Acute toxicity (LC$_{50}$) of ammonia to carp fish (Cyprinus carpio) at different pH Levels

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Abstract. Mass death of carp fish in reservoirs and lakes in Indonesia is due to the up-welling phenomenon of ammonia generated from the decomposition of fish feed remaining in the bottom of the lakes/reservoirs by microbes. The formation of ammonia gas is very dependent on pH. Most of the ammonia gas is formed at the high pH value. Ammonia concentration can be determined with indophenol blue method using UV-Vis spectrophotometer. Carp fish (Cyprinus carpio of 5-6 gr) was exposed to the three concentration of ammonia. Acute toxicity (LC$_{50}$) of ammonia (NH$_3$) was tested on similar sizes (5-6 g) of carp fish was maintained at three different pH levels within range of 7-9 for 96-h. Results showed that the concentration of ammonia increased at higher pH. The 96-h LC$_{50}$’s for exposure to ammonia were 60% (NH$_3$ 1.85 ppm at pH 8), 100% (NH$_3$ 2.16 ppm at pH 9), and insignificant result at NH$_3$ 1.68 ppm with pH 7.

1. Introduction

Ammonia occurs in natural water in unionized (NH$_3$) and ionized (NH$_4^+$) forms and can be a serious toxicant to fishes and other aquatic species. It is a product of biological metabolism and is the principal nitrogenous excretory product of freshwater teleost [1]. It also enters water systems from accumulated fish food which is not consumed by fish. The massive dead fish at Maninjau, Saguling, and Cirata lake in 2008, could be caused by fish feeding activity [2].

The toxicity of ammonia to different fish species has been extensively investigated [3,4]. The toxicity of NH$_3$ has been ascribed to the fact that this unionized form of ammonia can readily diffuse across gill membranes due to lipid solubility and lack of charge, whereas the ionized form occurs as a larger hydrated form with charged entities which cannot readily pass through the hydrophobic micropores in the gill membrane [5].

The carp fish (Cyprinus carpio), is on a world-wide basis, one of the most extensively cultivated fish species. Based on statistical data from Dinas Kelautan dan Perikanan in 2011, the carp fish production reaches 120 thousand tons per year, but the number of dead fish also high, reaching 30 thousand tons per year in West Java [6]. In view of suppress the dead fish number and the lack of information on ammonia toxicity to crap fish, the present investigation aimed to carried out determination of the acute toxicity of ammonia to carp fish at different pH levels. Cage culture is a system of cultivation in a container of floating net cage (FNC) placed in lakes, reservoirs, lagoons, straits and bays [7].

A mass death of carp fish happened in Maninjau, Toba, Cirata, and Saguling in December 2008-early 2009 [8]. This was due to the ammonia resulted from the decomposition of accumulated feed waste in the bottom part of the lake or reservoirs [9]. When temperature of lake surface is lower than the bottom part, up-welling phenomenon happened frequently in which ammonia gas arises from bottom part which is toxic for fish. LC$_{50}$ of ammonia based on NH$_4$Cl residual in the aquarium has
been studied to carp fish has been evaluated [10]. Current study aimed to determine of ammonia formed at various pH by using ammonia generator which was then absorbed by dilute sulfuric acid solution. The resulted gas absorbed at various pH was used to simulate LC50 to carp fish.

2. Experimental

2.1. Material

Carp fish was obtained from fisheries at Ciparay (West Java). Prior to test, the fish was maintained and acclimatization in tanks for about 96 hr. The average weight of the fish for testing was of 5-6 gr. Ammonium chloride, sodium hydroxide, sulfuric acid, phenol, trisodium phosphate, hydrogen chloride, methanol, sodium nitroprusside, sodium hypochloride were pro analysis grade (E.Merck).

2.2. Optimization ammonia formation at Different pH levels

A few volume of 1N NaOH solution was added into ammonia generator containing of 10 mL NH4Cl solution to produce 2 ppm of ammonia gases. The ammonia gas was then absorbed by 100 mL of 1N H2SO4 in the Impinger bottle into ammonium sulfate using a sampling pump with a flow rate of 0.5 L/min.

Determination of ammonium using indophenol method was carried out with spectrophotometric[11]. A calibration curve was made by preparing various ammonia chloride solution of 0.05 to 0.3 mg NH3-N/L. Its absorbance was measured at 630 nm. Optimization was carried out using the same method with calibration using 90 mL solution at pH 7-9 with the addition of 10 ml NH4Cl 25 ppm.

2.3. Acute Toxicity (LC50) Test

Before the start of each test, carp fish were placed in the aquarium in water of pH 7. Prepare 4 aquarium and adjust the pH of the water (10 L) in each aquarium with a variety of pH 7-10. Add the amount of volume (ml) of NH4Cl solution (scale-up from Preliminary test) in each aquarium. Put the 10 fish in each 4 aquarium. Observe the fishing mortality every 2 hr for 96 hr. Measuring pH, temperature, and DO every 2 hours for 96 hr. Specify LC50
3. Result and discussion
A calibration curve was made by preparing various ammonia chloride solution of 0.05 to 0.3 mg NH$_3$-N/L. Its absorbance was measured at 630 nm. Results of calibration process shown as a curve of ammonia concentration (Figure 1), with correlation coefficient is 0.9991, indicating its reliability for quantitative determination.

![Figure 2. Calibration curve for ammonia gas formation.](image)

Figure 2 shows that the ammonia gas formation was obtained of 1.09 ppm in 10 min and reached the maximum level of 2.16 ppm in 30 min. Its production was formed constantly after 30 min.

![Figure 3. Time requirement of ammonia generator for NH$_3$ formation.](image)

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Ammonia formation at different pH levels is shown in Figure 4. The ammonia concentration from the results of experiment using Impinger bottle showed an increase in ammonia concentration of 1.68 ppm at pH 7 becomes 2.35 ppm at pH10. The results of this experiment are used to determine LC50 in carp fish with and without the addition of ammonium chloride at different pH levels.
Table 1 shows that % mortality of carp fish reached 100% at pH 10 without ammonium chloride addition. At this point, all of fishes were no longer alive. This due to the excessive mucus with red spots on their gills and scales chipped. These characteristic revealed that all fish suffered from ammonia poisoning [12]. Moreover, LC50 value is shown in Table 1.

| No. | pH Solution in Aquarium | LC50-Mortality of Carp Fish (%) Without Adding of NH$_4$Cl 25 ppm | With Adding of NH$_4$Cl 25 ppm |
|-----|-------------------------|-----------------------------------------------------------------|-------------------------------|
| 1   | 7                       | 0                                                               | 0 (NH$_3$ 1.68 ppm)           |
| 2   | 8                       | 0                                                               | 60 (NH$_3$ 1.85 ppm)          |
| 3   | 9                       | 0                                                               | 100 (NH$_3$ 2.16 ppm)         |
| 4   | 10                      | 100                                                             | -                             |

Notes: - Water temperatur in aquarium 27-28 °C;
- Dissolved oxygen (DO) 3-5 ppm

The acute toxicity of NH$_3$ in 96 hr exposure times increased within higher pH Levels, which is indicated by the lethal fish in aquaria. The results showed that no mortality at pH 7 after 96 hr exposure but increased to 60 and 100% at pH 8 and pH 9, respectively.

4. Conclusion
It was concluded that the carp (C. carpio) are more sensitive to water containing ammonium chloride at a pH of 9 and 8 as compared to water pH 7. They show changes in behavior caused by bleeding in the gills and its scales chipped.

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