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Influence of *Edwardsiella ictaluri* Septicemia on Nitrite-Induced Methemoglobinemia in Channel Catfish (*Ictalurus punctatus*)

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For healthy channel catfish (Ictalurus punctatus), a consistent relationship exists between the molar ratio of nitrite:chloride in the water and percent methemoglobin in the blood (Schwedler and Tucker 1983). However, catfish are sometimes observed with methemoglobin levels inconsistent with ambient nitrite and chloride concentrations. To aid in diagnosis of the severity of the syndrome and to make subsequent treatments more efficaceous, we have attempted to identify the causes of these abnormal responses. In a previous report (Tucker and Schwedler 1983), we showed that lack of acclimation to nitrite can result in abnormally high levels of methemoglobin in nitrite-exposed channel catfish. We have also observed abnormal methemoglobin levels in fish when concurrent bacteremias are present.

Enteric Septicemia of Catfish is an acute bacterial disease caused by Edwardsiella ictaluri. Nitrite-induced methemoglobinemia and Enteric Septicemia of Catfish are both economically important diseases of commercially cultured channel catfish. Both diseases are most frequently encountered in the cooler spring and fall months and it is not uncommon for the diseases to occur concurrently. In the present study, we investigated the influence of acute infection with E. ictaluri on the level of methemoglobin in nitrite-exposed channel catfish fingerlings.

MATERIALS AND METHODS

Selected chemical characteristics of the water used in this study were: total hardness, 5 mg/L as $CaCO_3$; total alkalinity, 224 mg/L as $CaCO_3$, total filtrable residue, 250 mg/L; chloride, 20 mg/L; pH, 8.3. Channel catfish (mean weight 33.1 ± 1.6 g/fish) were obtained from a holding pond and acclimated to the test water for 96 h, without feeding, in a 500-L flow-through holding tank.

Four randomly selected fish were placed into each of twelve 65-L glass aquaria and held 24 h before testing. The test was conducted under static conditions at 24 ± 2° in O_2 saturated

water. Three aquaria were allocated for each of four treatments: 1) control, 2) fish injected with E. ictaluri, 3) nitrite added to test water, and 4) fish injected with E. ictaluri plus nitrite added to test water.

E. ictaluri isolate S82-1002 (Mississippi Cooperative Extension Service Stoneville Catfish Diagnostic Laboratory) was prepared using standard bacteriologic methods. Initial isolation was on trypticase soy agar (TSA) with 5% blood. Growth from TSA blood agar was suspended in 20 ml of fluid thioglycollate medium and incubated at 23° C for 16 h. After 16 h growth in thiogycollate, 6×10^4 viable bacteria (diluted in 0.85% sterile saline) were injected IP (0.2 ml total volume) into each fish. Fish used as controls were each inoculated with 0.2 ml of similarly diluted sterile thioglycollate medium.

Immediately after fish were injected with E. ictaluri, nitrite was added to the appropriate aquaria as reagent grade $NaNO_2$ to give a final concentration of 2.2 mg/L NO_2-N. The mean nitrite concentrations at the termination of the study, determined by diazotization (American Public Health Association et al. 1980), were: water with nitrite added, 2.25 $\pm$ 0.07 mg/L NO_2-N, water without nitrite added, 0.009 $\pm$ 0.0005 mg/L NO_2-N.

After 96 h, blood was collected from the caudal vessels of surviving fish in evacuated tubes containing sodium heparin. Blood samples were immediately placed on ice and all analyses were initiated within three hours. All hematological values except plasma nitrite concentrations were obtained using standard clinical methods as described by Wedemeyer and Yasutake (1977). Plasma nitrite concentrations were determined colorimetrically following diazotization of plasma diluted in distilled water.

Necropsies were performed on all fish including bacterial isolation from the posterior kidney on TSA with 5% blood and on Ordal's (Anacker and Ordal 1955) medium. Representative tissues were fixed in 10% neutral buffered formalin and histological sections were stained with hematoxylin and eosin. Blood smears were made from previously collected blood and stained with Wright-Giemsa stain (Humason 1972).

RESULTS AND DISCUSSION

E. ictaluri was recovered from the posterior kidney of all fish injected with the bacteria. No bacteria were isolated from fish in control or nitrite-only treatments. In the 72-96 h post-injection period, three fish in the E. ictaluri only and two fish in the E. ictaluri plus nitrite treatment died. All fish in the other two treatments survived the 96-h test period. All fish injected with E. ictaluri surviving to 96 h showed external lesions characteristic of acute E. ictaluri infection (Areechon and Plumb in press). Fish infected with E. ictaluri also had significantly ($P < 0.05$) lower hematocrits and red blood cell counts than uninfected fish. This was also observed

by Arrechon and Plumb (in press) and is characteristic of E. ictaluri septicemias.

Levels of methemoglobin in fish from both treatments with no added nitrite were about 5% (Table 1). This is characteristic of fish from waters with very low NO_2^-:Cl^- molar ratios (Schwedler and Tucker 1983). In fish from the nitrite-only treatment, the average methemoglobin level was 37%. Fish with E. ictaluri septicemias in aquaria with added nitrite had an average of only 20% methemoglobin.

Table 1. Selected hematological values for fish in the four treatments in this study. Infected = injected with E. ictaluri, Nitrite = 2.2 mg/L NO_2-N added to water. Values followed by different letters were determined to be significantly different at the 5% level by Tukey's honestly significant difference procedure (Steel and Torrie 1980).

	Treatment			
Parameter	Control	Infected	Nitrite	Infected + Nitrite
Methemoglobin (%)	5 a	5 a	37 c	20 b
Plasma nitrite (μM)	14 a	19 a	1039 c	625 b
Plasma chloride (mM)	128 b	112 a	134 b	112 a
Red blood cell count (No. x 10^{-6}/mL)	2.40 b	1.47 a	2.19 b	1.27 a
Hematocrit (%)	24 b	14 a	20 b	13 a
Hemoglobin (g/dL)	6.0 c	3.8 ab	5.2 bc	3.4 a

The fish from the E. ictaluri plus nitrite treatment had a 40% reduction in plasma nitrite concentrations compared with the nitrite only treatment. The magnitude of the reduction in plasma nitrite levels (40%) in fish from the E. ictaluri plus nitrite treatment compared to the nitrite only treatment was similar to the reduction in methemoglobin levels (46%) between the same two treatments. There was also a significant reduction ($P < 0.05$) in plasma chloride concentrations in fish infected with E. ictaluri.

It appears that monovalent anion balance is disturbed in fish with E. ictaluri bacteremias. This results in lower plasma nitrite concentrations and corresponding lower methemoglobin levels. There is evidence (Krous et al. 1982) that nitrite and chloride may be transported across gill epithelia by means of the same branchial anion exchange mechanism. Gill lesions associated with E. ictaluri infection have been reported

(Areechon and Plumb in press) and structural or functional changes in the gill cells involved in anion exchange could account for the decreased plasma chloride and nitrite concentrations. However, our histological observations failed to reveal major changes in gill structure to support this hypothesis.

The interaction between environment and infectious fish disease agents is of acknowledged importance (Sniezko 1974). In particular, the role of environmental stressors, such as low dissolved oxygen or high un-ionized ammonia concentration, as predisposing factors to infectious diseases has been demonstrated (Plumb et al. 1976; Walters and Plumb 1980). In contrast, the effect of an infectious disease organism on the severity of an environmentally induced disease has received little attention. Although toxicologists recognize that unhealthy fish may respond abnormally to toxicants, there is a paucity of quantitative data describing this interaction. The results of the present study demonstrate that concurrent infection with E. ictaluri profoundly effects the methemoglobin level in nitrite-exposed channel catfish. An abnormal level of methemoglobin in fish from commerical ponds may indicate the presence of an infectious disease organism. The fish disease diagnostician should be aware of this interaction and base diagnostic and treatment protocol accordingly.

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