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The effect of chicken eggshell lime application on peat water in the rearing media of Asian redtail catfish

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ABSTRACT

Peat water has a low pH and high organic acid content that inhibits the growth of Asian redtail catfish (*Hemibagrus nemurus*). This study aimed to evaluate the effect of chicken eggshell lime (CaO) on peat water quality and to determine the optimal dose to support the growth and survival of Asian redtail catfish. The study used a completely randomized design (CRD) with 5 CaO-equivalent lime dose levels (0, 5, 10, 15, and 20 mg/l) and 3 replications, over a 30-day rearing period. The results showed that increasing the lime dose significantly affected ($P < 0.05$) water quality and fish performance. The 20 mg/l dose treatment (P_4) produced the best water quality, with pH 6.2 – 7.3, alkalinity 30 mg/l, hardness 42 – 58 mg/l, calcium 12.9 – 13.8 mg/l, DO 6.31 – 6.40 mg/l, and the lowest CO_2 and ammonia levels (7 – 10 mg/l and 0.0998 – 0.1296 mg/l). The P_4 treatment also produced the highest absolute weight and length growth as well as the highest survival rate (83.33% ± 5.77%) compared to the control (66.66% ± 5.77%). It is concluded that chicken eggshell lime at a dose of 20 mg/l is effective in improving peat water quality and is recommended as the optimal dose for rearing Asian redtail catfish.

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1. INTRODUCTION

Format Indonesia has a very extensive area of peatland, reaching about 26 million hectares spread across Sumatra, Kalimantan, and Papua, with Riau Province being one of the regions with the largest area, namely about 3.8 million hectares or 60.1% of the total peatland in Sumatra [1]. This peatland has potential for use in aquaculture activities, but its water quality remains a major constraint because of its low pH, low mineral content, and high organic matter content [2]. Peat water is generally acidic with a pH ranging from 2 – 5 due to the high content of humic and fulvic acids resulting from the slow decomposition of organic matter, which causes low availability of nutrients and minerals in the water [2,3].

One freshwater fishery commodity with potential for development in peatland areas is the asian redtail catfish (*Hemibagrus nemurus*), which has high economic value due to its thick flesh, distinctive flavor, and relatively high selling price [4]. However, the success of its cultivation is strongly influenced by water quality conditions, especially pH. Asian redtail catfish have limited tolerance for

overly acidic water; low pH can disrupt respiration and osmoregulation, reduce appetite, cause stress, inhibit growth, and increase the risk of mortality if it persists for a long time [5].

The high acidity of peat water can also increase the solubility of toxic metals and disrupt water quality balance, so water quality improvement efforts are needed before it is used as a fish rearing medium [6]. Liming is commonly used to increase pH and alkalinity and stabilize water conditions. Dolomite and calcite lime have been widely used to neutralize acidic waters, but the use of alternative waste-based materials has begun to be developed because it is more economical and environmentally friendly [7,8].

One waste material with potential for use is chicken eggshell, which contains about 94% – 97% calcium carbonate ($CaCO_3$) and can be converted into calcium oxide (CaO) through a calcination process [9]. Rifai and Widiarti [10] reported that the addition of eggshell lime was able to increase the water pH up to 7.57, while a preliminary test on peat water showed that a dose of 10 mg/l was able to increase the pH from 3.5 to 7.8 within 24 hours. Nevertheless, information regarding the optimal dose of chicken eggshell lime for improving peat water quality as a rearing medium for asian redtail catfish remains limited. Therefore, this study evaluated the effect of chicken eggshell lime on peat water quality and determined the best dose to support the growth and survival of Asian redtail catfish.

2. RESEARCH METHODS

2.1. Time and Place

This study was conducted from August to September 2025 for 30 days, located at the Aquaculture Environmental Quality Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

2.2. Method

The study used a one-factor completely randomized design (CRD) with 5 treatment levels and 3 replications (15 experimental units). The treatment levels referred to the best dose from Tanbiyaskur et al. [11] namely golden apple snail shell lime equivalent to CaO 10 mg/l, which was effective in increasing the pH of lowland swamp water from 4.8 to 7.4 with 98% survival in catfish. The treatments tested were eggshell lime doses equivalent to CaO: P0 (0 mg/l), P1 (5 mg/l), P2 (10 mg/l), P3 (15 mg/l), and P4 (20 mg/l).

2.3. Procedure

2.3.1. Preparation of Eggshell Lime (Quicklime)

The preparation of eggshell lime referred to [12,13]. Chicken eggshells were collected and washed repeatedly with clean water until the rinse water was clear, then dried under sunlight for 5 days. The dried shells were ground using a blender, the shell membrane was separated, and the result was sieved using a coconut milk strainer to standardize the size before calcination. The eggshell powder was then placed into a porcelain crucible and calcined in a furnace at 900°C for 4 – 6 hours to convert $CaCO_3$ into CaO, indicated by a color change from yellowish-white to clean white. After calcination, the lime was ground with a mortar until powdery, sieved using a 100-mesh sieve shaker for uniformity, then stored in a thinwall container to maintain quality before being used as a neutralizer for the culture medium.

2.3.2. Container Preparation

The rearing container used was a $30 \times 30 \times 20\ cm^3$ aquarium with a water height of 11 cm, equipped with an aeration system. Before use, the aquarium was cleaned with a $KMnO_4$ solution, rinsed, and dried.

2.3.3. Liming Process

Before liming, the water pH was measured first, then the lime was weighed according to the dose of each treatment. Liming was carried out directly in a container containing 10 liters of water, with lime applied 3 times on days 0, 10, and 20. The lime was sprinkled into the container and aerated for 24 hours, after which the water pH was measured again [14].

2.3.4. Fish Rearing

The size and stocking density of Asian redtail catfish referred to [11,15], namely fish measuring ± 5 – 7 cm with a stocking density of 1 fish/l (10 fish/container). Feed was given ad satiation three

times a day (08:00, 12:00, and 16:00 WIB) for the 30-day rearing period. Length and weight were measured every 10 days in 10 fish per container, while survival was calculated at the study's end.

2.4. Parameters Measured

The water quality parameters observed included temperature and pH (measured daily using a thermometer and pH meter), dissolved oxygen/DO (measured at the beginning and end using a DO meter), as well as hardness, alkalinity, calcium, CO_2, and ammonia (NH_3), which were measured at the beginning and end of the rearing period [16]. Hardness and alkalinity were analyzed titrimetrically using 0.01 M Na_2EDTA with EBT indicator [17]. and 0.02 N H_2SO_4 with phenolphthalein / methyl orange indicator [18], respectively. Calcium levels were determined through a series of titrimetric precipitation tests [19], dissolved CO_2 was measured by carbonate titration using 0.0454 N Na_2CO_3 and PP indicator [20], while NH_3 was measured using a spectrophotometer based on a calibration curve [21]. Absolute weight and length growth and survival of Asian redtail catfish.

2.5. Data Analysis

Data on absolute weight growth, absolute length growth, and survival were analyzed using analysis of variance (ANOVA) based on the CRD model [22] with the help of SPSS. If the ANOVA results showed a significant effect ($P < 0.05$), it was followed by the Student–Newman–Keuls (SNK) test. Water quality data were presented in tabular form and analyzed descriptively.

3. RESULTS AND DISCUSSIONS

3.1. Water Quality Parameters

During the 30-day rearing period, water quality measurements included temperature, pH, DO, hardness, alkalinity, calcium, CO_2, and ammonia. The measurement results are presented in Table 1.

Table 1. Average results of water quality parameter measurements during the study.

Parameter	Result measurement					Optimal
	P0	P1	P2	P3	P4	
pH	4.4 – 7.1	5.2 – 7.1	5.6 – 7.2	5.6 – 7.2	6.2 – 7.3	6 – 8
Alkalinity (mg/l)	20 – 24	22 – 26	24 – 26	26 – 28	30	≥ 20
Hardness (mg/l)	20 – 48	26 – 50	30 – 46	36 – 56	42 – 58	50 – 250
Calcium (mg/l)	6.93 – 9.84	9.41 – 11.6	10.1 – 12.4	11.5 – 13.6	12.9 – 13.8	25 – 100
CO_2 (mg/l)	13 – 18	11 – 16	10 – 14	9 – 12	7 – 10	≤ 10
Ammonia (mg/l)	0.175 – 0.203	0.127 – 0.152	0.119 – 0.150	0.108 – 0.145	0.099 – 0.129	≤ 0.02
DO (mg/l)	6.11 – 6.13	6.21 – 6.22	6.22 – 6.26	6.23 – 6.27	6.31 – 6.40	≥ 5
Temperature (°C)	25 – 29	25 – 29	25 – 29	25 – 29	25 – 29	24 – 33

Based on Table 1, the P4 treatment showed the best water quality values for all measured parameters, with pH ranging from 6.2 – 7.3, alkalinity 30 mg/l, hardness 42 – 58 mg/l, calcium 12.9 – 13.8 mg/l, CO_2 7 – 10 mg/l, ammonia 0.0998 – 0.1296 mg/l, DO 6.31 – 6.40 mg/l, and temperature 25°C – 29°C. These results indicate that the addition of chicken eggshell lime was able to improve peat water quality as a rearing medium for Asian redtail catfish.

3.1.1. Degree of Acidity

pH measurements were carried out daily for 30 days of rearing with the results presented in Figure 1. The pH value in all limed treatments increased compared to the control (P0), with the pattern of increase following the increase in lime dose. Treatment P4 produced the highest and most stable pH (6.2 – 7.3), while P0 ranged from 4.4 – 7.1. The increase in pH occurs because CaO from calcination reacts with water to form $Ca(OH)_2$, which releases OH^- ions to neutralize the H^+ ions responsible for the acidity of peat water. In addition, the $CaCO_3$ contained in eggshell lime can dissolve under acidic conditions, thus increasing the availability of Ca^{2+} ions in the rearing medium [23-25].

During rearing, pH gradually decreases due to the release of H^+ from the peat water and an increase in CO_2 from fish respiration and the decomposition of organic matter, which forms carbonic

acid [23,26]. Changes in pH are also related to the availability of dissolved oxygen, where an increase or stability in pH can support an increase in DO, while increasingly acidic water conditions tend to reduce dissolved oxygen levels due to disruption of photosynthetic activity and biological processes in the water [27]. Higher lime doses provide greater alkalinity reserves and maintain pH stability longer, so lime reapplication every 10 days is necessary to sustain the medium's buffering capacity [28].

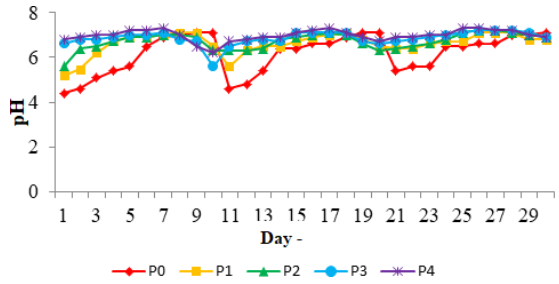


Figure 1. Graph of pH measurements during the study.

The near-neutral pH condition in P4 supports the fish's physiological processes, such as digestive enzyme activity and feed nutrient utilization, while low pH can cause stress and disrupt gill function, resulting in more energy being used for environmental adaptation [26,29]. Thus, the addition of chicken eggshell lime at a dose of 20 mg/l (P4) is the most effective treatment for improving peat water conditions, consistent with [11,30], who reported that increasing the dose of shell-based lime can increase the neutralization capacity of acidic water and support fish growth.

3.1.2. Alkalinity

Alkalinity was measured at the beginning and end of rearing (30 days), with the results presented in Figure 2. The alkalinity value increased from a range of 20 – 30 mg/l at the beginning to 24 – 30 mg/l at the end of the study, with P4 showing the highest value (30 mg/l) and P0 the lowest (24 mg/l), all of which were above the optimal range of ≥ 20 mg/l. The increase in alkalinity occurs because the reaction of CaO with water produces OH^- ions that increase the availability of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-), thus strengthening the water's buffering system and neutralizing the H^+ ions responsible for acidity [23,31]. In aquaculture, alkalinity plays an important role as a buffering capacity that maintains pH stability against sudden changes caused by CO_2 fluctuations influenced by photosynthetic and respiratory activity between day and night [32].

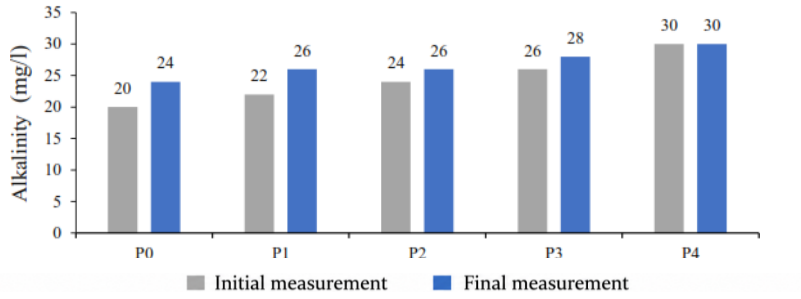


Figure 2. Histogram of alkalinity measurements.

The increase in alkalinity with increasing lime dose indicates that the higher the CaO dose, the greater the water's ability to maintain pH stability. Higher alkalinity conditions also support the nitrification process by reducing ammonia toxicity through the conversion of ammonia into a more stable form [11,31]. Conversely, low alkalinity causes pH to fluctuate more easily, which can increase physiological stress in fish [25]. Thus, treatment P4 (20 mg/l) is the most effective dose for increasing the buffering capacity of peat water, consistent with [11], who reported that the use of golden apple snail shell-based lime was able to improve alkalinity and water quality in catfish rearing media in swamp water.

3.1.3 Hardness

Hardness was measured at the beginning and end of rearing (30 days) with the results presented in Figure 3. The value increased from a range of 20 – 42 mg/l on day 1 to 46 – 58 mg/l on day 30, with P4 the highest (58 mg/l) and P0 the lowest (48 mg/l), both within the optimal range of 50 – 250 mg/l. This increase occurs because the reaction of CaO with water releases calcium ions (Ca^{2+}), which add dissolved minerals and improve the chemical characteristics of peat water, which is naturally low in hardness [31,33], so the higher the lime dose, the higher the resulting hardness.

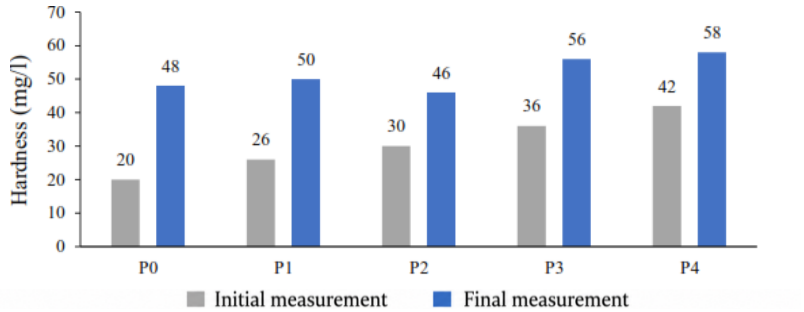


Figure 3. Histogram of hardness values.

Calcium ions support bone formation, enzyme activity, muscle contraction, and fish osmoregulation [34], so their adequate availability allows more feed energy to be allocated to growth rather than to maintaining physiological balance. Thus, P4 (dose of 20 mg/l) was the most effective treatment in increasing peat water hardness, consistent with [11], who reported similar results using shell-based lime.

3.1.4. Calcium

Hardness was measured at the beginning and end of rearing (30 days) with the results presented in Figure 4. Levels increased with increasing dose and rearing duration: on day 1, P4 was highest (12.9 mg/l) and P0 lowest (6.93 mg/l); on day 30, P4 reached 13.8 mg/l and P0 9.84 mg/l. This increase occurs because CaO from eggshell calcination reacts with water to form $Ca(OH)_2$ and release Ca^{2+} ions [11,35], which also contributes to increased pH, hardness, and alkalinity [31].

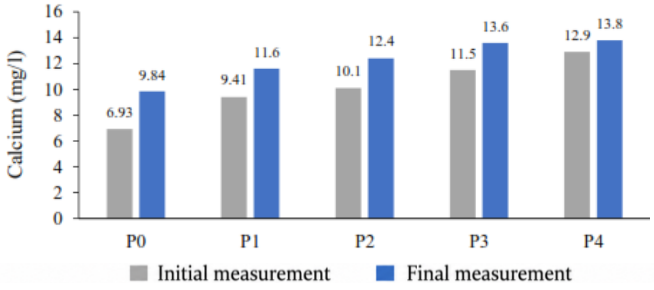


Figure 4. Histogram of calcium values.

Calcium serves as an essential mineral in osmoregulation, bone formation, muscle contraction, and fish enzyme activity [36]; its higher availability allows energy to be allocated more efficiently toward growth [37], consistent with P4, which produced the highest growth and survival rate in this study. Nevertheless, calcium levels in P4 (12.9 – 13.8 mg/l) are still below the optimal range for fish farming, namely 25 – 100 mg/l [38], so the increase that occurred enriched the minerals of the peat water without yet reaching ideal conditions. Overall, the addition of eggshell lime up to a dose of 20 mg/l was effective in increasing calcium levels while also supporting the improvement of peat water quality.

3.1.5. Carbon Dioxide (CO_2)

CO_2 was measured at the beginning and end of rearing (30 days) with the results presented in Figure 5. CO_2 levels decrease with increasing lime dose. On day 1, P0 had the highest CO_2 level (18

mg/l), while P4 was the lowest (10 mg/l); on day 30, CO_2 levels decreased further across all treatments, with the lowest value in P4 (7 mg/l) and the highest in P0 (13 mg/l). This decrease in CO_2 occurs because CaO from calcination reacts to form the alkaline $Ca(OH)_2$, thereby neutralizing H^+ ions and shifting the inorganic carbon balance from free CO_2 toward bicarbonate and carbonate forms [10,39]. In addition, CO_2 formed from fish respiration and the decomposition of humic and fulvic organic matter also affects the carbon balance in the water [25,40]. The increase in CO_2 from respiration is inversely related to pH, so CO_2 accumulation can cause a decrease in pH during rearing [41]. High CO_2 levels can also disrupt the acid-base balance and the efficiency of oxygen transport in fish blood [6]. With the lowest CO_2 level in P4 (7 mg/l), this treatment is thought to be able to create more stable water conditions, thereby supporting the growth and survival of asian redtail catfish. This result is consistent with [39], who stated that an increase in pH in peat water can reduce dissolved CO_2 concentration.

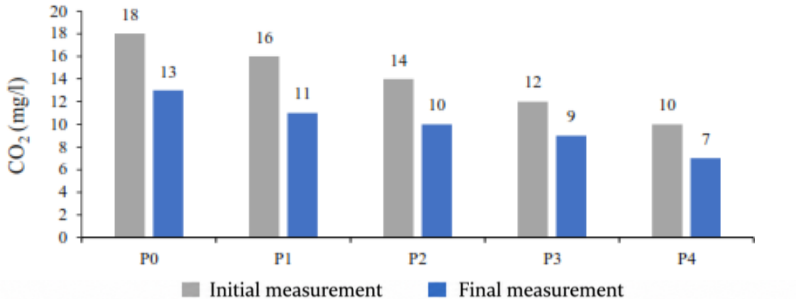


Figure 5. Histogram of CO_2.

3.1.6. Ammonia

Ammonia was measured at the start and end of the 30-day rearing period, with results shown in Figure 6. Ammonia levels increase with increasing lime dose. On day 1, P0 was highest (0.2032 mg/l) and P4 lowest (0.1296 mg/l); on day 30, ammonia levels decreased to 0.1751 mg/l in P0 and 0.0998 mg/l in P4. Ammonia in peat water forms from humic and fulvic organic matter decomposition and tends to accumulate because low pH limits nitrification [25]. The addition of CaO from eggshell lime increased pH and alkalinity, which functions as a buffer, supporting nitrogen transformation and the activity of nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) thereby reducing ammonia accumulation [42,43].

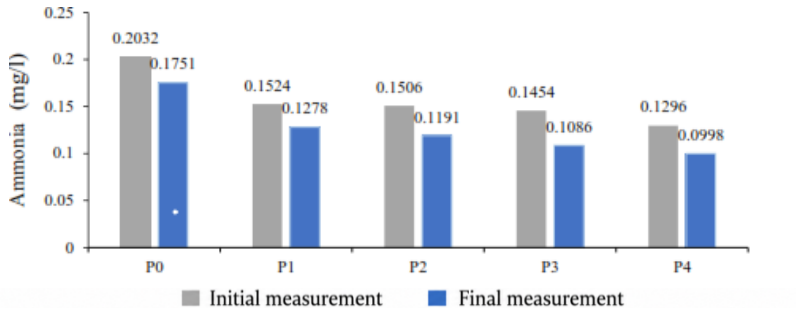


Figure 6. Histogram of ammonia values.

Un-ionized ammonia (NH_3) is toxic and can disrupt gill function, respiration, and the fish's ionic balance [44], and triggers metabolic stress that diverts feed energy from growth to detoxification [45,46]. With the lowest ammonia level (0.0998 mg/l), P4 created the most stable environment and supported energy efficiency for growth, consistent with [47], who reported that liming materials reduce ammonia through improvements in water pH and alkalinity.

3.1.7. Dissolved Oxygen (DO)

DO was measured at the beginning and end of the rearing period, and increased across all treatments over the 30 days. The highest value was found in P4 (6.31 → 6.40 mg/l), while the lowest was in P0 (6.11 → 6.13 mg/l), with all treatments falling within the optimal range (> 5 mg/l) for sup-

porting the respiration of tropical freshwater fish [48]. The increase in DO with increasing CaO dose is thought to occur because the increase in pH and alkalinity improves the balance of the carbonate system, thereby reducing the accumulation of CO_2 from respiration and the decomposition of organic matter [6]. More stable water conditions also support photosynthetic activity as an additional source of oxygen [49]. In addition, the use of aerators and the activity of decomposer microorganisms also play a role in increasing the diffusion and availability of oxygen in the rearing medium [50].

Adequate DO availability supports metabolism and the utilization of feed nutrients for fish growth, while low DO can cause physiological stress and reduce energy efficiency [51,52]. The highest DO value in P4 indicates that this treatment was able to create environmental conditions that better support the respiration and growth of asian redtail catfish.

3.1.8. Temperature

Water temperature is one of the environmental parameters that plays an important role in determining water quality because it affects the physical, chemical, and biological characteristics of an aquatic ecosystem [53]. Temperature changes can affect the physiological processes of aquatic organisms through their effects on water viscosity, mineral distribution, and dissolved oxygen solubility [30]. The temperature of the rearing medium in this study showed stable daily fluctuations across all treatments (P0 – P4), ranging between 25°C – 29°C, and was relatively uniform among treatments. This indicates that the addition of eggshell lime had no direct effect on changes in water temperature. This temperature range still falls within the optimal conditions for supporting tropical freshwater fish farming (25°C – 30°C), thus supporting the physiological activity of Asian redtail catfish [48]. As poikilothermic organisms, fish are highly dependent on ambient temperature because temperature affects enzyme activity, metabolic rate, digestive processes, and the efficiency of feed utilization for growth [54]. In addition, temperature has a close relationship with DO concentration, where an increase in temperature can reduce the water's ability to dissolve oxygen while simultaneously increasing the fish's metabolic oxygen demand [54]. Because the temperature during the rearing period remained within a relatively stable range, the temperature parameter was not a limiting factor for the growth and survival of asian redtail catfish. Thus, the success of the eggshell lime treatment in this study is more related to its ability to improve other water quality parameters, such as pH, alkalinity, hardness, and mineral availability [34].

3.2. Absolute Weight Growth, Absolute Length Growth and Survival Rate of Asian Redtail Catfish

Table 2 presents absolute weight and length growth and survival rate of Asian redtail catfish. Absolute weight and length growth were strongly influenced by optimal feeding and suitable water quality. Good water quality supports fish growth by influencing metabolic rate and energy assimilation [55]. The ANOVA results showed that the addition of chicken eggshell lime had a significant effect ($P < 0.05$) on the absolute weight and length growth of asian redtail catfish, with treatment P4 (20 mg/l) producing the highest growth compared to the other treatments. This indicates that eggshell lime improved peat water quality, creating conditions more supportive of fish growth.

Table 2. Absolute weight, absolute length, and survival rate values of Asian redtail catfish.

Treatment	Measured parameter		
	Absolute weight (g)	Absolute length (cm)	Survival rate (%)
P0 (Control)	$2,94 \pm 0,10^{a}$	$2,67 \pm 0,28^{a}$	$66,66 \pm 5,77^{a}$
P1 (5 mg/l)	$3,43 \pm 0,22^{b}$	$3,56 \pm 0,07^{b}$	$73,33 \pm 5,77^{ab}$
P2 (10 mg/l)	$3,83 \pm 0,09^{c}$	$4,18 \pm 0,32^{c}$	$73,33 \pm 5,77^{ab}$
P3 (15 mg/l)	$4,27 \pm 0,17^{d}$	$4,71 \pm 0,15^{d}$	$76,66 \pm 5,77^{ab}$
P4 (20 mg/l)	$5,09 \pm 0,21^{e}$	$5,61 \pm 0,32^{e}$	$83,33 \pm 5,77^{b}$

Note: Different superscript letters in the same column indicate a significant difference ($P < 0.05$).

Higher growth in P4 was associated with improved water chemistry following CaO addition, including increased pH and alkalinity that neutralized peat water acidity, greater calcium availability supporting tissue formation and physiological stability, and lower ammonia levels that reduced respiratory disturbance and fish stress [56-58]. Higher DO in P4 also supported fish metabolism, as oxygen

is needed to convert feed nutrients into energy for physiological activity and growth [59]. More stable media conditions allow more energy from feed to be allocated to growth rather than to maintaining the fish's physiological balance. In contrast, the control treatment (P0) showed the lowest growth because it was still in acidic, low-mineral peat water conditions with higher ammonia levels. This result is consistent with [11], who reported that the use of calcium-based lime materials could improve fish growth through improvements in pH, alkalinity, and mineral availability in the culture media.

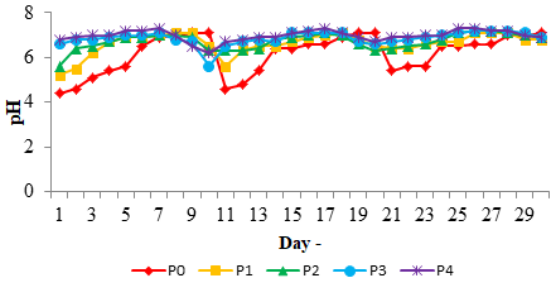


Figure 7. pH and mortality chart of Asian redtail catfish.

The ANOVA results showed that the addition of chicken eggshell lime had a significant effect ($P < 0.05$) on the survival rate of asian redtail catfish. P4 (20 mg/l) produced the highest SR (83.33% ± 5.77%), while P0 was the lowest (66.66% ± 5.77%), with the highest mortality in P0 (33.34%) and the lowest in P4 (16.67%). The increase in SR along with increasing CaO doses shows that improving the media conditions, making them more supportive of fish survival [48].

Mortality was inversely related to pH, increasing as pH declined around days 10 and 20 before lime reapplication. After day 23, mortality was rarely observed, suggesting that early rearing was a critical phase of fish adaptation to peat water (see Figure 7). Acidic conditions increased osmoregulatory energy expenditure and disrupted gill function and metabolism, raising mortality risk during prolonged exposure [60,61]. At higher CaO doses, stable pH, greater alkalinity and hardness, and lower ammonia reduced physiological stress and supported fish survival [28,62]. This result agrees with [11], who reported up to 98% SR for patin fish in swamp water limed with golden apple snail shells. Although SR was lower here, survival showed a similar increase with improved water quality. Overall, 20 mg/L chicken eggshell lime (P4) was the best treatment for improving Asian redtail catfish survival in peat water through stable pH from liming every 10 days, increased alkalinity and hardness, and reduced ammonia.

4. CONCLUSION

The application of chicken eggshell lime (CaO) to peat water medium had a significant effect ($P < 0.05$) on the water quality, growth, and survival of Asian redtail catfish (*Hemibagrus nemurus*). Increasing the lime dose was able to increase pH to 6.2 – 7.3, alkalinity to 30 mg/l, hardness to 42 – 58 mg/l, calcium level to 12.9 – 13.8 mg/l, and DO to 6.31 – 6.40 mg/l, as well as decrease CO_2 level to 7 – 10 mg/l and ammonia to 0.0998 – 0.1296 mg/l, while temperature remains stable in the range of 25°C – 29°C. The best treatment was obtained at a dose of 20 mg/l (P4), which produced the highest absolute weight and length growth as well as a survival rate of 83.33% ± 5.77%, higher than the control (66.66% ± 5.77%). Thus, chicken eggshell lime at 20 mg/l is an effective, economical, and environmentally friendly liming alternative for improving peat water quality and supporting Asian redtail catfish growth and survival.

AUTHOR CONTRIBUTIONS

Abigael Ruthmawati: Investigation, data curation, formal analysis, visualization, and writing—original draft. Saberina Hasibuan: Conceptualization, methodology, supervision, and writing—review and editing. Rodhi Firmansyah: Methodological refinement, validation, growth and survival evaluation, and writing—review and editing. All authors revised and approved the final manuscript.

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This research received no external funding.

DECLARATION OF AI USE

The authors declare that no AI tools were used in preparing this manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data supporting this study are included in the article. No separate datasets are available.

ETHICAL APPROVAL

Ethical approval was unnecessary, as no humans or animals were involved.

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