Effects of Pre-Exposure To Waterborne Selenium On Redox Homeostasis and Humoral Innate Immune Parameters in African Catfish, *Clarias Gariepinus* (Burchell, 1822) Experimentally Challenged With *Serratia Marcescens*

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Abstract

Bacterial infections have been associated with immune dysfunction and oxidative stress in cultured fish species while essential elements could boost immunity and exhibit antioxidant properties in fish. This study was therefore aimed at determining the effects of pre-exposure to waterborne selenium on humoral immunity and redox status of *Clarias gariepinus* experimentally challenged with *Serratia marcescens*. Juveniles *C. gariepinus* were pre-exposed to 50 µg/L selenium for 14 days after which they were challenged with 5×10^3 CFU/mL of *S. marcescens* via oral gavage for 24 or 48 h. The control fish were not exposed to selenium and not challenged with bacteria. Thereafter, fish were sacrificed, blood collected into EDTA bottles for the determination of plasma nitric oxide levels and respiratory burst, and the liver excised for the determination of reduced glutathione, lipid peroxidation, and activities of catalase, superoxide dismutase, and glutathione peroxidase. Fish that were challenged with bacteria without pre-exposure to selenium had elevated levels of nitric oxide, reduced glutathione, lipid peroxidation, and a higher activity of glutathione peroxidase and respiratory burst but a significant decrease in the activity of superoxide dismutase compared to the fish that were pre-exposed to selenium prior to bacterial challenge at both 24 and 48 h post-infection periods. The results from this study showed that infection with *S. marcescens* is capable of disrupting the immune system and redox homeostasis in *C. gariepinus*, while pre-exposure to selenium has the ability to improve the physiological status of fish that were challenged with bacteria probably through its antioxidant properties.

Highlight

The pre-exposure of *Clarias gariepinus* to waterborne selenium for 14 days improve the redox homeostasis and innate immunity of fish that were experimentally challenged with the bacterium, *Serratia marcescens*.

Introduction

Globally, fish are currently recognized as one of the cheapest source of animal protein accounting for up to 17% of dietary animal protein intake (Cazcarro et al. 2019). The important role of fish as the major source of animal protein has therefore resulted in increased fish production, and aquaculture continue to contribute to global fish production with global inland aquaculture production increasing from 29.9 million tons in 2007 to 41.9 million tons in 2012, although African countries contribute less than 2.7% of the global fish production (FAO 2018). However, in recent times, the investment in aquaculture is on the rise in African countries like Egypt, Nigeria, Uganda and Ghana (Cai et al. 2017; FAO 2018). In fact, a recent statistics showed that Africa experienced a growth rate of 15.5% in total fish production from 1995-2018 (FAO 2016).

The African catfish, *Clarias gariepinus* is widely cultivated in most African countries due to its tolerance to hostile environment such as turbid water, having high stocking density, fast growth rate etc. (El-Shebly 2006). In most African countries, local fish farmers cultivate their fish in earthen ponds, a system which

predisposes the fish in culture to infection by pathogens especially bacteria since the earthen ponds have been shown to harbor pathogenic organisms (Ampofo and Clerk 2010; Adeyemi et al. 2013). Notably, the infection of cultured fish by the bacteria *Serratia marcescens* has been widely reported in the literature. In the US, *S. marcescens* has been isolated in the white perch (Baya et al. 1992). In Malaysia, an outbreak of endemic disease from tilapia fish farms was linked to infection by *S. marcescens* (Chan et al. 2013). It has also been reported to cause high mortality and morbidity in guppy, *Poecilia reticulata* with skin and fin rot lesions (Dharmaratnam et al. 2017).

Fish are protected against pathogen infections by specific and non-specific immune element. Non-specific immune elements are those elements that act not only as first line of defense against all pathogens, but also play an instructive role in the development of acquired immune response (Biller-Takahasi and Urbinati 2014). The inhibition of specific immune responses and nonspecific defense mechanisms as a result of continuous stress by pathogen infections result in increased susceptibility to infections (Kotob et al. 2016). Aside the effects on immune systems, bacterial infections have been shown to result in redox imbalance in fish causing tissue lipid peroxidation and modulation of antioxidant enzymes (Adeyemi 2014; Roy et al. 2019; Souza et al. 2019). Fish like other vertebrates rely on complex antioxidant systems that include molecules such as glutathione and vitamin E, and antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase, thioredoxin reductase etc. to fight oxidative stress.

The introduction of essential elements either as a supplement in diets or incorporation into their environments has been shown to help in boosting the immune system of animals in response to infections (Jayawardena et al. 2020; Pecora et al. 2020). One typical essential element that has been shown to have great role in vertebrate immunity is selenium (Durigon et al. 2019). Selenium is an essential trace element in regular physiological processes of growing fish. Also, it is an important component of the antioxidant system and own a forceful immunomodulation and antimicrobial efficiency (Sarkar et al. 2015). It constitute an integral part of several selenoproteins such as glutathione peroxidase (GPx), iodothyronine deiodinase, thioredoxin reductase and selenophosphate synthetase. Although, it is an important components of normal physiological functioning, selenium have a thin margin between being essential and toxic. Some studies have it that selenium improves humoral and cell-mediated immune responses of fish (Hsu and Guo 2002; Biller-Takahashi et al. 2015).

Although, selenium has been reported to have the potential to boost the immune system of fish but has been shown to be also toxic to fish at elevated concentrations, as such there exist a knowledge gap about the actual role of selenium in fish physiology especially in circumstance of pathogen infections. Similarly, since selenium is a major constituent of important antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase, it is anticipated that routine supply of fish with moderate concentration of selenium could help in maintaining the redox homeostasis of fish. From the foregoing, this study was aimed at investigating the effects of pre-exposure to waterborne selenium on the innate immunity and redox status of African catfish (*C. gariepinus*) experimentally challenged with *S. marcescens*.

Materials And Methods

Experimental organism

The experimental animal used in this research was the African catfish, *Clarias gariepinus*. A total of 30 juvenile *C. gariepinus* (mean weight: 2.8 ± 0.13 g) were used in the study. The fish were purchased from a commercial farm within Akure metropolis, and were transported in open head plastic containers to the Research Laboratory, Department of Biology, Federal University of Technology, Akure. The fish were allowed to acclimatize to laboratory conditions in chlorine-free tap water for one week prior to commencement of experiments. The fish were fed with commercial fish pellets twice daily during the period of the acclimatization and experiment.

Pre-exposure of fish to selenium

The laboratory acclimatized fish were allocated into six tanks ($n = 5$ per tank); three of which were maintained in clean water for 14 days while the remaining tanks were exposed to 50 µg/L selenium (prepared from the stock solution of selenium) for 14 days. After the 14 days of selenium pre-exposure, one tank each from the unexposed (control) and selenium-exposed (Sel) were collected and the fish in those tanks were sacrificed, the liver excised, homogenized in ice-cold phosphate buffer (50 mM, pH 7.4), and the homogenates was used for the determination of oxidative stress parameters; levels of reduced glutathione, lipid peroxidation, and activities of catalase, superoxide dismutase, and glutathione peroxidase. The blood was collected into EDTA bottle, centrifuged at $10,000 \times g$ for 20 min at 4 °C to obtain the plasma and the plasma levels of nitric oxide and respiratory burst activity were determined.

Experimental challenge of fish with *S. marcescens*

Fish in the remaining tanks were challenged with 5×10^3 CFU/mL inoculums of *S. marcescens* via oral gavage for either 24 h or 48 h. The tanks were designated as: unexposed fish for 14 days but challenged with bacteria for 24 h or 48 h (Bact) and pre-exposed to selenium for 14 days and challenged with bacteria for 24 or 48 h (Sel + Bact). The fish in each group were collected at 24 and 48 h post-inoculation periods, sacrificed, the liver excised for the determination of oxidative stress parameters while the blood was collected for the determination blood plasma levels of nitric oxide and respiratory burst activity.

Plasma level of nitric oxide

Nitric oxide levels of the blood plasma were determined according to the method described by Guevara-Guzman et al. (1994), with a slight modification. Aliquot of 100 µL of the plasma was mixed with 100 µL Griess reagent and incubated for 10 min at room temperature. The absorbance was read by a spectrophotometer at 540 nm. The nitrite concentration was measured with the nitrite standard curve and expressed as µmol/mL.

Respiratory burst assay

The level of respiratory burst was determined by the method of Anderson and Siwicki (1995). A 100 μL of blood plasma was mixed with 100 μL of 0.2 % nitroblue tetrazolium (NBT) solution which was homogenized and incubated for 30 min at 25 °C. The NBT solution was prepared in phosphate buffered saline (PBS), prepared with NaCl (0.137 M), KCl (2.7 mM), KH_2PO_4 (1.5 mM), Na_2HPO_4 (8.1 mM), CaCl_2 (0.9 mM), MgCl_2 (0.49 mM) in distilled water Milli-Q qsp 1 litre), pH 7.4. 50 μL from the mixture was added to 1 ml of N, N-dimethyl formamide in a glass tube. The solution was properly homogenized and afterwards centrifuged at $3000 \times g$ for 5 min. The optical density of the supernatant was measured at 540 nm on a UV/visible spectrophotometer against a blank with same components except the blood was exchanged with distilled water.

Liver lipid peroxidation levels

Lipid peroxidation was determined by the thiorbarbituric acid reactive substances assay which quantifies the levels of malondialdehyde, the final product of lipid peroxidation. This method was based on the colorimetric determination of malondialdehyde (MDA) according to the method of Esterbauer and Cheeseman (1990). Homogenized suspension of liver was mixed with 2 volumes of cold 10% (w/v) trichloroacetic acid (TCA) to precipitate the protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was added to an equal volume of 0.67% (w/v) thiobarbituric acid and the mixture placed in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm. The concentration of MDA was expressed as nmol MDA mg protein⁻¹ using a molar extinction coefficient of 153,000 M⁻¹cm⁻¹.

Liver reduced glutathione levels (GSH)

The levels of reduced glutathione in the liver homogenate was estimated by the method of Ellman (1959). Briefly, 0.5 mL of tissue homogenate was precipitated with 2.0 mL of 5% TCA and centrifuged. To 1.0 mL of the supernatant, 3.0 mL phosphate buffer and 0.5 mL Ellman's reagent were added and incubated for 15 min at room temperature. The intensity of yellow color formed was measured at 420 nm. A series of standards (10– 50 μg GSH) were treated in a similar manner along with the blank containing 1.0 mL distilled water. The amount of GSH was expressed as nmol/mg protein.

Liver glutathione peroxidase activity

Glutathione peroxidase activity was determined according to the method of Hafeman et al. (1974). The tissue homogenate (approximately 0.5 mg protein) was incubated with 0.1 mL of 5 mM GSH, 0.1 mL of 1.25 mM H_2O_2 , 0.1 mL of 25 mM NaN_3 and phosphate buffer (0.05 mM, pH 7) in a total volume of 2.5 mL at 37 °C for 10 min. The reaction was stopped by adding 2 mL of 1.65 % HPO_3^{2-} and the reaction mixture was centrifuged at 1500 rpm for 10 min. 2 mL of the supernatant was mixed with 2 mL 0.4 M Na_2HPO_4 and 1 mL of 1 mM DTNB. The absorbance of the yellow colored complex was measured at 412 nm after incubation for 10 min at 37 °C against distilled water. A sample without the tissue homogenate processed in the same way was kept as non-enzymatic reaction.

Liver superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined according to the method of Beauchamp and Fridovich (1971). The reaction mixture comprised 0.01 mL of the tissue homogenate, 0.2 mL of 0.1 M EDTA (containing 0.0015% NaCN), 0.1 ml of 1.5 mM NBT and phosphate buffer (67 mM, pH 7.8) in a total volume of 2.6 mL. Later, 0.05 mL of riboflavin was added to the mixture, and incubated in the dark for 1 h after which the absorbance was measured at 560 nm using a UV/visible spectrophotometer. The tissue homogenate was replaced with distilled water to get the reading for the blank. SOD activity was expressed as $\mu\text{moles}/\text{min}/\text{mg}$ protein.

Liver catalase (CAT) activity

The catalase activity was determined according to the method described by Aebi (1984). Aliquot of 25 μL of tissue homogenate was added to 1.0 mL of phosphate buffer, and the enzyme reaction was started by the addition of 250 μL of H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30 seconds intervals for 3 min. The enzyme blank was run simultaneously with 250 μL of distilled water instead of hydrogen peroxide. The enzyme activity is expressed as nmoles of H_2O_2 decomposed/min/mg protein.

Protein determination

The protein levels in the tissue homogenate was determined according to the method described by Bradford (1976), using bovine serum albumen as the standard.

Statistical analyses

Data obtained were expressed as mean $\pm$ standard deviation. The data on GSH, MDA, CAT activity, SOD activity, GPx activity, nitric oxide, and respiratory burst were subjected to two-way analysis of variance with selenium pre-exposure (pre-exposed to selenium for 14 days versus not pre-exposed to selenium) and post-infection time (24 h versus 48 h) as factors. The means were separated using Tukey's multiple comparison tests. Statistical analyses were performed using GraphPad Prism software (version 5), and statistical significance was assumed at $p < 0.05$.

Results

Plasma levels of nitric oxide

The levels of nitric oxide in the blood plasma of *C. gariepinus* at 24 h or 48 h post-infection durations are shown in Figure 1A. There was a significant effect of pre-exposure to selenium on the levels of nitric oxide in the fish ($F_{3,40} = 55.26$, $p < 0.0001$) while there was no significant effect of post-infection duration ($F_{1,40} = 0.06$, $p = 0.8136$). At both 24 h and 48 h post-infection durations, the levels of nitric oxide was highest in the group that were challenged with bacteria without selenium pre-exposure (Bact). There was a significant reduction in the levels of nitric oxide in the group pre-exposed to selenium prior to bacterial

challenge (Sel + Bact) compared to the group challenged with bacterial without selenium pre-exposure (Bact). Also, the selenium pre-exposed group (Sel) has significant lower levels of nitric oxide compared to the control.

Respiratory burst activity

Figure 1B shows the respiratory burst in *C. gariepinus* challenged with *S. marcescens* with or without pre-exposure to selenium at 24 and 48 h post-infection durations. The pre-exposure of fish to selenium resulted in a significant difference in the respiratory burst activity of *C. gariepinus* ($F_{3,40} = 121.86, p < 0.0001$). The respiratory burst was significantly lower in the group pre-exposed to selenium prior to bacterial challenge compared to the group challenged with bacteria without selenium pre-exposure. The respiratory burst in the group pre-exposed to selenium was statistically the same as in control. The respiratory burst activity increased significantly with a longer post-infection duration (48 h) especially in the group challenged with bacteria without selenium pre-exposure ($F_{1,40} = 16.99, p = 0.0002$). There was a significant interactive effect of selenium pre-exposure and post-infection duration on the respiratory burst ($F_{3,40} = 6.76, p = 0.0009$).

Liver lipid peroxidation levels

The extent of lipid peroxidation in terms of the levels of malondialdehyde (MDA) in *C. gariepinus* challenged with *S. marcescens* with or without pre-exposure to selenium at 24 and 48 h post-infection durations was shown in Figure 2A. The levels of MDA in the liver of fish depended on both selenium pre-exposure ($F_{3,40} = 480.08, p < 0.0001$) and post-infection duration ($F_{1,20} = 106.21, p < 0.0001$). The levels of MDA was highest in the group that were challenged with bacteria without selenium pre-exposure. There was a significant reduction in the levels of MDA in the group pre-exposed to selenium prior to bacterial challenge compared to the group challenged with bacterial without selenium pre-exposure. Also, the selenium pre-exposed group (Sel) has significant lower level of MDA compared to the control. The level of MDA increased significantly in bacterial challenged groups (with or without selenium pre-exposure) at 48 h compared to 24 h post-infection period. There was a significant interactive effects of selenium pre-exposure and post-infection duration on the levels of MDA ($F_{3,40} = 44.60, p < 0.0001$).

Liver reduced glutathione levels (GSH)

Figure 2B shows the levels of reduced glutathione (GSH) in *C. gariepinus* challenged with *S. marcescens* with or without pre-exposure to selenium at 24 and 48 h post-infection durations. The levels of GSH in the liver of fish depended on both selenium pre-exposure ($F_{3,40} = 208.48, p < 0.0001$) and post-infection duration ($F_{1,20} = 105.51, p < 0.0001$). The levels of GSH was lowest in the groups challenged with bacteria at both 24 and 48 h post-infection durations. There was a significant interactive effects of selenium pre-exposure and post-infection duration on the levels of GSH ($F_{3,40} = 38.85, p < 0.0001$).

Liver glutathione peroxidase activity

The activity of glutathione peroxidase (GPx) in *C. gariepinus* challenged with *S. marcescens* with or without pre-exposure to selenium at 24 and 48 h post-infection durations was shown in Figure 2C. The activity of GPx in the liver of fish depended on both selenium pre-exposure ($F_{3,40} = 175.26, p < 0.0001$) and post-infection duration ($F_{1,20} = 61.35, p < 0.0001$). The activity of GPx decreased significantly in the group challenged with bacteria without selenium pre-exposure compared to the group that was pre-exposed to selenium prior to bacteria challenge. There was no significant difference in GPx activity among the control, fish pre-exposed to selenium alone, and fish pre-exposed to selenium prior to bacterial challenge. The GPx activity decreased significantly in bacterial challenged groups (with or without selenium pre-exposure) at 48 h compared to 24 h post-infection period. There was a strong interactive effects of selenium pre-exposure and post-infection duration on the activity of GPx ($F_{3,40} = 20.80, p < 0.0001$).

Liver superoxide dismutase activity

The activity of superoxide dismutase (SOD) in *C. gariepinus* challenged with *S. marcescens* with or without pre-exposure to selenium at 24 and 48 h post-infection durations was shown in Figure 2D. The activity of SOD in the liver of fish depended on both selenium pre-exposure ($F_{3,40} = 414.32, p < 0.0001$) and post-infection duration ($F_{1,20} = 317.60, p < 0.0001$). The activity of superoxide dismutase was not significantly different between the control and selenium group, but was statistically lower than the values for the groups challenged with bacteria (with or without selenium pre-exposure) especially at 48 h post-infection duration. The SOD activity increased significantly in bacterial challenged groups (with or without selenium pre-exposure) at 48 h compared to 24 h post-infection period.

Liver catalase activity

Figure 2E shows the activity of catalase (CAT) in *C. gariepinus* challenged with *S. marcescens* with or without pre-exposure to selenium at 24 and 48 h post-infection durations. There was a significant effect of pre-exposure to selenium on the activity of catalase ($F_{3,40} = 23.32, p < 0.0001$) while there was no significant effect of post-infection duration ($F_{1,40} = 1.83, p = 0.1843$). Catalase activity increased significantly in the groups challenged with bacteria (with or without selenium pre-exposure) compared to the control and the fish pre-exposed to selenium but not challenged with bacteria.

Discussion

The present study employed certain humoral immunity parameters to assess the effects of experimental challenge of *C. gariepinus* with a pathogenic bacterium, *S. marcescens*. The levels of nitric oxide has been used in various studies to evaluate the impact of exposure to stressors in fish. Nitric oxide acts as a signaling molecule in inflammatory reactions which are important components of immune system of fish (Pathak and Lal 2010; Baldissera et al. 2020). The significant elevated blood plasma levels of nitric oxide in fish challenged with *S. marcescens* in this study was consistent with results of other studies that also showed that the levels of nitric oxide increased in fish as a result of bacterial infection. Campos-Perez et

al. (2000) reported higher serum nitric oxide in rainbow trout infected with *Renibacterium salmoninarum*. This may be due to bacterial products such as lipopolysaccharide that are powerful inducers of nitric oxide synthase (iNOS), which enhance NO production, a fact supported by the high nitric oxide reported by Gopalakrishnan et al. (2011) in crab challenged with lipopolysaccharides.

Also, the results of this study showed that waterborne selenium improved immune responses and resistance of *C. gariepinus* to *S. marcescens* infection. The low level of nitric oxide in fish pre-exposed to selenium prior to bacterial challenge compared with fish challenged with bacteria without selenium pre-exposure supports this assertion. This showed that selenium protected the fish against bacterial challenge induced nitrogen species. Indeed, selenium has been reported to enhance survival and production of antibody in channel catfish that were challenged with pathogenic bacterium, *Edwardsiella ictaluri* (Wang et al. 1997).

Respiratory burst is an indication of tissue damage due to the production of reactive oxygen species (ROS) due to exposure to biotic and abiotic stressors (de Faria et al. 2021). The high respiratory burst recorded in bacteria only infected group might also be attributed to septicemia which might stimulate phagocytic action by antibodies in the blood. The result is in agreement with the findings of Reyes-Becerril et al. (2011) who reported high respiratory burst in *Sparus aurata* double injected with the Gram-negative bacteria. Other studies have also reported enhanced level of respiratory burst in fish after pathogen infection (Rodríguez et al. 2008; Raida and Buchmann 2009; Biller-Takahasi et al. 2013).

The results from this study showed that infection by *S. marcescens* resulted in oxidative stress in *C. gariepinus* evidenced by increased levels of lipid peroxidation, decreased levels of GSH and decreased activity of glutathione peroxidase. These results agreed with the reports of Adeyemi (2014) that showed a significant increase in the levels of tissues lipid peroxidation in response to experimental challenge with *Escherichia coli* and *Vibrio fischeri*. In another study the experimental challenge of grass carp with *Aeromonas hydrophila* caused a significant increase in the levels of lipid peroxidation in fish liver (Zhao et al. 2019). The increased levels of lipid peroxidation in the liver of fish challenged with bacteria showed that infection by *S. marcescens* weakened the antioxidant systems of the fish resulting in excessive accumulation of produced reactive oxygen species due to increased phagocytic and respiratory burst activities (Roy et al. 2019). This was supported by the results of the activity of glutathione peroxidase which decreased significantly in fish following challenge with bacteria. Glutathione peroxidase catalyzes the reduction reactions of hydrogen peroxide to water and oxygen as well as fatty acid hydroperoxides to fatty acid alcohols and oxygen at the cytosol and mitochondrial matrix (Day 2009).

It is evident from the results of this study that selenium plays a role in conferring some level of protection to African catfish challenged with *S. marcescens*. The activity of glutathione peroxidase (GPx) recorded in this study was higher in selenium and sel + bacteria groups compared to bacteria only and control. The high activity of GPx corroborated the findings of Araujo et al. (2021) that reported higher GPx activity in liver, kidney and plasma of Nile tilapia exposed to dietary nano-selenium and inorganic selenium. Similarly, Pacitti et al. (2013) reported that exposure of fish to selenium significantly increased the

expression of GPX-1 and GPx-4 which are the two forms of GPx found in the liver that have been shown to be capable of scavenging hydrogen peroxides radicals. The high GPx activity recorded in the sel and sel + bact groups might be indicative of the role of selenium in antioxidant defense system.

In conclusion, the results of this study showed that infection of *C. gariepinus* with *S. marcescens* causes redox imbalance in the infected fish probably due to breakdown of fish antioxidant systems. Also, our findings demonstrated the antioxidant capability of selenium in stressed fish. Although, the mechanism is not fully elucidated, it is believed that selenium constitute a major component of the antioxidant defense mechanism in fish. It is therefore recommended that selenium be incorporated into fish feed in doses below toxic threshold to booster fish immunity against pathogens.

Declarations

Ethics approval: The experimental procedures were in conformity with national and international standards on the use of laboratory animals. Also, the study was approved by institutional committee on the care and use of animal for experiments.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: All data sets, on which the conclusions of the manuscript rely on, are present in the results section in the manuscript.

Competing interests: The authors declare that they have no competing interest.

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Author's contribution: JAA and COA were involved in the design of the experiment. JAA, GAO, and OSB were involved in data acquisition and analyses. JAA, GAO and OSB were involved in writing the draft of the manuscript. JAA and COA were involved in writing the final draft of the manuscript.

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References

1. Adeyemi JA (2014) Oxidative stress and antioxidant enzymes activities in the African catfish, *Clarias gariepinus* experimentally challenged with *Escherichia coli* and *Vibrio fischeri*. Fish Physiol Biochem 40:347–354

2. Adeyemi JA, Ogunlowo KO, Oyedara OO (2013) Bacterial microflora in the gut, gill and skin of African catfish, *Clarias gariepinus* (Burchell, 1822) collected from earthen ponds in Oke-Baale, Osogbo, Nigeria. NISEB Journal 13:40–43
3. Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126
4. Ampofo JA, Clerk G (2010) Diversity of bacteria contaminant in tissue of a fish cultured in organic waste fertilized ponds. Open Fish Sci J 3:142–146
5. Anderson DP, Siwicki AK (1995) Basic haematology and serology for fish health programs. In Shariff M, Arthur JR, Subasinghe RP (eds.) *Diseases in Asian aquaculture ii. Fish health section*. Manila: Asian Fish Soc 85-202
6. Araujo JM, Fortes-Silva R, Pola CC, Yamamoto FY, Gatlin DM, Gomes CL (2021) Delivery of selenium using chitosan nanoparticles: Synthesis, characterization, and antioxidant and growth effects in Nile tilapia (*Oreochromis niloticus*). PLoS ONE 16:e0251786
7. Baldissera MD, Souza CF, Val AL, Baldisserotto B (2020) Involvement of purinergic signaling in the Amazon fish *Pterygoplichthys pardalis* subjected to handling stress: Relationship with immune response. Aquaculture 514:734481
8. Baya AM, Toranzo AE, Lupiani B, Santos Y, Hetrick FM (1992) *Serratia marcescens*: a potential pathogen for fish. J Fish Dis 15:15–26
9. Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287
10. Biller-Takahashi JD, Takahashi LS, Mingatto FE, Urbinati EC (2015) The immune system is limited by oxidative stress: Dietary selenium promotes optimal antioxidative status and greatest immune defense in pacu *Piaractus mesopotamicus*. Fish Shellfish Immunol 47:360–367
11. Biller-Takahashi JD, Takahashi LS, Saita MV, Gimbo RY, Urbinati EC (2013) Leukocytes respiratory burst activity as indicator of innate immunity of pacu *Piaractus mesopotamicus*. Braz J Biol 73:425–429
12. Biller-Takahashi JD, Urbinati EC (2014) Fish Immunology. The modification and manipulation of the innate immune system: Brazilian studies. An Acad Bras Cienc 86:1483–1495
13. Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
14. Cai J, Quagrainie K, Hishamunda N (2017) Social and economic performance of tilapia farming in Africa, 1130. FAO Fisheries and Aquaculture Circular (C
15. Campos-Perez JJ, Ward M, Grabowski PS, Ellis AE, Secombes CJ (2000) The gills are an important site of iNOS expression in rainbow trout *Oncorhynchus mykiss* after challenge with the Gram-positive pathogen *Renibacterium salmoninarum*. Immunology 99:153–161
16. Cazcarro I, López-Morales CA, Duchin F (2019) The global economic costs of substituting dietary protein from fish with meat, grains and legumes, and dairy. J Ind Ecol 23:1159–1171

17. Chan X, Chang CY, Hong K, Tee KK, Yin W, Chan K (2013) Insights of biosurfactant producing *Serratia marcescens* strain W2.3 isolated from diseased tilapia fish: a draft genome analysis. Gut Pathog 5:29. <https://doi.org/10.1186/1757-4749-5-29>
18. Day BJ (2009) Catalase and glutathione peroxidase mimics. Biochem Pharmacol 77:285–296
19. de Faria CFP, Martinez CBD, Takahashi LS, de Mello MMM, Martins TP, Urbinati EC (2021) Modulation of the innate immune response, antioxidant system and oxidative stress during acute and chronic stress in pacu (*Piaractus mesopotamicus*). Fish Physiol Biochem 47:895–905
20. Dharmaratnam A, Kumar R, Basheer VS, Sood N, Swaminathan TR, Jena JL (2017) Isolation and characterization of virulent *Serratia marcescens* associated with a disease outbreak in farmed ornamental fish, *Poecilia reticulata* in Kerala, India. Indian J Fish 64: 71–79
21. Durigon EG, Kunz DF, Peixoto NC, Uczay J, Lazzari R (2019) Diet selenium improves the antioxidant defense system of juveniles Nile tilapia (*Oreochromis niloticus* L.). Braz J Biol 79:527–532
22. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77
23. El-Shebly AA (2006) Evaluation of growth performance, production and nutritive value of the African catfish, *Clarias gariepinus* cultured in earthen ponds. Egypt Aquat Biol Fish 10:55–67
24. Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynoneal. Methods Enzymol 186:407–421
25. FAO (2016) The state of world fisheries and aquaculture: Contributing to food security and nutrition for all. FAO, Rome, p 200
26. FAO (2018) The state of world fisheries and aquaculture: Meeting the sustainable development goals. <http://www.fao.org/3/i9540en/i9540en.pdf>
27. Gopalakrishnan S, Chen F, Thilagam H, Qiao K, Xu W, Wang K (2011) Modulation and interaction of immune-associated parameters with antioxidant in the immunocytes of crab *Scylla paramamosain* challenged with lipopolysaccharides. Evid-Based Complementary Altern Med 2011:824962. <https://doi.org/10.1155/2011/824962>
28. Guevara-Guzman R, Emson PC, Kendrick KM (1994) Modulation of *in vivo* striatal transmitter release by nitric oxide and cyclic GMP. J Neurochem 62:807–810
29. Hafeman DG, Sunde RA, Hoekstra WG (1974) Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr 104:580–587
30. Hsu PC, Guo YL (2002) Antioxidant nutrients and lead toxicity. Toxicology 180:33–44
31. Jayawardena R, Sooriyaarachchi P, Chourdakis M, Jeewandra C, Ranasinghe P (2020) Enhancing immunity in viral infections, with special emphasis on COVID-19: A review. Diabetes Metab Syndr 14:367–382
32. Kotob MH, Menanteau-Ledouble S, Kumar G, Abdelzaher M, El-Matbouli M (2016) The impact of co-infections on fish: a review. Vet Res 47:98. doi:10.1186/s13567-016-0383-4
33. Pacitti D, Wang T, Page MM, Martin SA, Sweetman J, Feldmann J, Secombes CJ (2013) Characterization of cytosolic glutathione peroxidase and phospholipid-hydroperoxide glutathione

- peroxidase genes in rainbow trout (*Oncorhynchus mykiss*) and their modulation by *in vitro* selenium exposure. *Aquat Toxicol* 130-131:97–111
34. Pathak ND, Lal B (2010) Seasonality in expression and distribution of nitric oxide synthase isoforms in the testis of the catfish, *Clarias batrachus*: Role of nitric oxide in testosterone production. *Comp Biochem Physiol C* 151:286–293
 35. Pecora F, Persico F, Argentiero A, Neglia C, Esposito S (2020) The role of micronutrients in support of the immune response against viral infections. *Nutrients* 12:3198. doi:10.3390/nu12103198
 36. Raida MK, Buchmann K (2009) Innate immune response in rainbow trout (*Oncorhynchus mykiss*) against primary and secondary infections with *Yersinia ruckeri* O1. *Dev Comp Immunol* 33:35–45
 37. Reyes-Becerril M, López-Medina T, Ascencio-Valle F, Esteban M (2011) Immune response of gilthead seabream (*Sparus aurata*) following experimental infection with *Aeromonas hydrophila*. *Fish Shellfish Immunol* 31:564–570
 38. Rodríguez I, Novoa B, Figueras A (2008) Immune response of zebrafish (*Danio rerio*) against a newly isolated bacterial pathogen *Aeromonas hydrophila*. *Fish Shellfish Immunol* 25:239–249
 39. Roy D, Pal S, Ray SD, Homechaudhuri S (2019) Evaluating oxidative stress in *Labeo rohita*, infected asymptotically with native and invasive Aeromonads using biochemical indices. *Proc Natl Acad Sci India Sect B Biol Sci* 89:973–978
 40. Sarkar B, Bhattacharjee S, Daware A, Tribedi P, Krishnani KK, Minhas PS (2015) Selenium nanoparticles for stress-resilient fish and livestock. *Nanoscale Res Lett* 10:371. doi:10.1186/s11671-015-1073-2
 41. Souza CDF, Baldissera MD, Verdi CM, Santos RCV, Da Rocha MIU, Veiga MLD, Silva AS, Baldisserotto B (2019) Oxidative stress and antioxidant responses in Nile tilapia *Oreochromis niloticus* experimentally infected by *Providencia rettgeri*. *Microb Pathog* 131:164–169
 42. Wang C, Lovell RT, Klesius PH (1997) Response to *Edwardsiella ictaluri* challenge by channel catfish fed organic and inorganic sources of selenium. *J Aquat Anim Health* 9:172–179
 43. Zhao H, Luo Y, Wu Z, Zhou Y, Guo D, Wang H, Chen X (2019) Hepatic lipid metabolism and oxidative stress responses of grass carp (*Ctenopharyngodon idella*) fed diets of two different lipid levels against *Aeromonas hydrophila* infection. *Aquaculture* 509:149–158

Figures

Figure 1

The plasma levels of nitric oxide (A), and respiratory burst activity (B) in *C. gariepinus* challenged with *S. marcescens* with or without pre-exposure to selenium at 24 and 48 h post-infection durations. Each bar is the mean $\pm$ standard deviation ($n = 6$). Bars with different letters are significant different within the same exposure duration.

Figure 2

The levels of lipid peroxidation (A), reduced glutathione (B), activity of glutathione peroxidase (C), activity of superoxide dismutase (D), and activity of catalase (E) in *C. gariepinus* challenged with *S. marcescens* with or without pre-exposure to selenium at 24 and 48 h post-infection durations. Each bar is the mean $\pm$ standard deviation ($n = 6$). Bars with different letters are significant different within the same exposure duration.