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TOXICITY OF AMMONIA TO FINGERLING GRASS CARP, *CTENOPHARYNGODON IDELLA* (VALENCIENNES)

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Abstract: Experiments were conducted to establish the lethal dose concentration of un-ionized ammonia at which 50% and 100% mortality of fingerlings of *Ctenopharyngodon idella* (Valenciennes) occurred. Fingerlings of *C. idella* were obtained from Central Fish Seed Hatchery, Lahore and were stocked in 300 liters cemented indoor rectangular tanks. The tanks were supplied tube-well water (temperature 13-19 ^{0}C, pH 7.9-8.8; hardness 276-344 mg/l as $CaCO_3$, dissolved oxygen 5.0-8.1ppm with zero total ammonia nitrogen. Fingerlings were fed daily with standard fish feed. Median lethal concentrations (LC_{50}) were determined in glass aquaria each containing 9 fish (mean weight 12.7±1.4 to 7.2±1.31g) and 40 liters of test water. Total ammonia-N concentration was determined by direct Nesslerization**.** The 24 hour LC_{50} of un-ionized ammonia for *C. idella* fingerlings (mean body weight 2.50 ± 1.54g, at 15±0.1^{0}C and pH 8.0 ± 0.01) was 0.05mg/l. Lethal dose of un-ionized ammonia at which 100% *C. idella* fingerlings (mean body weight 2.96 ±0.8g, at 15±0.1^{0}C and pH 8.12 ± 0.02) showed mortality, was 0.075mg/l. Fish under experiment remained unharmed when ammonia concentration was 0.025 mg/l.

Key words: Ammonia, toxicity, fish survival, LC_{50}, LC_{100}.

INTRODUCTION

In the aquatic environment, ammonia originates from natural process by the ammonification of organic nitrogen compounds and from metabolic excretion of ammonia by aquatic animals. During winter ammonia can also accumulate in fish pond as an excretory product of the fish (Tomasso, 1994). According to Randall and Tusi (2002), ammonia is present in the aquatic environment due to agricultural runoff and decomposition of

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biological waste. When fish is reared at high stocking densities, there may be gradual accumulation of suspended solids and natural catabolites such as ammonia and carbon dioxide and a decrease in dissolved oxygen and pH, especially if water flow is restricted (Person-LeRujet *et al.,* 1997). In intensive aquaculture the toxicity of excreted nitrogen compounds is the single most important limiting parameter, once adequate oxygen level is maintained (Colt and Armstrong, 1981). High concentration of environmental ammonia will cause death of fish. High but sub-lethal environmental ammonia concentration have been associated with gill damage and reduced growth (Meade, 1985). In aquatic environment, ammonia exists in two forms as, ionized (NH_4) and unionized (NH_3-N). The un-ionized ammonia is toxic and is related to ammonia concentration, water temperature and water pH at any given time (Emerson and Russo., 1975; Bergerhouse, 1992; USEPA, 1999; Ip *et al.,* 2001). The un-ionized ammonia has adverse effects on fish, whereas, the ionized form is considered relatively non-toxic. Hence, most toxicity values are reported for un-ionized ammonia (Downing, 1955; Thurston and Philip, 1981).

The relationship between the fish and its sensitivity to ammonia is a complex process. Before absorption of the yolk, rainbow trout (*Salmo gairdneri)* withstand up to 50 times of ammonia concentration which is lethal to adults (Rice and Stokes, 1975). According to SECL (1992) the start of feeding life stage of fish is the period of highest ammonia sensitivity. Ruffier and Boyle (1981) explained the mechanism of ammonia toxicity as osmo-regulatorty imbalance causing kidney failure, superficial excretion of endogenous ammonia resulting in neurological and cytological failure and gill epithelia damage leading to suffocation. Ammonia intoxication in fishes may cause hyperventilation, erratic swimming, increased gills ventilation, loss of balance and equilibrium (Hillaby and Randall, 1979; Knoph, 1996; Ip *et al.,* 2001). Fish response to short term exposure to ammonia, includes, erratic movement, loss of equilibrium lack of foraging and even mortality (Meade, 1985, Russo and Thurston, 1991).

It is necessary to determine the safe level of each potentially limiting factor, hence, considerable attention has been paid to investigate the effects of ammonia (Haywood, 1983; Mead, 1985; Russo and Thruston, 1991; Wood, 1993). Sub-lethal ambient ammonia concentration can cause physiological disturbance in fish that can impair the recovery of largemouth bass, *Micropterus salmoides* from exercise (Suski *et al.,* 2007).

The transgenic carps are less tolerant of un-ionized ammonia than non-transgenic carp (Guan *et al.,* 2010). A significant linear relationship between chub *Leuciscus cephalus* larva susceptibility to ammonia toxicity and both body weight and body length was reported. The critical level of un-ionized ammonia nitrogen for chubb larvae was suggested as 0.49mg/l (Gomulko *et al.,* 2011).

Available knowledge of short-term and long term response of ammonia in major and Chinese carps is patchy. The aim of the present study was to estimate LC_{50}, LC_{100} ammonia concentration at which 50% and 100% mortality of *C. idella* occur and to measure the toxic and safe concentration of ammonia for fingerling of *C. idella.*

MATERIALS AND METHODS

Fingerlings of *C. idella* were obtained from Central Fish Seed Hatchery, Manawan Lahore. Fish were stocked in 300 liters cemented indoor rectangular tanks. The tanks were supplied tube well water (temperature 13-19 °C, pH 7.9-8.8; hardness 276-344mg/l as $CaCO_3$, dissolved oxygen 5.0-8.1 ppm with zero total ammonia nitrogen contents. Fish were fed daily with standard commercial formulated fish feed (22% protein, 8% fat and 1% premix vitamin).

Determination of Lethal Concentration

Median lethal concentrations (LC_{50}) were determined in glass aquaria each containing 9 fish (mean weight 12.7±1.4 to 7.2±1.31g) and 40 liters of test water. Fish were allowed to acclimatize to the aquaria for 24 hours before addition of ammonia (ammonium chloride). The ammonia experiment was replicated three times.

Carp nominal NH_3-N (as ammonium chloride) in experimental aquaria was 6.26g NH_4Cl/40 L and 9.4g NH_4Cl/40 L resulting in ammonia nitrogen 0.05 mg/l and 0.075 mg/l. Each experiment consisted of five experimental aquaria that were containing ammonia and one control aquarium. Fish were offered feed during the exposure.

Water quality was monitored at the beginning and end of each experiment. pH was measured by Orion model 301pH meter. Dissolved oxygen and temperature was determined with YSI Oxygen meter Model 57. Total ammonia-N concentration was determined by direct

Nesslerization (APHA 1992). The concentration of un-ionized ammonia–N was calculated from Tables based on Emerson and Russo (1975).

RESULTS

Determination of LC_{50} by un-ionized ammonia in C. idella

Water quality variables from the experimental aquaria, containing fish was determined before addition of ammonium chloride. During the study, all physiochemical parameters were in suitable range. The result of this experiment has been shown in Table I. The fish (*C. Idella)* was exposed to 0.05 mg/l NH_3-N. After 24-h exposure, water quality was checked to detect, how much ammonia-N mg/l was present in water (Table II). The fish was exposed to 0.05 mg/l NH_3-N. After 24 hours when 50% of the experimental fish were dead due to un-ionized ammonia nitrogen, mortality was recorded. Mortality varied from 50 to 65.5% which is significant (Table III). After exposure to 0.05 mg/l NH_3-N for 24 hours the water quality variables from aquaria were determined (Table IV).

Table I: Water quality variables before application of NH_4Cl during determination of LC_{50} of fingerling *C. idella.*

Parameter	Treatments		
	T1	**T2**	**T3**
Temperature °C	17.2±0.5	18.1±1.2	18.2±1.5
pH	8.0 ±0.2	7.9±0.2	8.2±0.3
Dissolved oxygen (mg/l)	5.2 ±0.90	5.5±0.82	6.9±0.2
Total alkalinity as $CaCO_3$ (mg/l)	412 ±17.20	425±10.12	430±17.31
Total hardness as $CaCO_3$ (mg/l)	260 ± 20.5	284±8.10	302±11.94
Calcium (mg/l)	88.5 ±2.50	102±15.10	100±8.15
Magnesium (mg/l)	109.6 ±2.4	95.6±2.10	91.2±10.2
Chloride (mg/l)	26.12± 1.8	30.2±5.10	39.8±1.8
Free CO_2	2.4 ±0.24	2.9±1.5	3.1±1.8

Table II: Water quality variables during 24 hours exposure of fingerling *C. idella* to ammonia-N.

Time (hrs.)	No. of fish in aquaria	Temp. °C	pH	NH_3-H µgm/l	Percent Nominal
0	6	15.0±0.35	8.23±0.45	60	112±=0.5
24	6	16.4±0.24	8.0±0.39	59	118±1.03
0	6	12.1±0.25	8.0±0.39	59	110 ±1.05
24	6	14.23±0.21	7.9±0.94	54	118±10.5
0	6	14.3±0.04	7.9±0.012	53	106±1.02
24	6	16.8±0.12	8.1±0.047	55	110±1.05

(Values for temperature and percent Nominal are mean = SD and values for pH are average. The percent Nominal values represent the percentage of target concentration measured during the test).

Table III: Toxicity test for determination of LC_{50} for fingerling *C. idella* by un-ionized ammonia.

Sr. No	Weight (g)	Length (cm)	Total no. of fish	Dead	Alive	Mortality	Dose of Tan, mg/l
1	1.86±0.52	5.15±1.2	18	12	6	65.50	0.05
2	2.78±1.54	5.57±1.45	18	10	8	55.55	0.05
3	3.75±2.53	6.55±2.45	18	09	0	50.00	0.05

Determination of LC_{100} for C. idella

Water quality variables from experimental aquaria having grass carp (mean weight 2.95±0.41g) before the addition of ammonium chloride were observed (Table V).

Grass carp was exposed to 0.075mg/l NH_3-N. After 24 hours exposure the water quality was observed to detect how much ammonia was present in water. Amount of un-ionized ammonia in mg/l is represented in Table VI. Experimental fish died due to NH_3-N. Mortality was observed which varied from 89 to 100 % (Table VII).

Grass carp was exposed to 0.075 mg/l. After 24 hours exposure the water quality was observed to detect how much ammonia was present in water. Amount of un-ionized ammonia is mg/l. After 24 hours exposure water quality variables were observed (Table VIII).

Table IV: Water quality variables after 0.05mg/l NH_4Cl treatment of fingerling *C. idella.*

Parameters	Treatments		
	T1	T2	T3
Temperature °C	16.4 ±0.4	14.2±1.2	16.6±2.3
pH	8.0±0.3	7.9±0.3	8.1±0.4
Dissolved Oxygen (mg/l)	6.20±.91	5.8±0.82	7.1±1.3
Total alkalinity as $CaCO_3$(mg/l)	428±10.7	437±12.1	411±10.5
Total hardness as $CaCO_3$ (mg/l)	265±18.17	274±10.15	315±5.41
Calcium (mg/l)	75.54±.52	100±11.51	98.±10.12
Magnesium (mg/l)	112±2.6	95.6±10.5	1105±6.16
Chloride (mg/l)	36.4±2.15	45.4±5.17	58.1±1.9
Free CO_2	4.15±1.15	4.5±0.5	4.0±2.1

Table V: Water quality variables before addition of NH_4Cl for determination of LC_{100} of *C. idella.*

Parameter	T1	T2	T3
Temperature °C	12.1±0.08	12.0±0.20	12.5±0.12
pH	8.33 ±0.97	8.23±0.47	7.93±0.47
Dissolved oxygen (mg/l)	6.5 ±0.25	6.2±0.27	8.75±0.29
Total alkalinity as $CaCO_{3\,(}$mg/l)	403 ±32.29	388.66±16.22	458.3±3.29
Total hardness as $CaCO_{3\,(}$mg/l)	277 ± 31.53	274±8.89	281±4.25
Calcium (mg/l)	85 ±5.15	95.14±9.75	96±8.15
Magnesium (mg/l)	115 ±9.17	137.4±5.15	120±10.2
Chloride (mg/l)	24.35± 1.7	36.6±7.12	42.2±5.31
Free CO_2	3.2. ±0.31	2.1±1.5	4.2±1.7

Table VI: Water quality variables during 24 hours exposure of *C. idella* to ammonia-N. (for LC_{100})

Time (hrs)	No. of fish in aquaria	Temp.°C	pH	NH_3-H µgm/l	Percent Nominal
0	6	12.0±0.1	8.33±0.97	89	118.6±0.2
24	6	12.4±0.2	8.21±0.31	80	106.6±0.3
0	6	12.0±0.20	8.20±0.39	68	90.66 ±5.4
24	6	13.4±0.21	8.10±0.15	82	109.33±4.27
0	6	12.5±0.12	7.0±0.04	64	85.33±9.31
24	6	13.8±1.2	8.0±0.00	69	92±0.2

Values for temperature and percent Nominal are mean±SD, and values for pH are average. The percent Nominal values represent the percentage of target concentration measured during the test).

Table VII: Toxicity test for determination of LC_{100} by un-ionized ammonia on *C. idella*

Sr. No.	Weight (g)	Length (cm)	Total no. of fish	Dead	Alive	Mortality	Dose of Tan, mg/l
1	2.86 ± 1.02	4.42 ±2.2	18	18	0	100	0.075
2	2.75 ± 0.9	5.13 ± 0.05	18	17	1	94.4	0.075
3	2.88 ± 0.8	6.55 ±1.45	18	16	2	89.0	0.075

Table VIII: Water quality variables after 0.075mg/l NH_4Cl treatment of *C. idella*

Parameters	T1	T2	T3
Temperature °C	13.3±0.01	13.4±0.02	13.8±1.2
pH	8.2±0.31	8.1±0.1	8.0±0.2
Dissolved Oxygen (mg/l)	7.2±0.37	6.9±0.20	6.9±1.2
Total alkalinity as $CaCO_{3(}$ mg/l)	411±20.15	425±12.15	398±11.10
Total hardness as $CaCO_3$ (mg/l)	257±10.15	298±15.57	301±10.15
Calcium (mg/l)	77.35±.52	105±11.52	98.4±3.12
Magnesium (mg/l)	105±15.25	112±12.60	151.5±1725
Chloride (mg/l)	40±1.05	52±2.1	50.2±1.15
Free CO_2	1.9± 0.5	1.9±0.6	2.1±1.0

DISCUSSION

The 24 hours LC_{50} of unionized ammonia nitrogen for *C. idella* fingerling was 0.05mg/l NH_3-N. Similarly the 24 hours LC_{100} for *C. idella* was 0.075mg/l NH_3-N. The median lethal concentration of ammonium chloride has shown to be species specific (Eleister Rani *et al.*, 1997). A narrow range of ammonia tolerance exists within the test population or the test animals (Redner and Stickeny, 1979). At chronic toxicity level one to two days before death *C. idella* looked swollen and weak but without the loss of equilibrium which was observed during this study. This is the indication of slow ammonia poising action as indicated by Person-Lruyet, *et al.* (1995). Boyd (1992) has reported that for the best warm water fish culture, decline in dissolved oxygen content decrease tolerance of fishes to ammonia. There are number of fish species that can tolerate high environmental ammonia (Randall and Tusi, 2002). Richardson (1997) reported 96 h, LC_{50} at 15ºC and pH 7.5 and pH 8.1 ranged from 0.75 to 2.35mg NH_3/l for seven freshwater fish species in New Zealand. He concluded that these fishes were more tolerant than native invertebrate species. *C. idella* fingerlings showed more sensitivity towards unionized ammonia compared to seven native fish species of New Zealand as reported by Richardson (1997). Our results are comparable to Yong Xin *et al.* (1986) who reported maximum allowable toxicant ammonia for grass carp fingerling as 0.054-0.099mg/l NH_3/l as judged from the toxic effects of ammonia on their gills structure. Starved fishes are more sensitive to external ammonia than fed fish (Randall and Tusi, 2002). Mean 48h LC_{50} value of unionized ammonia (NH_3) for larval tilapia, *Oreochromis niloticus* was 1.009±0.02mg/l and for fingerlings this value was 7.40±0.01mg/l (Caglan *et al.*, 2005). Transgenic carps are less tolerant to un-ionized ammonia than non-transgenic (Guan *et al.*, 2010), whereas, 0.49mg/l un-ionized ammonia nitrogen was suggested for chub larvae as critical value by Gomulko *et al.* (2011).

The free CO_2 concentration never varied from 4mg/l in this study. Toxicity of a solution of ammonium chloride could be decreased by increasing the level of free CO_2 which reduced the pH value until a concentration of free CO reached which was itself toxic to fish. In water ammonia exists in equilibrium between two forms ionized and un-ionized. The percentage of lethal ammonia that is un-ionized at any given time is related to both temperature and pH of water. It is well known fact that growth rate of fish in acidic waters is usually less than that under alkaline condition. In the present study the main objective was NH_3-N toxicity so no proper emphasis could be given on pH effects.

The alkalinity varied from 398 to 425 in water during experiments. Alkalinity (bicarbonate concentration) only affects the toxicity of ammonia by its part in determining the pH value of the water in conjunction with the level of free carbon dioxide present, (Lloyd, 1961).

More work is required for investigation of NH_3 intoxication and NH_3 autointoxication because various symptoms of NH_3 poisoning in fish are often indistinguishable from those of other poisons or infections. More work is needed to be done to establish with certainty safe levels for long term growth of *C. idella* as well as to determine the adoptive physiological mechanism involved in ammonia toxicity.

In the carp culture system in the province of Punjab, a true safe, maximum acceptable concentration of ammonia-N or lethal ammonia are not known. The present study has illustrated that increase in NH_3-H will increase the mortality. 0.075mg/l NH_3-N cause 100 % mortality in grass carp. Uptake of ammonia by algae and its volatilization to atmosphere which is a typical characteristic of a pond culture system has not been emphasized so more research work is needed to determine different nutrient path ways under various environmental conditions.

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