Bull. Environ. Contam. Toxicol. (1989) 43:295–301

Environmental Contamination and Toxicology

Nitrite–Induced Anemia in Channel Catfish, *Ictalurus punctatus* Rafinesque

C. S. Tucker,[1] R. Francis-Floyd,[2] and M. H. Beleau[2]

[1]Mississippi Agricultural and Forestry Experiment Station and [2]College of Veterinary Medicine, Delta Branch Experiment Station, Stoneville, Mississippi 38776, USA

Since 1983 numerous cases of anemia have been reported in populations of channel catfish *Ictalurus punctatus* Rafinesque cultured in the southeastern United States (Klar et al. 1986; Plumb et al. 1986). Many of these outbreaks in Alabama and Georgia have been attributed to abnormal folate metabolism in fish related to the ingestion of feed contaminated with pteroic acid, a folic acid-breakdown product (Butterworth et al. 1986). However, many cases of anemia in channel catfish cultured in Mississippi do not appear to be feed-related because only one pond may contain anemic fish, although fish in all ponds on a farm receive feed from a common feed storage bin. Further, most outbreaks of anemia in Mississippi occur in late fall and early spring (MacMillan 1985), whereas feed-related anemias appear most frequently in late spring and summer (Klar et al. 1986).

Environmental nitrite-nitrogen concentrations of 4 mg/L or more occur sporadically in channel catfish culture ponds, and the frequency of occurrence is greatest in the fall and spring. We have observed that some cases of anemia in populations of pond-raised channel catfish follow prolonged exposure to high concentrations of environmental nitrite. However, there was no evidence that exposure of channel catfish to environmental nitrite was the cause of the observed anemia. Hemolytic anemia following nitrite exposure has been described for sea bass *Dicentrarchus labrax* (L.) (Scarano et al. 1984) and rainbow trout *Salmo gairdneri* Richardson (Margiocco et al. 1983), but not for channel catfish.

In the present study we show that a variable, but generally mild, anemia develops in channel catfish exposed to nitrite. We also offer a management procedure for preventing the development of anemia during periods of elevated environmental nitrite concentrations.

Send reprint requests to C. S. Tucker at the above address.

MATERIALS AND METHODS

Channel catfish fingerlings were obtained from a nursery pond at the Delta Branch Experiment Station, Stoneville, Mississippi. Fish were held in 900-L fiberglass tanks supplied with well water with the following characteristics: calcium, <0.1 mg/L; magnesium, <0.1 mg/L; sodium, 90 mg/L; chloride, 20 mg/L; total alkalinity, 3.9 mEq; pH, 8.3-8.5. Fish were fed a commercial pelleted feed (32% crude protein) at a rate of about 2% of initial body weight per day.

Feeding was discontinued after a 4-wk acclimation period, and fish were individually weighed and randomly distributed among four 280-L fiberglass tanks. Each tank was stocked with 38 fish weighing 100 $\pm$ 11 g (mean $\pm$ SD). Tanks were supplied with water (2.0 L/min per tank) from the same source as the holding water. Airstones were used to maintain dissolved oxygen concentrations near saturation. During the test, un-ionized ammonia-nitrogen concentrations were <0.05 mg/L and the water temperature was 18-23C.

Fish were held in the test tanks for 7 days before the initial sampling. On the day of initial sampling (Day 0), six fish were netted from each tank. Blood was collected from the caudal vessels in evacuated tubes containing sodium heparin. Hematocrit, total hemoglobin, and percent methemoglobin were determined using standard clinical methods (Tietz 1970). Functional hemoglobin was calculated by subtracting total methemoglobin (percent methemoglobin x total hemoglobin) from total hemoglobin. Sampled fish were not returned to test tanks.

Immediately after the initial sampling, solutions of either distilled water or reagent grade sodium nitrite dissolved in distilled water were added to the water supply of each tank using a peristaltic pump. Fresh stock solutions of sodium nitrite were prepared every other day. We attempted to maintain nitrite-nitrogen concentrations of 0, 1.0, 2.0, and 3.0 mg/L. Actual nitrite concentrations were measured at least every other day by the azo-dye method (Strickland and Parsons 1972).

Six fish were removed from each tank after 7, 14, and 21 days of exposure. Blood was collected and analyzed as previously described.

The Statistical Analysis System (SAS Institute, Inc 1985) PROC REG program was used to partition Type 1 sum of squares into linear and quadratic components. Regressions were performed both within nitrite concentrations to evaluate trends over time and within exposure periods to evaluate dose-responses. We established an alpha = 0.05 as the level of significance for all tests.

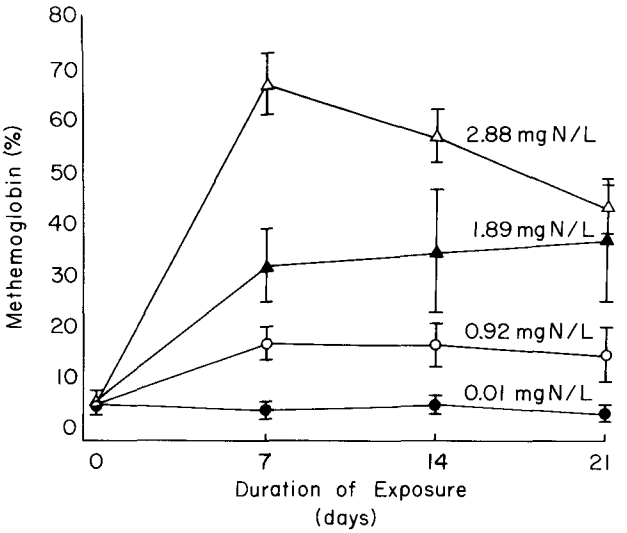

Figure 1. Percent methemoglobin (mean ± standard deviation) in channel catfish fingerlings after 0, 7, 14, and 21 days of exposure to four concentrations of environmental nitrite.

RESULTS AND DISCUSSION

The mean nitrite-nitrogen concentrations (+SD) for the four treatments were 0.01 ± 0.004, 0.92 ± 0.18, 1.89 ± 0.32, and 2.88 ± 0.40 mg/L. Using these mean nitrite concentrations and a constant chloride concentration of 20 mg/l for the test water, the average nitrite:chloride molar ratios for the four treatments were 0.00, 0.12, 0.24, and 0.37.

Environmental nitrite is concentrated in the blood of channel catfish by the active transport system in the gills which normally transports chloride (Lewis and Morris 1986). The most thoroughly studied toxic effect of nitrite is the oxidation of hemoglobin to methemoglobin, a brown-colored product that is incapable of reversibly binding oxygen. The overall mean percent methemoglobin in fish sampled prior to nitrite exposure (Day 0) was 5% of total hemoglobin. This is considered normal for channel catfish in the absence of environmental nitrite (Lewis and Morris 1986). Percent methemoglobin after nitrite exposure was proportional to environmental nitrite concentrations (Fig. 1).

There were significant ($P<0.05$) linear or quadratic trends of decreasing mean hematocrit, total hemoglobin, and functional hemoglobin concentration over time for fish exposed to average environmental nitrite-nitrogen concentrations of 1.89 or 2.88 mg/L (Table 1). A significant ($P<0.05$) quadratic dose-response was found for these three blood variables after 7 days of exposure. The dose-response was generally most evident

Table 1. Mean hematocrit, and total and functional hemoglobin levels in channel catfish fingerlings after 0, 7, 14, and 21 days of exposure to four concentrations of nitrite. R-squared = coefficient of determination for the quadratic regression; LSD = least significant difference; n.s. = not significant at the 0.05 level; asterisk = trend is significant at the 0.05 level. Six fish were sampled at each time period and nitrite concentration.

Mean nitrite concentration (mg NO_2-N/L)	Duration of exposure (days)				Trend			LSD
	0	7	14	21	Linear	Quadratic	R^2	(5%)
	Hematocrit (%)							
0.01	27	26	25	27	n.s.	n.s.	0.06	4
0.92	25	25	23	25	n.s.	n.s.	0.04	3
1.89	30	27	17	21	*	*	0.44	5
2.88	27	19	13	18	*	*	0.60	5
Linear	n.s.	*	*	*				
Quadratic	n.s.	*	n.s.	n.s.				
R-squared	0.04	0.31	0.65	0.48				
LSD(5%)	5	5	5	5				
	Total hemoglobin (g/100 mL)							
0.01	7.4	6.5	6.9	6.6	n.s.	n.s.	0.05	1.5
0.92	6.4	7.3	7.4	6.4	n.s.	*	0.25	1.0
1.89	7.1	7.6	5.6	5.6	*	n.s.	0.23	1.7
2.88	6.4	5.7	3.8	5.1	*	n.s.	0.26	1.8
Linear	n.s.	n.s.	*	*				
Quadratic	n.s.	*	n.s.	n.s.				
R-squared	0.04	0.25	0.50	0.24				
LSD(5%)	1.2	1.6	1.8	1.4				
	Functional hemoglobin (g/100 mL)							
0.01	7.0	6.2	6.5	6.4	n.s.	n.s.	0.05	1.5
0.92	6.2	6.1	6.2	5.5	n.s.	n.s.	0.10	1.0
1.89	6.8	5.2	3.8	3.6	*	n.s.	0.50	1.7
2.88	6.0	1.9	1.7	2.9	*	*	0.83	0.9
Linear	n.s.	*	*	*				
Quadratic	n.s.	*	n.s.	n.s.				
R-squared	0.08	0.72	0.75	0.67				
LSD(5%)	1.1	1.4	1.4	1.2				

at 14 days of exposure, as indicated by the higher R-squared values for the regressions on that day.

With one exception there were no significant linear or quadratic trends over time for mean hematocrit, or total and

functional hemoglobin concentrations in fish exposed to 0.92 or 0.01 mg/L nitrite-nitrogen. The one exception was the significant ($P<0.05$) quadratic trend over time for total hemoglobin in fish exposed to 0.92 mg/L. Mean total hemoglobin concentrations for this group inexplicably increased, then decreased, over the course of the study. However, the final mean total hemoglobin concentration (6.4 g/100 mL) was identical to that for the initial sampling.

Although the mean hematocrits and total hemoglobin concentrations in fish exposed to the two highest nitrite concentrations indicate only moderate anemia, there was considerable variation among fish within these groups, and some fish were severely anemic. For example, on Day 14 the mean hematocrit was 13% for fish exposed to 2.88 mg/L nitrite-nitrogen. However, the individual hematocrit values for the six fish sampled were 4, 9, 11, 14, 18, and 20% of total blood volume. We have examined channel catfish from several commercial culture ponds with chronically high environmental nitrite concentrations and noted similar wide ranges in the degree of anemia in fish from the same pond population.

The anemia that develops in nitrite-exposed fish is thought to be related to the high energy requirement for methemoglobin reduction. The major methemoglobin-reducing system in mammalian red blood cells is NADH-methemoglobin reductase. This system has been demonstrated in channel catfish (Huey and Beitinger 1982), and presumably is the major system for methemoglobin reduction in fish. Much of the total metabolic energy of the red blood cell is used to generate NADH for the reductase system and to maintain cell membrane integrity (Smith 1975). Under chronic oxidative stress, such as prolonged nitrite exposure, an inordinate share of the cell's energy resources is used for methemoglobin reduction. This results in shortened mean red blood cell lifespan and an increased rate of cell hemolysis (Scarano and Saroglia 1984; Scarano et al. 1984). Several factors affect the state of the antioxidant defense system of red blood cells (Smith 1975). Differences in the nutritional status (Scarano and Saroglia 1984), genetic background, or other factors could affect the susceptibility of individual fish to nitrite-induced hemolytic anemia. This could account for the wide range of hematocrits that developed among fish exposed to the highest nitrite concentration.

The development of both methemoglobinemia and anemia caused a marked reduction in functional hemoglobin in fish exposed to the two highest nitrite concentrations (Table 1). On Days 7 and 14, blood from fish exposed to 2.88 mg/L nitrite-nitrogen averaged less than 30% of the functional hemoglobin content of control fish. The decreased vascular oxygen carrying capacity in fish with reduced levels of functional hemoglobin could result in peripheral hypoxia, particularly if environmental

dissolved oxygen concentrations are suboptimal. Waters in commercial channel catfish culture ponds are eutrophic, and environmental dissolved oxygen concentrations are often less than 50% of saturation in the early morning during the warmer months of the year. Thus, commercially cultured channel catfish exposed to nitrite are at high risk to death by either anoxia or the secondary effects of prolonged hypoxia, such as stress-related bacterial infection.

Anemia resulting from exposure of channel catfish to environmental nitrite is preventable by decreasing the rate of nitrite uptake through the gills into the bloodstream. Environmental chloride competes with nitrite for transport across gills of channel catfish and under the conditions usually encountered in fish culture ponds, the rate of nitrite uptake and the amount of methemoglobin formed is proportional to the molar ratio of nitrite:chloride in the water (Schwedler and Tucker 1983). In the past, nitrite toxicosis of pond-raised channel catfish was treated by adding chloride salt (usually NaCl) to the pond water to maintain a nitrite:chloride molar ratio of about 0.25. This recommendation was based upon the results of short-term tests (Bowser et al. 1983) which demonstrated improved resistance of fish to hypoxia at this nitrite:chloride ratio compared to fish exposed to higher nitrite:chloride ratios. Our results show that prolonged exposure (>7 days) of channel catfish fingerlings to nitrite:chloride ratios of about 0.25 or higher results in a generally moderate anemia, presumably hemolytic in nature, which exacerbates the effect of methemoglobinemia on the oxygen carrying capacity of the blood. Channel catfish exposed to 0.92 mg/L nitrite-nitrogen (nitrite:chloride molar ratio = 0.12) did not become anemic and developed only mild methemoglobinemia. Sodium chloride is inexpensive and, in culture ponds with little water exchange, the treatment is long-lasting. Therefore during episodes of elevated environmental nitrite concentrations, we recommend increasing the amount of chloride salt added to commercial catfish ponds to obtain a nitrite:chloride molar ratio of about 0.1 or less. This should maintain functional hemoglobin at near normal levels and diminish the adverse effects of chronic nitrite exposure on fish health.

Acknowledgments. This material is based in part on work supported by the United States Department of Agriculture under Agreement 84-CSRS-2-2363. We thank Fay Hagan and Walter Drapala for conducting and interpreting the statistical analyses of the data.

REFERENCES

Bowser PR, Falls WW, VanZandt J, Collier N, Phillips JD (1983) Methemoglobinemia in channel catfish: methods of prevention. Prog Fish-Cult 45:154-158

Butterworth CE, Plumb JA, Grizzle JM (1986) Abnormal folate metabolism in feed-related anemia of cultured channel catfish. Proc Soc Exp Biol Med 181:49-58
Huey DW, Beitinger TL (1981) A methemoglobin reductase system in channel catfish (Ictalurus punctatus). Can J of Zool 60:1511-1513
Klar GT, Hanson LA, Brown SW (1986) Diet related anemia in channel catfish: case history and laboratory induction. Prog Fish-Cult 48:60-64
Lewis WM, Morris DP (1986) Toxicity of nitrite to fish: a review. Trans Am Fish Soc 115:183-195
MacMillan JR (1985) Infectious diseases. In: Tucker CS (ed) channel catfish culture, Elsevier Science Publishers, Amsterdam, pp 405-496
Margiocco C, Arillo A, Mensi P, Schenone G (1983) Nitrite bioaccumulation in Salmo gairdneri Rich. and hematological consequences. Aqua Toxicol 3:261-270
Plumb JA, Horowitz SA, Rogers WA (1986) Feed-related anemia in cultured channel catfish (Ictalurus punctatus). Aquaculture 51:175-179
SAS Institute Inc. (1985) SAS user's guide: basics. SAS Institute, Inc., Cary, North Carolina
Scarano G, Saroglia MG (1984) Recovery of fish from functional and hemolytic anemia after brief exposure to a lethal concentration of nitrite. Aquaculture 43:421-426
Scarano G, Saroglia MG, Gray RH, Tibaldi E (1984) Hematological responses of sea bass Dicentrarchus labrax to sublethal nitrite exposures. Trans Am Fish Soc 113:360-364
Schwedler TE, Tucker CS (1983) Empirical relationship between percent methemoglobin in channel catfish and dissolved nitrite and chloride in ponds. Trans Am Fish Soc 112:117-119
Smith RP (1975) Toxicology of the formed elements of the blood. In: Casarett LJ, Doull J (eds) Toxicology: The basic science of poisons, MacMillan Publishing Co, Inc, New York, pp 225-243
Strickland JDH, Parsons TR (1972) A Practical Handbook of Seawater Analysis, 2nd edition. Fisheries Research Board of Canada, Ottawa
Tietz NW (1970) Fundamentals of Clinical Chemistry. WB Saunders, Philadelphia

Received August 1, 1988; accepted January 31, 1989.