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Effect of water acidity on the growth performance, survival, and hematology condition of the barramundi fish *Lates calcarifer* (Bloch, 1790) fingerling

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ABSTRACT

Global warming is caused by increased carbon emissions into the atmosphere resulting from burning oil, gas, and other fossil fuels. Subsequently, the carbon gas enters the waters through a diffusion process facilitated by the concentration of gases in the air, which is higher than in the waters. The outcome of this process is a decrease in water acidity, leading to a lower pH, which can disrupt the life of aquatic biotas. Therefore, this study aims to analyze the effect of decreasing of pH on the growth, survival, and physiological conditions of barramundi (*Lates calcarifer*). To achieve this objective, seven pH levels were tested, namely pH of 7.24 (control), pH 6.74, pH 6.24, pH 5.74, pH 5.24, pH 4.74, and pH 4.24. Every treatment was performed with four replications, and the fish was reared for 30 days in the respective tested pH. The reared madia is sea water with a salinity of 22 ppt. The experiment was conducted in the laboratory of fish breeding Faculty of Marine and Fisheries, Universitas Syiah. The results showed that a decreasing in pH had a significant effect on the growth performance and hematological condition of barramundi ($P<0.05$), but its had no significant effect on survival ($P>0.05$). The experimental fish could survive at pH 4.24, but their growth and hematological conditions were disrupted below 6.24. Therefore, it was concluded that the lower threshold value of pH for barramundi was 6.24.

Introduction

Climate change is caused by global warming, which has increased the average temperature on earth in the last decades (Lineman *et al*., 2015; Nguyen *et al*., 2011). Based on the Meteorology, Climatology, and Geophysics Agency of Indonesia, there is an increasing in temperature of 0.03°C every year (BMKG, 2019). Global warming is caused by increased carbon gas emissions in the atmosphere due to burning petroleum, gas, and fossil fuels. Subsequently, carbon gas enters the waters through a diffusion process due to higher gas concentration in the air than in the waters. This causes a decrease in water acidity, and threatening aquatic biotas, including fish (Pachauri *et al*., 2014). Extreme conditions, including increased water temperature, low dissolved oxygen concentrations, and decreased water pH, can disrupt fish life by reducing the metabolic rate, growth, and spawning due to disrupting the endocrine system and changing migration patterns (Roessig *et al*., 2004).

Ocean acidification is a common term used to describe the decrease in the pH of seawater, which is currently occurring. Dissolving carbon gas in the oceans causes an increase in the concentration of hydrogen ions (H^+), resulting in a decrease in the pH of seawater. According to Jacobson (2005), the pH decreased from 8.25 to 8.14 during 1751 - 2004. Moreover, Orr *et al.* (2005) stated that since the industrial revolution, the pH of the oceans has decreased by approximately 0.1 unit, equivalent to a 30% increase in hydrogen ions, and is expected to continue decreasing from 0.3 - 0.4 units in 2100. Ocean acidification occurs because the pH of

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seawater is experiencing decreases due to increased CO_2 levels dissolved in the sea. This CO_2 leads to the formation of hydrogen ions, resulting from the breakdown of carbonic acid, ultimately leading to a decrease in pH levels and an acidic ocean (Mochammad, 2021). Fish and other aquatic biotas can tolerate a pH between 6.0-9.0 (Boyd, 2017), but the optimum ranges of pH for most species is between 6.5 and 8.5 (Joko *et al*., 2013), this is depending on age and physiological conditions.

Barramundi *Lates calcarifer* is one of commercial commodities of marine fisheries worldwide. This fish has high economic value and is one of the popular animal protein sources with increased market demand. As an illustration in Indonesian context, the import volume of Barramundi in 2012 to European countries, such as Italy, Spain, and France, reached 14,285 tons and increased to 18,572 tons in 2014 (Hardianti *et al*., 2016), excluding exports to Japan and USA.

Currently, the majority of barramundi production in Indonesia originates from wild sources. However, concerns exist regarding the potential decline in production due to the effects of climate change. In this regard, Amir *et al.* (2022) have conducted a laboratory-scale investigation to evaluate the impact of climate change, including rising water temperatures, on the survival, growth, and physiological condition of barramundi. The findings indicate that while the increased water temperature did not significantly affect the survival rate, it contributed to the physiological stress experienced by the fingerling. Moreover, the critical temperature limit of barramundi was 42 °C, and at this temperature, Barramundi fingerling can last for 70 min only. The water temperature changes significantly affect the survival and physiological stress in freshwater fighting fish *Betta rubra* Perugia (Nur *et al*., 2020). In regards to water pH, Marimuthu *et al.* (2019) studied the effect of water pH on the hatching and survival rate of African catfish *Clarias gariepinus* larvae, and they found that the larvae can survive at low pH (pH 3.7 and 4.5), but the survival rate was decreased. However, the effect of acidification or pH changes on the physiology, survival, and growth performance of fish especially barramundi (*L. calcarifer*) has not been studied. This information is important to predict the impact of climate change on fish life, specifically barramundi, to develop a mitigation plan. Therefore, it is necessary to carry out laboratory-based experiments to analyze the effect of reducing the pH of the water on the growth, survival, and physiological response of barramundi (*L. calcarifer*).

Materials and Methods

Experimental design

The experimental method with a completely randomized design with 7 levels of treatments was used in this study. The tested treatments were pH 7.24 as a control, pH 6.74, pH 6.24, pH 5.74, pH 5.24, pH 4.74, and pH 4.24, performed with four replications. The study was conducted in the Laboratory of Fish Breeding, faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia.

Preparation of medium and experimental fish

The plastic container with a volume of 20L was used as a reared medium, and 280 fish fingerlings with an average length of 4.2 cm were purchased from the Brackish Water Aquaculture Fishery Center (BPBAP) Ujung Batee Aceh Besar, Indonesia. Furthermore, the fish was acclimatized to a salinity of 22 ppt for 7 days before use in the experiment. During the acclimatization and experimental periods, the fish were provided with a commercial diet that contained 48% crude protein and was fed at a rate of 5% of their body weight twice daily, precisely at 9:00 AM and 17:00 PM.

Tested pH

The plastic containers (Vol. 20L) were filled with 10L of seawater with a salinity of 22 ppt, then 10 fish was stocked into every container. The sea water was collected from Ujung Batee Beach, Aceh Besar district, and the seawater was precipitated for one week before use in the experiment. To obtain the tested pH level, seawater filled in a container was added with a nitrite acid solution (HNO_3), as shown in Table 1. The experimental fish were fed a commercial diet containing 48% crude protein *ad libitum* twice daily for 30 days. Dissolved oxygen, temperature, and salinity were measured and kept at optimal limits for barramundi.

Table 1. The amount of nitric acid (HNO_3) used for each level tested pH.

Tested pH	HNO_3 used (ml)
7.24	-
6.74	1.00 ml
6.24	2.75 ml
5.74	4.50 ml
5.24	6.25 ml
4.74	8.00 ml
4.24	9.50 ml

Blood examination

At the end of the experiment (day-30), three fish were taken randomly from each container and anesthetized using MS-222 solution (100 mg L^{-1})

based on Muchlisin *et al.* (2004). The blood was taken from the caudal venipuncture section using a syringe and then pooled in a tube. A glucometer (Easy Touch GCU 3in1) calculated hemoglobin and blood glucose levels, while erythrocytes and leukocytes were counted using a hemacytometer.

Growth performance and survival

The weight gain of the fish was measured at the end of the experiment. All fish that were still alive at the end of the experiment were weighed and measured individually. The weight gain was calculated based on Muchlisin *et al.* (2016) as follows: W = Wt – Wo, where W, Wt, and Wo are the weight gain (g), body weight at the end of the experiment (g), and body weight at the start of the experiment (g). The daily growth rate was measured based on Muchlisin *et al.* (2016) as follows: DGR (g day^{-1})= (Wt-W0)/t, where DGR is the daily growth rate (g day^{-1}), Wt is the body weight at the end of the experiment (g), Wo is the body weight at the start of the experiment (g), t is the length of time of the experiment (days). Furthermore, the specific growth rate (SGR) was calculated based on Muchlisin *et al.* (2017) as follows: SGR (% day^{-1})= (Ln Wt - Ln Wo)/t x 100, where SGR is the specific growth rate (% day-1), Wt is the body weight at the end of the experiment (g), Wo is the body weight at the start of the experiment (g), t is the length of time of the experiment (days). The survival rate was calculated based on Muchlisin *et al.* (2016) as follows: SR (%) = (No-Nt)/No x 100, where SR = Survival (%), Nt is the number of the fish that died during the study, No is the number of the fish at the start of the experiment.

Fish behavior observation

Observation of fish behavior was carried out every day before, during and after feeding. The observed behavior was fish movement, swimming activity, and feeding activity conducted two hours every day for six days.

Data analysis

The growth performance, survival, and blood profile data were subjected to one-way Analysis of Variant (one-way ANOVA) followed by Duncan's multirange test using SPSS software ver. 20.0. The fish behavior was analyzed descriptively by comparing the data to related studies and reports.

Results

Growth performance and survival

The ANOVA test showed that a decrease in water pH had a significant effect on weight gain, daily growth rate, and specific growth rate of barramundi (*Lates calcarifer*) fry ($P<0.05$) but had no significant effect on survival rate ($P>0.05$). Duncan's multiple range test showed that the highest weight gain and daily growth rate were recorded at pH 7.24 but not significantly different from pH 6.74. The highest specific growth was found in control treatment (pH 7.24), and this value was significantly different from other treatments. Similarly, the highest survival was also found in the pH 7.24, but this value was not different from the pH 6.74, 6.24, and 5.74 treatments, as shown in Table 1. In general, the lower values of all parameters were recorded in fish reared at pH 4.24 but were not significantly different from pH 6.24 to 4.74 except for survival rate (Table 2).

Table 2. Growth performance and survival rate of Barramundi *Lates calcarifer* reared at different water pH for 30 days. The mean values±SD at the same column with different superscript are significantly different ($P<0.05$).

Water pH treatment	Weight gain (g)	Daily growth rate (g day^{-1})	Specific growth rate (% day^{-1})	Survival rate (%)
7.24	0.533 ± 0.005 ^{c}	0.018± 0.000 ^{c}	1.537 ± 0.181 ^{d}	95.000 ± 7.071 ^{a}
6.74	0.517 ± 0.071 ^{c}	0.017 ± 0.002 ^{c}	1.192 ± 0.273 ^{c}	90.000 ± 0.000 ^{a}
6.24	0.336 ± 0.529 ^{b}	0.011 ± 0.001 ^{b}	0.869 ± 0.155 ^{b}	90.000 ± 0.000 ^{a}
5.74	0.201 ± 0.174 ^{a}	0.007 ± 0.000 ^{a}	0.473 ± 0.363 ^{a}	93.333 ± 5.773 ^{a}
5.24	0.259 ± 0.033 ^{ab}	0.009 ± 0.000 ^{ab}	0.639 ± 0.074 ^{ab}	85.000 ± 7.071 ^{b}
4.74	0.332 ± 0.702 ^{b}	0.011 ± 0.001 ^{b}	0.804 ± 0.213 ^{ab}	85.000 ± 7.071 ^{b}
4.24	0.281 ± 0,057 ^{ab}	0.009 ± 0.001 ^{ab}	0.711 ± 0.115 ^{ab}	76.666 ± 15.275 ^{b}

Table 3. The blood profile of the Barramundi fingerling (*Lates calcarifer*) reared at different water pH for 30 days. The mean values±SD at the same raw with different superscript are significantly different (P<0.05).

Parameter	pH						
	7.24	6.74	6.24	5.74	5.24	4.74	4.24
Hemoglobin (g 100 mL^{-1})	6.63±0.2^{a}	7.60±0.2^{b}	7.86±0.6^{b}	7.90±0.6^{b}	8.30±0.1^{b}	8.26±0.6^{b}	8.33±0.6^{b}
Glucose (mmol L^{-1})	3.73±0.5^{a}	5.50±0.6^{b}	6.03±0.2^{bc}	6.73±0.2^{cd}	6.90±0.4^{cd}	7.20±0.2^{d}	7.43±0.5^{d}
Erythrocytes (x10^{6}) (cell/mm^{3})	6.62±0.2^{a}	7.59±0.2^{b}	7.80±0.7^{b}	7.89±0.6^{b}	8.29±0.1^{b}	8.26±0.6^{b}	8.32±0.6^{b}
Leukocytes (x10^{4}) (cell/mm^{3})	3.72±0.5^{a}	5.50±0.5^{b}	6.02±0.2^{bc}	6.72±0.2^{cd}	6.89±0.4^{cd}	7.19±0.2^{d}	7.42±0.5^{d}

Table 4. The behavior of Barramundi *Lates calcarifer* fingerling reared at different water pH.

pH treatment	Behavior and symptoms
7.24	All the fish swim normally, have normal feeding activity, and no symptom was detected
6.74	All the fish swim normally, have normal feeding activity, and no symptom was detected
6.24	All the fish normally swim, had normal feeding activity, and no symptom was detected
5.74	Most fish are restless and swim hyperactively, and their appetite has decreased
5.24	Most of the fish displayed non-active swimming, stayed at the bottom of the tank, and their appetite decreased.
4.74	Some fish rise to the surface, the operculum moves fast, and some fish stay at the bottom, and their appetite has decreased.
4.24	Most fish rise to the surface, the operculum moves fastly, and of fish still stay at the bottom. Most of the fish were not feeding actively.

Table 5. Main water quality parameters measured during 30 days of the experiment.

pH treatment	Parameters		
	Temperature (°C)	DO (mg L^{-1})	Salinity (ppt)
7.24	28.0	7.1	22.0
6.74	28.0	7.2	22.0
6.24	28.0	6.9	21.0
5.74	28.0	6.9	22.0
5.24	28.0	7.1	22.0
4.74	28.0	6.6	22.0
4.24	28.0	6.6	22.0

Hematology

The ANOVA test showed that the decrease in water pH significantly affected the total hemoglobin, glucose, erythrocytes, and leukocyte counts of fingerling Barramundi *L. calcarifer* blood (P<0.05). The total hemoglobin increased with the acidification level, where the higher counts were obtained at the pH 4.24 treatment (8.33 g 100 mL^{-1}). However, this value was not significantly different from other treatments except for the pH of 7.24. The highest blood glucose was also found at pH 4.24, and this value was not significantly different from the pH 5.74, 5.24, and 4.74 treatments but different from the pH 7.24, 6.74, and pH 6.24. The highest number of erythrocytes was also found in pH 4.24, and this value was significantly different from the other treatments except for 7.24. Moreover, the higher leukocytes were also recorded at pH 4.24, and this value was not significantly different from pH 5.74, 5.24, and 4.74 (Table 3).

Fish behavior

The direct observations showed that at pH 7.24 to 6.24, the barramundi fingerlings were swimming actively. However, at pH 5.74 to 5.24, the fish showed non-active swimming or tended to be passive and stayed at the bottom of the tank. At pH 4.74 to 4.24, most fish swim to the water's surface probably to take oxygen, and some remain at the bottom of the tank (Table 4). However, this study showed that most snapper fingerling could survive at a pH of 4.74 (survival >90%), but when the pH decreased to 4.24, more fish was dead (survival< 77%).

Main water quality parameters

Temperature, dissolved oxygen, and salinity were measured two times a day in the morning (07.30 AM) and afternoon (16.30 PM) 30 min before feeding. There was no significant change in values during the study, where dissolved oxygen and salinity ranged

from 6.6 mg/L to 7.5 mg/L and 21.0 ppt to 22.0 ppt. Meanwhile, the temperature was stable at 28.0 °C, and the pH persisted at the tested levels (Table 5).

Discussion

The results showed that barramundi fingerling can tolerate the decreasing in pH up to 4.24, with survival rates ranged from 75% to 93%. However, at pH 5.74 and below, the mortality rate increased. According to Jones (1964), fish have been shown to tolerate a pH range of 4 to 11. Since the test pH falls within this tolerance range, it can be concluded that most fish are capable of surviving under such conditions (>75%). In term of growth performance, the tolerance limit of pH is 6.24, while at a lower pH, the growth rate decreases. Even though most barramundi fingerlings can survive up to pH 4.24, their growth has been disturbed. This was confirmed by changes in blood glucose concentration which increased twice (7.43 mmol L^{-1}) compared to normal conditions (3.70 mmol L^{-1}).

The fish showed inactive behavior at pH 4.74 - 4.24, and some moved to the surface water to obtain more oxygen supply. This indicated damage to the gill tissue, as confirmed by histological analysis. Therefore, the optimal lower threshold for barramundi fry was 6.24, where the growth and survival are high and slightly under control at this pH. Moreover, at a pH of 6.24, the blood and histological profile of the gills showed better performance. Murni *et al.* (2006) stated that a pH value lower or higher than the optimum limit can cause fish to stress and experience physiological disturbances, resulting in death. Even though fish can still tolerate pH 6.0 – 9.0 (Boyd, 2017) with the optimum range of pH 6.5-8.5 (Joko *et al.*, 2013), while the lowest optimal point for the survival of barramundi fingerling was pH 5.74.

Apart from being influenced by the acidity of the water, the growth and survival of barramundi fingerlings are also influenced by environmental factors, such as temperature and dissolved oxygen (Honcharova *et al.*, 2021). The water temperature range in this study was 28 °C, and dissolved oxygen was 6.6-7.2 mg L^{-1}. The optimum water temperature for fish is 25-30 °C, and dissolved oxygen is not less than 2 mg L^{-1} (Payne *et al.*, 2016; Hidayat, 2014). Therefore, the temperature and dissolved oxygen are still within the optimum range for fish. Blood glucose is the main energy source for most vertebrates, and the concentration is influenced by hormones, feed, and temperature (Bartonkova *et al.*, 2016). Based on the results, the higher glucose concentration was 7.4 mmol L^{-1} at pH 4.24 and was 3.5 mmol L^{-1} before treatment. The normal fish blood glucose levels are 2.2 to 5.0 mmol L^{-1} (Shabrina *et al.*, 2018; Hertika *et al.*, 2021). Therefore, the level was passed the tolerance limit for fish. The high blood sugar levels decrease fish appetite (Falcinelli *et al.*, 2016), and indicating the fish is under stress condition (Suma *et al.*, 2023) then reducing the growth performance as recorded in this study. According to Rachmawati (2010), glucocorticoids increase during stress, increasing blood glucose levels to overcome high energy needs during times of stress.

Hemoglobin plays an important role in the circulatory system, transporting oxygen from the gills throughout the body, supplying nutrients to cells, and also playing a role in removing the rest of the metabolism (Ciaccio *et al.*, 2022). According to Andersen *et al.* (2021), the hemoglobin concentration in fish can indicate their health status and aid in detecting stress. The results showed that decreased water pH was associated with increased hemoglobin levels. For instance, the concentration of fish before treatment was 6.3 g 100 mL^{-1}, which increased to 7.9-8.3 g 100 mL^{-1} following the experiment. The normal range for hemoglobin concentration in fish is between 5.2-9.0 g 100 mL^{-1} (Andersen *et al.*, 2021), hence the values obtained were within the normal range.

The number of erythrocytes and leukocyte cells in barramundi fingerling increased with decreased pH. The lowest and highest erythrocyte count was at pH 7.24 and 4.24, and its normal ranged are from 1 - 3 x 10^6 cells/mm^3 (Fitria *et al.*, 2019). The main function of erythrocyte is to transport Hemoglobin (Hb), which carries oxygen from the gills to the fish's body cells (Witeska *et al.*, 2013). Furthermore, stress is indicated when the erythrocyte level in fish is excessively high. This is because the fish may require more oxygen in anticipation of unfavorable conditions, increasing erythrocyte levels (Shen *et al.*, 2018; Ortega-Villaizan *et al.*, 2022). At low and high pH conditions, leukocytes were 3.6 x 10^4 cells/mm^3 and 6 x 104 to 7.5 x 10^4 cells/mm^3. The primary role of leukocytes is to act as a defense system or immune response to eliminate pathogens (Minaka *et al.*, 2012). In general, the number of erythrocytes and leukocytes is species-depending and is also influenced by age, sex, environmental conditions, dissolved oxygen, and food (Yanto *et al.*, 2015; Ejraei *et al.*, 2015).

Conclusions

Water acidity affected the growth performance and hematological condition of barramundi *L. calcarifer* fingerling ($P<0.05$), but had not significant effect on survival rate ($P>0.05$). The best growth

performance was obtained at pH 7.24 and 6.74. Most barramundi fingerlings survived at pH 4.24, but their growth and hematological conditions were disrupted below 6.24. The glucose concentration, hemoglobin, erythrocytes, and leukocytes tend to increase with decreasing pH. Therefore, it was concluded that the lower threshold value of pH for barramundi was 6.24.

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