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The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to *Streptococcus iniae* under stress in low and high temperatures

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Abstract

Mozambique tilapia *Oreochromis mossambicus* acclimated to 27 °C were then held at 19, 23, 27 (control), 31 and 35 °C, and were examined for non-specific cellular and humoral responses after 12–96 h. Total leucocyte count decreased significantly when fish were transferred to 19 and 23 °C after 48 and 96 h, and when transferred to 35 °C over 12–96 h, respectively. Respiratory burst decreased significantly when fish were transferred to 19, 31 and 35 °C over 24–96 h, whereas phagocytic activity and phagocytic index decreased significantly when fish were transferred to low temperatures (19 and 23 °C) and high temperatures (31 and 35 °C) over 12–96 h. Lysozyme activity decreased significantly when fish were transferred to 19 °C after 12–96 h, but increased significantly when transferred to 31 and 35 °C over 48–96 h. Alternative complement pathway (ACH_{50}) also decreased significantly when transferred to 19 and 23 °C after 12 h, but increased significantly when transferred to 31 and 35 °C after 24 h. In another experiment, tilapia reared at 27 °C were injected intraperitoneally with *Streptococcus iniae* at a dose of 1×10^7 colony-forming units (cfu) $fish^{-1}$, and then reared onward at water temperatures of 19, 23, 27 (control), 31 and 35 °C. Over 48–168 h, the cumulative mortality of *S. iniae*-injected fish held in 19 and 35 °C was significantly higher than that of injected-fish held in 23, 27 and 31 °C. It is concluded that transfer of tilapia *O. mossambicus* from 27 °C to low temperatures (19 and 23 °C) after 12 h, and transfer of fish from 27 °C to high temperatures (31 and 35 °C) reduced their immune capability. Moreover, tilapia under temperature stress at 19 and 35 °C from 27 °C decreased its resistance against *S. iniae*.

Keywords: Mozambique tilapia; *Oreochromis mossambicus*; Temperature; Challenge; Non-specific cellular response; Non-specific humoral response; *Streptococcus iniae*

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1. Introduction

Tilapia is the third most commonly farmed fish after carp and salmonids with global production of 1.49 million metric tonnes (mmt) in 2002, and is expected to grow to 2.0 mmt in 2010 [1]. Tilapia can tolerate low temperature of 6–10 °C for short periods, and tolerate temperature as high as 35–42 °C. Growth occurs between 20 and 35 °C, whereas above 37 °C increasing mortalities are likely to occur [2].

Tilapia is extremely hardy but may be stressed by excessively high or low temperatures which increase susceptibility to pathogens. Among the common disease agents, flexibacteria are likely to cause skin lesion syndrome under high or low temperatures, and protozoans like *Ichthyophthirius* and *Trichodina*, and virus *Lymphocystis* are likely to cause white spot at low temperatures [3,4]. The adverse effects of the bacterium *Streptococcus iniae* in many farmed fresh water fish including tilapia have been reported [5,6]. This bacterium causes heavy losses from mortality, reduced growth as well as unmarketable appearance in fish. Tilapia is one of the most susceptible fish to *S. iniae* infection [6,7]. Therefore, the effect of temperature stress on the immune system of fish and its susceptibility against invading pathogens are of primary concern [8].

Several reactive oxygen species (ROS) are produced by fish phagocytes during the respiratory burst. Once bacteria or fungi are engulfed by leucocytes, the host's NADPH-oxidase is activated, which in turn increases oxygen consumption and subsequently produces ROS such as superoxide anion ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$) [9]. The release of superoxide anion is known as the respiratory burst, and together its derivates are bactericidal [10].

Lysozyme found in cutaneous mucus, peripheral blood and certain tissues rich in leucocytes, is an enzyme which catalyses the hydrolysis of *N*-acetyl muramic acid and *N*-acetyl glucosamine of peptidoglycan in bacterial cell walls [11]. This protein plays a crucial role in the defense system. Alternative complement pathway, also a non-specific humoral response, exhibits lytic, proinflammatory, chemotactic and opsonic activities in teleosts [12].

In freshwater fish, changes in temperature have been reported to affect cytotoxic cell activity, macrophage activity, respiratory burst and leucocyte count in common carp *Cyprinus carpio* [13–17]. Change in temperature has also been reported to affect lysozyme activity, phagocytic activity and the immunoglobulin (IgM) level of tilapia *Oreochromis aureus* and *Oreochromis niloticus* [18–20]. This study was aimed at determining the immune parameters of Mozambique tilapia *Oreochromis mossambicus* and its resistance against *S. iniae* when fish were subjected to change of low and high temperatures. For the former purpose, total leucocyte count (TLC), respiratory burst (release of superoxide anion), phagocytic index, phagocytic activity, lysozyme activity and alternative complement pathway were examined.

2. Materials and methods

2.1. Fish

O. mossambicus brood stock obtained from Lu Kang Branch, Taiwan Fisheries Research Institute, were shipped to our laboratory, and held in circular freshwater tanks supplied with a filter and an aeration system. About 450 fish were used for this experiment. Fish were acclimated at 27 °C for three weeks. For the susceptibility experiment, there were 10 treatments (five challenged test groups and five unchanged control groups). The test and control groups comprised 10 fish in triplicate tanks. For the experiment of immune parameter assays, there were 25 treatments (five temperatures at 19, 23, 27, 31 and 35 °C combined with five exposure times at 0, 12, 24, 48 and 96 h). Tests were carried out in duplicate test groups consisting of three fish each in 60 l glass aquaria containing 20 l of water. In all tests, the fish were fed twice daily with a formulated diet (Tairoun Feed Company, Taipei, Taiwan) during the experiment. The fish ranged from 34 to 49 g, averaging 41.2 ± 5.03 g (mean ± SD) with no significant size difference among the treatments. During the experimental periods, dissolved oxygen ranged from 6.93 to 9.12 mg l^{-1}, pH ranged from 7.8 to 8.2 and ammonia–N was lower than 0.01 mg l^{-1}.

2.2. Formalin-killed Escherichia coli

E. coli (DH5α) was grown overnight in 100 ml tryptic soy broth (TSB) at 37 °C. Formaldehyde (37%) was added to give 2% final concentration and the culture was shaken at 22 °C overnight. Stock cultures were centrifuged at 700 × *g* for 10 min at 4 °C. The supernatant fluid was removed and the bacterial pellet washed twice with 50 ml PBS (NaCl,