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Chronic Effects of Formalin on Erythrocyte Counts and Plasma Glucose of Nile Tilapia, *Oreochromis niloticus*

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Abstract

The effects of sublethal concentrations of formalin on the erythrocyte counts and plasma glucose of Nile tilapia, *Oreochromis niloticus*, were investigated using static bioassays and continuous aeration during a 12-week exposure at concentrations of 25.0, 6.25, 3.125, 1.56 and 0.00 (control) mg·l^{-1}. The toxicant led to anemic and hyperglycemic responses in the test fish. The severity of these conditions was directly proportional to the toxicant concentration. However, at 1.56 mg·l^{-1} concentration no significant effects ($P>0.05$) were observed in the test fish. Precautions in the successful use of formalin to control ectoparasites on fish is discussed.

Introduction

Formalin has long been a traditional treatment of fish ectoparasites and it is usually efficient at concentrations of 167-250 $mg \cdot l^{-1}$ for 1 hour (Roberts 1978). It is applied by bath, flush or flowing treatment methods and has proved effective against most ectoparasitic protozoans and some monogenetic trematodes. A concentration of 500 $mg \cdot l^{-1}$ for 30 minutes has proved effective against the salmon louse *Lepeophtheirus salmonis* (Hastein and Bergsjo 1976). Meyer and Collar (1964) reported that it could be used at 25 $mg \cdot l^{-1}$ for an indefinite length of time in bath treatment.

Many chemical agents used in the treatment of external infestations of fish have been known to have cumulative adverse effects on the fish. Wedemeyer (1971) attempted to quantify the stress of chemical treatments in rainbow trout, *Oncorhynchus mykiss*, and coho salmon, *O. kisutch,* and there seemed little doubt that repeated use of disinfectant chemicals such as formalin might cause considerable damage to gill epithelia. Smith and Piper (1972) reported that 167

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$mg \cdot l^{-1}$ of formalin destroyed and desquamated the gill epithelium of rainbow trout.

Nile tilapia, *Oreochromis niloticus*, is a common fish in tropical freshwaters, and is used widely in aquaculture in Africa and Asia. Chronic effects of sublethal doses of formalin on hematology of Nile tilapia have not been reported in the tropics.

The objective of this investigation was to evaluate the effects of sublethal doses of formalin on the erythrocyte counts and plasma glucose levels of Nile tilapia.

Materials and Methods

Fingerlings of Nile tilapia of the same broodstock collected from Rock Water Fish Farm, Jos, Nigeria, mean weight 1.8 g, were acclimated to laboratory conditions for seven days prior to exposure. Mortality was less than 2% during the acclimation period. Fifteen fingerlings were stocked in each test aquarium with dechlorinated, aerated tap water. Feeding was done once daily (0800 hours). The experimental set-up consisted of 12 30-l aquaria, using a pelleted diet at 4% body weight of the fish. The water was changed once weekly to remove accumulated fecal material and unconsumed feed.

Formalin was obtained as 40% formaldehyde. Using dilution of this solution, preliminary runs were done over 96 hours to determine the sublethal concentrations. The following sublethal concentrations were delivered into each of the first six aquaria; 25.0, 12.5, 6.25, 3.125, 1.56 and 0.00 $mg \cdot l^{-1}$. The remaining six aquaria served as replicates.

The exposure period was 12 weeks, during which the following water quality parameters in each test aquarium were monitored weekly using methods described by APHA (1980): temperature, dissolved oxygen, free carbon dioxide, alkalinity, pH and unionized ammonia. Two fish from each test aquarium were sacrificed and analyzed at 2-weekly intervals for erythrocyte counts and hematocrit values. The mean values were recorded. Blood was collected by severing the tail of the fish according to the method of Grizzle (1977).

Erythrocyte count was done using a standard hemocytometer. Modified Dacie's fluid was used as the diluting fluid using the method of Blaxhall and Daisley (1973). Blood was drawn directly into RBC pipettes just beyond the 1.0 mark, touching the tip with absorbent tissue to reduce the volume to exactly the 1.0 mark. Thereafter, the diluting fluid was drawn to the 101 mark (1:100 dilution) and mixed for 1-2 minutes. The diluted blood was then introduced into a standard hemocytometer. Erythrocytes were counted in five 1-mm squares under a microscope with x100 objective. Three counts for each diluting pipette were taken and their mean value recorded. The erythrocyte count in 1 ml of blood was obtained using a formula given by Blaxhall and Daisley (1973).

Fresh blood samples were collected into heparinized microhematocrit tubes (Blaxhall and Daisley 1973) sealed with plasticine at one end and centrifuged for five minutes at 3,000 rpm. The centrifuged plasma was

transferred into a test tube and made up to 2 ml using distilled water. The glucose content was quantified using the anthrone method as modified by Wedemeyer and Yasutake (1977).

Analysis of variance with the Duncan's multiple range F test (Duncan 1955) was determined for each of the sampling periods.

Results

The water quality parameters in the test aquaria did not vary significantly from those of the control aquaria (Table 1). All were within suggested tolerance range (Mackereth 1963). Nevertheless, the formalin led to significant depletion ($P<0.05$) in the erythrocyte counts of the exposed fish (Table 2), the depletion being proportional to the toxicant concentrations and sampling periods. Significant increases ($P<0.05$) in plasma glucose were observed in exposed fish

Table 1. Mean water quality parameters* obtained during exposure of the Nile tilapia, *Oreochromis niloticus*, to sublethal concentrations of formalin for 12 weeks.

Parameters	Formalin concentrations ($mg \cdot l^{-1}$)					
	25.00	12.50	6.25	3.125	1.56	0.00 (control)
Temperature (°C)	24.04 (0.06)	24.09 (0.07)	24.25 (0.06)	24.27 (0.06)	24.56 (0.03)	24.23 (0.06)
Dissolved oxygen ($mg \cdot l^{-1}$)	6.91 (0.04)	6.90 (0.06)	7.02 (0.06)	6.95 (0.05)	6.92 (0.04)	6.93 (0.07)
Carbon dioxide ($mg \cdot l^{-1}$)	4.72 (0.05)	4.96 (0.04)	4.86 (0.03)	4.82 (0.04)	4.64 (0.07)	4.94 (0.03)
Alkalinity ($mg \cdot l^{-1}$)	32.97 (0.03)	33.49 (0.30)	36.88 (0.08)	34.17 (0.04)	34.19 (0.20)	32.83 (0.20)
pH	6.76 (0.05)	6.63 (0.04)	6.58 (0.02)	6.65 (0.03)	6.54 (0.03)	6.74 (0.03)
NH_3 (unionized)	0.22 (0.01)	0.23 (0.03)	0.22 (0.01)	0.23 (0.01)	0.22 (0.01)	0.22 (0.01)

*Mean values with standard deviation in parentheses.

Table 2. Mean erythrocytes ($x10^6$ cells$\cdot ml^{-1}$)* of the Nile tilapia, *Oreochromis niloticus*, exposed to various sublethal concentrations of formalin for 12 weeks

Concentrations	Exposure period (weeks)					
($mg \cdot l^{-1}$)	2	4	6	8	10	12
25.00	6.5 (0.02)	5.4 (0.02)	5.0 (0.01)	4.5 (0.01)	4.3 (0.01)	4.1 (0.01)
12.50	7.2 (0.02)	6.6 (0.40)	5.8 (0.02)	5.6 (0.02)	5.3 (0.01)	5.1 (0.02)
6.25	7.5 (0.01)	7.3 (0.02)	6.9 (0.01)	6.4 (0.02)	6.0 (0.02)	5.8 (0.01)
3.125	8.5 (0.03)	8.0 (0.01)	7.8 (0.02)	7.5 (0.01)	7.2 (0.01)	7.2 (0.01)
1.56	9.9 (0.04)	9.7 (0.02)	9.4 (0.01)	8.9 (0.01)	8.6 (0.01)	8.2 (0.02)
0.00 (control)	11.3 (0.03)	11.1 (0.03)	11.1 (0.01)	10.8 (0.03)	11.4 (0.04)	11.1 (0.02)

*Mean values with standard deviations in parentheses.

Table 3. Mean plasma glucose ($mg \cdot ml^{-1}$)* of the Nile tilapia, *Oreochromis niloticus*, exposed to various sublethal concentrations of formalin for 12 weeks.

Concentrations	Exposure period (weeks)					
	2	4	6	8	10	12
25.00	0.061 (0.01)	0.065 (0.09)	0.071 (0.02)	0.088 (0.02)	0.088 (0.01)	0.092 (0.03)
12.50	0.053 (0.01)	0.059 (0.04)	0.063 (0.05)	0.072 (0.06)	0.075 (0.02)	0.081 (0.02)
6.25	0.042 (0.08)	0.045 (0.02)	0.051 (0.08)	0.058 (0.01)	0.063 (0.04)	0.065 (0.01)
3.125	0.032 (0.04)	0.036 (0.14)	0.042 (0.01)	0.048 (0.02)	0.053 (0.09)	0.056 (0.03)
1.56	0.030 (0.01)	0.030 (0.08)	0.033 (0.01)	0.038 (0.06)	0.040 (0.01)	0.043 (0.01)
0.00 (control)	0.029 (0.02)	0.027 (0.04)	0.032 (0.01)	0.031 (0.02)	0.030 (0.01)	0.031 (0.02)

*Mean values with standard deviations in parentheses.

(Table 3), the increase being proportional to the toxicant concentrations and sampling periods. The erythrocyte counts and plasma glucose of fish exposed to 1.56 $mg \cdot l^{-1}$ formalin were not significantly different ($P<0.05$) from those of the control groups.

Discussion

Sublethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of the aquatic organisms. However, they have significant effects which result in several physiological dysfunctions as earlier reported by Edwards (1970) and Omoregie et al. (1990). A dose-dependent impairment in the erythrocyte count and plasma glucose of *O. niloticus* was observed when the fish were exposed to various sublethal concentrations of formalin for 12 weeks in static bioassays with continuous aeration system. The most adverse effects were observed in the group of fish exposed to 25 $mg \cdot l^{-1}$ of the toxicant. The fish had a progressive decrease in erythrocyte count and increase in plasma glucose as exposure time increased.

Reduction in erythrocytes reported in this investigation indicated that *O. niloticus* exposed to sublethal concentrations of formalin became anemic, which according to Wedemeyer et al. (1984) is due to hemodilution resulting from impaired osmoregulation across the gill epithelium. Smith and Piper (1972) had earlier reported that 167 $mg \cdot l^{-1}$ of formalin led to the destruction and desquamation of the gill epithelium which, according to Davies et al. (1976), leads to impaired gaseous exchange across the gill lamellae of rainbow trout. Similar reductions in the erythrocytes of Baltic salmon *Salmo salar*, and channel catfish *Ictalurus punctatus*, exposed to Malachite green were reported by Glagoleva and Malikova (1968) and Grizzle (1977), respectively; and in Nile tilapia exposed to Gammalin 20 and Actellic 25 EC by Omoregie et al. (1990). Changes in erythrocyte count were reported by Wedemeyer and Yasutake

(1977) and Clarke et al. (1979) to be a strong indicator of stress due to presence of toxicants in the aquatic environment.

The progressive accumulation of plasma glucose reported in this investigation indicated that *O. niloticus* exposed to sublethal concentrations of formalin became hyperglycemic. Wedemeyer (1973) documented that fish show marked hyperglycemic response to stressful environmental conditions, as a result of incomplete metabolism of the blood sugar due to impaired osmoregulation (Omoregie et al. 1990). Earlier reports by Oguri and Nace (1966) and McLeay and Brown (1975) subscribe to the observation of this investigation.

Formalin is widely used by fish farmers in the tropics to treat ectoparasitic infestations of fish. Though this is legal, the deleterious consequences on the health of fish subjected to nominal chronic exposure calls for more research and precautions in its usage. It is vital to crosscheck calculations of dosage and to monitor the prescribed exposure time for the beneficial use of the compound.

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