The Acute Toxicity of Ammonia on Tilapia (Oreochromis niloticus L.) Larvae and Fingerlings

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Abstract: The aim of the present study was to determine median lethal concentrations (LC 50) of unionised ammonia (NH_3) for the larvae and fingerlings of Tilapia (*Oreochromis niloticus* L. 1758). The mean 48 h LC50 values were determined as 1.009 ± 0.02 mg/l for larvae and 7.40 ± 0.01 mg/l for the fingerlings. Larvae exposed to different concentrations of ammonia moved very rapidly, lost equilibrium in water and showed sideways swimming. In the case of the fingerlings, an increase in their movements, ventilation, convulsions, spiral swimming, efforts to swallow air from the surface of water, increase in the mucous secretion in the gills and on the body surface, haemorrhage in the gills, and darkening in the eye and on the skin were observed. The histological study showed hyperplasia and fusion of the lamella on the gills.

Key Words: Tilapia, *Oreochromis niloticus*, unionised ammonia (NH_3), toxicity

Tilapia Balığı (*Oreochromis niloticus* L.) Larva ve Yavruları Üzerine Amonyağın Akut Toksisitesi

Özet: Bu çalışmada, Tilapia (*Oreochromis niloticus* L. 1758) larva ve yavrularında iyonize olmamış amonyağın (NH_3) ortalama öldürücü konsantrasyonları (LC50) saptanmıştır. 48 saat ortalama LC50 değerleri larvalarda $1,009 \pm 0,02$ mg/l ve yavrularda $7,40 \pm 0,01$ mg/l olarak belirlenmiştir. Farklı amonyak konsantrasyonlarına maruz kalan larvaların çok hızlı hareket ettiği, suda dengesiz ve yan yattıkları gözlenirken, yavru balıkların hareketlerinde artma, titreme, düzgün olmayan yüzme şekli, suyun dışından hava yutmaya çalışma, solunum hızında artma, vücut yüzeyinde ve solungaçlarda mukoz salgıda artma gözlenirken, solungaçlarda hemorraji, göz yuvarında ve deride koyulaşma belirlenmiştir. Histolojik olarak incelenen solungaçlarda ise hiperplazi ve lamellar yapışma (füzyon) saptanmıştır.

Anahtar Sözcükler: Tilapia, *Oreochromis niloticus*, iyonize olmamış amonyak (NH_3), toksisite

Introduction

The economics of most modern aquaculture operations require that animals be cultured at high densities. A consequence of high-density aquaculture is the increased probability of exposure of the animals to elevated concentrations of nitrogenous wastes, particularly to ammonia and nitrite (1,2). Ammonia, an end product of the protein catabolism, represents 60% to 80% of nitrogenous excretion of fish (3). In addition, ammonia can enter natural waters from sewage effluents, industrial wastes and agricultural materials (4,5).

In intensive fish farming, excessively high doses of ammonia, issuing either from excretion or from external pollution can cause reductions in fish growth or even death (3,6,7). For these reasons, toxicity of ammonia to fish has been intensively investigated in numerous of fish species (6-8). Of the species tested, salmonids were found to be the most sensitive, whereas coarse fish like carp and catfish were the most resistant (9).

It was demonstrated that the toxicity of ammonia depends principally upon the presence of NH_3 and the toxicity of NH_3 was considered to be relatively independent of pH and temperature while NH_4^+ was regarded as having little or no toxicity (10). The toxicity of NH_3 is ascribed to the fact that this unionised form (NH_3) of ammonia can readily diffuse across the gill membranes due to its lipid solubility and lack of charge, whereas the ionised form (NH_4^+) occurs as a larger hydrated form with charged entities which cannot readily

pass through the hydrophobic micropores in the gill membrane (5). However, it has been shown that NH_4^+ may also have considerable toxicity under low pH conditions (7).

It has been reported that tilapia can withstand very polluted environment, feeding on animal manure and even sewage sludge (11). Thus, the intensification of Nile tilapia farming needs to optimise all the production factors, especially the quality of water. Although the toxicity of ammonia was studied for many species, the complete lack of data for Nile tilapia (*Oreochromis niloticus* L.) on this problem led us to carry out a study of ammonia toxicity for growing stages of Nile tilapia.

Materials and Methods

Fish Storage Conditions

This study concerns Nile tilapia (*Oreochromis niloticus* L.) obtained from artificial reproduction and weighing 0.05628 ± 0.0083 g (larvae) and 10.114 ± 0.045 g (fingerlings) in Ankara University Fisheries Research Centre. Totally 240 larvae and 240 fingerlings were used in the experiments.

The larvae were randomly selected from hatch tanks and transferred to one of the twelve 3-l jars just before the experiment (10 larvae/jar).

The fingerlings were taken from the tanks where they were usually stocked and put into the experimental system (10-l aquarium) at least 10 days before the beginning of the experiment (10 fingerlings/aquarium). During this acclimatisation period, water in the tanks was aerated continuously and renewed in every 24 h. Water temperature and pH value were maintained at the same values as the prescribed experimental conditions. Fingerlings were fed with a commercial pellet twice a day. Feeding was stopped 48 h prior to the experiment. The photoperiod was maintained on an 18 h L: 6 h D cycle. The initial and final concentrations of ammonia, temperature (mercury thermometer) pH, (Metrohm Herisau Digital pHmeter E 532) and O_2 values (YSI Model 51 B) in each jar and aquarium were determined 5 times a day The pHmeter and oxygenmeter were recalibrated before each measurement. Temperature, DO, pH, total hardness and NO_2 were 23 ± 1 $^{\circ}$C, 7.2 ppm, 8.0 ± 0.2, 13.2 $^{\circ}$F and 0.001 mg/l, respectively.

Renewal (semi-static) 48-h tests were carried out to determine the lethal concentrations for 50% of the fish (48-h LC50). The experimental technique followed that of APHA (12), Reish and Oshida (13), and ISO (14). The experimental medium was changed every 24 h with fresh solution. Water was aerated by compressed air to maintain the oxygen concentration above 7 mg/l.

Ammonium chloride (NH_4Cl) was used as a source of ammonia and 1 M stock solutions were prepared. The pH of ammonia stock solution was kept constant at the desired test value by adjusting the level of $NaCO_3$ (7). All chemicals used were analytical reagent grade. Same procedures outlined above were followed in the experiments with larvae and fingerlings.

Experiment 1

Initially, a range finding test was carried out to determine the main experimental concentrations. The test concentrations were obtained by serial dilution of the ammonia stock solution. In this test, wide intervals as 1, 1/10, 1/100, 1/1000, 1/10,000 and a control group were used in two replicates (15). The tests were conducted for both larvae and fingerlings.

Experiment 2

Second experiments were conducted for 5 different concentrations of ammonia and a control group in two replicates to determine median lethal concentrations (LC50) both larvae and fingerlings. These five different main experimental concentrations were determined based on 0%-100% death of fish in 24 h in the first experiments and then were selected from a logarithmic scale (12,13). In these experiments main concentrations were ranging from 0.018, 0.0155, 0.0135, 0.0115, 0.0075 g NH_4Cl/l for larvae and 0.18, 0.155, 0.115, 0.075, 0.037 g NH_4Cl/l for fingerlings. The concentrations of ammonia in the water were measured spectrophotometrically with phenate method (12) and calculated from the measured values, the pH and the temperature using the formulae of Emerson et al. as given elsewhere (7).

Median lethal concentration values (LC50) for both larvae and fingerlings were calculated using probit analysis method (13).

In order to illustrate the disorder caused by ammonia the behaviour of the larvae and fingerlings were observed. In addition, the gills, fins, skin, spleen, kidney and liver of the fingerlings were examined macroscopically and the branchial lamellae of fish were taken for microscopic preparation (Crossmon triple stain) (16).

Results

LC50 values

The unionised ammonia concentrations were 0.59, 0.83, 1.01, 1.25 and 1.52 mg/l, and 0.61, 0.81, 1.04, 1.22 and 1.55 mg/l in the larvae jars, and 2.18, 4.79, 7.58, 9.09 and 11.33 mg/l, and 2.28, 5.17, 7.47, 8.75 and 12.05 mg/l in the aquariums of fingerlings in the first and the second replicates, respectively.

The acute toxicity of ammonia (48 h) of larvae of Nile tilapia (*Oreochromis niloticus* L.) under semi-static test conditions varied between 1.007 and 1.01 mg/l NH_3. In fingerlings these values were found to be 7.39 and 7.41 mg/l NH_3, for the first and second replicates, respectively.

Mortalities caused by increased ammonia concentrations for the larvae and the fingerlings are presented in Figures 1 and 2.

Behaviour of fish and clinical signs

During the toxicity experiments, both the larvae and the fingerlings showed similar reactions, nevertheless the behaviours of fingerlings were observed more clearly than those of the larvae. The larvae exposed to different concentrations of ammonia moved rapidly, lost equilibrium in water and began to sideways swimming contrary to the control group.

In the fingerlings, an increase in their movements, convulsions, spiral swimming, efforts to swallow air from the surface of water, increase in ventilation and death were observed compared to the control group. The mouth and the gills of the dead fish were gaping. The external observations of Nile tilapia fingerlings during the tests allowed us to define the following clinical signs compared to the control group: an increase in the amount of mucus secretion in the gills and on the body surface, haemorrhage in the gills and darkening in the eye and on the skin. However, there were no external differences observed in the liver, the spleen or the kidney.

Microscopic observations

In the experiments, the gill lamellae of the fingerlings exposed to different ammonia concentrations were also studied histologically, resulting in distinguishable (Figure 3a, 3b, 3c) deformations compared to the control group (Figure 3d). Besides, gill hyperplasia and lamella fusion on the lamellae were established as given in Figures 3a, 3b, and 3c. No histological changes were observed from branchial lamellas of the tested groups exposed to various ammonia concentrations.

Discussion

Although the effects of the toxicity of ammonia on fish were studied in numerous fish species, it is difficult to make a direct comparison amongst the available results, since the methods of calculating the LC50 are not the same and fish size and species are different. In this study, the acute toxicity of ammonia (48-h LC50) on Nile tilapia (*Oreochromis niloticus* L.) larvae and fingerlings were determined as 1.007-1.01 mg/l and 7.39-7.41 mg/l in two different replicates, respectively. Similarly, Daud et al. (17) reported 6.6 mg/l for 48-h LC50 in hybrid Tilapia species (*O. mossambicus x O. niloticus*). The discrepancy can be attributed to the difference in the average size of the fish, which was 8.9459 ± 0.0152 cm in the current study, whereas Daud et al. (17) used 2.13
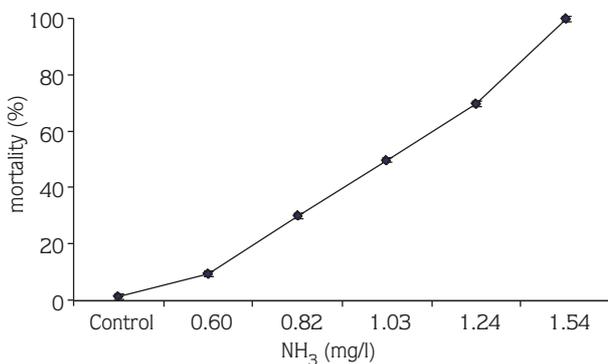

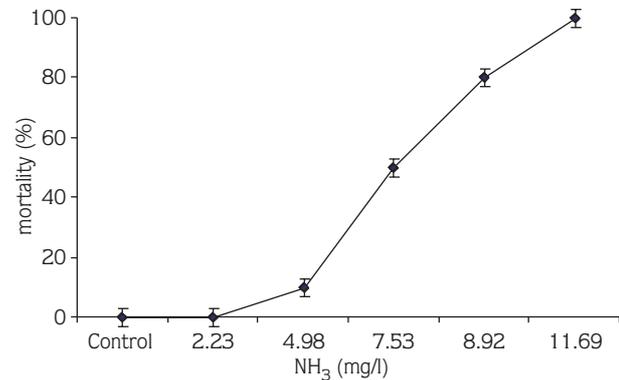Figure 1. Mean mortality in tilapia larvae exposed for 48 h to different NH_3 concentrations.

Figure 2. Mean mortality in tilapia fingerlings exposed for 48 h to different NH_3 concentrations.
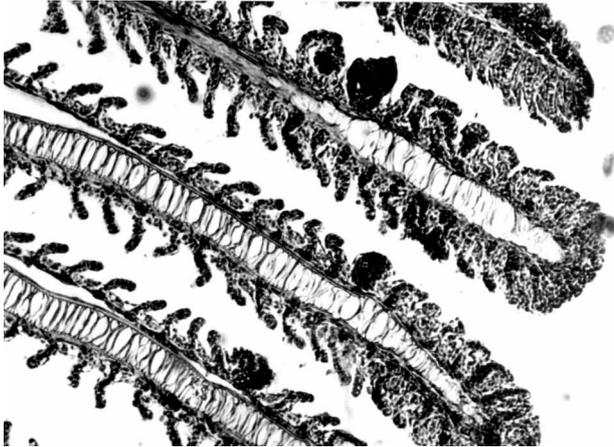
Figure 3a. Gill lamella fusion of tilapia exposed to ammonia for 48 h at 2.18 mg/l (200x).
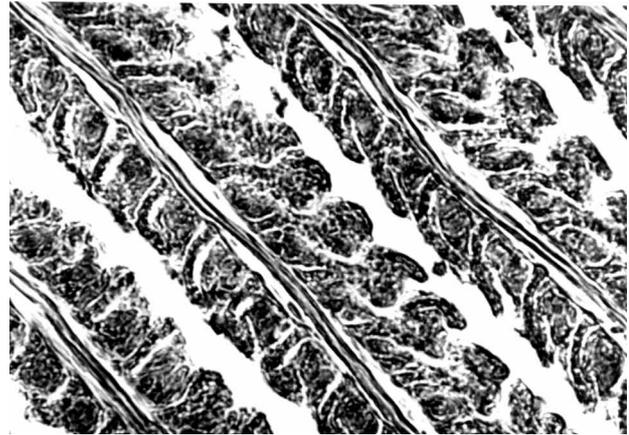

Figure 3b. Gill lamella fusion and hyperplasia of tilapia exposed to ammonia for 48 h at 4.79 mg/l (200x).
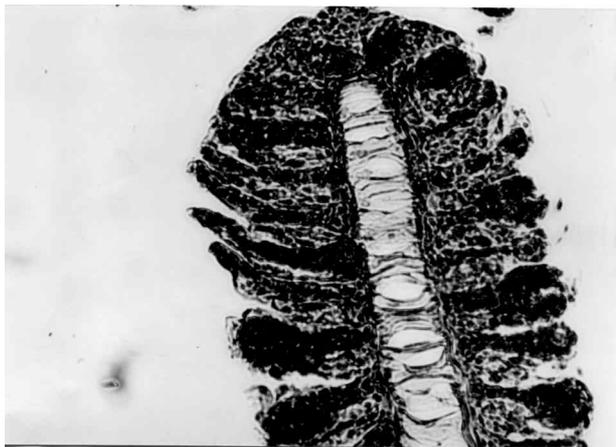

Figure 3c. Gill lamella fusion of tilapia exposed to ammonia for 48 h at 4.79 mg/l (400x).
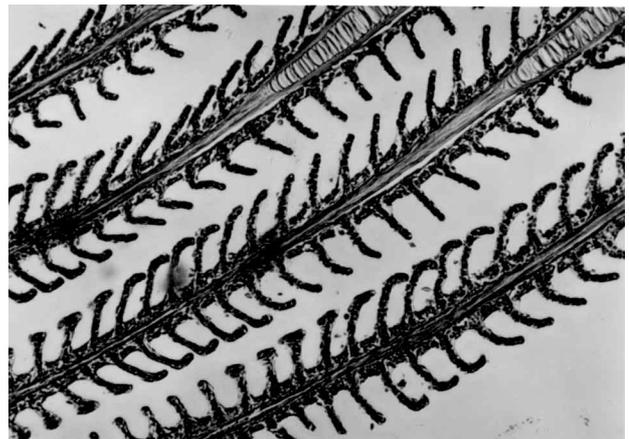

Figure 3d. Gill lamella of control group (200x).

± 0.35 cm fishes. Besides, the tilapia species used in both studies are different. The most sensitive species to ammonia is known as rainbow trout (*Oncorhyncus mykiss*) and LC50 values ranged between 0.068 and 0.62 mg/l NH_3 according to life stage (18). Common carp (*Cyprinus carpio*) with LC50 range of 0.43-2.1 mg/l (4) and channel catfish (*Ictalurus punctatus*) with LC50 range of 1-3.8 mg/l can be counted as the more tolerant species (7). So, based on the findings of this study, it is apparent that tilapia is the most tolerant species to ammonia. Besides, Wong (11) reported that tilapia can withstand a very polluted environment, feeding on animal manure and even sewage sludge.

The results of the current study indicate that tilapia larvae are less tolerant to ammonia level than the fingerlings. The differences in sensitivity to ammonia based on the growing stage have been already observed in other species. For instance, Reinbach-Klinke found out that fry is more sensitive to ammonia than the larger trout (6). In addition, Salin and Williot (3) observed that the youngest fish (60 to 260 mg) are the ones which show the highest sensitivity to ammonia with a 24-h LC50 value of 1 mg NH_3/l, whereas for the large fish (about 450 g), this value increase to 2.5 mg/l NH_3. Similar results were reported by many other researchers for the cases of species such as rainbow trout (15), common carp (19) and green sunfish (20).

When the behaviours of the fish were examined, both the larvae and the fingerlings showed similar reactions. But, the behaviour of fingerlings was observed more

clearly than that of the larvae. The larvae exposed to different concentrations of ammonia moved more rapidly, lost equilibrium in water and began to show sideways swimming. The fingerlings, on the other hand, showed increase in their movements, ventilation and death with convulsions, spiral swimming and efforts to swallow air from the surface of water. The mouth and the gills of the dead fish were gaping. The behaviour of intoxicated fish was similar to that described by other authors (17,21,22). Daud et al. (17) observed that red tilapia fry swim erratically prior to death. Knoph (22) recorded coughing, hyperventilation followed by sporadic ventilation, twisting, loss of equilibrium, spiral swimming, convulsions and death following a short period in a coma-like state, in which there were no body motions except weak movements of the gills. At death, the mouth was usually gaping and the gills were widely extended. Smart (21) determined that increasing the unionised concentrations in water increased the oxygen consumption 3.3-fold. Similar reactions were also observed for Siberian sturgeon (*Acipencer baeri*): increased ventilation, loss of equilibrium, swimming on the back and finally a very violent tendency followed by death.

When the clinical symptoms were evaluated, an increase in mucus secretion in the gills and on the body surface, haemorrhage in the gills and darkening in the eye and on the skin were observed. Increase in mucus secretion in the gills and on the body surface also appeared as a symptom of gill necrosis (4) and was observed by Salin and Williot (3) on Siberian sturgeon. Darkening on the eye and on the skin was thought as a reaction of fish to the toxicant. Gill haemorrhage was also observed in acute toxicity study by Daud et al. (17) on red tilapia and a sublethal toxicity study by Kucuk (23) on blue tilapia (*Oreochromis aureus*).

Finally, gills were studied histologically and some lamella deformations were observed. Gill hyperplasia and lamella fusion were also reported. Although Westers used 0.0125 mg/l NH_3-N as the maximum allowable concentration for fish culture, Burrows reported extensive hyperplasia of gill epithelium in chinook salmon, *Oncorhyncus tshawytscha*, after exposure to only 0.005 mg/l of NH_3-N for 6 weeks (6). So it is clear that the first problem would appear on the gill tissue when the ammonia concentration in water increases as proven also in this study. Furthermore, Kirk and Lewis (24) reported that the gills of the rainbow trout exposed to 0.1 mg/l ammonia for 2 h exhibited deformation of the lamellae. The filamental and lamellar epithelium was covered with shallow, circular depressions in which the integrity of microridges was maintained. Salin and Williot (3) observed that Siberian sturgeon (270 g) exposed to more than 60 mg/l of ammonia reveal a modification of the epithelium of the secondary lamellae and the base of the filament is slightly turgescent. Similar results were also confirmed by Mitchell and Cech (25), who studied channel catfish.

Consequently, it was clearly shown that tilapia tolerated very polluted conditions. And considering other published data mentioned above, it could be considered superior to other fish species in this respect. Chronic effects of ammonia on blood (haematology) and tissues (histopathology) of Nile tilapia should also be examined.

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