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EFFECTS OF AMMONIA ON GROWTH OF *CTENOPHARYNGODON IDELLA* (VALENCIENNES) FINGERLINGS

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Abstract: An experiment was conducted to find out growth rate of a commercial carp, *Ctenopharyngodon idella* (Valenciennes) at two concentration of ammonia; 0.027mg/l and 0.029 mg/l of un-ionized ammonia. The experiment was conducted in eight, 350 liter fiberglass tanks. The experiment continued for 84 days. The initial mean body weight of fingerling ranged from 71.2 to 74.16 g. The fish was fed daily with commercial formulated feed (22% protein, 8% fat and 1 % premix vitamin). The water quality was monitored on weekly basis. The rate of growth was monitored at the end of experiment by weighing individual fish in each batch. The faster growth rate was viz. 15 mg/g/day in control tank. The fish in tanks with 0.027 mg/l and 0.029 mg/l of un-ionized ammonia showed growth at the rate of 12.5 mg/g/day and 11.2 mg/g/day respectively. This study revealed that ammonia has major effect on growth of pond fish, such as *C. idella.*
Keywords: ammonia, growth, grass carp, fingerlings.

INTRODUCTION

Production of grass carp, *Ctenopharyngodon idella* in polyculture system has increased in the last years. China, Pakistan, Bangladesh, India and Sri Lanka are now the main grass carp producing counties in Asia. In pond aquaculture the water quality control may be critical in order to maximize production as well as to maintain the well being of the fish. Poorly maintained fish pond or poor welfare of fish is known to cause many production related problems. Such condition reduces both capacity and efficiency of rearing facility. Water quality is one of the most

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important factors to fish health and stress level. It is therefore, important and essential to establish the tolerance limits at which a fish species have disturbances related to physiological process and reduced growth from any unwanted level of such parameter. Oxygen and Ammonia are the first and second limiting factors for fish reared in intensive aquaculture respectively. Ammonia is the main end product of nitrogen metabolism in teleost and can be extremely toxic to fish if allowed to accumulate in pond or water body. Ammonia excretion is directly related to protein intake and time after feeding (Handy and Poxton, 1993).

In water, ammonia exists in an equilibrium between two forms ionized and unionized. The percentage of lethal ammonia that is un-ionized at any given time is related to concentration of ammonia, water temperature and water pH (US EPA, 1980; Emerson and Russo, 1975; Bergerhouse, 1992). The un-ionized fraction of ammonia is primarily responsible for its harmful effects. The ionized fraction is considered relatively non-toxic (Downing and Merkens, 1955). Because un-ionized ammonia is more toxic than the ionized ammonia, most toxicity values are reported for un-ionized ammonia (Thurston and Philip, 1981). Signs and symptoms of ammonia intoxication in fishes may include such as hyperventilation, erratic swimming, gulping air at the surface of water, increased gills ventilation, loss of balance and equilibrium (Smart, 1976, Hillaby and Randall, 1979; Knoph, 1996). High but sub-lethal environmental ammonia concentration have been associated with gill damage and reduced growth. In short term exposure to ammonia fish response include, erratic movement, loss of equilibrium, lack of foraging and even mortality (Meade, 1985, Russo and Thurston, 1991).

Ashe *et al.*, (1996) suggested that growth of fingerling of white bass, *Morone chrysops* should not be inhibited if the fish are continuously exposed to un-ionized ammonia nitrogen at 0.06mg/l or less. The 24 hours median lethal concentration of un-ionized ammonia for *C. idella* fingerling was reported as 0.05mg/l and lethal dose of un-ionized ammonia was 0.075mg/l (Kosour, 2001). Nitrite stress cause alteration in activities of various tissues and serum enzymes in the fingerling of *Labeo rohita* and so stresses the need for proper management of nitrite in water during culture (Das *et al*, 2004). 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 short daily ammonia peaks may result in negative effect on growth equivalent to that found under chronic ammonia exposure in juvenile turbot, *Scophthalmus maximus* (Foss *et al.,* 2009).

The aim of this study was to study long term effect of sub-lethal ambient un-ionized ammonia nitrogen concentration on survival and growth rate of grass carp fingerling.

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 276-344 mg/l as $CaCO_3$, dissolved oxygen 5-8 ppm and zero ammonia nitrogen). Fish were fed daily with standard commercial formulated fish feed (22 % protein, 8% fat and 1% premix vitamin). Three different batches of *C. idella* mean weight 71.2 to 74.16 ± 1.2 g, at pH 8.3 and temperature 15-25 °C were used for growth test. Experiment was conducted in 8 fiberglass tanks capacity 350 liters containing 10 fish in each tank. Two different ammonia concentrations were used in each experiment with one replicate for control and two replicate for test. 27.3 g NH_4Cl/350 liters was added to get the 0.027 mg/l ammonia-nitrogen. In the other three tanks 29.84 g NH_4Cl/350 liters water was added to get 0.029 mg ammonia-nitrogen/l. All fish were fed daily with standard formulated commercial feed. Total ammonia nitrogen was measured weekly (minor changes in pH and salinity). The concentrated NH_4Cl was added to stabilize un-ionized ammonia nitrogen concentration. After 84 days fish were weighed. The rate of growth was determined as milligram per gram per day by applying the formula; Ln=(Wf - Wi) x 100/days. Wf = final weight of the fish; Wi= Initial weight of the fish; Days = duration of the experiment.

RESULTS AND DISCUSSION

Effect of un-ionized ammonia on growth of C. idella

The experiment was conducted to see the effect of un-ionized ammonia on growth. The experiment continued from 1st February to 25th April. The rate of growth decreased as the amount of ammonia increased.

The results are given in Table I and II. After 84 days the fish were weighed finally to see the growth which is faster *viz.*, 15 mg/gm/day in the control as there was no ammonia. As the concentration of ammonia nitrogen increases the rate of growth decreased. The survival was 100% in all three groups, which indicated that the fish was well maintained in each tank and was safe.

Carps show maximum growth at 27 °C. In this study temperature varied from 12-25.6 °C. Increase in water temperature also increases the proportion of un-ionized ammonia in an ammonia solution. Besides the absence of un-ionized ammonia, temperature is also a factor due to which the rate of growth in control tank is 15mg/g/day. As the water used in the present experiment had same alkalinity and hardness, they are very productive regarding their use in aquaculture. In this experiment, there was no ammonia nitrogen in the control tank hence the rate of growth was same as in control tank.

The maximum growth rate was observed in control tank which has no NH_3-N. In one group where the un-ionized ammonia concentration was 0.027mg/l the growth rate was 12.5mg/g/day. In third group, lowest growth rate of 11.2mg/g/day was observed at the highest concentration of un-ionized ammonia 0.029 mg/l. This may be due to loss of appetite. There are evidences that accumulation of metabolites in medium affect appetite (Paul and Smith, 1990; Cia and Summerfet, 1992). Fish subjective to long term exposure to pollutants exhibited reduction in appetite (Heath, 1987).

Table I: Water quality parameters of experimental tank before and during application of ammonium chloride.

Parameter	Mean±SD
Temperature °C	15.6±1.5
pH	8.5±0.5
Dissolved oxygen mg/l	6.7±1.5
Total alkalinity as $CaCO_3$ mg/l	420±6
Total hardness as $CaCO_3$ mg/l	296±7
Calcium mg/l	89±1.5
Magnesium mg/l	110±2
Chloride mg/l	27.5±1.5
Free CO_2 mg/l	2.45±0.05

Table II: Effect of ammonium chloride (group $C_{1(0.027mg/l)}$ and group $C_{2\ (0.029\ mg/l)}$) on grass carp after 84 days.

Days	pH	Weight (g)	Water Vol.	Temp. °C	Control	C_1	C_2	NH_4Cl (g/L)
84 (n=2)	8.30±0.3	74.2±1.6	350	15.25±0.3	-	-	-	0.00
84 (n=3)	8.29±0.2	71.2±1.2	350	15.53±0.3	-	0.22	0.21	0.078
84 (n=3)	8.2±0.27	73.3±1.9	350	15.92±0.2	-	0.03	0.02	0.084

Mean with standard errors are in mg/l and length in cm

Table III: Mean±SE of growth and survival of grass carp.

Parameters	Control	Unionized ammonia-nitrogen (mg/L)	
		0.027	**0.029**
Initial weight (g)	74.16±1.03	71.2±1.2	73.3±1.9
Final weight (g)	195.5±8.5	158.3±6.9	146.7±7.8
Survival	100%	100%	100%
Rate of growth	15mg/g/day	12.5mg/g/day	11.2mg/g/day

Decreased feeding rate may be due to ill effects of the components of ammonium chloride. The decreased food intake may be due to damage caused to taste receptors. The absorption of food decreased with increasing concentration of ammonium chloride in relation to the consumption of food. It is known that gastric concentration is an important factor in the regulation of appetite and food intake in the fish. So due to the inhibition of intestinal absorption the efficiency of absorption of digested food decrease and this will result in the retention of food in the alimentary canal. Consequently the rate of growth will also decrease. Briggs and Fing-Smith (1996) stated that ammonia accumulation affects growth through several mechanisms such as reduction of appetite and switching of urea excretion. In semi-intensive fish culture the maximum observed rate of growth is > 20mg/g/day at 28 °C (best temperature for growth).

Many workers have reported the effects of ammonia on number of fish species. *Labeo rohita* fingerlings subjected to sub-lethal un-ionized ammonia (0.0132mg/l) for 30 days exhibited significant changes in physiological metabolism (as described in introduction) the metabolic recovery was not observed within 30 days of exposure (Achraya *et al.*, 2004). Nitrite stress cause alteration in activities of various tissues and serum enzymes in the fingerling of *L. rohita* and so stresses the need for proper management of nitrite in water during culture (Das *et al*, 2004). Largemouth bass, *Micropterus salmoides* in sub lethal ambient ammonia concentration can have physiological disturbance that can impair the recovery of fish from exercise (Suski, *et al.,* 2007). The negative effect on growth and hyper-oxygenation under chronic exposure to ammonia is possible to be harmful in juvenile turbot (Foss *et al.,* 2007). The short daily ammonia peaks may result in negative effect on growth equivalent to that found under chronic ammonia exposure in juvenile turbot (Foss *et al.,* 2009)

In the present study maximum growth rate was 15mg/g/day. It may be due to low temperature of experimental water. As the growth experiments were conducted during February and April when temperature ranged from 15 °C to 25 °C. This study has contributed to the establishment of safe and acceptable levels of ammonia for long term growth of *C. idella.*

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