

Effect of Sublethal Unionized Ammonia on Mortality Rate of Red Tilapia (*Oreochromis Spp.*) Fingerlings in *Aeromonas hydrophila* Infection

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Abstract

A study was carried out to investigate the effect of sublethal unionized ammonia concentration on the mortality rate of tilapia fingerlings in *Aeromonas hydrophila* infection. Mono-sex tilapia about 5 cm in length was used in this experiment. Sublethal unionized ammonia concentrations were determined and 1.1, 1.7, 2.2, 3.6 and 4.6 mg/L ammonium chloride was chosen for the experiment. The sublethal effects of unionized ammonia concentration on *A. hydrophila* infection in fish fingerlings were then determined. There were 2 mortalities each at ammonia concentrations of 2.2 and 3.6 mg/L in fingerlings infected with *A. hydrophila*. However, there seemed to be no association between concentration of unionized ammonia and fingerling mortality suggesting that sublethal unionized ammonia concentrations do not cause death in fingerlings exposed to *A. hydrophila* infection.

Keywords: Unionized ammonia, Tilapia, *A. hydrophila*, mortality rate

Introduction

The accumulation of ammonia in aquatic systems is due to the metabolism of protein from uneaten feed and organic matter inside the pond. However, fish themselves, either being fed or starved are the primary source that excrete ammonia as their byproduct (Francis-Floyd *et al.*, 2005). Fishes exposed to sublethal ammonia concentrations displayed histopathologic alterations in the gills, liver and kidney. Gill tissues showed hyperemia, chloride cell proliferation, fusion in secondary lamella, and telangiectasis. Liver tissue revealed cloudy swelling and hydropic degeneration, whereas in kidney tissues, hyperemia and glomerulonephritis were observed (Aysel *et al.*, 2008). *Aeromonas hydrophila* and other aeromonads are among the most common bacteria in freshwater habitats throughout the world. The clinical signs shown by affected fishes varied from septicemia, ascites, erosion,

ulceration, detachment of scale and exophthalmia. The postmortem findings varied from congestion to focal lesions in the liver, spleen, and kidney. This study was to investigate the effect of sublethal concentrations of unionized ammonia on the susceptibility of tilapia fingerling to *A. hydrophila* infection based on mortality rate.

Materials and Methods

Sublethal concentration

Unionized ammonia

Five hundred mono-sex red tilapia fingerlings (*Oreochromis* spp.) about 5 cm in length were bought from a farm at Sg. Buah, Bangi, Selangor, Malaysia. The fishes were placed in two separate tanks with aeration for 4 d before experimentation. Ammonium chloride (NH_4Cl) was used as a source of ammonia (Xu *et al.*, 2005). The presence of ammonia in water was calculated based on the molecular weight. In this experiment, the sublethal concentration of unionized ammonia (UIA), that is where no mortality occurred in the fingerlings, was determined. Ammonium chloride was added to the fish tanks at the rate of 40, 75, 120 and 250 mg/L and left for 4 d. The concentrations of unionized ammonia in the respective tanks were 1.1, 1.7, 2.2, 3.6 and 4.6 mg/L.

Aeromonashydrophila

Three concentrations (5×10^4 , 5×10^5 and 5×10^6 cfu/mL) of *A. hydrophila* were used as sublethal concentrations.

Effect of Ammonium chloride on A. hydrophila infection

This experiment was carried out in fibre plastic aquariums. The aquarium was filled with a 7 L of water each. Each aquarium housed 14 fingerlings supplied with aerations using compressed air via an air-stone. Sublethal concentrations of ammonium chloride and *A. hydrophila* were added to the respective tanks. One tank each served as the negative control (without NH_4Cl or *A. hydrophila*). The fingerlings were fasted for 24 h prior to experiment and left for 4 days. The aquariums were duplicated. The temperature and pH was measured at the first day of experiment in order to determine the value from the table of fraction of unionized ammonia. The dead fingerlings were recorded and removed from the aquarium each day.

Statistical analysis

Chi-square test using SPSS 16 was used in order to see the relationships between the different concentrations of unionized ammonia and the mortality rate of tilapia fingerlings in *A. hydrophila* infection.

Results

Effect of sublethal concentration on tilapia fingerlings

Unionized ammonia

In the determination of sublethal concentration of unionized ammonia, there were 3 mortalities on day one, at 4.6 and one at 1.7 mg/L NH_4Cl concentrations. On the second day, there were three mortalities one each at 1.1, 2.2 and 4.6 mg/L NH_4Cl . No mortality was observed on day three. On day 4 there were two mortalities, one each at 1.1 and 2.2 mg/L.

A. hydrophila

In the second experiment to determine the sublethal concentration of *A. hydrophila*, the highest number of fingerling that died was in the lowest concentration of bacteria (5×10^4 cfu/mL) which was 3 of 14 fingerlings. One fingerling died in medium concentration (5×10^5 cfu/mL). No fingerling died in high bacteria concentration (5×10^6).

Unionized ammonia and A. hydrophila infection

The third experiment was to investigate the effect of sublethal ammonia concentration on susceptibility of tilapia fingerling to *A. hydrophila* infection. The result showed that there were no deaths at 0 and 1.1 mg/L unionized ammonia. However, at 2.2 and 3.6 mg/L, there were two deaths each.

Discussion

The result from the first experiment showed that the number of fingerling deaths were not consistent for each concentration of unionized ammonia. Deaths were observed at all concentration of ammonia except at 3.6 mg/L. Therefore, the cause of death remains uncertain. If the fingerlings died because of unionized ammonia, then there should also be more mortalities as 3.6 mg/L NH_4Cl . For the sublethal concentration of unionized ammonia determination, the range between 1.1 to 3.5 mg/L was chosen. This range is comparable to one study that showed that the median lethal concentration within 48 h for tilapia fingerlings to be 7.1 mg/L unionized ammonia (El-Sherif *et al.*, 2008).

The result for second experiment showed, that the highest number of fingerling that died was in the lowest bacterial concentration. No fingerlings died in high concentration of bacteria. This result was similar to the previous result in which death was observed mainly at low instead of high concentrations of ammonia. There is a possibility that other factors such as stress due to transportation and sudden changes in environment may be the contributing factors to the mortalities of the fingerlings. From observation, the fingerlings that dead was smaller in size than those that lived.

The result for third experiment showed there was no mortality at 0 and 1.1 mg/L NH_4Cl . However, at 2.2 and 3.6 mg/L NH_4Cl , there were two deaths for each concentration. This shows that even at 3.6 mg/L unionized ammonia very few fingerlings died. Most of the mortalities occurred in first two days of the experiment. Another study conducted by Evans *et al.* (2005) on the susceptibility of *Streptococcus* infection due to ammonia exposure showed that the median lethal concentration (LC_{50}) for tilapia was 1.46 mg/L unionized ammonia at 24 and 48 h post-exposure, 1.33 mg/L at 72 h post-exposure, and 0.98 mg/L at 96 h post-exposure. However, 93–100% mortalities were observed within 24 h among fish exposed to 2.0, 3.0, or 4.0 mg/L UIA. Their study showed that even sublethal concentration of ammonia had significant effects on mortality rate in *Streptococcus* infection. We failed to show a similar effect with *A. hydrophila* infection. The result also showed that there was no association between fish fingerling mortality and concentration of unionized ammonia.

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