# The Evaluation on the Use of Formalin to Neutralize Ammonia in Freshwater and Saltwater and its Effect on Hybrid Catfish and Giant Tiger Prawn (*Penaeus monodon* Fabricius)

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## ABSTRACT

Laboratory scale experiment was done to determine the dosage of formalin needed to neutralize ammonia both in freshwater and saltwater and to study the effect of this treatment method on hybrid catfish (*Clarius macrocephalus* X *C. gariepinus*) and giant tiger prawn (*Penaeus monodon* Fabricius). The concentrations of ammonia used in this experiment were between 0.3 and 2.0 mg-N/l. The relationships between the dosages of formalin (Y) and the concentrations of ammonia nitrogen (X) are "Y = 13.6 + 76.3X" for freshwater, "Y = 11.63 + 107.25X" for 20 ppt saltwater and "Y = 23.9 + 115.0X" for 30 ppt saltwater. The neutralization of ammonia by formalin at the concentrations of ammonia of 1.5 and 2.0 mg-N/l resulted in 15.0 and 30.0% 96 hr-mortality of 2.05 g hybrid catfish and the neutralization of ammonia by formalin at the concentrations of ammonia of 1.0, 1.5 and 2.0 mg-N/l resulted in 15.0, 33.3, and 40.0% 96 hr-mortality of 1.56 g of *P. monodon*. When chronic toxicity tests were conducted in the period of 12 weeks, declining in growth rates of tested species were observed when formalin was used to neutralize ammonia at the concentrations of ammonia >0.8 mg-N/l for hybrid catfish and >0.5 mg-N/l for *P. monodon*. Survival rates of test animals decreased when formalin was used to neutralize ammonia at the concentrations of ammonia >1.0 mg-N/l for hybrid catfish experiment and >0.8 mg-N/l for *P. monodon* experiment.


**Key words:** formalin, ammonia, hybrid catfish, giant tiger prawn

## INTRODUCTION

In aquaculture ponds ammonia is released from the decomposition of leftover feed, animal feces and other organic matters. Fish and shrimp can also directly excrete ammonia into the water. Ammonia is highly toxic to fish and shrimp and is considered to be one of the most important limiting factors affecting fish and shrimp production. At high concentration, ammonia can be fatal to aquatic animal. Short term exposure of fishes to high concentration of ammonia causes increased gill ventilation, hyper-excitability, loss of equilibrium, convulsion, and then death (Smart, 1978; Thurston *et al*., 1981). Chronic exposure of fishes to lower concentrations of ammonia decreases growth rate and survival rate (Thurston *et al*., 1984, 1986). Ammonia reacts with formaldehyde to form hexamethylenetetramine. Formalin (37-40% formaldehyde) has been widely used to treat fungal infections and parasites of fish and shrimp. It is one of a few chemicals approved by USFDA for aquaculture (Seldon and Goodwin,

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2003). Formaldehyde is low toxic to fish and shrimp. This study was done to evaluate the possibility of using formalin for the neutralization of ammonia in aquaculture ponds.

## MATERIALS AND METHODS

**Determination of formalin dosage for the neutralization of ammonia**

The experiment was done in 40 l glass aquaria. Dechlorinated tap water was used for freshwater experiments and saltwater with 20 ppt and 30 ppt salinity was used for saltwater experiments. Since ammonia concentration in aquaculture ponds is rarely excess 2.0 mg-N/l, test concentration of ammonia were assigned to be between 0.3 and 2.0 mg-N/l. Ammonium chloride was used to prepare ammonia stock solution for the preparation of test water with concentrations of ammonia of 0.3, 0.5, 0.8, 1.0, 1-5 and 2.0 mg-N/l. Formalin was added to the aquaria at 10 ppm intervals to roughly estimate the dosages that can neutralize each concentrations of ammonia within 5 minutes. Then the exact dosages were determined by testing with 1 ppm interval of formalin concentration. Three replications were set for each experimental unit.

**Acute toxicity test**

Static bioassay test was done in 40 l glass aquaria containing 30 l test water to determine acute toxicity of the end product from the reaction of ammonia and formalin on 2.05 g hybrid catfish (*Clarius macrocephalus* X *C. gariepinus*) and 1.56 g *P. monodon*. Saltwater with 30 ppt salinity was used for shrimp experiment. Test water were prepared to get ammonia concentrations of 0.3, 0.5, 0.8, 1.0, 1-5 and 2.0 mg-N/l. Then formalin was added into each aquarium to neutralize ammonia using the dosages obtained from the first experiment. The solution were mixed and let stand for 5 minutes to allow the reaction to complete. Then 20 test animals were transfer into each aquarium. Control units with no chemical treatment were also set. Three replications were set for each treatment unit. No aeration was provided. There was no feeding for hybrid catfish test but shrimp were fed with artemia nauplii to prevent cannibalism. Mortality rates were checked at 96 hours.

**The study for the effect on growth rate and survival rate**

The study for the effect on growth rate and survival rate of the end product from the reaction of ammonia and formalin on 2.05 g hybrid catfish and 1.56 g *P. monodon* were carried out in 40 l glass aquaria. Saltwater with 30 ppt salinity was used for shrimp experiment. Stocking rate of test animals was 90 per aquarium. Each aquarium was filled with 30 l of test water. Ammonium chloride solution was added to obtain ammonia concentrations of 0.3, 0.5, 0.8, 1.0, 1.5 and 2.0 mg-N/l. Then formalin was added to neutralize ammonia using dosages obtained from the first experiment. After mixing for 5 minutes, test animals were stocked. Control units without chemical treatment were also set up. Three replications were set for each treatment. Aeration was provided for each aquarium. Test animals were fed by pellet feed. Feces and leftover feed were siphoned off and 50% of test water was changed daily. Test water was prepared daily using same concentrations of ammonia and formalin. Test period was 12 weeks. Mortality rate and growth rate of test animals were determined.

Test water was analyzed for salinity, pH, alkalinity, hardness, dissolved oxygen and ammonia. Salinity of water was measured by salinometer ATAGO S/MILL. Dissolved oxygen concentration was measured by oxygen meter YSI 550A. pH of water was measure by pH meter YSI 50. Ammonia concentration was determined by method recommended by Grassholf (1976). Alkalinity and hardness were determined by titration methods (APHA *et al*., 1992).

## RESULTS AND DISCUSSION

### Dosage of formalin for the neutralization of ammonia

The amount of formalin needed to neutralize ammonia at the concentrations of 0.3, 0.5, 0.8, 1.0, 1.5 and 2.0 mg-N/l were 44, 48, 67, 97, 118 and 173 ppm for freshwater, 54, 63, 88, 121, 163 and 235 ppm for 20 ppt saltwater, and 63, 77, 110, 145, 196, and 254 ppm for 30 ppt saltwater. When salinity of water increased, higher dosage of formalin was needed to neutralize the same concentration of ammonia (Table 1). The relationship between the dosages of formalin (Y) and the concentrations of ammonia (X) were represent by simple linear regression; $Y = 13.63 + 76.26X$ ($r = 0.9862$) for freshwater, $Y = 11.63 + 107.25X$ ($r = 0.9921$) for 20 ppt saltwater, and $Y = 23.89 + 115.03X$ ($r = 0.9979$) for 30 ppt saltwater.

**Table 1** Dosages of formalin needed to neutralize ammonia at different water salinity levels.

| Water salinity | Ammonia concentration (mg-N/l) | | | | | |
|---|---|---|---|---|---|---|
| | 0.3 | 0.5 | 0.8 | 1.0 | 1.5 | 2.0 |
| Freshwater | $44^a$ | $48^a$ | $67^a$ | $97^a$ | $118^a$ | $173^a$ |
| 20 ppt saltwater | $54^b$ | $63^b$ | $88^b$ | $121^b$ | $163^b$ | $235^b$ |
| 30 ppt saltwater | $63^c$ | $77^c$ | $110^c$ | $145^c$ | $196^c$ | $254^c$ |

**Remark:** Average values with different letter in the same column are statistically significantly different ($P<0.05$)

**Table 2** Properties of water used for the determination of the dosages of formalin for the neutralization of ammonia.

| | Water quality | | |
|---|---|---|---|
| | Salinity ( ppt ) | pH | Temperature ( $^oC$ ) |
| Freshwater | 0 | 8.0 | 27 |
| Saltwater | 20 | 8.1 | 27 |
| Saltwater | 30 | 8.1 | 27 |

### Short term effect on test species

When 2.05 g hybrid catfish were stocked in test aquaria containing freshwater with 0.3, 0.5, 0.8, 1.0, 1.5 and 2.0 mg-N/l of ammonia which were neutralized by 44, 48, 67, 97, 118 and 173 ppm of formalin, 15.0 and 30.0% 96 hr-mortality of fish were observed in aquaria with ammonia concentrations of 1.5 and 2.0 mg-N/l. When the test was done with 1.56 g *P. monodon* using 30 ppt saltwater with the same series of the ammonia concentrations treated with formalin at the dosages of 63, 77, 110, 145, 196 and 254 ppm, 96 hr-mortality rates of shrimp were 15.0, 33.0 and 40.0% in aquaria with ammonia concentrations of 1.0, 1.5 and 2.0 mg-N/l.

**Table 3** Mortality rates of test species within 96 hr test period (%)

| Ammonia ( mg-N/l )/ formalin ( ppm ) | Hybrid catfish | Ammonia ( mg-N/l )/ formalin ( ppm ) | *P. monodon* |
|---|---|---|---|
| control | $0^{a}$ | Control | $0^{a}$ |
| 0.3/44 | $0^{a}$ | 0.3/63 | $0^{a}$ |
| 0.5/48 | $0^{a}$ | 0.5/77 | $0^{a}$ |
| 0.8/67 | $0^{a}$ | 0.8/110 | $0^{a}$ |
| 1.0/97 | $0^{a}$ | 1.0/145 | $15.0^{b}$ |
| 1.5/118 | $15.0^{b}$ | 1.5/196 | $33.3^{c}$ |
| 2.0/173 | $30.0^{d}$ | 2.0/254 | $40.0^{d}$ |

**Table 4** Properties of water used for short term effect studies

| Water quality | Test species | |
|---|---|---|
| | Hybrid catfish | *P. monodon* |
| Salinity ( ppt ) | 0 | 30 |
| pH | 8.0 | 8.2 |
| Temperature ( $^{o}C$ ) | 27.0-29.0 | 26.5-29.0 |
| Dissolved oxygen ( mg/l ) | 3.4-6.0 | 3.7-6.0 |
| Alkalinity ( mg/l as $CaCO_3$ ) | 197 | 138 |
| Hardness ( mg/l as $CaCO_3$ ) | 127 | 5,675 |

## Effect on growth rate and survival rate of test species

At 12 weeks of experimental period, the neutralization of 0.8 mg-N/l of ammonia by 67 ppm formalin did not have any effect on survival rate of hybrid catfish but survival rate of fish decreased to 83.3, 80.0 and 70.0% when 97, 118 and 173 ppm of formalin were added to neutralize 1.0, 1.5 and 2.0 mg-N/l of ammonia. Considering from the change in average weight of fish, the effect on growth rate of fish occurred when 0.8 mg-N/l of ammonia was neutralize by 67 ppm of formalin. The neutralization of higher concentrations of ammonia with higher dosages of formalin resulted in more decrease of the growth rates of experimental fish (Table 5).

The neutralization of 0.5 mg-N/l 0f ammonia by 77 ppm formalin did not have any effect on survival rate of *P. monodon* but survival rate of shrimp dropped down to 90.0, 76.7, 66.7 and 65.0% when 110, 145, 196 and 254 ppm of formalin were added to neutralize 0.8, 1.0, 1.5 and 2.0 mg-N/l of ammonia. The effect on growth rate occurred when 0.5 mg-N/l of ammonia was neutralize by 77 ppm of formalin. The neutralization of higher concentrations of ammonia with higher dosages of formalin resulted in more decrease on the growth rates of experimental shrimp (Table 6).

**Table 5** Average weight(g) and average survival rate (%) of hybrid catfish culture in freshwater containing 0.3 to 2.0 mg-N/l of ammonia which have been neutralized by formalin ( initial weight = 2.05 g ).

| Ammonia ( mg-N/l )/ formalin ( ppm ) | Weeks | | | Survival rate at 12 weeks |
|---|---|---|---|---|
| | 4 | 8 | 12 | |
| control | 8.88[a] | 15.20[a] | 18.44[a] | 100.0[a] |
| 0.3/44 | 8.86[a] | 15.16[a] | 18.41[ab] | 100.0[a] |
| 0.5/48 | 8.83[a] | 15.12[a] | 18.27[ab] | 100.0[a] |
| 0.8/67 | 8.61[b] | 15.02[ab] | 18.16[b] | 100.0[a] |
| 1.0/97 | 8.86[b] | 14.86[bc] | 18.00[c] | 83.3[b] |
| 1.5/118 | 8.36[c] | 14.78[c] | 17.85[c] | 80.0[c] |
| 2.0/173 | 8.32[c] | 14.68[c] | 17.79[c] | 70.0[d] |

**Remark:** Average values with different letter in the same column are statistically significantly different (P<0.05)

**Table 6** Average weight (g) and average survival rate (%) of *P. monodon* in 30 ppt saltwater containing 0.3 to 2.0 mg-N/l of ammonia which have been neutralized by formalin (initial weight = 1.56 g).

| Ammonia ( mg-N/l )/ formalin ( ppm ) | Weeks | | | Survival rate at 12 weeks |
|---|---|---|---|---|
| | 4 | 8 | 12 | |
| Control | 4.86[a] | 8.44[a] | 10.67[a] | 100.0[a] |
| 0.3/63 | 4.79[a] | 8.43[a] | 10.62[ab] | 100.0[a] |
| 0.5/77 | 4.73[ab] | 8.20[b] | 10.42[bc] | 100.0[a] |
| 0.8/110 | 4.59[b] | 8.20[b] | 10.38[c] | 90.0[b] |
| 1.0/145 | 4.29[c] | 7.95[c] | 10.16[d] | 76.7[c] |
| 1.5/196 | 4.17[c] | 7.82[c] | 9.95[d] | 66.7[d] |
| 2.0/254 | 4.11[c] | 7.87[c] | 9.99[d] | 65.0[d] |

**Remark:** Average values with different letter in the same column are statistically significantly different (P<0.05)

**Table 7** Properties of water during 12 weeks studies for the effect on growth rate and survival rate of test animals.

| Water quality | Test species | |
|---|---|---|
| | Hybrid catfish | *P. monodon* |
| Salinity ( ppt ) | 0 | 30 |
| pH | 7.3-7.9 | 8.2-8.3 |
| Temperature ( $^{o}C$ ) | 26.0-30.5 | 27.0-29.0 |
| Dissolved oxygen ( mg/l ) | 3.9-5.6 | 5.2-6.6 |
| Alkalinity ( mg/l as $CaCO_3$ ) | 110-143 | 157-187 |
| Hardness ( mg/l as $CaCO_3$ ) | 176-205 | 845-885 |

According to the results of this study, the amount of formalin needed to neutralize ammonia increased as salinity of water increased. The dosage of formalin for the neutralization of 0.3 mg-N/l of ammonia increased from 44 ppm for freshwater to 54 ppm for 20 ppt saltwater and to 63 ppm for 30 ppt saltwater. The dosages of formalin needed for the neutralization of ammonia can be estimated using the relationship between dosages of formalin (Y) and concentrations of ammonia (X) which were represented by simple linear regression; $Y = 13.63 + 76.26X$ ($r = 0.9862$) for freshwater, $Y = 11.63 + 107.25X$ ($r = 0.9921$) for 20 ppt saltwater, and $Y = 23.89 + 115.03X$ ($r = 0.9979$) for 30 ppt saltwater.

Considering from the long term effect on survival rate and growth rate of test animals, the neutralization of 0.5 mg-N/l ammonia by 48 ppm formalin can be done without any adverse effect on survival rate and growth rate of hybrid catfish and the neutralization of 0.3 mg-N/l of ammonia by 63 ppm formalin can be done without any adverse effect on survival rate and growth rate of *P. monodon*. However, formalin treatment may cause oxygen depletion in aquaculture ponds because it is highly toxic to phytoplankton (Allison, 1962). Pond managers should monitor dissolved oxygen concentrations after formalin treatments and provide aeration if necessary.

The reaction between ammonia and formaldehyde results in the formation of hexamethylenetetramine. Hexamethylenetetramine, also called hexamine or methenamine, is a heterocyclic organic compound. It is crystalline and white in appearance. Particle size ranges from 80-800 micrometers. It is moderately soluble in water and very soluble in most organic solvents. It is hydrolyzed in an acid medium into ammonia and formaldehyde. Methenamine is used as a preservative for fish, meat and prickles. It may act as a skin, eye or respiratory irritant. $LD_{50}$ for rat by intravenous injection was 9200 mg/kg (Zimmerman and Zimmerman, 1977; Joint FAO/WHO Expert Committee on Food Additives, 1965). There is no report on the toxicity of this substance on aquatic animals.

## CONCLUSIONS

According to the results of this study, the neutralization of ammonia by formalin in freshwater can be done without any effect on growth rate and survival rate of hybrid catfish if the concentrations of ammonia being neutralize is equal to or lower than 0.5 mg-N/l. In 30 ppt saltwater, the neutralization of ammonia by formalin can be done without any effect on growth rate and survival rate of *P. monodon* if the concentrations of ammonia is 0.3 mg-N/l or lower.

The dosage of formalin needed to neutralize ammonia can be estimated from the following equation:

$Y = 13.63 + 76.26X$ for freshwater

$Y = 11.63 + 107.25X$ for 20 ppt saltwater and

$Y = 23.89 + 115.03X$ for 30 ppt saltwater

Where: $Y$ = Dosage of formalin needed to neutralize ammonia

$X$ = Concentration of ammonia in water (mg-N/l)

However, more investigation is needed in order to understand the behavior of methenamine in aquatic environment. Toxicity of methenamine on aquatic organisms is also needed to be verified.

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