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INFLUENCE OF CALCIUM AMMONIUM NITRATE ON THE BLOOD CHLORIDE OF *LABEO ROHITA* (HAM.) FINGERLINGS

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The fish *Labeo rohita* when exposed to sublethal level of calcium ammonium nitrate, exhibited significant increase in blood chloride concentration at day 20 (13% over control, $P < 0.05$). At 25 and 30 days, the blood chloride concentration decreased by 11.0 - 7.3% over control ($P < 0.05$).

INTRODUCTION

Since the work of Krogh (1939), investigations on the influence of exposure to agricultural chemicals upon the blood chloride regulation of fish attracted a number of workers (Schales & Selma, 1941; Black, 1957; Doyle & Donna, 1961; Sarkar, 1992a). The presence of nitrogen compounds in blood for longer periods exhibited several physiological abnormalities (Srivastava & Srivastava, 1980) and the effects of urea and ammoniun sulphate on the gill damage of fresh water fishes have been reported (Srivastava & Srivastava, 1980 & 1982; Sarkar, 1992b). Because the gills are responsible for chloride regulation in fishes, gill damage might be accompanied by an impairment of the salt balance. The blood chloride is an important extracellular anion for regulation of cation-anion balance and osmotic pressure. Therefore, it is necessary to find out whether gill damage is associated with the fluctuation of blood chloride content. In the present investigation, Calcium Ammonium Nitrate (CAN) - a nitrogen - containing fertilizer, was considered to determine effects of sublethel level of CAN on blood chloride concentrations of a fresh water fish, *Labeo rohita*.

MATERIALS AND METHODS

Fingerlings of *L. rohita* (17.5 ± 1.3 g) collected from a pond, were acclimated for a week under laboratory conditions. Sublethal concentration of analytical reagent grade CAN previously determined at 30^0C (Sarkar, 1993), was selected for the study. The LC_{50} value for CAN was determined by the method of Litchfield & Wilcoxon (1949). Groups of eight fishes (2 fish in 15 liter glass jar) were subjected to sublethel level of CAN (LC_{50} = 151.2 mg/l at 30^0C) for 10, 15, 20, 25 and 30 days. Tests were conducted in 15 liter glass jars, each containing 10 liter of unchlorinated borehole water (dissolved oxygen 6.5 mg/l, pH 7.5, total alkalinity 165 mg/l, hardness 135 mg/l). Tests were repeated three times accompanying with control. For determination of blood chloride in fish, 0.2 ml of blood was taken from control and treated fish in a small conical flask containing 1.8 ml of distilled water. The solution was then titrated with standard mercuric nitrate solution using 0.06 (4 drops) of diphenyl carbazone as indicator. Two replicate tiitrations were made on each sample and calculated according to the following formula : Amount of mercuric nitrate used (mg) x 100/A = meq Cl/l [A = Number of $HgNO_3$ mol. required for 2 ml of NaCl solution].

RESULTS AND DISCUSSION

Fish when exposed to sublethal level of CAN, exhibited significant increase in blood chloride concentration at day 20 (13% over control, $P < 0.05$, Fig. 1). At other days (25 and 30 days), the

blood chloride concentration decreased by 11.0 - 7.3% over control ($P < 0.05$).

In aquatic ecosystems where calcium ammonium nitrate is used as a potential source of nitrogen for fish culture, the concentration of blood chloride will affect the fish physiology and accumulation of metabolites of CAN is closely associated with the alteration of blood chloride level of fish. Decrease of blood chloride level in fish is due to decrease in the activity of carbonic anhydrase (Thomas & Murthy, 1976) and interference of cortisol (Srivastava & Srivastava, 1988). However, it is also possible that fish exposed to sublethal level CAN for prolonged period exhibited hypoclorinemia in blood. It has been reported that Tilapia (*Oreochromis mossambicus*) when exposed to sublethal level of urea (LC_{50} = 1,340 mg/l), exhibited significant increase in blood chloride concentration at day 15 while at days 20 and 30, the blood chloride level decreased (Sarkar, 1992a).

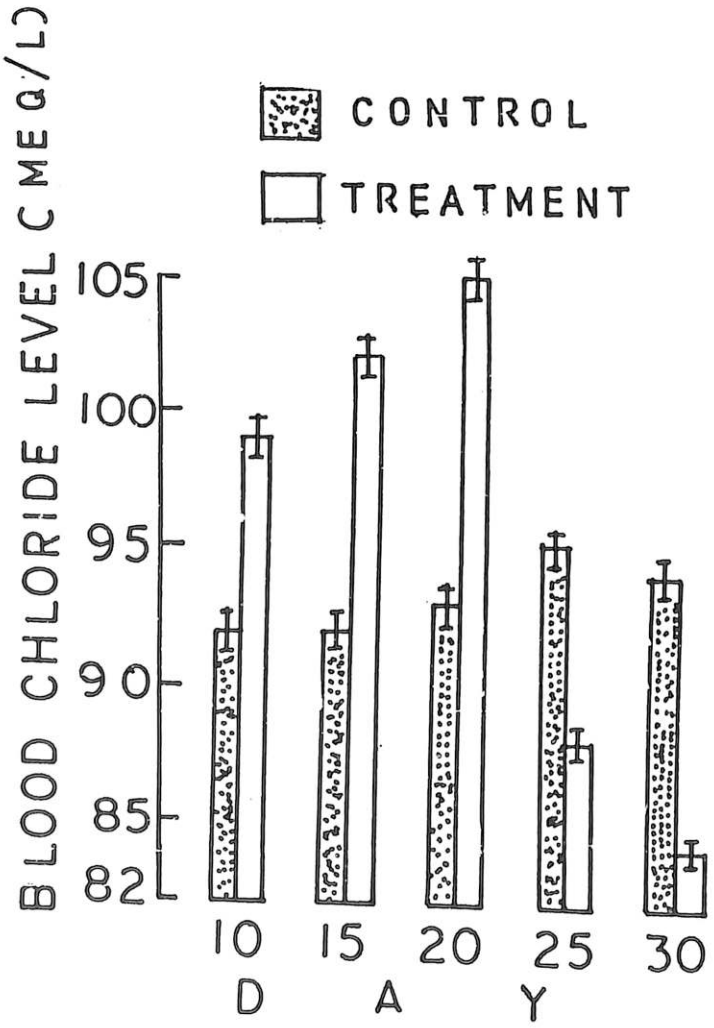

Fig. 1 : Blood chloride level of *L. rohita* during exposure to sublethal level of CAN (LC_{50} = 151.2 mg/l at 30^0C) at different time intervals.

One might assume that gill destruction would produce changes in the chloride level of blood; however, results from previous experiment (Srivastava & Srivastava, 1982) indicate that fish gills were damaged when exposed to sublethal level of urea for 4 days. In the present study fish exposed to CAN up to 20 days will undoubtedly damage fish gills but did not affect the blood chloride level. Subsequent decrease in chloride level of blood indicates that the adjustment in blood chloride level was achieved and therefore, this concentration of CAN should be considered as the upper physiological limit for this species of fish. The shock effect of abrupt chloride increase in fish culture ponds may produce an increase in the blood chloride level but once the adjustment of blood chloride is achieved, a new concentration of blood chloride in fish is established.

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