed during the present study suggests that the detoxification/ degradation of the toxicant either took place partially in the blood itself or involved the serum protein. This fall further suggests a progressive protein degradation or biochemical transformation of the protein Nitrogen into other nitrogenous products as suggested by Rao *et al.* (1984). Recently Jha (2009) and Poonam *et al.* (2010), Singh and Chaudhary (2011), Koley and Kumar (2012), Ahsan (2016) has observed similar result under the exposure of Nuvan, fenvalerate, Atrazine and Eklaux in various fresh water fishes.

We know that the plasma protein provide colloid, osmotic pressure, transport hormone and also act as buffer. It is likely that the metal could have caused drastic changes in the permeability of the membrane of the fish body surface thereby greatly altering water and ionic balance. Rao *et al.* (1983) found significant fall in Na^+, K^+ and Ca^{2+} in *Channa punctatus* exposed to parathion.

Again we know that the serum proteins of the fish are of hepatic origin. A depletion in the total serum protein level may be due to possible reduction or stoppage of their synthesis by liver cells due to direct toxic actions of the ammonia on the hepatocytes. It is possible that in *C. batrachus* part of the depletion may be due to the utilization of serum protein as energy reserves to meet the higher energy demands of fish under ammonia exposure.

Significant decrease in total plasma protein was recorded and it was found that the percent decrease was higher in 120 days exposure period (68.2%) then 96 hour (46.4%) in fish *Channa punctatus* exposed to 11.12 ppm sublethal concentration of cadmium (Sastry & Shukla 1990). In agreement with Sastry & Shukla (1990) decrease in 30 days of exposure of cadmium chloride at sublethal concentration of 1.32 ppm to *C. punctatus* was recorded.

Apoptosis seems the probable cause as another explanation also in which “decision” for apoptosis can come from the cell itself, from the surrounding tissue or from a cell that is part of immune system. In these cases apoptosis were functioning to remove the damaged cell, preventing it from sapping further nutrients from the organism.

SERUM CHOLESTEROL:

The present investigation exhibited hypercholesterolemia (Table-1) under the toxic influence of ammonium chloride and in agreement with the findings of Verma *et al.*2002; Ayyaduri, 2004; Das *et al.* 2004; Prakash & Maheshwari, 2005; Prakash *et al.*,2006; Kalaivani *et al.*,2008 and Jha & Jha, 2010 ; Jha (2009) and Poonam *et al.* (2010); Singh and Chaudhary (2011), Koley and Kumar (2012) and Pratibha & Kumar, 2013, Sunita Rani, et al.2015, and Dilip, M. and Vidya B. 2016) has observed similar result under the exposure of Nuvan, fenvalerate and Eklaux in various fresh water fishes.

The elevated blood cholesterol level may be due to the hypermetabolic state of the fish or due to the impaired liver function as suggested by Holmberg *et al.* (1972). Again the increase in cholesterol may be due to its increased synthesis in the liver as the precursors for interval hormones caused by the toxicant. Part of this increased cholesterol store might have found its way into the blood as suggested by Ahsan & Ahsan 1988; and Madhavan P, Elumalai K. 2016.

The carbohydrate (glucose) also is converted by adipose tissue into fatty acids and stored as triglyceride. These two process- up take of fatty acids and glucose from blood and their synthesis into triglyceride are enhanced by the action of the hormone “insulin”. Several hormones such as epinephrine, norepinephrine, glucagons, ACTH, GH and thyrotropic hormone stimulates the release of fatty acids from adipose tissue (Popjak and Grant, 1963).

Triglycride synthesis & Lipolysis do not follow the same path way in the adipose tissue. Adipose tissue does not possess ‘glycerokinase enzyme’, hence it has to depend on the production of de- hydroxyacetone phosphate by glycolysis.

Lipolysis in adipose tissue is brought about by “hormone-sensitie-lipase” mediated by C. AMP in that tissue increasing FFA in plasma. Adipokinin secreted by anterior pituitary also enhance the FFA levels by increasing lipolysis of fat depot. Glucose or insulin decreases free fatty acid level as in diabetes mellitus where as EN or NEP from adrenal or ACTH and TH all increases FFA level in plasma.

This interrelationship between FFA and glucose level (as we have seen in the case of diabetes in the present experiment) in plasma and their up take & utilization by adipose tissue & other tissues is referred as “Glucose-Fatty – Acid-Cycle”.

CONCLUSION:

It could be concluded that *C. batruchus* with average weight 30.0± 4.0 g, were more suitable to culture at water fertilizer, ammonium chloride concentration of < 20 mg/l for optimum growth performance and survival rate than other water conditions. Therefore, it can be recommended to be carried out under the similar experimental conditions.

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