INHIBITION OF AMMONIA AND NITRITE TOXICITY TO CHANNEL CATFISH

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Abstract: Ammonia is the primary nitrogen-containing waste product of fish. Under natural conditions, ammonia is converted first to nitrite and then to nitrate. Ammonia and nitrite are toxic to fish at low levels, but nitrate is relatively non-toxic. Ammonia has a variety of toxic effects, including reduced growth rates, whereas nitrite primarily interfers with oxygen transport. The literature pertaining to toxic levels and effects of environmental ammonia and nitrite as well as experimental methods of inhibiting these toxicities are reviewed. Prevention of elevated pH for high ammonia conditions, and the addition of sodium chloride for elevated nitrite levels are suggested as possible inhibitors of these toxicities in channel catfish production.

Proc. Ann. Conf. S.E. Assoc. Fish & Wildl. Agencies 33:600-605

The economic pressure to increase production in the culture of channel catfish emphasizes the importance of managing water with high levels of metabolic wastes. Ammonia is the primary nitrogenous waste product of fishes and may reach toxic levels in intensive culture situations if not removed from the environment or biologically oxidized by bacteria to nitrate, a less noxious compound. During the biological oxidation of ammonia to nitrate, an intermediate compound, nitrite, may reach toxic levels due to an imbalance in the populations of nitrifying bacteria. The purpose of this paper is to (1) review the literature concerning toxic effects of ammonia and nitrite, (2) review known methods of inhibiting these toxicities, and (3) describe management practices that may prove valuable in inhibiting these effects in channel catfish (*Ictalurus punctatus*) culture systems.

TOXIC EFFECTS

Ammonia

Environmental ammonia exists in either the ionized (NH_4+) or un-ionized (NH_3) form. The un-ionized form is the form toxic to fishes because it radily diffuses across gill membranes (Wuhrmann and Woker 1949). The percentage of the total ammonia present in the un-ionized form is directly related to increasing pH and temperature (Emerson et al. 1975).

Median lethal concentration (LC_{50}) values (1-4 days) for un-ionized ammonia range from 1.39 to 3.80 mg/liter for channel catfish (Colt and Tchobanoglous 1976, Robinette 1976, Tomasso et al. 1980a). This wide range of values is probably due to differences in certain water quality characteristics--particularly pH and hardness (Tomasso et al. 1980a). Preliminary experiments in our laboratory indicate that during lethality tests most mortalities occur during the first 6 hours, indicating either a wide range of tolerance to ammonia within the population of test fish, or that the fish acclimate in some way (Redner and Stickney 1979) to the elevated ammonia levels. This leveling off of the LC_{50} after a few hours of exposure was also noted in coho salmon (*Oncorhynchus kisutch*), fingerlings (Buckley 1978).

Channel catfish exposed to sublethal concentrations of ammonia have shown reduced plasma sodium levels (Tomasso et al. 1980a). In various species of fish ammonia has been shown to cause degenerative tissue damange to gills and kidneys (Burrows 1964,

Reichenbach-Klinke 1967, Smart 1976, Thurston et al. 1978), reduced oxygen carrying capacity of hemoglobin (Sousa and Meade 1977), increased oxygen consumption, respiratory rate, heart rate (Smart 1978), and incrased urine output (Lloyd and Orr 1969). While all of these actions are probably contributing mechanisms to ammonia toxicity, the major toxic mechanism has not yet been identified. The suggestion by Smart (1978) that ammonia may act as an uncoupler of oxidative phosphorylation is currently being investigated in our laboratory and may prove to be the primary toxic mechanism.

Un-ionized ammonia levels do not generally reach lethal levels in channel catfish culture systems, but levels are frequently reached that affect growth. Robinette (1976) reported that growth was significantly reduced in fish continuously exposed to 0.12 mg/liter un-ionized ammonia. A linear decrease was observed in growth of channel catfish cultured in the continuous presence of 0.048-0.989 mg/liter un-ionized ammonia (Colt and Tchobonoglous 1978), whereas 1.162 mg/liter un-ionized ammonia resulted in complete mortality during a 31-day growth trial. Andrews et al. (1971) reported a correlation between percent gain and ammonia levels during a high density culture experiment.

Nitrite

Only recently has nitrite come to be recognized as a distinct water quality problem in channel catfish production (Konikoff 1975, Lovell 1979). As with amonia, nitrite exists in either the ionized (NO_2^-) or the un-ionized (HNO_2) form, with the relative percentages depending on the pH and temperature of the water. In the case of nitrite, however, the percentage in the un-ionized form increases with decreasing pH. Both forms are toxic although the un-ionized form probably crosses the gills more readily.

Relatively little work has been reported on toxic levels of nitrite to channel catfish. Konikoff (1975) reported 24, 48, 72, and 96 hour LC_{50s} of 33.8, 28.8, 27.3 and 24.8 mg/liter, respectively. A 24 hour LC_{50} of 4.99±2.1 mg/liter was determined in our laboratory (Tomasso et al. 1980b). This large difference in results can probably be attributed to variation in water quality parameters, and points out the need for their consideration in any toxicity evaluation. For example, the pH during the first 24 hours of Konikoff's test varied from 7.2 to 7.8, whereas in our tests, the pH was buffered to pH 7.0±0.1. The maintenance of a lower pH in our tests resulted in exposure to more of the toxic un-ionized form of nitrite.

The primary toxic effect of nitrite is the formation of methemoglobin, an oxidized form of hemoglobin not capable of transporting oxygen. Methemglobin gives the blood a brown color, hence the name "brown blood disease". Huey et al. (1980) found that fish could survive methemoglobin levels of up to 85% for short periods of time, and that in fish exposed to equal concentrations at differing pHs, fish developed higher methemoglobin levles as pH decreased. Gill damage has also been reported as a result of chronic nitrite exposure in steelhead trout (*Salmo gairdneri*) (Wedemeyer and Yasutake 1978). To date, no studies on the effects of sublethel nitrite exposure on growth have been reported.

INHIBITION OF TOXIC EFFECTS

Ammonia

By far the best method of alleviating ammonia stress is to remove the environmental ammonia, either by filtering or changing the water. Rapid reversibility of symptoms of ammonia toxicity was observed in trout transferred to ammonia free water (Smart 1978). A similar observation was made in our laboratory when moribund channel catfish survivors of ammonia toxicity tests were transferred to ammonia free water.

When water exchange is not a practical approach, downward adjustment of pH is an alternative. Fig. 1 illustrates the 24 hour LC_{50} of total and un-ionized ammonia to channel

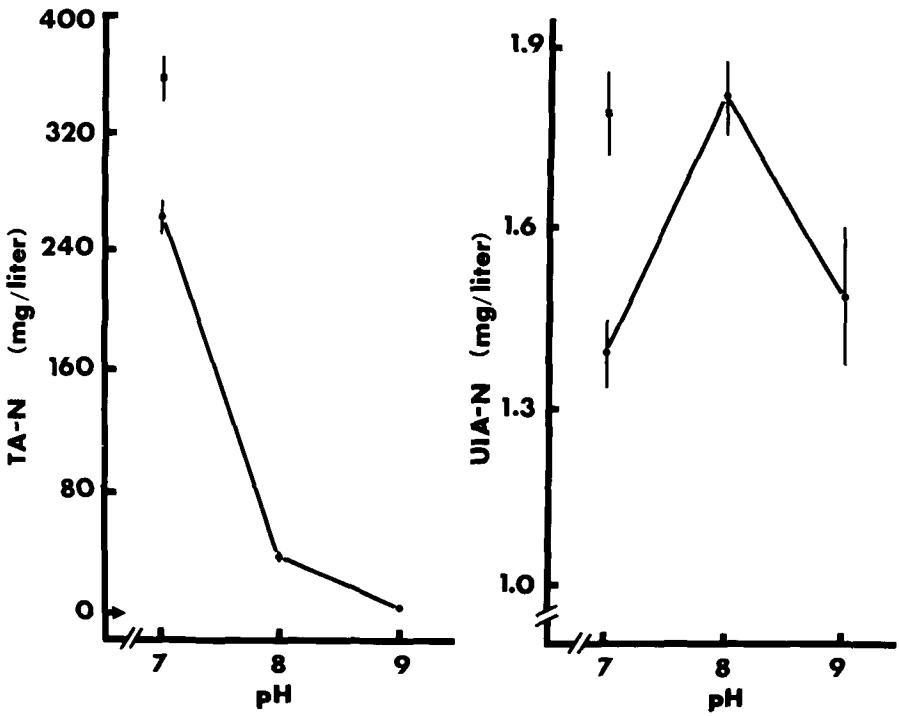

Fig. 1. Twenty-four hour median lethal concentrations of total ammonia nitrogen (TA-N) and un-ionized ammonia nitrogen (UIA-N) for channel catfish at pH 7, 8 and 9. Circles = tap water; squares = calcium enriched water. (From Tomasso et al. 1980a).

catfish at pH 7,8 and 9 (Tomasso et al. 1980a). At pH 7 the lethal limit is probably so high that it should not be significant in culture situations, but in more alkaline waters, ammonia may be a severe problem in terms of inhibition of growth and other sublethal effects. Increasing water hardness (40-440 mg/liter) offers some protective effect (Fig. 1), probably by decreasing gill membrane permeability (Potts and Fleming 1979).

Nitrite

Stress from nitrite may be alleviated in channel catfish by addition of sodium chloride, calcium chloride, potassium chloride or sodium bicarbonate (Huey et al. 1980) The monovalent anion component of each of these compounds is responsible for the stress alleviation, probably by competing with nitrite at the gills for entrance into the fish (Perrone and Meade 1977). A molecular chloride:nitrite ratio of 16 completely inhibits elevation of methemoglobin levels in nitrite-exposed catfish (Tomasso et al. 1979). This ratio was determined in water containing 1 meq/liter bicarbonate which probably

contributed to the protective effect. Methemoglobin levels for fish exposed to 5 mg/liter nitrite in Memphis tap water (pH = 7, alkalinity = 47 mg/liter) were 77%, whereas fish exposed to the same concentration of nitrite at Stuttgart, Arkansas, (pH = 8, alkalinity = 250 mg/liter) developed methemoglobin levels of 9%, which were not different from control levels.

Addition of chloride to nitrite-containing water was as effective as transferring fish to fresh water in reversing nitrite-induced methemoglobinemia (Fig. 2). The rapid rate of recovery indicates that fish probably have an enzyme system that reduces methemoglobin (Cameron 1971), rather than recovery depending on the synthesis of new red blood cells. Unlike results with steelhead trout (Wedemeyer and Yasutake 1978), calcium chloride is no more effective than sodium chloride in inhibiting methemoglobinemia or death (Tomasso et l. 1980b).

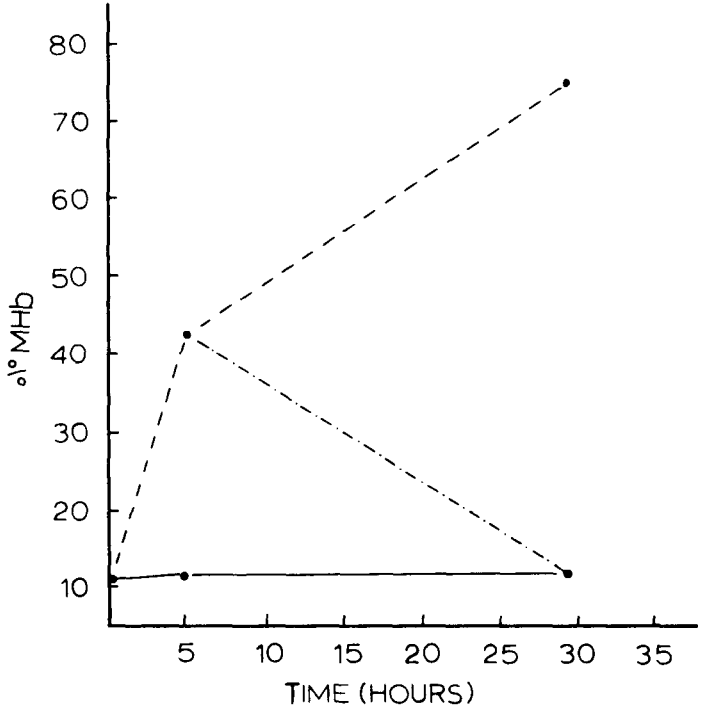

Fig. 2. Methemoglobin levels of channel catfish exposed to 5 mg/liter nitrite for 5 hours and then placed in either fresh water or 5 mg/liter nitrite and 100 mg/liter sodium chloride for 24 hours. ——— = control, - - — — = 5 mg/liter nitrite, -.-.-. = fresh water or 5 mg/liter nitrite and 100 mg/liter sodium chloride. (From Tomasso et al. 1979).

MANAGEMENT PRACTICES

Ammonia

Ammonia is most likely to become a problem in ponds when the carrying capacity is approached. Special care must be taken to prevent the build-up of excessive nutrients that cause the development of dense plankton blooms. In such cases wide variations in oxygen and pH may occur over a 24 hour period. Although early morning oxygen depletions are

of primary concern during this period, the associated high pH levels that may develop in mid-afternoon can increase the amount of the existing ammonia in the toxic un-ionized form by several fold. Careful management of feeding rates, and the addition of fresh water to reduce nutrient loads as well as diluting ammonia levels will reduce the likelihood of ammonia becoming a problem.

Nitrite

As with ammonia toxicity, water replacement is one of the most effective management techniques available. If this is not possible, addition of chloride may offer a practical alternative. The amount of chloride required will vary with the concentration of nitrite and bicarbonate in the water. In our experimental systems (Tomasso et al. 1979) 25 mg/liter sodium chloride was adequate to protect fish exposed to 1 mg/liter nitrite at pH 7. This method is practical for small culture systems and is probably practical for ponds. Salt concentrations in the range required are not prohibitively expensive and should not be detrimental to the dynamics of the culture system. Care must be taken to protect equipment from the corrosive action of salt. In our recirculating systems we add salt during the conditioning of the biological filters and dilute it out after the filters have reduced any nitrite build-up.

Maintenance of high dissolved oxygen lelvels may also help to increase tolerance to nitrite by increasing oxygen loading tension in the blood, and increasing dissolved oxygen in the plasma.

Interactions

A final consideration is the possible toxic interaction of environmental ammonia and nitrite. Although this interaction has not been demonstrated, L. Marking and T. Bills of the Fish Control Laboratory in La Crosse, Wisconsin, have demonstrated a toxic interaction between nitrite and the lampricide TFM. If a toxic ammonia-nitrite interaction does exist, the detrimental limits of both parameters may be much lower than currently thought.

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