E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(2): 332-335

www.fisheriesjournal.com
Received: 16-01-2017
Accepted: 17-02-2017

G Narayana Naidu
Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India

PPN Vijay Kumar
Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India

U Shameem
Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India

Correspondence
PPN Vijay Kumar
Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India

Acute and sub acute toxic effect of ammonia on behavioral and haematological responses of Indian major carp *Labeo rohita* Ham, 1822

G Narayana Naidu, PPN Vijay Kumar and U Shameem

Abstract
Work has been carried out to study the acute and sub acute toxicity of ammonia on behavioral and haematological responses of Indian major carp *Labeo rohita* from culture ponds. Acute dose was found to be 12.5 ppm, which was determined by exposing the fish for 96 hrs. to various concentrations of ammonia i.e. 11, 12, 13, 14, 15, 16, 17 ppm and by subjecting the results to probit analysis. 1/3rd of the acute dose was considered as sub-acute dose for undertaking sub-acute toxicity studies. *L. rohita* showed erratic behavior when exposed to acute dose, but no such abnormal behavioral changes were noticed at sub-acute dose. However, a gradual decrease in some haematological parameters like RBC, Hb %, and HCT% an increase in the WBC count was noticed both at acute and sub-acute levels during different post exposure days.

Keywords: Ammonia, Sub acute toxicity, acute toxicity, Lc_{50} value, *Labeo rohita*

1. Introduction
During the present study an attempt has been made to determine the acute and sub - acute effect of un-ionized ammonia on the behavioral and haematological responses of Indian major carp *L. rohita*. It is the natural inhabitant of the riverine system of northern and central India, and also the rivers of Pakistan, Bangladesh and Myanmar. The traditional culture of this carp goes back to hundreds of years where its culture is recorded in small ponds of Eastern Indian states. Aquatic pollution by ammonium compounds, has important place among the environmental pollutants due to their large scale production and indiscriminate usage for agricultural and aquaculture farms besides, industrial effluents, bio degradation of waste products and food remains also contribute for the formation of considerable quantity of ammonia in the environment.
In aquaculture systems control of ammonia and nitrite is the second most important factor following dissolved oxygen for the proper survival and growth of cultured organisms and therefore for the growth of aquaculture industry [1]. Nevertheless, the buildup of nitrogenous waste products from feed decomposition and organism excretion can lead to reduced productivity, sometimes resulting in the collapse of an entire aquaculture system. Ammonia, the main nitrogenous waste produced by fish exists in two forms, ionized and un-ionized. Though it is a urinary waste product, it is also excreted through the gill membrane by a Na^+/NH_4^+ exchange system [2, 3]. Elevated levels of ammonia in the body have a large number of deleterious effects due to its effect on the central nervous system [4]. Following acute ammonia intoxication, convulsions and death soon follow in all vertebrates. The un-ionized form easily diffuses across biological membranes, and can be lethal to fish. Blood is a good bioindicator or a diagnostic tool to study the problem in organ function. The measurement of haematological changes in blood of fish under exposure to any toxicant may be used to predict effects upon acute, sub-lethal and chronic exposure.

2. Materials and Methods
The present work was conducted in the laboratory of Zoology Department, Andhra University. To undertake experimental work fish were collected from various culture ponds located in and around Visakhapatnam and Vizianagaram districts Andhra Pradesh, with a weight range of the 25-30 grams and total length 10 - 13 cm.

96 hrs acute toxicity studies with unionized ammonia using *L. rohita* was carried out to determine the lethal concentration (LC_{50}) by maintaining water conditions of pH (7 - 8.5), temperature (30 °C), salinity (0.01 ppt) and hardness (60 - 1800 ppm) of water. Plastic tanks of 100 liters volume, were used to carry out the experimental study. Fifty fish were maintained in each tank and were acclimatized to laboratory conditions for one week before the commencement of the experiment. Fish feed prepared in the laboratory with the following composition (40% of Groundnut oil cake, 33% of Rice bran, 20% of Soybean meal, 5% of Fish meal and 2% of Minerals & vitamin mixture) was used ad libitum. Leftover feed and fecal wastes were removed from the tanks through vacuum pumps. Ten fish were used in each test dose and for each dose three replicates were maintained. Stock solution of ammonia at a concentration of 1mg/ml was prepared and stored in a dark and cool place. Subsequent concentrations were prepared freshly from the stock solution whenever required for the experimental study. Range finding test was carried out with different doses of ammonia (0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 ppm) by taking into consideration the dose ranges of un-ionized ammonia for other fish species from the literature. Each batch of fish was maintained in separate tubs for 96 hrs and the mortality of fish was noted at time intervals of 12hrs, 24hrs, 48hrs, 72hrs and 96hrs. Based on the value of range finding test, the dose for LC_{50} experimental toxicity trial was determined. The data on percent fish mortality, obtained during 96 hour lethal concentration trial were analyzed by employing the probit analysis method [5]. A separate batch of 50 fish were maintained without adding un-ionized ammonia as controls.

The stress effect of ammonia was measured in terms of study of clinical signs, changes in haematological parameters of blood. Blood samples were collected after 12hrs, 24hrs, 48hrs, 72hrs and 96 hrs post exposure period and analyzed for various haematological parameters were done following the methods of Hendricks and Shaw and the diagnostic protocol of diagnostic kits made by BMK Laboratories, India [6, 7].

The dosage for the sub-acute toxicity test was determined based on the LC_{50} value as 5 ppm and the sub-acute toxicity test was conducted for 60 days with the sub-acute dose. 50 fish of equal size and length were exposed to 5ppm of un-ionized ammonia in 50 liter tanks. Blood samples were collected after 7 days, 14 days, 21 days, 30 days, 45 days and 60 days, post exposure period and analyzed for haematological parameters. The blood was drawn from the fish by cardiac puncture with 1ml disposable syringe rinsed with anticoagulant (EDTA) and was stored in refrigerator untill further use in airtight microcentrifuge tubes to prevent haemolysis.

Statistical analysis was performed using one-way Analysis of Variance with the "General Linear Model" procedure. Duncan's multiple-range test for variables was used to check the data for significant difference.

3. Results

During the range finding test, no mortality was noted at 10ppm, however, 10percent mortality at 11ppm, 40percent mortality at 12ppm, 70percent mortality at 13ppm and 100percent mortality at 16ppm was observed. Based on these observations, doses from 11ppm to 17ppm were taken into consideration for conducting median lethal concentration test. Lc_{50} trials were conducted with six different concentrations of un-ionized ammonia viz., 11, 12, 13, 14, 15, 16 and 17 ppm. Fish were observed for 96 hrs and the percent mortality was noted after every 24 hrs and the calculated LC_{50} value was found to be 12.5 ppm.

The following changes were observed in behavioural responses and haematological responses of *L. rohita* during the 96 hr. acute toxicity studies with a lethal dose of 12.5 ppm and during 60 days sub-acute toxicity studies with a sub-acute dose of 5 ppm un-ionized ammonia.

3.1 Behavioral responses

Several behavioral responses like erratic swimming activity, excessive mucus secretion, rapid gill opercular movements, coughing and increase in the frequency of surfacing were noted during exposure of fish to acute toxic doses of 12.5 ppm. These changes were more pronounced during the early phase of exposure rather than during the later phase.

However, changes in behavioural responses of fish during exposure to sub-acute toxic doses of 5ppm of un-ionized ammonia were not as prominent as those observed during acute toxicity trials. It was noted that due to continuous exposure for 60 days, the fish rather got acclimatized to the presence of ammonia and exhibited little change in their behavioral response, especially during the later part of exposure period. Fish were without any marked clinical signs, except that they appeared weak and anaemic by the end of exposure period. No such behavioral changes were noticed in control fish, which remained normal and healthy, throughout the study period.

3.2 Haematological responses

Significant changes were noticed in haematological parameters of *L. rohita* exposed to 12.5 ppm of un-ionized ammonia. The data collected on various haematological parameters like total RBC, WBC, Hb and HCT% at different post exposure intervals i.e. 12, 24, 48, 72 and 96 hours are presented in Fig. 1.

In the exposed fish a gradual decrease in the level of red blood cell counts was noted from 12 hrs to 96hrs, over control fish. In control fish, the total RBC count was noted as $0.519X10^6/mm^3$ (0.32-0.64), whereas in exposed fish it was noted as $0.516X10^6/mm^3$ (0.36-0.79) at 12hrs, $0.382X10^6/mm^3$ (0.16-0.47) at 24hrs, $0.373X10^6/mm^3$(0.9-0.47) at 48hrs, $0.207X10^6/mm^3$(0.13-0.32) at 72hrs and $0.199X10^6/mm^3$ (0.16-0.36) at 96 hrs. On the other hand, a gradual increase in total WBC was noted during post exposure periods over control. The total WBC count in the control group was found to be $131.04X10^4/mm^3$ (129.28-136.02), followed by $409.082X10^4/mm^3$ (393.34-429.17) at 12hrs, 492.74 $X10^4/mm^3$ (393.7-492.0) at 24hrs. A gradual increase in the values was noted at 48, 72 and 96 hours, recording $502.09X10^4/mm^3$ (493-512) at 48hrs, $521.77X10^4/mm^3$ (497-529) at 72hrs and $560.46X10^4/mm^3$ (491-568) at 96hrs.

A gradual decrease in the percentage of haemoglobin (Hb%) was noted from 12hrs to 96 hrs, over unexposed control fish. In control fish the values were 2.84gm/dl (1.9-3.3),whereas in exposed fish at different intervals of 12, 24, 48, 72 and 96 hours the Hb% was recorded as 1.96 gm/dl (1.9-2.2) at 12hrs, 1.73gm/dl (1.1-2.5) at 24hrs,1.44gm/dl (0.7-1.8) at 48hrs, 1.31 gm/dl (1.1-1.6) at 72hrs, and 1.02gm/dl (0.6-1.4) at 96hrs. Hematocrit percentage (Hct%) showed a gradual decrease from 12hrs to 96hrs in exposed fish from 6.36%(4.7-7.2) at 12hrs to 6.05% (5.6-7.4) 96hrs with control fish which showing 6.36% (3.4-7.2). The Hct% values in exposed fish at different intervals of 12, 24, 48, 72 and 96 hours were found to be 6.05% (5.6-7.4), 5.46% (3.1-6.2), 3.99% (2.2-5.2),

3.95% (2.8-4.7) and 3.23%(2.6-4.4) respectively.

Observations made on various haematological parameters during exposure to sub acute level of 5 ppm un-ionized ammonia, has indicated a gradual decrease in the total RBC count, Hb and Hct percentages at different post exposure intervals, whereas WBC values showed increase. The mean data on various haematological parameters is presented in the Fig. 2. In the control fish the total RBC count was recorded as 0.515 X10^6/mm^3(0.4-0.6) and for post exposure days the values were 0.501 x10^6/mm^3 (0.24-0.9) at 7 days, 0.47 x 10^6/mm^3 (0.3-1) at 14 days, 0.33 x 10^6/mm^3 (0.2-0.4) at 21 days, 0.196 x 10^6/mm^3 (0.1-0.9) at 30 days,0.11 x 10^6/mm^3 (0.1-1.02) at 45 days and 0.054 x 10^6/mm^3 (0.01-0.08) at 60 days. Variations in WBC values were more pronounced between control and exposed group a slight increase was noted at all the intervals from 7 to 60 days post exposure period. The WBC count in control fish were recorded as 142.04 x 10 ^{4}/mm^3 (138-143) and at different intervals they were found to be 146.693 x $10^4$$mm^3$ (121.3-175.6) at 7 days, 147.76 x 10 ^{4}/mm^3 (143-168.2) at 14 days, 148.2 x 10 ^{4}/mm^3(133.5-168.3) at 21 days, 151.82 x 10^4/mm^3 (139.2-163.7) at 30 days, 152.88 x 10^4/mm^3(130.9-179.2) at 45 days and 167.41 x 10^4/mm^3(142.3-192.6) at 60 days. A gradual decrease in the amount of Hb% was noted during different post-exposures intervals. A sudden drop was noted in the haemoglobin concentration during all days of exposure. In the control group the haemoglobin concentration was found to be 2.876gm/dl (1.9-2.2), whereas at different post exposure intervals the values were1.89gm/dl (0.2-2.1) at 7 days, 1.82gm/dl (1.5-3.3) at 14 days,0.64gm/dl (0.4-0.7) at 21 days, 0.55 gm/dl (0.2-1.3) at 30 days,0.22 gm/dl(0.1-1.3) at 45 days and 0.15gm/dl (0.1-0.9) at 60 days. A similar situation was noted even in the case of hematocrit percentage. The hematocrit percentage was found to be 5.81(4.9-6.1) for control group fish and for the exposed fish the values were 5.02% (2.1-7.6) at 7 days, 4.2% (3.6-5.3) at 14 days, 3.9% (2.4-5.3) at 21 days, 3.6% (3.1-4.6)at 30 days, 2.8% (2.3-6.2) at 45 days and 1.95% (1.8-2.3) at 60 days. Statistical analysis of the data using single factor ANOVA followed by Duncan's multiple range test, showed the differences in means to be significant between control and all other exposure periods (p<0.05).

Table 1: Empirical Probit and approximate expected probits for the above data is 12.5 ppm

S. No	Dose ppm	Mortality %	X Log Concentration	y Empirical Probit	Y Expected Probit
1	11	10	1.0413	3.7184	3.469
2	12	40	1.0791	4.75	4.546
3	13	70	1.1139	5.52	5.537
4	14	80	1.1461	5.84	6.455
5	15	90	1.1760	6.28	7.307
6	16	100	1.2041	8.71	8.1079
7	17	100	1.2041	8.71	8.1079

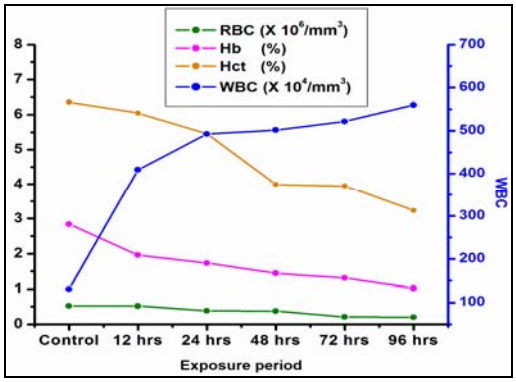

Fig 1: Heamatological parameters of *L. rohita* exposed to acute toxic dose of 12.5 ppm un-ionized ammonia, during different post exposure interval

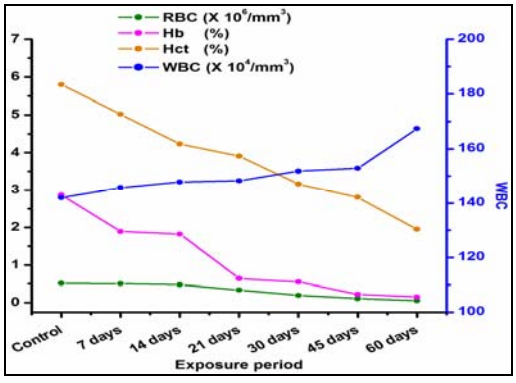

Fig 2: Heamatological parameters of *L. rohita* exposed to sub acute toxic dose of 5 ppm un-ionized ammonia, during different post exposure intervals

4. Discussion

The present work has been undertaken to determine the effect of acute and sub-acute toxicity of commercial grade un-ionized ammonia (NH_3) in *L. rohita* an edible fish reared for its high nutritive and economical value in culture ponds throughout North Coastal Andhra Pradesh. The behavioral responses of fish exposed to acute and sub acute levels of ammonia include erratic swimming activity, excess mucus secretion, puffing of gill opercula, coughing and loss of equilibrium [8]. These changes were more pronounced during the initial stages of exposure, rather than during the later stages. This is particularly true in the fish exposed to acute concentration of un-ionized ammonia. The reason for these sudden behavioral changes may be due to the irritating effect of the toxicant at high concentration. In the sub-acute toxicity, behavioral changes were not very prominent, may be due to the exposure of fish for longer duration which makes it to get acclimatized to the presence of ammonia in later phase such changes were also noticed by earliest investigators. Changes in swimming behavior (disorientation and erratic swimming), in gill ventilation and the colour of fish (darkened skin) were observed within two hours after addition of ammonia by Lemarie, *et al* [9].

The overall observations made during the present study on the behavioral cha nges of *L. rohita* exposed to acute (96 hrs) and sub-acute levels (5ppm) of un-ionized ammonia solution shows that *L. rohita*, though sensitive during the initial stages of exposure, can become tolerant after prolonged exposure to sub-acute doses of ammonia.

The LC_{50} value obtained during the present study is in comparison to those reported by earlier investigators for similar size group of fishes. The toxicity of ammonia to aquatic animals particularly fishes, has been extensively

investigated and fairly reviewed as early as in 1980 by Alabaster and Lloyd [10]. The variations in the LC_{50} values of ammonia reported during several studies undertaken over a period of time can be attributed to variations in the individual characters of the host involved such as size, weight, sex and biological behavior which play an important role in determining the LC_{50} values of any toxicant. Thus the median lethal concentration of ammonia, causing 50 percent mortality of the exposed fish, varies depending on the size and species of the fish host involved. However, the mortality of fish is both a time and dose dependent response.

The knowledge of the hematology is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes [11]. The significant decreases in RBC, Hct and haemoglobin (Hb%) observed during the present study were in line with observations made by others. The observed reduction in number of RBC and distorted shape of red blood cells (RBCs) during the present study may be due to the hypoxic condition that results in tissues of fingerlings exposed to acute dose of unionized ammonia concentration. When ammonia concentrations increase in water, ammonia excretion by fish decrease and the levels of ammonia in the blood tissue increase. High levels of ammonia increase oxygen consumption by tissues, damage gills, reduce ability of blood to transport oxygen. Increased level of ammonia in water increases its content in the blood of fish through diffusion and poisoning of central nervous system [12]. In the present investigation reduction of hemoglobin may be due to inhibition of aerobic glycolysis in the stressed fish. Prolonged reduction in hemoglobin content is deleterious and may lead to degeneration and could be ascribed as pathological conditions in exposed fish. Saeed also recorded a marked reduction in RBC count and haemoglobin (Hb) concentration after the exposure of *O. niloticus* to acute ammonia concentrations [13]. The decrease in these parameters can be attributed to haemolysis of RBC or may be due to decrease in the erythropoietic activity of kidney or the haemo-dilution resulting from impaired osmo-regulation across the gill epithelium [14]. Further, the observed reduction in the haemoglobin content may be due to the prevailing anoxic condition, as depression and exhaustion in the haemopoietic potential occurs under such conditions [15].

An increase in the TLCs occurs as a protective response to stress [16] and such an increase in TLCs occurs by an increase in lymphopoisis and/or enhanced release of lymphocytes from lymphoid tissue [17]. It is well known fact that the leucocytes are involved in the regulation of immunological function of body [14]. However a prolonged exposure to higher concentration of toxicants causes failure of such TLCs production, leading to a decrease in the non-specific immunity of fish [18]. The observed increase in TLCs suggests that the body tried to enhance the TLCs as a protective mechanism against the stress due to toxicant exposure.

5. Acknowledgements

The authors gratefully acknowledge the assistance extended Department of zoology Andhra University for providing facilities for undertaking the studies.

6. References

1. Ebeling JM, Timmons MB, Bisogni JJ. Engineering analysis of the stoichiometry of photoautotrophic, auto trophic, and heterotrophic removal of ammonia nitrogen in aquaculture systems. Aquaculture. 2006; 257:346-358.
2. Tomasso JR. Toxicity of nitrogenous wastes to aquaculture animals. Rev Fish Sci, 1994; 2:291- 314.
3. Fuller SA, Henne JP, Carmichael GJ, Tomasso JR. Toxicity of ammonia and nitrite to the gilla trout. N AM J AQUACULT. 2003; 65:162-164.
4. Ip YK, Chew SF, Leung IAW, Jin Y, Lim CB, Wu RSS. The sleeper *Bostrichthys sinensis* (Family Eleotridae) stores glutamine and reduces ammonia production during aerial exposure. J. Comp. Physiol. B. 2001a; 171:357-367.
5. Finney DJ. Statistical Method In Biological Assay (3rd Ed), Cambridge University Press. London, 1978, 508.
6. Hendricks LJ. Erythrocytes counts and haemoglobin determinations for two species of suckers, genus Catostomas, from Colorado. Copeia. 1952; 4:265-266.
7. Shaw AF. A direct method for counting the leukocytes, thrombocytes and erythrocytes in bird's blood. J. Pathol. Bacteriol. 1930; 33:833-835.
8. Aysel CKB, Gulten K. The acute toxicity of ammonia on tilapia (*Oreochromis niloticus* L.) larvae and fingerlings. Turk J Vet Anim Sci. 2005; 29:339-344.
9. Lemarie G, Dosdat A, Coves D. Effect of chronic ammonia exposure on growth of European seabass (*Dicentrarchuslabrax*) juveniles. Aquaculture, 2004; 229:479-491.
10. Alabaster JS, Lloyd R. Ammonia. In: Water quality criteria for fish. Alabaster, J.S. and R. Lloyd (Eds.). Butterworths, London, 1980, 85-102.
11. Kori-Siakere O, Ake Jeg, Idoge E. Hemtologial Characteristics of The African Snakehead, *Parachanna obscura*. Afr. J.Biotechnol. 2005; 4(6):530.
12. Svobodova Z, Machhova J, Kroupova H, Smutna M, Groch L. Ammonia Autointoxication Of Common Carp, Case Studies. Aquacullt. Int. 2007; 15:227-286.
13. Saeed RMA. Toxicity of ammonia to the Nile tilapia, *Oreochromis niloticus.* J. Egypt. Ger. Soc. Zool. 1997; 23(A):125-144.
14. Santhakumar M, Balaji M, Ramudu K. Effect of sublethal concentration of monocrotophos on erythropoietic activity and certain haematological parameters of fish Anabas testudineus (Bloch). Bull Environ Contam Toxicol. 1999; 63:379-384.
15. Sawhney AK, Johal MS. Erythrocyte alterations induced by malathion in *Channa punctatus* (*Bloch*). Bull Environ Contam Toxicol, 2000; 64:398-405.
16. Sastry KV, Sharma K. Mercury induced haematological and biochemical anomalies in Ophiocephalus *Channa punctatus*. Toxicology Letters, 1980; 5(3-4):245-249.
17. Johansson-Sjobeck ML, Larsson A. The effect of cadmium on the hematology and on the activity of delta-amino leverlinic acid dehydratase (ALA-D) in blood and hematopoietic tissues of the flounder, *Platichthys flesus* (L.). Environ. Res. 1978; 17:191-204.
18. Svobodova Z, Vykusova B, Machova J. The effects of pollutants on selected haematological and biochemical parameters in fish. In: Muller, R. and Lloyd, R. (Eds.), Sublethal and chronic effects of pollutants on freshwater fish. FAO Fishing News Books, Oxford, 1994, 39-52.