Stress response of Asian redtail catfish (*Hemibagrus nemurus*) fingerlings subjected to various pH exposure

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**Abstract.** Asian redtail catfish (*Hemibagrus nemurus*) is one of the economic freshwater fish species in Indonesia. There is still a lack of information regarding water quality tolerance of Asian redtail catfish. Therefore, this study was conducted to obtain information on the stress response of Asian redtail catfish under various pH exposures. The experimental fish was the second generation of domesticated Asian redtail catfish from the Cirata population (Length: 11.7 ± 0.6 cm; Weight: 13.2 ± 1.6 g). The pH treatment in this experiment was low pH (4-5), optimal pH (6-9), and high pH (10-11) with three replicates in each treatment. Stress response was observed by measuring blood glucose and plasma cortisol. Moreover, fish behavior and survival were also observed. Results showed that glucose level of Asian redtail catfish fingerlings was higher at low pH treatment (258.8 ± 75.1 mg/dL) than optimal (58.7 ± 7.5 mg/dL) and high pH treatment (151.0 ± 21.5 mg/dL) (P<0.05). Cortisol level at low (34.02 ± 3.16 IU) and high pH (32.88 ± 13.85 IU) was significantly higher than optimal pH treatment (12.56 ± 5.18 IU) (P<0.05). Low and high pH exposure to Asian redtail catfish increased their cortisol and glucose levels, which will lead them to mortality.

1. Introduction

Water quality is the most critical parameter for aquatic organisms [1]. In terms of fish, mostly, they live in the water with pH value around neutral. The exposure of low or high pH will lead to a negative impact on their physiological aspects [2,3,4,5,6]. It was related to their acid-base regulation, which has been studied before [5,7,8,9,10]. Several fish species have low or high water pH tolerance [6,11,12,13]. However, there is still less observation on the physiological response of some native fish species in Indonesia regarding several pH levels exposure.

One of the freshwater native fish species in Indonesia with good economic value is Asian redtail catfish (*Hemibagrus nemurus*). This species is popular among the people in some areas of Java and Sumatra. Therefore, the aquaculture technique of this species should be developed continuously. It is one of the potential commodities for aquaculture because of its environmental tolerance and high fecundity. It is also adaptive in ponds and human-made swamps [14].

Limiting factors such as water quality parameters will have a negative impact on diseases and mortality. Chronic exposure that exceeds the tolerance limit will negatively affect fish health and stability. Thus, fish will become maladaptive [15,16]. In terms of aquaculture ponds management,
physical and chemical conditions of the rearing environment should be observed regarding fish physiology and behavior. Changes in water pH levels could disturb fish growth and metabolism. Previous studies regarding the effects of various pH on fish growth and physiological response have been conducted [17-26]. Nevertheless, the study of pH exposure on Asian redtail catfish has not conducted yet. Hence, in this study, we provide an observation on the stress response of Asian redtail catfish fingerlings subjected to various levels of pH.

2. Materials and methods
This study was conducted at Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFAFE), Bogor. The experimental fish used in this study was the second generation of domesticated Asian redtail catfish from Cirata population [27]. Fish (Length: 11.7 ± 0.6 cm; Weight: 13.2 ± 1.6 g) were exposed to water with low pH (pH: 4-5), optimal pH (pH: 6-9), and high pH (pH: 10-11) condition with three replications in each treatment. The experiment was carried out by inserting the 20 fish in each treatment aquarium (50 × 40 × 30 cm; water volume: 30 L). The low pH water condition was set by adding CH₃COOH, whereas a high pH water condition was set by adding NaOH. Parameters obtained in this study were behavior, mortality, survival rate, and stress parameters (glucose and cortisol). Blood glucose and cortisol parameter were obtained by blood collection from three random fish from each treatment. Blood glucose level was analyzed by using a glucose reader (Accu-Chek Active, Roche, Germany). Cortisol level was analyzed by using ELISA kit (Bioassay Technology Laboratory, China) along with microplate photometer (Biosan HiPo MPP-96, Latvia). Data were analyzed statistically using one-way ANOVA with a 95% confidence level.

3. Results and discussion
The results showed that at low pH conditions, fish exhibited irregular swimming activity after more than three hours, excreted mucus, moved irregularly, and then lost equilibrium. Fish started to die in the third hour, and all fish finally died after nine hours. Almost similar to low pH conditions, all fish died at sixth hours on high pH treatment. Meanwhile, fish at optimal pH conditions did not show any abnormal symptoms during 48 hours of observation (Table 1 and Figure 1). Overall, after continuous observation for 48 hours, survival rate at low, optimal, and high pH treatments were found at 0.0 ± 0.0%, 100.0 ± 0.0%, and 0.0 ± 0.0%, respectively (P<0.05).

| Time (hour) | Treatments | Treatments | Treatments |
|------------|------------|------------|------------|
| 0          | Swim normally | Swim normally | Swim normally |
| 3          | Fish excreted mucus from their bodies | Swim normally | Several fish were collapsed and died |
| 6          | Fish suffered a blister on their bodies; Several fish died | Swim normally | All fish died |
| 9          | All fish died | Swim normally | - |
| 12         | - | Swim normally | - |
| 18         | - | Swim normally | - |
| 24         | - | Swim normally | - |

Table 1. Behaviour, clinical symptoms, and mortality of Asian redtail catfish (Hemibagrus nemurus) fingerlings under various pH exposures.
The results of pH exposure experiments showed that acid and base pH affected the survival of Asian redtail catfish fingerlings. Exposure to acidic and alkaline pH in fish forced them to adapt to their environment for survival. Exposure to pH levels beyond the threshold would result in fish mortality. A previous study stated that changes in freshwater pH would lead to large mucus production, which increases diffusion resistance, reduces gas transfer, and generates hypoxemia [28]. Acid water exposure will decrease NaCl levels on blood and develop blood acidosis [29,30].

A previous study stressed that low calcium levels exacerbate ion loss across the gills on acid water conditions. In contrast, high calcium levels in water result in acidosis because of the impairment of ion regulatory mechanisms are improved [2]. Similar to low pH exposure, high pH levels could damage fish gills and skins. Changes in pH on freshwater fish would disturb many physiological processes by reducing oxygen uptake by the gills. Another impact is the structural changes of hemoglobin, which are disrupted the acid-base balance. A short term of high pH exposure has triggered blood alkalosis, reduce sodium influx, and reduce the excretion of ammonia [31].

Based on previous studies with tilapia, survival, and plasma content of sodium decreased due to exposure to acidic pH [18]. Fathead minnow still survived at low pH of up to 4.5. However, little pH exposure will produce abnormal behavior, lower fecundity, and lower hatching rate [32]. In other experiments, perch could survive at pH 10. However, they died at pH 11 [33]. Studies on rainbow trout indicated that these fish could survive at pH ≤10 for a long time. The survival ability is due to the adjustment process of acid-base balance in the body, ion exchange in the respiratory system, as well as the excretion and production of ammonia [34,35].

The impact of various pH exposures on the stress response of Asian redtail catfish fingerlings was shown in Table 2. During the study, the glucose level was higher at low pH treatment than optimal and high pH treatment (P<0.05). Furthermore, the cortisol level of Asian redtail catfish fingerlings at low and high pH was significantly higher than optimal pH treatment (P<0.05). The value from both treatments was not significantly different (P>0.05).

Table 2. Glucose and cortisol level of Asian redtail catfish (*Hemibagrus nemurus*) fingerlings during pH treatments.
Various exposures of pH levels had become an environmental stressor for Asian redtail catfish fingerlings in this study. The impact of environmental stressors had been studied regarding several important parameters on fish, such as growth and metabolism, reproduction, health, and survival. Based on the information, blood properties measurements had become popular methods to analyze the stress response according to environmental stressors [36].

As mentioned before, changes in pH level would bring the acid-base imbalance. Consequently, the blood glucose and cortisol level carried out in this experiment showed that Asian redtail catfish could not tolerate low and high pH conditions despite already making physiological adaptations to cope with those conditions. In the present study, blood glucose levels increased with low and high pH exposure. Blood glucose levels have been used as stress indicators in fish [37-38]. Under stress conditions, hyperglycemia provides additional energy to respond to the pH water changes [39].

In terms of the fish stress response, elevations of blood glucose levels would be accompanied by a rapid increase in plasma cortisol. Plasma cortisol mediates glycogenolysis for the regulation of plasma osmolarity. This fact was proven in several studies (40,41,42).

Rapid changes in pH level resulted in a physiological and behavioral disturbance that lead to death. Cortisol and glucose level tends to increase by low and high pH exposure beyond its optimal range. Fish acid-base compensation was depending on metabolic strategies from fish itself to regulate pH by adjusting acid-base equivalents between the body and environment [43,44,45]. Generally, most of the fish will control environmental pH changes through increased H+ excretion or decreased HCO₃⁻ secretion. Therefore, they can accumulate HCO₃⁻ ions. The regulation may vary depending on individual species [7].

A previous study [46] stated that increasing cortisol and blood glucose levels in fish during stress conditions was the result of catecholamine action on stored glycogen in the liver and other tissues. Low and high pH exposure as the stressor had triggered the hypothalamus pituitary-interrenal axis, which will induce fish secreted the cortisol [47]. Furthermore, cortisol will stimulate the increase of blood glucose and liver gluconeogenic capacity [48,49]. Hence, cortisol and glucose level are commonly used for fish stress indicators [4, 16, 50]. In this study, low and high pH levels increased the stress of Asian redtail catfish fingerlings. It observed from their behavior before the mortality occurred. Exposure to acidic or basic treatment will decrease the fish's appetite, increase the movements to escape, exhaust them, and finally lead them to mortality because of acclimatization failure. During the study, measurements of water quality parameters, including temperature, dissolved oxygen, and pH, were shown in Table 3. The results showed no significant differences in temperature and dissolved oxygen (P>0.05).

| Parameters                  | Treatments          |
|-----------------------------|---------------------|
|                             | Low pH (4-5)        |
|                             | Optimal pH (6-9)    |
|                             | High pH (10-11)     |
| Glucose (mg/dL)             | 258.8 ± 75.1<sup>c</sup> |
|                             | 58.7 ± 7.5<sup>a</sup> |
|                             | 151.0 ± 21.5<sup>b</sup> |
| Cortisol (IU)               | 34.02 ± 3.16<sup>b</sup> |
|                             | 12.56 ± 5.18<sup>a</sup> |
|                             | 32.88 ± 13.85<sup>b</sup> |

Description: Different superscript letters indicated significant differences between treatments (p<0.05).

Table 3. Water quality on rearing media of Asian redtail catfish (Hemibagrus nemurus) fingerlings during treatments.
Water quality is a variable that would affect the survival, reproduction, growth, and production of fish [51]. Temperature and dissolved oxygen in each treatment were on the optimal range [52,53] and not significantly different. The level of pH played a role as a limiting factor in the survival of Asian redtail catfish fingerlings, as stated by other previous studies [54]. At low or high pH beyond its limit, fingerlings in the present study were failed to acclimatize with external environment conditions through acid-base regulation, which leads them to death.

4. Conclusion

Exposure of low or high pH conditions will induce the increase of stress indicator (cortisol and glucose level) on Asian redtail catfish fingerlings, which could lead to death. The results of this study could be used as a reference for water quality management of this species.

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