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UREA INDUCED PROTEIN CONTENT IN LIVER OF AIR BREATHING FISH BY FOLIN METHOD

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ABSTRACT

Fertilizers find a perennial application in agriculture. Extensive use of urea in croplands affects the life and sustainability of other living creatures like fishes, amphibian and reptiles etc directly or indirectly. Urea is a most commonly used nitrogenous fertilizer and this fertilizer affects the skin and respiratory organs like gills of cat fishes which are generally found in or near the drainage water of croplands. The affects were so profound that it can easily be studied under light microscope.

KEY WORDS: Protein content, Urea, Air breathing fishes, homozenization-liver tissue.

INTRODUCTION

Farmers distribute fertilizer extensively on croplands, pastures and orchards. These fertilizers are important to high-yield agriculture. It is assumed that one-third of the country's annual production of food and natural fiber is attributed to fertilizers. Their extensive use, however, creates some problems. The effects of urea on mortality and behavior of an air breathing fish was studied by Sah and Thakur in 1988. The effluents from industry adversely affect the natural quality of water bodies. Drainage of agrochemicals from agricultural lands (Mishra et.al., 1994, Thakur and Pandey, 1989). Use of biocidal plant extracts for fish capture (Ojha et.al., 1989, Ojha and Singh, 1992), and also the presence of numerous pathogens. Contaminated water from such disturbed aquatic environments continuously ventilates the gills of fishes.

Urea (NH_2-CO-NH_2) is a commonly used nitrogenous fertilizer. It is readily soluble in water and may pass into various water bodies through irrigation water and surface runoff especially during rains.

MATERIALS AND METHODS

Live specimens of *Clarias batrachus* (average body weight 58.60 gm ± 5 gm, length=19cm ±3) were collected from local fish market of Lumding. Healthy fishes were acclimatized in the laboratory for 7 days. They were transported to the Department of Zoology, Lumding college at the early hours of the morning (6.00-8.00 hour) in a large plastic container. The fishes were acclimatized for 14 days during which they were fed to satiation with commercial fish feed pellets twice daily. Left over feed and faeces were siphoned off promptly and dead fishes were promptly removed to avoid contamination. The percentage of death recorded during the acclimatization was less than 2%. They were then transferred to the experimental plastic aquaria (10 fish/40L aquarium).

Urea was procured from local agrochemical dealers. The 96 hrs LC_{50} was calculated by regression analysis was found to be 10 mg/ 8 liter.

Experimental procedure: Forty litres capacity plastic aquaria were maintained throughout the exposure period. Ten juveniles were placed in the aquarium. Bore-hole water was used during acclimatization and exposure period. Feeding regime (800 and 1800hr) during the exposure period wasthe same as that of acclimatization period. In order to monitor the toxicant strength, level of dissolved oxygen, effects of evaporation, ammonia concentration and reduced stress during the experiment, the test media were replaced by 50% prepared-concentrations of the same quality after removing its equivalent along with the undigested food and defaecation every 48 hr to maintain the requisite level and potency of the concentration. The exposure period lasted for 14 days during which some water quality parameters were monitored after 48 hr with the exception of temperature which was determined every 24 hr using the method described in APHA(1998). Increasing time period of urea exposure changes the hematological parameter in air breathing fishes (Thakur and Sah, 1988). At the start (0hr) of the experiment ten fishes were sacrificed and analyzed for the biochemical parameters.

The fishes were sacrificed, the desired organ (liver) was removed and pulverized in a laboratory mortar and pestle. while extractions were prepared by adding 2ml of

sucrose solution before been centrifuging (Mahoba, 1987)and stored in another test-tube in the refrigerator until analyses.

RESULTS

Biochemical Parameters: The biochemical parameters were determined by using colorimetric method, which was used to determine the total protein (Biuret reaction) content of the studied organs.

Data Analysis: All data were presented using statistical techniques like means, average etc. Control values obtained at the beginning and at the end of 30-day exposure period were not significantly different.

Preparation of Standard Curve

Took a protein solution (BSA) of 0.25 mg/l strength and prepared the following incubation mixture-

Incubation Mixture

Reagents	Blank	S_1	S_1	S_2	S_2	S_3	S_3
Sample BSA(ml)	0	0.1	0.1	0.2	0.2	0.3	0.3
Water(ml)	0.5	0.4	0.4	0.3	0.3	0.2	0.2
Concn.of Protein(ug/ml)		50	50	100	100	150	150
Protein reagent(ml) wait for 40/	5	5	5	5	5	5	5
Folin reagent(ml) wait for 15/	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Observation

	Vol. taken (ml)	OD at 650 mu	Average optical density
S_1	0.1	0.075	0.0625
S_1	0.1	0.05	
S_2	0.2	0.095	0.10
S_2	0.2	0.11	
S_3	0.3	0.175	0.18
S_3	0.3	0.19	
E_1	0.2	0.185	0.164
E_1	0.2	0.15	
E_2	0.4	0.185	0.164
E_2	0.4	0.15	
E_3	0.5	0.169	0.169
E_3	0.5	0.168	

CALCULATION

From standard curve- 0.452 O.D.=0.25mg/protein ml

So for E_1 0.164 O.D.=0.22mg

It is diluted by 50 so that protein content=0.22 x 50 x 5 =55.0 mg/gm

For E_2 0.164 O.D.=0.155mg

It is diluted by 100 so that protein content=0.155 x 100 x 5 =75.5 mg/gm

For E_3 0.169 O.D.=0.155mg/gm

It is diluted by 500 so that protein content=0.155 x 500 x 5 =75.5 mg/gm

Average protein content= 75.5 x75.5 x 55.0/3=1045mg protein

So,if 0.46 gm fish liver contains 1045mg protein then,

1 gm fish liver contains = 1045mg/ 460mg=2.26 mg protein

So protein content of treated *Clarias batrachus* liver is 2.26mg protein/gm wet wt of liver, whereas, the controlled fish liver contained protein was 29.32±0.67 mg /100mg wet tissue.

CONCLUSION

The present investigation on the freshwater fish Clarias batrachus treated with urea, the organic fertilizer revealed the succeptibility of the fish to the toxic stress (LC_{50}=10mg/8liter). The variation of protein profile sreves as a tool to monitor the pathological status of treated fish. The inhibition in protein synthesis might be due to tissue necrosis which leads to loss of intracellular enzymes or other proteins (Jyothirmayee *et al*, 2005). Kumari and Kumar (1966) observed inhibited biosynthesis of protein in the olfacory epithelium leading to depletion of the protein content. This substantiates the present investigation. Accumulation of urea in the water body primarily affects the non-target organisms especiallt fish and get deposited. These fish through food chain affects humans and cause deleterious effects. Hence, the usage of pesticides should be restricted to have a healthy ecology (Helen *et al*., 2015).

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