EGYPTIAN JOURNAL OF AQUATIC RESEARCH **ISSN: 1687-4285**
VOL. 33 NO. 1, 2007: 455-465

ACUTE AND CHRONIC AMMONIA TOXICITY TO NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGS

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Keywords: Nile tilapia, ammonia, acute, chronic toxicity

ABSTRACT

The effect of acute and chronic unionised ammonia (UIA) exposure on survival and growth performance in Nile tilapia fingerlings were examined. Median lethal concentrations (LC50) values of unionised ammonia at 24, 48, 72 and 96 hours post exposure were 1.67, 1.44, 1.19 and 0.93 mg/l, respectively. The chronic experiment was conducted over 60 days. Nile tilapia fingerlings with a mean initial weight of 10.43 g were reared in four stable ammonia concentrations [0.006 (control), 0.098 (low), 0.15 (medium) and 0.24 mg UIA/l (high)]. No mortality was observed during the whole experiment. No significant differences was found in the SGR between the control group (1.25) and low-concentration group (1.20) displaying higher growth rates than both the medium (0.58) and high-concentration group (0.31) through the experimental period ($P<0.05$). Increasing ammonia concentration affects feed conversion ratio (FCR) of fish exposed to 0.15 mg UIA/l and 0.24 mg UIA/l, while did not affect those exposed to 0.098 mg UIA/l. The no-observable effect concentration was 0.098 mg UIA/L. Increasing the concentration of ammonia higher than this value negatively affects the growth rate of fish. However, it could be concluded that although Nile tilapia fingerlings can tolerate the high ammonia polluted conditions, ammonia concentration should be kept lower than 0.098 mg UIA/L to obtain a good conditions for growth.

1. INTRODUCTION

There is a worldwide growing interest in the control of water quality within the fish farming process in order to improve the productivity of fish production systems and to enhance fish quality (Dosdat *et al*., 2003). It is well known that one of the most important limiting factors in intensive culture systems is the build up of toxic nitrogenous waste (Kir *et al*., 2004; Haywood, 1983; Person-Le Ruyet *et al*., 1997a).

Ammonia and urea are the two main nitrogenous products excreted by teleost fish (Forster and Goldstein, 1969), with ammonia usually presenting 60-90% of nitrogen excretion (Salin and Willot, 1991; Handy and Poxton, 1993). Ammonia which mainly is excreted through the fish gills primarily dependant on protein intake, and on the metabolic efficiency of the fish, which is species specific and is affected by increasing levels of ambient ammonia.

Total ammonia nitrogen (TAN) is composed of un-ionised (NH_3-N) and ionised forms $(NH_4)^+$ (Losordo *et al*., 1992; Masser *et al*., 1992). Both the ionised and unionised forms of total ammonia nitrogen are toxic to fish, but the unionised form seems to be much more toxic to fish. The equilibrium between the two forms is highly dependent on pH, salinity and temperature (Handy and Poxton, 1993). The NH_3 molecule is nonpolar and readily soluble in liquids. It is 300-400 times more toxic than NH_4^+ (Thurston *et al*., 1981; Haywood, 1983; Chin and Chen, 1987;

Frias-Espericueta *et al*., 1999). Ammonia is toxic, not only to fish but also to all aquatic animals (Zhao *et al*., 1997; Harris *et al*., 1998), especially in pond aquaculture at low concentrations of dissolved oxygen (Alabaster *et al*., 1983).

Accumulation of ammonia in such confined areas deteriorates water quality, and may show down the growth, increase oxygen consumption and ammonia-N excretion, affect hemolymph and free amino acid levels and may even cause high mortalities (Chen and Lin, 1992; Chen *et al*., 1994).

Acute toxicity of ammonia to fish has been investigated in a number of species (Person-Le Ruyet *et al*., 1995; Abdelmoez and Abdalla, 1998; Sampaio *et al*., 2002; Kir *et al*., 2004; Evans *et al*., 2006; Karasu Benli and Koksal, 2005). But studies on the effects of chronic un-ionised ammonia exposure in fish are scarcer. Chronic UIA exposure may affect fish and other organisms in several ways, e.g. gill hyperplasia (Smart, 1976), changes in mucous production, growth and stamina (Lang *et al*., 1987), muscle depolarisation (Taylor, 2000) and may also act directly on the central nervous system, causing hyperventilation (McKenzie *et al*., 1993), hyperexcitability, coma, convulsions and finally death (Ip *et al*., 2001).

Reduced growth rates due to UIA exposure have been reported in several species, (Wajsbrot *et al*., 1993; Person-Le Ruyet *et al*., 1997a,b; Frances *et al*., 2000; Dosat *et al*., 2003; Foss *et al*., 2003; Lemarie *et al*., 2004; Foss *et al*., 2004; El-Shafai *et al*., 2004). Of the species tested, salmonids were found to be the most sensitive whereas carp and catfish were the most resistant (Richardson, 1991).

It has been reported that tilapia can withstand high levels of ammonia. Abdelmoez and abdalla (1998) suggested that Nile tilapia has tolerance to un-ionized ammonia similar to that of other tilapia species, somewhat greater than that of channel catfish, and greater than that of many other warm water fish and salmonids. Although the toxicity of ammonia was studied for many species, the complete lack of data for Nile tilapia (*Oreochromis niloticus*) is evident (Karasu Benli and Koksal, 2005).

The purpose of this study is to investigate the effect of acute toxicity and chronic exposure to ammonia on survival and growth of Nile tilapia fingerlings in two consecutive experiments.

2. MATERIALS AND METHODS

2.1. FISH SPECIES AND EXPERIMENTAL CONDITIONS

Nile tilapia *Oreochromis niloticus* fingerlings (mean weight 10.43±1.47 g) used in these experiments were produced from broodstock kept in the fish research unit Faculty of Agriculture, Cairo University. The fish were acclimated to the rearing conditions for 15 days prior to the toxicity tests. During this acclimation period, water in tanks was aerated continuously to maintain dissolved oxygen above 6.5 mg/L and renewed in every 24 h. Water temperature and pH values were 28 ± 2 °C and 7.5 ± 0.4, respectively.

Ammonium chloride (NH_4CL) was used as a source of ammonia. Ammonia stock solutions were prepared by dissolving required amounts of ammonium chloride (NH_4CL) (Merck reagent grade) in fresh water.

The total ammonia nitrogen concentration was determined using the boric acid-sulphuric acid titration method (APHA, 1998). Un-ionised ammonia nitrogen (UIA-N) concentrations were calculated using the general equation of bases (Albert, 1973):

$$NH_3 = \frac{[NH_3 + NH_4^+]}{[1+10^{(pka-pH)}]}$$

In fresh water, the calculation of pk_a is based on the equation developed by Emerson *et al*. (1975):

$pk_a = 0.09018 + 2729.92/\ T$

(T= Kelvin= 273 +T °C)

pH was measured daily in all tanks using a digital pH meter. Dissolved oxygen and temperature was measured using oxygen meter (YSI model 55).

2.2. EXP. 1: LETHAL MEDIAN CONCENTRATION (LC_{50}):

A number of 308 Nile tilapia fingerlings (10.43 ±1.47 g) were used to determine the short-term LC_{50} (median lethal concentration) toxicity tests according to the methods described by the American Public Health Association (1998). The fish were stocked in 60-L aquaria (14 fish / aquarium). Ten different concentrations of ammonia and the control group in two replicates were used to determine the tolerance limits of TAN and NH_3-N. Experimental concentrations of TAN ranged from 5.22 to 52.7 mg/l. The unionized ammonia concentrations were 0.24, 0.47, 0.72, 0.93, 1.19, 1.44, 1.67, 1.89, 2.13 and 2.43.

The experimental medium was changed every 24 h with fresh solution. Water was aerated continuously by compressed air to maintain the oxygen concentration above 6.5 mg / L. The pH values ranged from 7.1 to 7.9 during the whole experiment. The temperature was the same in all tanks, ranging from 26-30°C during this experiment. During this experiment, fingerlings were not fed. Fish mortality observation was made at 12-h intervals up to 96 h.

2.3. EXP. 2: CHRONIC EXPOSURE

During this experiment which lasted 60 days, 56 fingerlings were distributed evenly among eight 60-L continuously aerated aquaria (7 fish / aquarium). The aquaria were static systems cleaned by suction daily, where approximately 10% of the water in the aquaria was replaced daily. Water was exchanged completely every three days. Fish were fed extruded pellets (30% protein).

Three different concentrations of ammonium chloride (7.64, 15.28 and 22.92 mg/L) were fed into the aquaria in order to obtain a range of three ambient TA-N concentrations, subsequently three UIA concentrations from 10% to 30% 96-h LC_{50}. The measured TAN-N values for the control, treatment 1, 2 and 3 were 0.17±0.02, 2.70±0.09, 4.3±0.21 and 6.80±0.09, respectively. The calculated unionised ammonia was 0.006±0.001, 0.098±0.019, 0.157±0.031 and 0.248±0.089 mg UIA-N/l in the control, treatment 1, 2 and 3, respectively. Except ammonia, the environmental conditions were stable in all tanks. The pH values ranged from 7.1 to 7.9 during the whole experiment. Dissolved oxygen never fell below 6.5 mg/l in all tanks. The temperature was the same in all tanks, ranging from 23-31°C during the experiment.

Mean final wet weight of fishes was determined. Specific growth rate (SGR) was determined as:

$$SGR = \frac{(\ln W_f - \ln W_i) \times 100}{T}$$

and daily weight gain (DWG) was determined as :

$$DWG = \frac{W_f - W_i}{T}$$

Where W_f is the mean wet weight in grams at the end of experiment, W_i is the initial mean wet weight and T the duration of the experiment. Mortality rate was determined during the experiment.

2.4. STATISTICAL ANALYSIS

All statistical analysis was carried out using the SPSS program version 8.0. Data were tested for significant differences by one-way analysis of variance followed by the Duncan's multiple range tests. P values < 0.05 were considered to be significant.

3. RESULTS

3.1. ACUTE TOXICITY EXPERIMENT

3.1.1. Behaviour of fish

During the acute toxicity experiment, no change in behavior occurred in the control group. Nile tilapia fingerlings subjected to ammonia concentrations in the other treatments showed increasing movements, erratic swimming, efforts to swallow air from the surface of water, increase in ventilation and death. Also, changes in the color (darkened skin), an excessive mucus secretion on the body and in the gills and gaping in the mouth and gills of the dead fish were observed.

3.1.2. LC50 data

The median lethal concentration values (LC_{50}) data are shown in fig.1. No fish died in the control treatment. Tolerance of *O. niloticus* fingerlings to TAN and NH3-N showed a decrease with the increase in concentration. After 24, 48, 72 and 96 h of exposure, the LC_{50} values were 36.3, 31.19, 25.82 and 20.31 mg/L, respectively for TAN and 1.67, 1.44, 1.19 and 0.93 mg/L, respectively for NH_3-N.

3.2. CHRONIC TOXICITY EXPERIMENT

No mortality was observed in any of the experimental groups throughout the experimental period. Table (1) showed the growth performance of Nile tilapia fingerlings during the experiment. The mean values of specific growth rate (SGR) of Nile tilapia fingerlings were 0.31, 0.58, 1.20 and 1.25 for treatments 1, 2, 3 and the control. No significant differences was found between the control group (0.006 mg UIA/L) and low-concentration group (0.098 mg UIA/L) displaying higher growth rates than both the medium (0.15 mg UIA/L) and high-concentration group (0.24 mg UIA/L) through the experimental period ($P<0.05$). Ammonia concentration affected significantly feed conversion ratio (FCR) of fish exposed to 0.15 mg UIA/L and 0.24 mg UIA/L, while did not affect those exposed to 0.098 mg UIA/L (Fig. 2).

Table (1): Growth performance of Nile tilapia fingerlings exposed to different concentrations of ammonia toxicity.

Parameter	Control (0.006 mg UIA/L)	Low (0.098 mg UIA/L)	Medium (0.157 mg UIA/L)	High (0.248 mg UIA/L)
Initial body weight (g/fish)	10.67±1.62	9.82±1.60	10.89±1.25	10.35±1.32
Final body weight (g/fish)	22.75 ± 3.92^a	20.17 ± 3.14^b	15.55 ± 2.15^c	12.51 ± 1.61^d
Weight gain (g/fish)	12.07 ± 2.35^a	10.35 ± 1.84^b	4.65 ± 0.97^c	2.16 ± 0.41^d
Daily weight gain (g/fish/day)	0.20 ± 0.03^a	0.17 ± 0.03^b	0.077 ± 0.016^c	0.036 ± 0.006^d
SGR (%)	1.25 ± 0.073^a	1.20 ± 0.12^a	0.58 ± 0.06^b	0.31 ± 0.03^c
FCR (%)	1.4 ± 0.12^a	1.46 ± 0.22^a	3.48 ± 0.28^b	7.08 ± 1.02^c

-Mean±SD

-Means in the same row with different letters are significantly different ($P<0.05$)

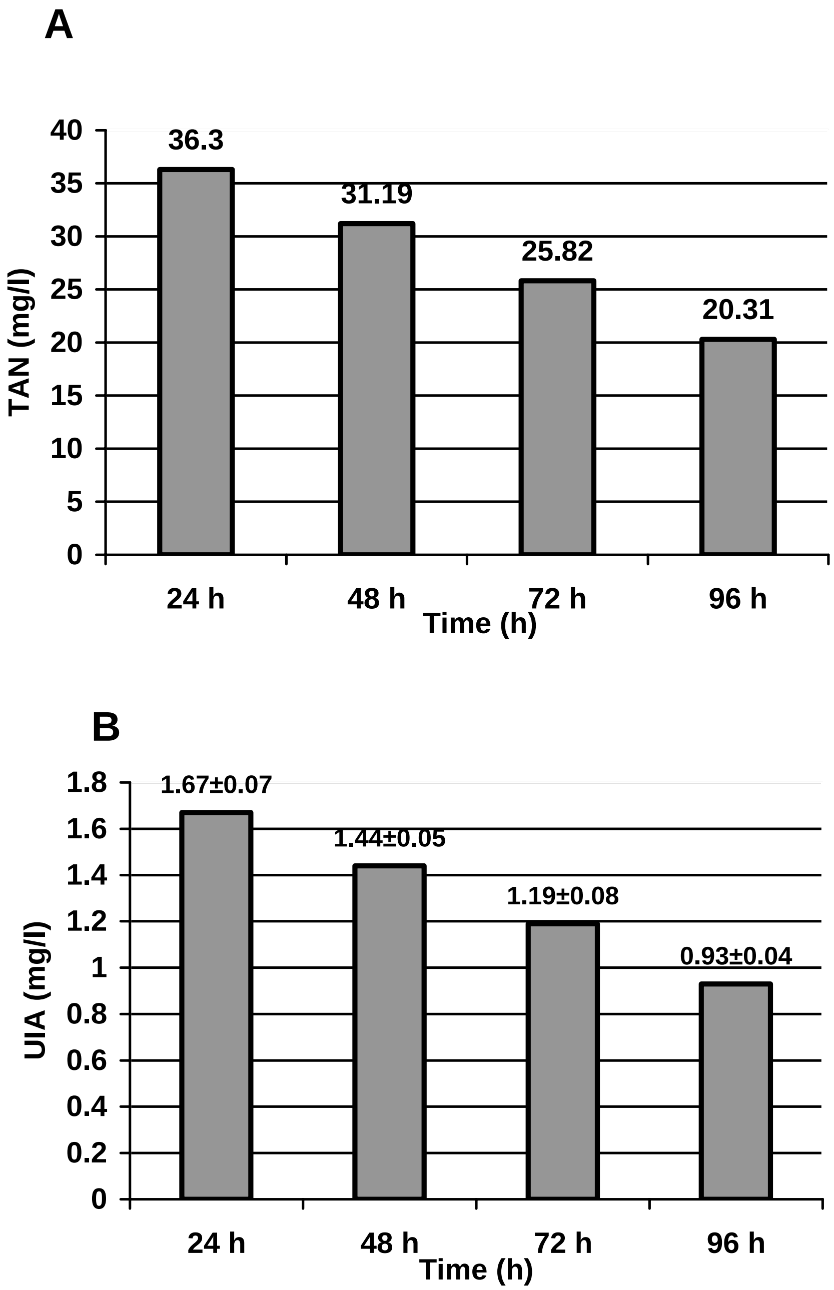

Fig. (1): LC50 (median lethal concentration) of total ammonia nitrogen (mg TAN/L) (A) and unionised ammonia (mg UIA- N/L ± SD) (B) for Nile tilapia fingerlings exposed to different concentrations.

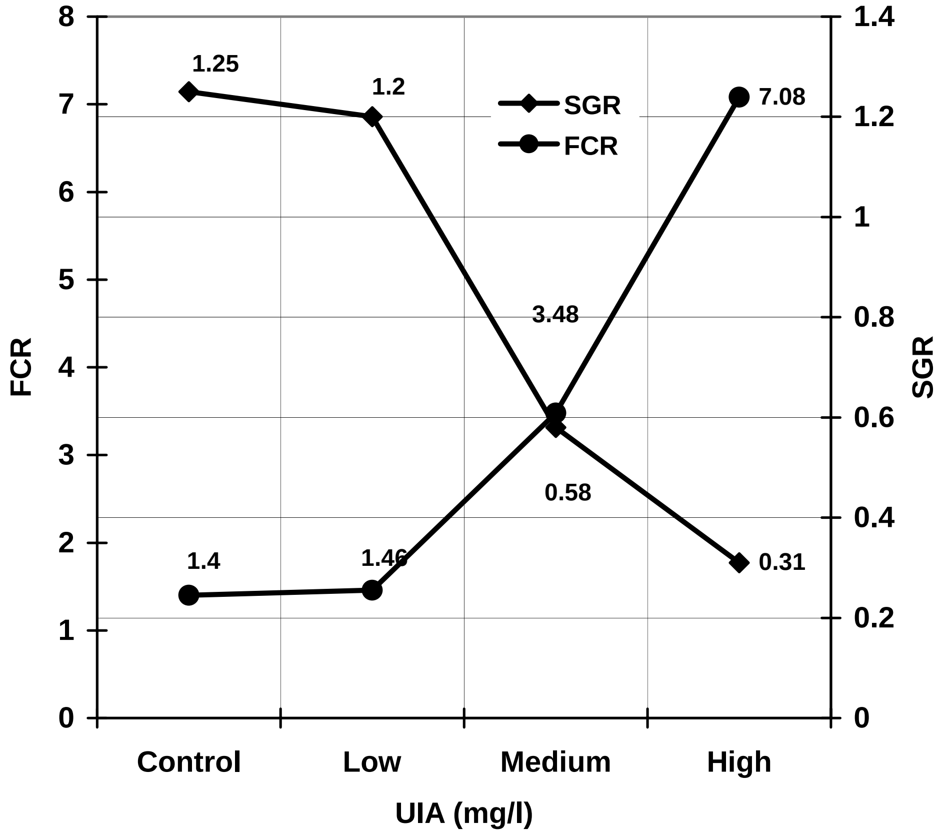

Figure 2. Specific growth rate (SGR) and Feed conversion ratio (FCR) in relation to UIA concentration

4. DISCUSION

The present results indicated that the acute toxicity of ammonia (24h, 48h, 72h and 96h LC_{50}) on Nile tilapia fingerlings were 1.67, 1.44, 1.19 and 0.93 mg UIA/L. Similar results were reported by Evans *et al*. (2006) which indicated that LC_{50} values were 1.46, 1.46, 1.33 and 0.98 mg/L UIA for Nile tilapia at 24, 48, 72 and 96 hours post exposure. On the other hand, Karasu Benli and Koksal (2005) and Abdelmoez and Abdalla (1998) reported 7.39 and 1.36-2.65 at 48 and 96 hours post exposure of Nile tilapia, respectively. These differences may be due to the differences in the average size of fish, method of calculating LC_{50} and the experimental conditions such as temperature and pH values. In red tilapia species (*O. mossambicus* × *O. niloticus*) the 48-h LC_{50} was determined as 6.6 mg UIA/L by Daud *et al*. (1988). For other warm water fish species, the LC_{50} values ranged between 0.43-2.1 mg/L NH_3 for common carp (Hasan and Machintosh, 1986) and 1-3.8 mg/L NH_3 for channel catfish (EPA, 1998).

The observed effects on the changes in behavior were similar to these reviewed by Haywood (1983), Montfort *et al*. (2000), Lemarie *et al*. (2004) and Karasu Benli and Koksal (2005). The increase in oxygen consumption movement and the abnormal swimming at the surface were observed. They explained these observations due to the deformations in the gill lamella and the buildup of ammonia in the blood and other tissues (brain, liver, muscle), which has negative effects on synaptic connections of the central nervous system and the *N*-methyl-*D*-aspartic acid (NMDA) receptor activity.

In the chronic experiment, Nile tilapia fingerlings were exposed to ambient UIA concentrations ranging from 0.098 to 0.24 mg/l for 60 days to determine the chronic effect of ammonia. These concentrations were used to obtain a range of UIA from 10% to 30% of 96-h LC_{50}s reported in the acute toxicity experiment. Lemarie *et al*., (2004) used a rang of concentrations which represent from 10% to 40% of the 96-h LC_{50}s reported for sea bass.

No mortality was detected up to a level of 0.24 mg UIA-N/l which represents 25.8% of the 96LC_{50} value. Nasr *et al*. (1998) and El-Shafai *et al*. (2004) reported no mortality with ammonia exposure up to 0.45 and 0.434 mg UIA/l for Nile tilapia juveniles, respectively.

In this experiment, growth performance of Nile tilapia fingerlings was significantly influenced by UIA concentration except the low concentration treatment (0.098 mg UIA/l) which reported to be the no-observable effect concentration. This value is closed to 0.068 mg UIA/L observed in Nile tilapia fingerlings (El-Shafai *et al*., 2004). However, Szumski *et al*. (1982) suggested that the non-effect ammonia criterion of 0.08 mg UIA/l could be applied to warm water fish.

As in our results, El-Shafai *et al*. (2004) did not record a reduction in feed intake in ammonia-exposed Nile tilapia fingerlings up to 0.43 mg UIA. Ammonia affects the internal physiology of the fish. So, the fish may consume feed but do not assimilate it. However, several studies demonstrated the different reasons of reduction in the growth rate during ammonia exposure. Rasmussen and Korsgaard (1996) and Foss *et al*. (2003) found that reduced growth was attributed to a decrease in food intake with increasing UIA concentrations and to a reduced food conversion efficiency for juveniles turbot and spotted wolfish, respectively. Otherwise, food conversion efficiency was unaffected by ambient UIA concentration.

5. CONCLUSIONS

The present study demonstrated that Nile tilapia fingerlings can tolerate relatively the high ammonia concentration. However, ammonia concentration should be kept lower than 0.098 mg UIA/L. increasing the concentration of ammonia above this level may negatively affect the growth rate of fish.

ACKNOWLEDGMENTS

The authors would like to thank Dr. M. A. Elnady for valuable comments on an earlier version of this manuscript.

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