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Studying the Effect of High Water Acidity on the Tilapia Fish *Oreochromis aureus in Vitro*

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ABSTRACT

A significant increase in the concentration of carbon dioxide in the atmosphere, as well as in the fresh water from the decomposition of organic and inorganic materials, is related to decreased pH values. The current study aimed to expose the blue tilapia fish, *Oreochromis aureus*, to different concentrations of acidity using ice carbon dioxide gas, as well as to examine the physiological, biochemical, and histological indicators of the fish. The findings presented an increase in the activity of the ALT and ALP enzymes in the fish exposed to acidic concentration (pH 6.5), while the measurements of the AST enzyme in the treated fish showed a decrease in the activity level. Histopathology examination of the tested fish exhibited a slight damage in fish gills within one hour of exposure, and it increased with increasing the time period. An observable influence was noted in the secondary gill lamellae, represented by their bending and curling in numerous specimens. It was deduced that histopathological symptoms increased with prolonged exposure to the acid.

INTRODUCTION

The harmful effects of increased emissions of carbon dioxide and other greenhouse gases have led to the rise in the acidity of the waters in the Arabian Gulf including the northwestern region over time. The biological effects of the ocean acidification phenomenon in the Arabian Gulf and Iraqi territorial waters were exclusively very clear, as in the case of the coral bleaching, which indicates the need to move toward the necessary measures to protect the marine environment. Where the previous studies indicated a state of rapid deterioration in the waters of the Arabian Gulf, which may affect the water chemistry and the biogeochemical cycle earlier than expected. This study showed that the value of the water pH in the Arabian Gulf under normal circumstances is greater than 7.7 and decreases as the temperature rises to become less than 7.7 **(Uddin *et al*. 2012)**. In addition, there is another environmental problem, which is eutrophication, as it increases the carbon dioxide inputs into coastal waters. The reason is that the anthropogenic inputs of nutrients have increased massive algal blooms, which deplete oxygen from the bottom waters and increase the release of carbon dioxide **(Sunda & Cai,**

2012). On the other hand, the differences in the environmental conditions and the exposure to climate changes as well as the geographical variability in response to climate changes control the future changes in phytoplankton productivity **(Lincoln *et al*., 2021)**. However, the decline in the finfish population in the north of the Arabian Gulf is driven by the progressive hyper-salination **(Ben-Hasan *et al*. 2018)**. Acidification of freshwater environments may have passive impacts, where changes in the pH due to CO2 addition can pose physiological challenges that may completely alter the ecosystem's synthesis and function. It has been found that acidification affects the activities of microbes, plants, and animals in freshwater ecosystems. Whereas, organisms can be directly affected by changes in the pH during both short and long-term acidic periods. The acidification in the freshwater environment supports the abundant growth of filamentous algae, alters the balance of diatoms, reduces the occurrence of neutral species, and greatly reduces the diversity of zooplankton species.

Moreover, snails, crayfish, clams, and freshwater shrimp are sensitive to acids. It is possible to trace the historical progression of acidification by examining the remains of diatoms in sediment accumulations. While the species diversity of diatoms is reduced, the biomass and productivity of dinoflagellates are only slightly affected. On the other hand, acidification is responsible for fish loss in large parts of the world as fish populations become unbalanced. Acidified freshwater leads to the accumulation of manganese and mercury in fish. Short periods of severe acidity can result in significant fish mortality. The distribution of fish-eating birds may be affected by the impact of acidification on their prey, leading to the accumulation of mercury and cadmium. This accumulation is directly and indirectly related to acidification in fish-eating mammals **(Muniz, 1990)**.

A significant increase was reported in the concentration of carbon dioxide in the atmosphere, which in turn affects freshwater systems. Freshwater bodies receive carbon dioxide from the decomposition of organic and inorganic materials, leading to a related decrease in the pH value by approximately 0.3 **(Martinez *et al.*, 2005; Duarte *et al.*, 2020)**. Therefore, the current study aimed to expose the blue tilapia fish, *Oreochromis aureus*, to different concentrations of acidity using the ice carbon dioxide gas and to examine the physiological, biochemical, and histological indicators of the fish.

MATERIALS AND METHODS

Experimental animals and exposure condition

The study was conducted on the *O. aureus*, with a total mean length of 16± 1.2cm and a mean weight of 39.03± 1.6g, as mean ± S.E. The *O. aureus* specimens were collected from the Marine Science Center farms, University of Basrah, Basrah, Iraq. The fish were acclimatized for a week in experimental glass tanks (60 x 30 x 30cm) containing 20 liters of dechlorinated tap water under laboratory conditions. The temperature was maintained at 21± 2°C, with a constant 12:12 light: dark photoperiod. A continous aeration system was provided to ensure that dissolved oxygen levels remained at 7.2± 0.2mg/ L. The fish were fed a dry pellet diet containing a crude protein content of 25%. Dead organisms were removed regularly by hand nets. After that, they were exposed to two different levels of acidity (7.8, and 6.5) using ice carbon dioxide made by Zamzam Company for the production of carbon dioxide and dry ice, Baghdad, Iraq. The exposure durations were half, 1, and 2 hours.

Enzyme activity determination

After exposing the fish to the different time periods, five fish were taken from each aquarium and used as replicates. They were anesthetized directly in ice water, then blood samples were collected from the caudal vein of each fish, as mentioned by **Zhang *et al*. (2015)**, using a 3ml syringe and separating the serum from the clot by using a universal centrifugation. Moreover, the serum samples were preserved in 2ml Eppendorf tubes and frozen at -20°C until analysis was conducted. The activity of enzymes of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), in UL unit, were calculated by UV test technique **(Rahimikia, 2017)**.

The gill tissues were examined for control samples. Moreover, the samples were exposed to different concentrations of acid. They were fixed and dried with graded concentrations of ethanol, then immersed and embedded in paraffin, cut to a size of 7 microns, and then stained with hematoxylin and eosin. The tissues were evaluated according to the method of **Triebskorn *et al*. (2008)**.

RESULTS AND DISCUSSION

Biochemical parameters

The biochemical measurements recorded in Table (1) and Fig. (1) show an increase in the activity of the ALT and ALP enzymes in fish exposed to an acidic concentration of pH 6.5, which were 1302.8± 0.16 and 1061.6U/ L after a 2-hour exposure period, respectively. These levels are higher than those of the control group (94.5± 0.05 and 20.97U/ L, respectively). Meanwhile, the measurement of the AST enzyme in the treated fish was 739± 0.13U/ L, which is lower than that recorded in the control fish group (1075.2± 0.09U/ L). It has been demonstrated that exposure of fish to foreign chemicals and substances results in an increased activity of liver enzymes (AST, ALT), which correlates with both the concentration and duration of exposure to these substances. This increase is attributed to liver damage, leading to the release of enzymes from the liver cytosol into the bloodstream **(Abdel-Tawwab *et al*. 2016, Aldoghachi *et al*., 2019, Aldoghachi, 2022)**.

Table 1. Biochemical measurements of ALT, AST and ALP (Activity U/ L) in *Oreochromis aureus* exposed to pH 7.8 and 6.5 for an exposure period of half, 1, and 2 hour(s)

	Control (pH 7.8)	**Exposure (Half an hour)**	**Exposure time: 1 hour**	**Exposure time: 2 hours**
ALT	94.5	1245.0	488.0	1302.8
AST	1075.2	739.9	711.5	1061.6
ALP	20.97	20.45	31.7	163.4

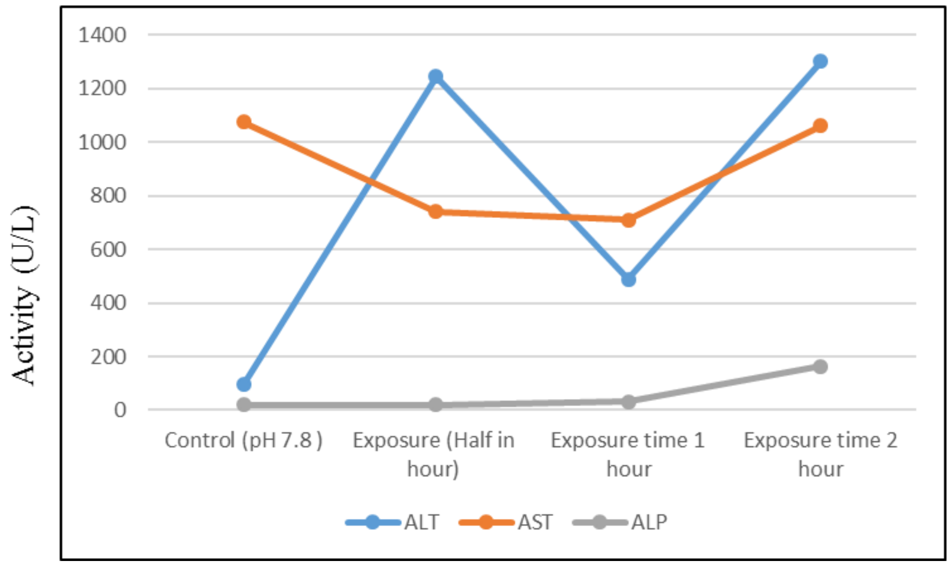

Fig. 1. Activity of blood serum ALT, AST and ALP in *Oreochromis aureus* exposed to pH 7.8 and 6.5 for an exposure period of half, 1, and 2 hour(s)

Measuring blood chemistry variables is key to understanding how fish respond to natural environmental fluctuations and the challenges presented by anthropogenic change (such as pollution and climate change). Correct blood chemistry data can be vital in revealing the health and performance of individuals, populations and entire ecosystems. In fish, blood parameters have been measured to inform a wide range of physiological purposes, such as broad health status, biochemistry, immunology, hematology, reproduction, nutrition, and ecotoxicology **(Davison *et al*., 2023)**.

The study of **Wang *et al.* (2019)** on *Scophthalmus maximus* fish proved that changes in the level of the water pH values (6.3- 8.8) affect the fish's blood physiology and distress the activity of blood enzymes total superoxide dismutase activity (T-SOD) and alkaline phosphatase activity, in addition to its effect on total nuclear anomalies. Additionally, they also found that the hemoglobin content (HBC) was higher in acidic conditions than in alkaline conditions.

Some studies have proven that high carbon dioxide in water affects the organism's physiology as well as the behaviors associated with stimuli and thus affects the organism's physical activity **(Verschoor *et al.*, 2004; Ellis *et al*., 2017)**.

Phillips *et al*. (2015) expected that the reaction of carbon dioxide in the atmosphere will lead to acidification of water, with an expected change of about 0.5- 3 units in the pH in the Great Lakes in Laurentian Germany during the next 35 years, which indicates that fresh water can acidify faster than ocean water. In addition, their study explained the increase in the amounts of dissolved organic carbon in fresh water due to the increased global warming and the rise in carbon dioxide in the atmosphere.

The quality of water in fish ponds and lakes is affected by the interaction of several chemical components, such as carbon dioxide, pH, alkalinity, and hardness, and can have fundamental effects on fish productivity, stress level, fish health, availability of oxygen, and ammonia poisoning. High concentrations of carbon dioxide cause a decrease in the pH values of the water since carbon dioxide reacts with water to produce carbonic acid (H2CO2) and then lowers the pH value, limiting the ability of fish blood in the gills to carry oxygen **(Wurts & Durborow, 1992)**.

Histological examination of fish gills

The regular shape of the gill fibers of fish appears with a regular arrangement of the gill filaments and secondary filaments (Fig. 2). Histopathology of the gills of fish tested and treated with acidic concentration (pH 6.5) showed a slight damage within one hour of exposure and it increased with increasing the exposure period. However, we have observed that the gills suffer from enlargement due to the mucous cells and vacuolation in the gill membrane. A clear effect was observed in the secondary gill lamellae represented by their bending and curling in many specimens (Fig. 3) which causes congestion and hemorrhage of gills, especially when exposed continuously to pH 6.5 for periods of exposure of more than an hour. In addition, the presence of necrosis in the pavement cells was detected. With increasing exposure to the acidic medium for a period of two hours, a bulb shape was formed from the fusion of the peripheral cells of the filaments (Fig. 4). This is due to the method of increasing the surface area to protect against the positive ions resulting from the ions of the acidic medium.

Fish gills play many vital activities including respiratory functions, osmoregulation, and secretion. Moreover, gills have close contact with the surrounding environment and are mostly sensitive to changes in water quality and, therefore, are considered the main target of pollutants **(Pereira *et al*., 2013)**.

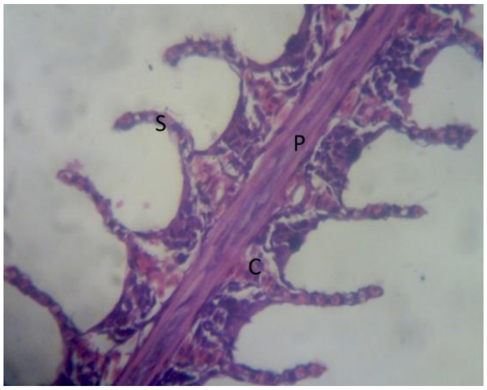

Fig. 2. A micrograph image of the gills of tilapia fish *Oreochromis aureus* for control samples, which show **(P)** the primary gill filaments, **(S)** secondary gill lamellae, and **(C)** pavement cells with a regular arrangement (X40) (H&E)

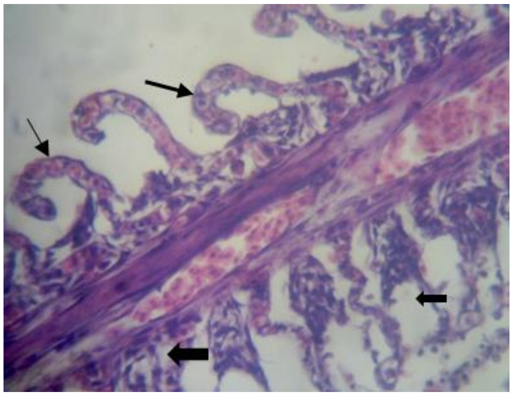

Fig. 3. A micrograph image of the gills of the tilapia fish, *Oreochromis aureus*, exposed to an acid concentration of 6.5 for the first hour, showing curling of the secondary gill lamellae (line arrow) and necrosis of parts of the secondary lamellae (black arrow) (X40) (H&E)

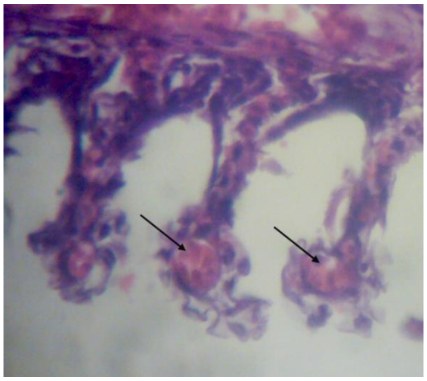

Fig. 4. A micrograph image of the gills of the tilapia fish, *Oreochromis aureus*, exposed to an acid concentration of 6.5 over 2 hours, showing bulb shape in the end of the secondary gill lamellae (line arrow) (X40) (H&E)

Damage to the epithelial membranes is an initial reaction of the gills to the ions of various pollutants, and they compete for union and passage through the chloride cells, which are important cells in the process of ionic balance, which causes damage to those cells, thus affecting the process of osmotic regulation of fish, which results in multiple damages, such as electrolyte imbalance, disruption, and necrosis of the gill tissues, and ultimately decreased oxygen absorption, followed by suffocation and death **(Hwang 2011; Shih *et al*., 2022)**.

Weiss *et al*. (2018) documented the effects of high levels of carbon dioxide on freshwater crustaceans in the lower part of the world. For the food web of two species of the genus *Daphnia*, known as water fleas, the increase in carbon dioxide levels above the maximum observed in fresh water makes it difficult for to the formation of shells, and also it leads to dull their senses, thus affecting their defense mechanism, and reducing the ability to sense predators, making them more vulnerable to predation.

The change in the level of carbon dioxide is related to the change in the water temperature, water density, pH, distribution of ion types, as well as the total inorganic content.

CONCLUSION

Increasing CO_2 in the water increases the acidity of fresh water and reduces pH values with increasing exposure periods. It has effects on the enzymatic activity of the blood of the tilapia fish, represented by an increase in the enzymatic activity of ALT and ALP, while the activity of AST decreases. There are also histopathological effects on the gills of the tilapia fish, which increase with an increase in the time period of exposure to acid. Therefore, the emission of CO_2 gases to the aquatic environment, which causes an increase in the acidity of fresh water, must be reduced.

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