



PHYSIOLOGICAL STRESS ON CLARIAS BATRACHUS DUE TO FERTILIZER DIAMMONIUM PHOSPHATE

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ABSTRACT

In the blood serum of *Clarias batrachus*, cholesterol, total protein, globulin and urea nitrogen, on its exposure to 50, 100 and 200 ppm of Diammonium phosphate in the aquatic medium for 15 days registered an increase of 21.89%, 33.82% and 70.85%, 7.02%, 14.37% and 26.12%, 41.34%, 80.26% and 139.91%, 15.14%, 40.05% and 63.74% respectively above controls while the serum albumin on similar exposure suffered a decline of 14.36%, 24.54% and 38.95% respectively below controls.

KEYWORDS : Fertilizer, Diammonium Phosphate, Physiological Stress, Fish

INTRODUCTION

The effect of a variety of agrochemicals including fertilizers and pesticides introduced into inland waters with agricultural run off upon the haematological parameters viz. Haemoglobin content, haematocrit, erythrocyte count, total and differential leucocyte count has been reported by a number of workers (10,16,18). The present work seeks to investigate the effects of the fertilizer Diammonium phosphate (Nitrogen 18%, Phosphoric acid 46%) upon important biochemical parameters like cholesterol, total proteins, albumin, globulin and urea nitrogen of blood serum of the fresh water fish *Clarias batrachus*.

MATERIALS AND METHODS

Live and healthy specimens of *Clarias batrachus* weighing around 70±3 g and measuring 22±3 cm, collected from the river Gomti through fish catchers were maintained in laboratory in a tank containing 250 litres of tap water for a week in order to acclimatize them to laboratory conditions. They were fed at regular intervals of 48 hrs on a diet containing mustard oil cake and rice bran (50% of each) at the rate of 2% of their body weight. The water of the tank was regularly renewed every 24 hrs. The fish were then divided into four groups of ten each and each group was kept in a separate aquarium containing 50 litres of clean fresh water. The first group was used as control, while the second, third and fourth used for tests were exposed to 50, 100 and 200 ppm of Diammonium phosphate respectively for a period of 15 days. Regular feeding and renewal of the medium was continued throughout the experimental period.

At the expiry of the period, the blood of each fish of the respective groups was collected through caudal vein in separate tubes; after allowing 5 minutes for clotting, the tubes were centrifuged at 3000 r.p.m. for 20 minutes and the serum was sucked out by pipettes into small clean test tubes separately. The control and the test sera samples were processed for estimation of cholesterol (21) total serum proteins and blood urea (2,20).

OBSERVATIONS AND RESULTS

The serum cholesterol, total proteins, globulins and urea nitrogen of the test groups of fish, on exposure to 50, 100 and 200 ppm of Diammonium phosphate in the aquatic medium for 15 days consistently increased; the serum cholesterol rose by 21.89%, 33.82% and 70.85%, total protein by 7.02% 14.37% and 26.12%, globulin by 41.34%, 80.26% and 139.91% and urea nitrogen by 15.14%, 40.05% and 63.74% respectively above controls (Table-1). Thus with increasing concentrations of Diammonium phosphate (DAP) in the aquatic medium of fish, there occurred a progressive rise in the serum urea nitrogen accompanied with increasing degrees of hypercholesterolaemia and hyperproteinemia. On the other hand, the serum albumin in the three test groups of fish in the same experiment on exposure to 50, 100 and 200 ppm of DAP registered a fall of 14.36%, 24.54% and 38.95% respectively below controls showing that increasing concentration of the pollutant caused, increasing degrees of hypoalbuminemia (Table-1).

DISCUSSION

A rise in serum cholesterol in the fresh water fish *Channa punctatus* occurred on exposure to a medium contaminated with 2,3,4 - triaminobenzene, Malathion and Cadmium (9); serum cholesterol in *Clarias batrachus* increased on treatment with Rogor (8). In teleosts,

lipid mobilisation is stimulated by thyroid hormones while radiothyroidectomy has been found to cause visceral lipid deposition (13,14). In case of humans, serum cholesterol increases in a number of diseases including hypothyroidism and lipid nephrosis (3). In our experiment, hypercholesterolaemia in *Clarias batrachus* is neither genetic nor diet induced but environment induced, it appears that PO₄ ions derived from DAP in the aquatic medium, enter the fish body fluids and somehow cause a partial or total break down of factors which inhibit the enzymatic activity of HMG CoA reductase (β -hydroxy- β -methyl glutaryl CoA reductase) catalyzing the reaction constituting the rate limiting and regulatory step in the biosynthesis of cholesterol. Thus, with the curbs on the biosynthetic rate limiting reaction loosened, cholesterol synthesis continues uninhibited. However since a number of vital organs viz. Liver, pancreas, thyroid and kidney have been implicated in hypercholesterolaemia in fishes as well as humans (19), only a detailed biochemical investigation can elucidate the set of actual causes leading to the elevated levels of serum cholesterol in the fish under the effects of the experimental pollutant. Further, since hypercholesterolaemia in humans is associated with a number of diseases, its occurrence in fish may pertinently be considered a negative health factor which may ultimately lead to a gradual decline in the total fish yield.

Total protein content increased in the fresh water teleost *Cyprinus carpio* on exposure to 50 ppm of the fertilizer urea to the aquatic medium for periods ranging from a fortnight to two months (17). In our experiment, a progressive increase in the total serum proteins in *Clarias batrachus* on exposure to 50, 100 and 200 ppm of DAP in the aquatic medium may be due to a decline in their utilization as nutrients by body tissues and a diminished rate of protein degradation; there is certainly a loss of equilibrium between production and destruction of body proteins (4) reflecting a general disturbance in protein metabolism.

Prolonged oral administration of the fertilizer Sodium dihydrogen phosphate to rats (*Rattus norvegicus*) caused hyperglobulinemia accompanied with hypoalbuminemia (11). In our experiment with the fish *Clarias batrachus*, exposure to DAP in the aquatic medium resulted in hyperglobulinemia along with hypoalbuminemia and the changes in the serum globulin and serum albumin levels occurred in opposite directions, a pattern of variation characteristic of certain diseases such as liver cirrhosis in humans (3). Such marked alterations in serum protein levels generally reflect a diseased state with disturbed vital functions (19).

Urea nitrogen levels in blood serum invariably registered a rise in the fresh water teleost *Channa punctatus* on its exposure to a variety of pollutants in its aquatic medium such as 2,3,4-triaminobenzene, endosulfan (both insecticides) and Manganese (1,13). Blood urea levels also rose in *Clarias batrachus* on exposure to Rogor in the aquatic medium (8). In all these cases, kidney damage has been held to be the cause of rise in the urea nitrogen level in the blood serum. In the present investigation, exposure of *Clarias batrachus* to different concentrations of DAP in the aquatic medium caused an elevation in the urea nitrogen level of serum. Increase in blood urea in teleosts certainly reflects an impaired renal physiology. Excess of NH₄⁺, H and PO₄⁻ ions in the medium and their inevitable introduction into the fish body fluids through the extensively exposed permeable membranes (skin, buccopharynx and the entire gastro-enteric lining) and the

consequent stress on acid-base balancing mechanism and hence an undue pressure of work on the kidney is quite likely to cause such a renal insufficiency leading to the observed pathological rise in the blood urea nitrogen. A change in osmolarity of the surrounding medium and the concomitant effect on inter-renals possibly leading to some extent of dehydration may also have played a contributory role in bringing about the observed elevation in the blood urea nitrogen (5,15). Under different stress conditions significant toxicity was observed (22-27)

TABLE - 1 : Effect of fertilizer Diammonium phosphate (DAP) on biochemical parameters of serum of the teleost fish *Clarias batrachus* (Values are given in mean \pm S.D.) (The sign + or - indicates increase or decrease over control)

Parameters	Control	Concentration of DAP (15 days exposure)		
		50 ppm	100 ppm	200 ppm
Cholesterol (mg/dl)	150.70	186.20 \pm 16.31	202.90 \pm 12.32	259.30 \pm 20.98
Change (%)	\pm 10.95	(+ 21.89)	(+33.82)	(+70.85)
Total Protein (g %)	8.84 \pm 0.29	9.37 \pm 0.41	10.05 \pm 0.22	11.04 \pm 0.35
Change (%)		(+ 7.02)	(+14.37)	(+26.12)
Globulin (g %)	3.44 \pm 0.37	4.60 \pm 0.47	5.88 \pm 0.20	7.81 \pm 0.40
Change (%)		(+41.34)	(+80.26)	(+139.91)
Albumin (g %)	5.45 \pm 0.23	4.69 \pm 0.14	4.17 \pm 0.23	3.32 \pm 0.20
Change (%)		(-14.36)	(-24.54)	(-38.95)
Urea nitrogen (mg/dl)	12.32 \pm 0.21	14.49 \pm 0.22	17.65 \pm 0.25	20.66 \pm 0.90
Change (%)		(+15.14)	(+40.05)	(+63.74)

Number of observations 10 in each case.

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