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Formaldehyde Residue in the Muscle of Nile Tilapia

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Abstract

Formaldehyde residues in muscle tissue were determined for Nile tilapia *Tilapia niloticus* which had been treated indefinitely with formalin at rates of 25 mg·l^{-1}, and bath treatments of 125 mg·l^{-1} for 1.5 h or 250 mg·l^{-1} for 1 h. In the indefinite treatment, formaldehyde in treated fish in-creased slightly from 48 to 72 h, and levels were back to normal at 96 h. There were, however, no statistical differences in the formaldehyde residue between formalin indefinitely treated fish and control fish. In the bath treatments, there were no significant differences in muscle formalde-hyde residue between treated and control fish, and between fish treated with 125 and 250 mg·l^{-1} formalin. Formalin degraded more quickly at high temperature than at low temperature. No effect of salinity on formalin degradation was found in water at 25 ± 1°C.

Introduction

Nile tilapia *Tilapia niloticus* is a valuable fish cultured in ponds, cages and raceways. The fish is highly regarded by producers and consumers in many tropical and subtropical countries since it can produce high yields in a short period on relatively low inputs (Pullin and Lowe-McConnell 1982).

Although tilapia is more resistant to diseases than many other cultured species, many disease problems still occur. A wide range of parasites has been reported in tilapia, particularly protozoa and monogenic trematodes (Balarin 1979; Roberts and Sommerville 1982). These problems cause low growth rates, and decreased production and survival.

Formalin is one of the therapeutic chemicals effective in treating protozoa and monogenetic trematodes. Formalin was approved by the Food and Drug Administration in the USA as a parasiticide for use on trout, salmon, catfish, largemouth bass *Micropterus salmoides* and bluegill *Lepomis macrochirus* at 25 $mg \cdot l^{-1}$ in ponds and up to 250 $mg \cdot l^{-1}$ for 1 h in tanks and raceways (Schnick et al. 1989). However, formalin is still not approved for use in tilapia.

There is no information in the literature on formaldehyde residue in tilapia tissues following use of formalin. This study presents the results of formaldehyde residue in Nile tilapia muscle after fish were exposed to formalin solution. The effect of water temperature and salinity on formalin persistence in water was also investigated.

Materials and Methods

Experiments were conducted using formalin as an indefinite treatment at a concentration of 25 $mg \cdot l^{-1}$ and as a bath treatment at a concentration of 125 $mg \cdot l^{-1}$ for 1.5 h or 250 $mg \cdot l^{-1}$ for 1 h (equal to 9.25, 46.25 or 92.50 $mg \cdot l^{-1}$ of formaldehyde, respectively) to determine formaldehyde residue in tilapia muscle. In the indefinite treatment, formalin was added to the water and allowed to dissipate.

For the indefinite treatment, 84 one-year-old fish, 17.1 $\pm$ 1.0 cm (mean $\pm$ SD) in length and 91.5 $\pm$ 11.4 g in weight, were kept in four 500-l fiberglass tanks at a temperature of 20.1 $\pm$ 2.5°C. Two tanks were treated and two served as a control, with 21 fish in each tank. The fish were acclimated in the tanks 5-7 d before the experiment. Then 100% formalin (commercial formulation of 37% formaldehyde, Fisher Scientific, Pittsburgh, Pennsylvania, USA) was added to the treated tanks to get 25 $mg \cdot l^{-1}$ formalin in water. Three fish were sampled from each tank at 0, 24, 48, 72, 96, 120 and 144 h after exposure to the formalin solution. To minimize the loss of formalin from the aqueous solution, neither aeration nor water was provided to the tanks during formalin exposure.

For the bath treatment, six styrofoam boxes were filled with 40 l water. Seventy-two fish, 17.8 $\pm$ 0.6 cm in length and 102.8 $\pm$ 9.5 g in weight, were divided into six groups and one group was kept in each box. Formalin was added to two boxes to get 125 $mg \cdot l^{-1}$ formalin and to two other boxes to get 250 $mg \cdot l^{-1}$. The fish were exposed 1.5 h in the 125 $mg \cdot l^{-1}$ formalin solution or 1 h in the 250 $mg \cdot l^{-1}$ solution. Treated and control fish were released into separate tanks with flowing freshwater. The treated and control fish were sampled at 0, 12, 24, 48, 72 and 96 h after bath treatment. After the fish were killed and skinned, the muscles were sliced off from the trunk-tail region in both sides with a fillet knife. A muscle sample from each fish was analyzed separately to determine formaldehyde residue.

Sixteen 1-l glass jars were used to test formalin degradation at 15, 25, 30 and 35°C. Four jars were kept in a water bath set to the required temperature with two containing formalin solution and the other two containing water as controls. The 25 $mg \cdot l^{-1}$ formalin solution was made with deionized water. Water samples were collected from each jar at 0, 24, 48, 72 and 96 h.

Ten 50-l aquaria were used to test salinity effects on 25 $mg \cdot l^{-1}$ formalin degradation. Salt (Flint River Mills, Inc., Georgia, USA) was added to water to make 3, 6, 9, 12 and 16‰ salinity. Formaldehyde was added to each aquarium to make 25 $mg \cdot l^{-1}$ formalin solutions. Two aquaria were used at each salinity and two water samples were analyzed from each aquarium at 0, 24, 48 and 72 h after adding formaldehyde.

The Nash test has been one of commonly used methods to determine formaldehyde residue in aquatic animal tissues (Castell and Smith 1973; Sillis 1979; Hose and Lightner 1980). In this experiment, formaldehyde in muscle or water samples were analyzed employing the Nash test in a prior study (Xu and Rogers 1993). Briefly, one part fish muscle was homogenized with two parts distilled water in a Waring blender at high speed for 30 sec. To 12 ml of fish muscle homogenate, 12 ml of 20% sulfuric acid was added, and stirred with a

Vortex mixer at high speed for 5-10 sec to accomplish extraction of formaldehyde.

The resulting acidic extracts were filtered through Whatman no. 40 filter paper. Five-ml of aliquots of the filtered extracts were added to 10 ml distilled water. These extracts were adjusted to pH 6.0 with a Corning pH meter (Corning Medical, Medfield, Massachusetts, USA) with 10 N NaOH or 1 N acetic acid and further diluted to 25 ml with distilled water.

To 2.5 ml diluted extracts, 2.5 ml of double-strength Nash reagent was added, and incubated for 5 minutes at 60°C. Optical density was then read at 415 nm with a Spectronic 20D spectrophotometer (Milton Roy, Analytical Products Division, New York, USA).

Formaldehyde residue in the fish muscle was determined by comparing with the standards, and recorded as micrograms of formaldehyde per gram of fish muscle ($\mu g \cdot g^{-1}$). Formalin in water was determined with similar procedures used for muscle assay except no extraction was made with 20% sulfuric acid.

For the standards, 3 ml of the formalin stock solutions were added to 9 ml distilled water to provide known aqueous solutions containing 0, 5, 10, 15, 20, 25 and 50 $mg \cdot l^{-1}$ formalin. Formalin stock solutions (0, 20, 40, 60, 80, 100 and 200 $mg \cdot l^{-1}$) were prepared immediately before use.

To evaluate the accuracy of the assay procedure, samples of fish muscle were spiked with formaldehyde at concentrations of 1.9, 7.4 and 18.5 $\mu g \cdot g^{-1}$ (equivalent to 5, 20 and 50 $\mu g \cdot g^{-1}$ formalin), and the accuracy was calculated from the test results as the percent recovery of formaldehyde. The precision of the procedure was determined by the degree of agreement among individual tests, and expressed as the coefficient of variation. Standard curves were used to check the linearity at levels of 0, 5, 10, 15, 20, 25 and 50 $mg \cdot l^{-1}$ formalin.

Water quality features were measured during the experiments. Dissolved oxygen, monitored with a YSI oxygen meter (Yellow Spring Instruments, Yellow Spring, Ohio, USA), was usually higher than 7.0 $mg \cdot l^{-1}$. The pH, measured with a Corning pH meter, ranged from 7.1 to 7.6. Total alkalinity and hardness, determined with a Hach water quality test kit (Hach Chemicals, Loveland, Colorado, USA), were 28.5 $mg \cdot l^{-1}$ and 22.8 $mg \cdot l^{-1}$, respectively. Average temperature during the experimental period was 21-23°C. The data were analyzed and tested by analysis of variance procedures (SAS Institute 1982). Probabilities of 0.05 or less were considered statistically significant.

Results and Discussion

In the indefinite treatment, formaldehyde residue increased in tilapia muscle after exposure to 25 $mg \cdot l^{-1}$ formalin solution. Reaching the highest value after 48 h when treatment was conducted, the residue level then decreased gradually and was back near normal after 96 h at 21-22°C (Fig. 1). There were, however, no statistical differences in formaldehyde residue between treated and control fish. An average 1.9 ± 0.1 $\mu g \cdot g^{-1}$ of formaldehyde ($N = 42$) was present in control fish, and 2.2 ± 0.3 $\mu g \cdot g^{-1}$ was found in treated fish.

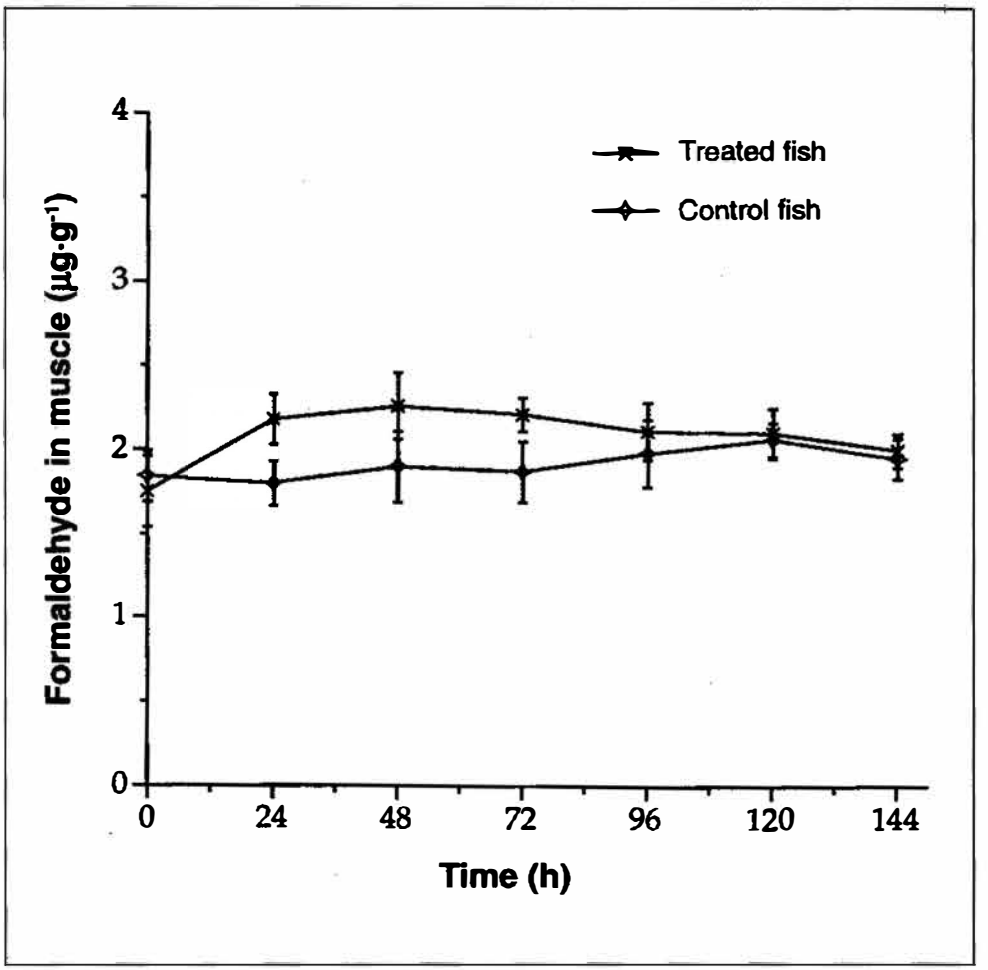

Fig. 1. Formaldehyde residue in muscle of Nile tilapia treated indefinitely with 25 mg·l^{-1} formalin at 21-22°C.

In the bath treatment, formaldehyde residue in muscle was 1.34 ± 0.29 µg·g^{-1} (mean ± SD, N = 36) for control fish, 1.30 ± 0.25 µg·g^{-1} for 125 mg·l^{-1} formalin-treated fish, and 1.57 ± 0.2 µg·g^{-1} for 250 mg·l^{-1} formalin-treated fish. There were no significant differences in formaldehyde residue between treated and control fish or between 125 mg·l^{-1} treated fish and 250 mg·l^{-1} treated fish (Fig. 2).

Formalin degraded more quickly at high temperature than at low temperature in water (Fig. 3). It took 72 h for formalin to degrade completely at 30 and 35°C. No difference was found in formalin degradation time between 30 and 35°C. However, it took much longer for formalin to degrade completely at low temperature and only 20% (5 mg·l^{-1}) of formalin was depleted at 15°C after 96 h (Fig. 3). No effect of salinity on formalin degradation was found in water. After 72 h, formalin was almost completely degraded in water with different salinities at 25 ± 1°C (Fig. 4).

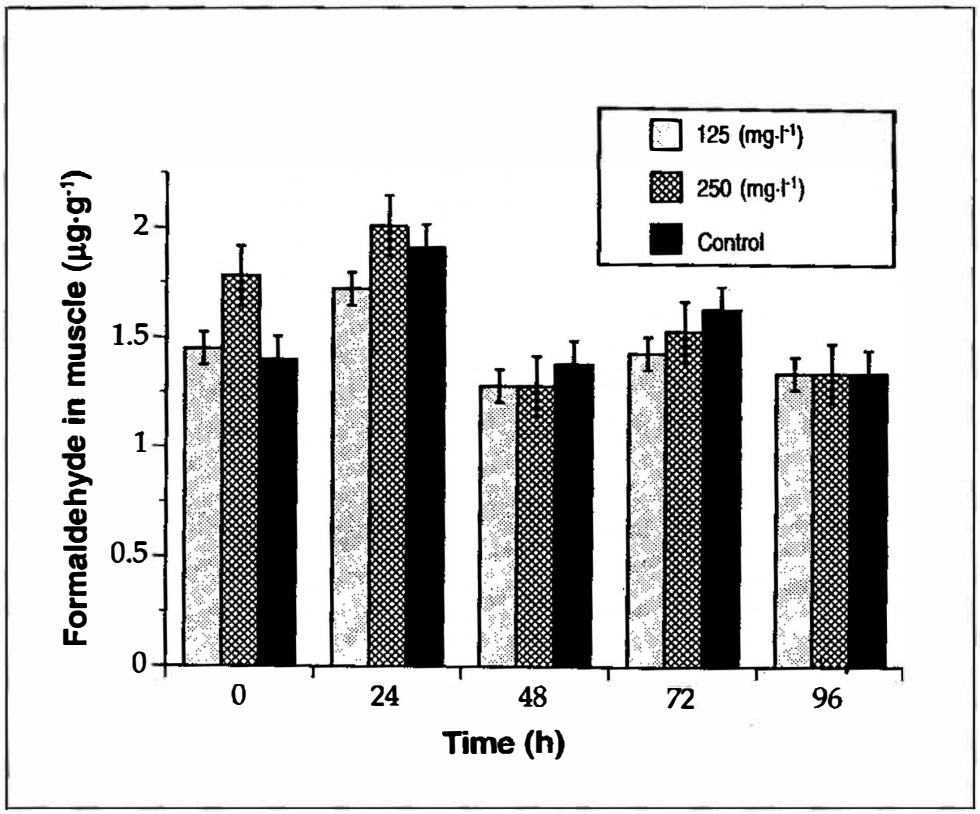

Fig. 2. Formaldehyde residue in muscle of Nile tilapia treated with 125 mg·l⁻¹ formalin for 1.5 h or 250 mg·l⁻¹ formalin for 1 h at 21-22°C.

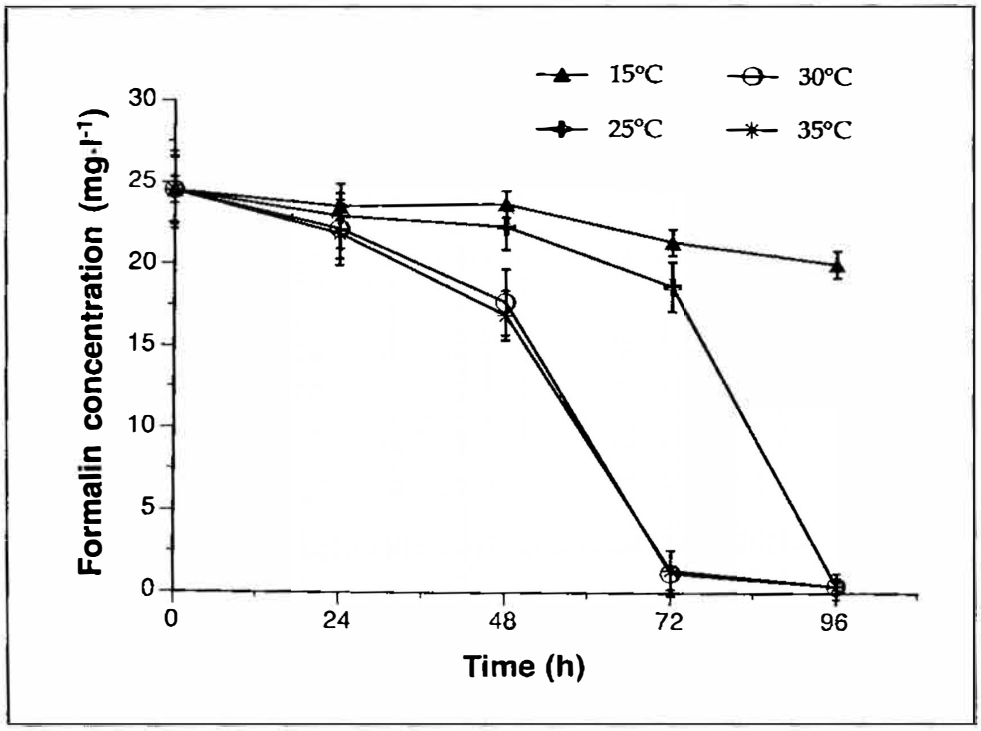

Fig. 3. Degradation of 25 mg·l⁻¹ formalin solution in water with different temperatures.

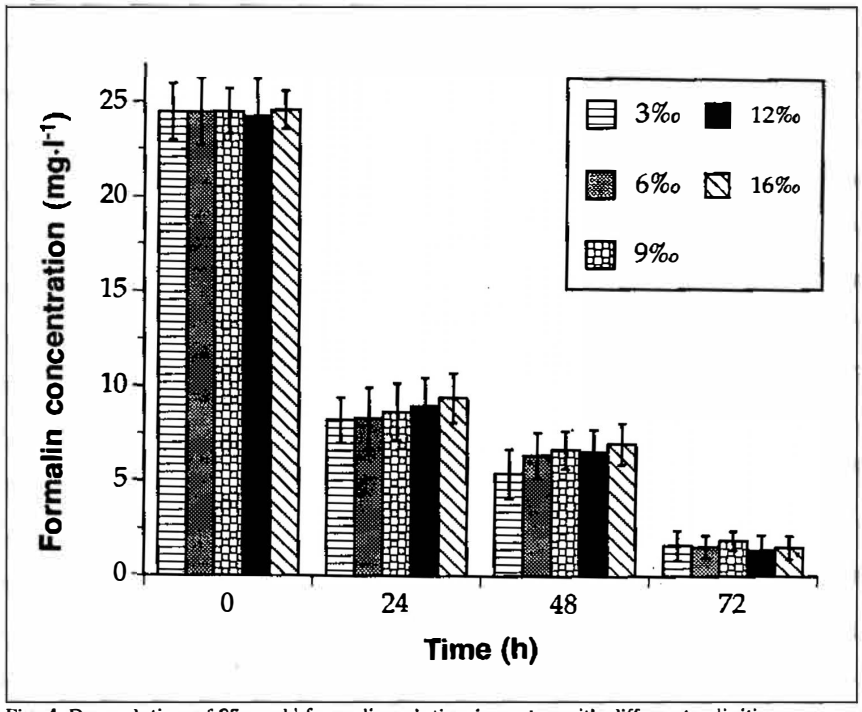

Fig. 4. Degradation of 25 mg·l^{-1} formalin solution in water with different salinities.

For muscle tissues spiked with formaldehyde at concentrations of 1.85, 7.4 and 18.5 μg·g^{-1}, recovered formaldehyde concentrations were 1.9 ± 0.1 μg·g^{-1} (mean ± SD), 5.3 ± 0.3 μg·g^{-1} and 12.6 ± 0.4 μg·g^{-1}; formaldehyde recoveries were 103.3, 71.8 and 67.9%; and coefficients of variation of formaldehyde recovered were 10.7, 6.7 and 5.4%, respectively. The optical density was directly proportional to the formalin concentration from 0.50 mg·l^{-1} in standard curves. The regression line was $Y = -0.0062 + 0.0040X$ ($r^2 = 0.9942$).

Formaldehyde is a normal cellular metabolite and levels in mammalian cells range from 1.5 to 15 μg·g^{-1} (Feinman 1988). The endogenous levels of 3-12.8 μg·g^{-1} formaldehyde was found in fish (Frontier Feeds 1987; cited by Schnick 1987). In our experiments, an average of 1.62 μg·g^{-1} formaldehyde, ranging from 1.24 to 2.07 μg·g^{-1} was found in control fish. This concentration should not have been greatly influenced by postmortem change because formaldehyde residue in the muscle was assayed immediately after fish were killed.

There are few reports on the determination of formaldehyde residue following use of formalin in aquatic animals. Sillis (1979) treated channel catfish and largemouth bass with 300 mg·l^{-1} formalin solution for 3 h; coho salmon and rainbow trout for 1 h; and channel catfish and largemouth bass with 35 mg·l^{-1} formalin indefinitely. He found no residues of formaldehyde in fish exposed to formalin. Hose and Lightner (1980) treated cultured blue shrimp *Penaeus stylirostris* with 50 and 150 mg·l^{-1} formalin for 6 or 24 h. No formaldehyde residues in these shrimp were found to result from the therapeutic use of formalin. These authors, however, used processed standards in their assays, i.e., formaldehyde stock solution was added to tissue homogenate to make the standards. Since formaldehyde is present naturally in animal cells, the amount of formal-

dehyde naturally present in cells was not detected with processed standards. In a recent paper, Subasinghe and Yusoff (1993) reported that tropical fish *Puntius gonionotus* (Bleeker) retained 5.75 ppm formaldehyde following exposure to 50 ppm formalin for 24 h; *Clarias batrachus* (L.) retained 14.03 and 18.72 ppm formaldehyde following exposure to formalin at 50 and 100 ppm for 24 h, respectively. No formaldehyde residue, however, was detected in exposed fish after 24 h in clean water.

In a prior experiment, Xu and Rogers (1993) exposed striped bass *Morone saxatilis* to a static solution of 25 $mg \cdot l^{-1}$ formalin in water. They found that formaldehyde residue was higher in treated fish than in control fish at 48 and 72 h after applying formalin, but no differences of formaldehyde residue were noticed in muscle between treated and control fish at other sampling time. In this study, no statistical differences were found in formaldehyde residue in muscle of Nile tilapia between fish treated indefinitely with 25 $mg \cdot l^{-1}$ formalin and control fish, between fish treated with 125 $mg \cdot l^{-1}$ formalin for 1.5 h and control fish, and between fish treated with 250 $mg \cdot l^{-1}$ formalin for 1 h and control fish.

Nile tilapia is euryhaline and can grow and reproduce in fresh, brackish and seawater, so there is a need to use formalin in water with different salinities and temperatures. Fish gills and skin are the most likely routes for formalin absorption when formalin is dissolved in water. The concentration and persistence of formalin in water affect formalin absorption into fish. The degradation tests in this study show that salinity has no effect on degradation of formalin in water, but temperature does. Formalin degraded more slowly at low temperature than at high temperature. As a result, fish have a longer exposure time to formalin at low temperature when formalin is used in water indefinitely. Since temperature also affects fish, it is necessary to consider both fish metabolism and formalin persistence in water when conducting a formalin residue study in fish.

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