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# Impact of Aeromonas Hydrophila Infection on Oxidative Stress in Nile Tilapia

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**Abstract** Background and Aim: The lack of disease control has the potential of being the limiting factor of fish production. Improving disease resistance of cultured fish are major challenges facing fish culturists, especially *Aeromonas Hydrophila* (*A. Hydrophila*) bacterial infections that cause mass mortalities. There was a positive link between bacterial infection and stimulation of oxidative stress in fish. The aim of the current study was to assess the effect of *A. Hydrophila* on the oxidative stress parameters in Nile tilapia. Materials and methods: Nile tilapia (n = 110) with average size between 70 and 90gwere obtained from local farms in Kafrelsheikh. After 2 weeks of acclimation, fish were intraperitoneally injected by *A. Hydrophila* to get two divergent lines of fish in response to disease: one susceptible/diseased and the other resistant to disease. Results: The level of the antioxidant parameters (CAT, GSH and SOD) and the lipid peroxidation marker malondialdehyde (MDA) were measured in the spleen of infected and control fishes. There was a marked increase in the splenic level of MDA and a marked decrease of antioxidant GTH and antioxidant enzymes (SOD, and CAT) activities in susceptible fishes. Conclusion: This suggests a positive link between bacterial infection and stimulation of oxidative stress in susceptible fish. However, this link was negative in resistant fish.

**Keywords** Nile Tilapia, *A. Hydrophila*, Oxidative Stress

## Introduction

The tilapia is a group of cichlid fishes, which includes three economically important genera namely, Tilapia, Oreochromis, and Sarotherodon. It is an important commodity, ranking ninth in global aquaculture production [1-3]. China, Egypt, Indonesia, Philippines, and Thailand are the principal producing countries of tilapia. The world tilapia production had been growing increasingly in recent years with 1.7 million metric tons in 2016 [4].

The ubiquitous bacterium *A. Hydrophila* is a freshwater, facultatively anaerobic, chemoorganoheterotroph [5] and the etiologic agent of disease in amphibians, birds, fishes, mammals, and reptiles, with the most common forms of disease being gastroenteritis, septicemia, and necrotizing fasciitis [6]. Virulence in *A. hydrophila* is multifactorial, with disease resulting from the production and/or secretion of virulence factors, such as adhesins, cytotoxins, hemolysins, lipases, and proteases as well as the capacity to form biofilms, use specific metabolic pathways, and mediate virulence factor expression through quorum sensing [7]. Oxidative stress is the steady state level of oxidative damage in a cell, tissue, or organ; caused by ROS (Redox imbalance). A particular molecule, or the whole organism can be affected due to this damage [8-9]. Oxidative stress occurs as a result of oxidation exceeds the antioxidant systems in the biological system secondary to a loss of balance between them [10-12]. The disturbed balance leads to oxidative stress which can be responsible for damaging DNA, proteins and lipids. An inefficient repair mechanism may finally trigger cell necrosis, or neoplastic transformation [13]. There was positive link between bacterial infection and stimulation of oxidative stress in susceptible fish. The aim of the current study is to assess the effect of *A. Hydrophila* on the oxidative stress parameters in Nile tilapia.

## Material and Methods

### Fish and Rearing Conditions

This work was approved by the Animal Care and Welfare Committee of Kafrelsheikh University which follow the general guidelines of the Canadian Council on Animal Care. Genetically unrelated Nile tilapia (n = 110, weight 60± 2.90 g) with average size between 70 and 90 g were obtained from local farms in Kafr El- Sheikh. After 2 weeks of acclimation, fish reared in fiberglass tanks (20 fish per tank). These tanks filled with appropriate amount of de-chlorinated water at pH ranged from 6.5-7, temperature at a range of 26-28 °C, and light cycle of 13:00 hours light and 11:00 hour dark. Fish were fed a commercial ration with 30.0% crude protein, 4.0% crude lipid, 4.4% pure crude fiber have total energy of ≥3754 kcal/kg at a rate of 3% body weight daily.

### Bacterial Challenge Test and Fish Groups

Bacterial challenge using *A. Hydrophila*, a virulent strain obtained kindly from the Fish Disease Lab, National Institute of Oceanography and Fisheries (NIOF), Alexandria branch, Egypt, was done to get two divergent lines of fish in response to disease: one susceptible/diseased and the other resistant to disease. *A. hydrophila* was cultured in Bactotryptic soy broth and identical solid media containing 1.5% Bacto agar at 25°C with rotation at 250 rpm. Fish (n = 90) were challenged intraperitoneally with *A. hydrophila* ($0.5x10^6$ CFU/fish) and kept under observation for 14 days to record symptoms and survival rate daily. During observation period, 20 challenged-fish were dead and 40 out of the remaining 70 showed the symptoms (and was considered as diseased/susceptible group), while the remaining 30 showed no symptoms (and was considered as resistant group). Control (non-challenged) fish was intraperitoneally injected by PBS (n = 20). Re-isolation of injected bacteria from freshly dead fish during the period of observation was confirmed.

### Sampling

Following collection of blood samples, the fish were sacrificed by spinal cord transection and spleens were immediately removed and rinsed in PBS to remove blood. Pieces from spleen were homogenized in PBS, centrifuged and the obtained supernatants were used for biochemical analysis (MDA, GSH and antioxidant enzymes). Spleen tissue homogenate was prepared as follow: a weight of 100 mg spleen was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2-8°C. The supernatant was removed and assayed immediately. Alternatively, aliquot and store samples at -20°C or -80°C.

**Determination of Reduced Glutathione (GSH)**

Reduced glutathione was measured calorimetrically using the method described by Beutler et al [14]. The method based on the reduction of 5.5 dithiobis 2-nitrobenzoic acid (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

**Determination of Lipid Peroxide (Malondialdehyde)**

Peroxide (Malondialdehyde) was measured colorimetrically using the method described by Ohkawa et al. [15]. Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95 $^{o}$C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm.

**Determination of Superoxide Dismutase Activity (SOD)**

Superoxide dismutase activity (SOD) was determined by colorimetric method according to Nishikimi et al [16]. This assay relies on the ability of the enzyme to inhibit the phenazinemethosulphate-mediated reduction of nitrobluetetrazolium dye.

**Determination of Catalase (CAT)**

Catalase activity was measured by colorimetrically according to Fossati et al [17]. Catalase reacts with known quantity of $H_2O_2$. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase (HRP), remaining $H_2O_2$ reacts with 3,5-Dichloro- 2-hydroxybenzene sulfuric acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample.

**Statistical Analysis**

The statistical analysis was carried out by Graph Pad Prism 7 (Graph Pad Software, Inc., LaJolla, CA, USA) using one way Analysis of Variance (ANOVA) method followed by Duncan Multiple Range Test (DMRT) for examination of the significance of the differences among different groups. Data were presented as mean ± standard error of mean (SEM) and significance was declared at $P < 0.05$.

**Results**

The spleen MDA level was significantly increased in the susceptible fish than the control and resistant fish. The spleen SOD level was significantly increased in the resistance fish than the control and susceptible fish. Moreover, the susceptible fish showed a significant lower SOD level than the control fish. The spleen GSH level was significantly decreased in the susceptible fish than the control and resistance fish. However, the resistance fish showed insignificantly lowered GSH level than the control fish. The spleen CAT level was significantly decreased in the susceptible fish than the control and resistance fish. However, the resistance fish showed insignificantly lowered CAT level than the control fish (Table 1 & Figure 1).

**Table 1:** Levels of spleen MDA, GSH level, SOD and CAT in Control, resistant and susceptible tilapia

| | **Control** | **Resistant** | **Susceptible** |
|---|---|---|---|
| MDA (nmol/g tissue) | 42.17±1.32 | 43.89±1.11 | 55.26±1.25 **** |
| SOD (U/g tissue) | 204.11±5.72 | 251.70±6.5 **** | 151.82±4.89 **** |
| GSH (U/g tissue) | 12.02±0.74 | 11.81±0.93 | 7.83±0.52 ** |
| CAT (U/g tissue) | 11.23±0.57 | 10.48±0.50 | 7.86±0.43 **** |

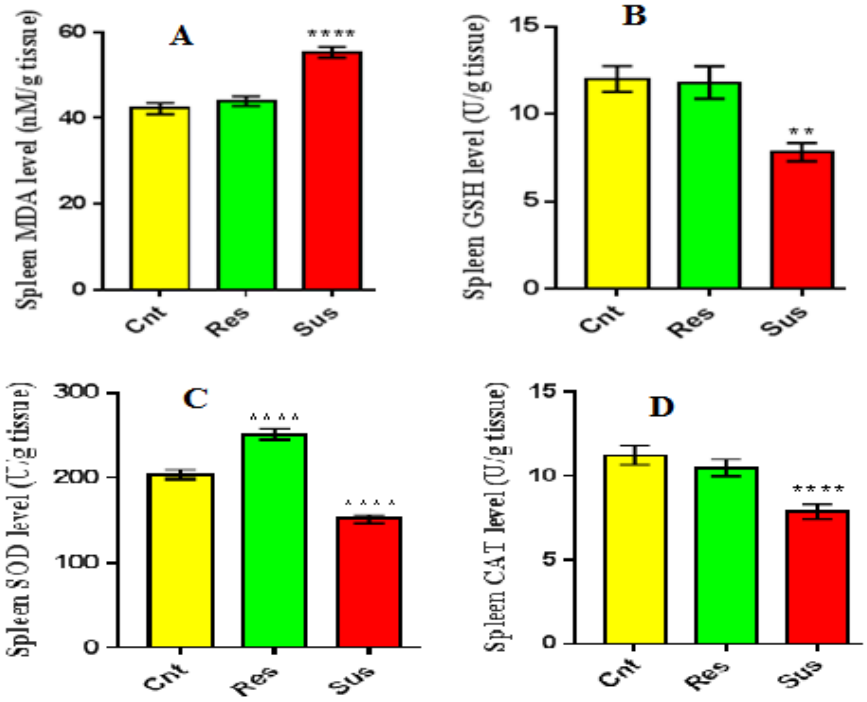


*Figure 1A: Levels of spleen MDA, GSH level, SOD and CAT in Control, resistant and susceptible tilapia. Cnt: Control tilapia; Res: resistant tilapia; Sus: susceptible tilapia*

**Discussion**

The lack of disease control has the potential of being the limiting factor of fish production. Improving disease resistance of cultured fish are major challenges facing fish culturists, especially *A. hydrophila* bacterial infections that cause mass mortalities. In fish, the innate cellular defense includes macrophages, granulocytes (mainly neutrophils) and nonspecific cytotoxic cells. Both macrophages and neutrophils (called phagocytic cells in fish) act as antibacterial defenses because they have the ability to engulf bacteria and kill them by production of reactive oxygen species (ROS) during the process called respiratory burst. During this process, many oxygen radicals are released including superoxide anion ($O_2$), $H_2O_2$ and the hydroxyl free radical ($OH^-$), which has potent bactericidal activity. In addition, neutrophils can kill bacteria and produce bactericidal hypohalite ions in the presence of halide ions and $H_2O_2$because it contains myeloperoxidase (MPO) in their cytoplasmic granules [18]. On the other hand**,** neutrophils and macrophages contain lysozyme and other hydrolytic enzymes in their lysosomes [19]. Thus, it is possible that there is a link between ROS and immunity. The obtained data revealed that the antioxidant enzymes (CAT and SOD) activities and GSH level in the spleen were significantly increased in the control and resistant fish than the susceptible fish. However, the lipid peroxidation marker MDA level in the spleen was significantly increased in susceptible fish than the control and resistant fish.

CAT is one of the primary antioxidant enzymes involved in ROS removal [20]. In contrast to our findings, Fei et al. [21] reported that the activity of CAT decreased significantly in infected fish as compared to the control fish. This contradictory results may be attributed to variation in fish and type of bacterial infection.

SOD is one of the main anti-oxidant defense enzymes generated in response to oxidative stress, which converts the highly toxic superoxide anions into hydrogen peroxide. SOD can scavenge the excessive ROS release [22]. An increase in the superoxide anion production against pathogens is considered to be beneficial after exposing shrimp to immunostimulants [23]. Change in SOD level following infection indicates the important role played by this enzyme not only in removal of excessive ROS but also in immunity. In consistent with our results, Ying Tang et al. [24].

found that the activity of SOD decreased significantly after *A. hydrophila* challenge in diseased fish. This suggests a positive link between bacterial infection and stimulation of oxidative stress in susceptible fish as evidenced by induction of lipid peroxidation (as marked by high level of MDA) and inhibition of antioxidant enzymes (SOD, GPx and CAT) activities. However, this link was negative in resistant fish.

**Conclusion**

The level of the antioxidant parameters (CAT, GSH and SOD) in the spleen were significantly increased in the control and resistant fish than the susceptible fish. However, the lipid peroxidation marker MDA level in spleen was significantly increased in susceptible fish than the control and resistant fish.

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