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The effects of alkalinity on production performance and biochemical responses of spiny lobster *Panulirus homarus* reared in a recirculating aquaculture system

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Abstract

Spiny lobsters (*Panulirus* sp.) were valuable and one of the most popular Indonesian export commodities. Some approaches were made to increase the quantity and quality of cultivated spiny lobsters. Land-based mariculture with Recirculating Aquaculture System (RAS) was applied to increase lobster harvesting and optimize environmental quality by adjusting water alkalinity. This study aimed to determine the optimum level of alkalinity for spiny lobsters *Panulirus homarus* rearing in RAS. This study investigated the effects of applying four water alkalinity levels (Control, 125, 200, and 275 mg L^{-1} $CaCO_3$) on the biochemical responses of *P. homarus* observed in the hemolymph in terms of Total Hemocyte Count (THC), glucose, total protein, calcium, and pH levels.

Furthermore, we also studied the alkalinity effects on lobster production performance parameters in terms of body weight gain, body length, Survival Rate (SR), Specific Growth Rate (SGR), and Feed Conversion Ratio (FCR). Lobsters with an initial weight rate of 58.05±1.69 g and an initial total length rate of 115.33±1.52 mm were reared for 60 days in a recirculation system. Results of water quality parameters such as ammonia, nitrite, nitrate, dissolved oxygen, temperature, and salinity during the study were available for lobster rearing. Different alkalinity levels affected the biochemical responses and production performance of lobsters. The best alkalinity level to reared *Panulirus* sp. in the recirculation system during this study was 200 mg L^{-1} $CaCO_3$ so that it could achieve the highest survival rate of 86.67% with SGR 0.60±0.01 % day^{-1}.

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Introduction

Indonesian spiny lobsters *Panulirus homarus* have high economic value and are potential to be developed as marine culture commodities. The availability of spiny lobsters (Palinuridae) in nature tends to decline yearly (Booth and Kittaka 2000; Jones 2010). Hence, to overcome this challenge, aquaculture can be used as an alternative attempt to increase lobsters' quantity and also to provide a sufficient number of lobsters for market demand. Vietnam and Indonesia are known for their great development of lobster cultivation (Phillips 2013). Lobsters that are widely cultivated in Indonesia are sand lobsters *P. homarus* and pearl lobsters *Panulirus ornatus*. Currently, lobster cultivation activities in Indonesia are carried out in floating net cages. However, cultivation using floating net cages face several obstacles, including high cannibalism, low survival rate, low growth, high production costs, lack of water quality management, and uncontrolled feed residue. Efforts that have been made to increase lobster production are through indoor land-based aquaculture using a recirculation system. Several research has been conducted to study the effects of shelters application and modification during lobster rearing (Adiyana et al. 2014; Supriyono et al. 2017), shelters to the lobster ratio (Djai et al. 2017), and the effects of higher stocking density on their growth (Subhan et al. 2018).

Lobster production can be increased by optimizing the quality of the aquatic environment. According to Verghese *et al.* (2007), an optimal aquatic environment could improve spiny lobster *P. homarus'* immunity response. One of the aquatic environment parameters that can be optimized in a closed aquaculture system is alkalinity. Alkalinity functions as a buffer against a drastic decreasing in pH value. Alkalinity can be increased and maintained by $CaCO_3$, $MgCO_3$, and $NaHCO_3$ supplementation (Boyd 2016). Lime can be used to increase alkalinity and pH so that the mortality rate of sensitive organisms' acid can be reduced (Weatherley 1988). In the recirculation system, alkalinity is an important factor during the nitrification process (Summerfelt et al., 2015). Colt (2006) observed that the nitrification process slowed down at low pH.

Moreover, Eding et al. (2006) also found that nitrification stopped completely at pH 5.5. To support nitrification when the water exchange rate is at its lowest, Chen et al. (2006) recommended maintaining the alkalinity at 200 mg L^{-1} as $CaCO_3$. Alkalinity neutralizes hydrogen ions (H^+) in water, so it induces the binding of hydrogen ions (H^+) with carbonate ions (CO_3^{2-}) to form bicarbonate ions (HCO_3^-). Furthermore, bicarbonate ions (HCO_3^-) are used by nitrifying bacteria to convert ammonia to nitrite and nitrate (Timmons and Ebeling 2007).

Each aquatic organism has optimal alkalinity levels to support its metabolism. The optimal alkalinity levels can help the development of *Oryzias latipes* (Yao et al. 2010) and also reduce heavy metal accumulation in *Labeo rohita* fish (Adhikari et al. 2006). Certain alkalinity level plays a significant role in acid-base regulation of *Homarus gammarus* lobster (Middlemis et al. 2016), increased growth and survival rates for *Rhamdia quelen* (Andrade et al. 2007), *Macrobrachium rosenbergii* shrimp (Gonzales-Vera and Brown 2007), and *Litopanaeus vannamei* shrimp (Furtado et al. 2014; Maica et al. 2018).

Alkalinity is presumed can influence the biochemical responses of aquatic organisms so alkalinity can be proposed as a biota stress indicator. Several parameters that are recorded to determine lobster stress level are hemolymph glucose, Total Hemocyte Count (THC), and total protein level. Glucose is a source of energy that can be directly utilized by crustaceans, and its concentration in hemolymph can increase until reaching hyperglycemia. Stress due to environmental conditions could increase glucose levels in Panulirus interruptus lobsters (Ocampo et al., 2003). Total Hemocyte Count (THC) levels are closely related to crustacean health, especially to non-specific immune system mechanisms through the phagocytosis process (Johansson et al. 2000). Meanwhile, protein is the largest element of hemolymph, and it is an antimicrobial forming material in crustaceans (Fredrick and Ravichandran, 2012).

The total protein is also a major source of energy in metabolism, and it has the capability to maintain homeostatic conditions (Lorenzon et al., 2007).

P. homarus lobsters reared in individual compartments could live in alkaline conditions within the range of 32.1-241.3 mg L^{-1} $CaCO_3$ (Pratiwi 2016). Meanwhile, Aji et al. (2019) conducted a preliminary study on the effects of different alkalinity levels (0, 160, and 240 mg L^{-1} $CaCO_3$) to lobster growth; they stated that *P. homarus* lobsters would show better growth and survival rates if reared at alkalinity level of 160 mg L^{-1} $CaCO_3$. Still, they showed no significant difference after treatment without $CaCO_3$ addition (control). Therefore, based on their preliminary study, it is necessary to conduct further research to determine which is the best level of alkalinity within the range from 68 (control) to 275 mg L^{-1} $CaCO_3$ for *P. homarus* lobsters reared in a recirculation system. This study aimed to determine the best alkalinity level for *P. homarus* lobsters reared using a recirculation system and determine the effect of alkalinity on water quality, biochemical responses, and production performance of lobsters.

Materials and Methods

Research design

The study on the effects of alkalinity on production performance and biochemical responses of spiny lobsters used *P. homarus* lobsters with an average initial weight of 58.05±1.69 g and an average total length of 115.33±1.52 mm, then 180 lobsters were randomly divided into 3 treatments of different alkalinity concentrations and one control with three replications. The levels of alkalinity treatments were 125, 200, and 275 mg L^{-1} $CaCO_3$. To create a certain alkalinity level, some $CaCO_3$ (lime) in water was added, whereas the control treatment did not have $CaCO_3$ addition. The $CaCO_3$ addition method referred to research by Furtado *et al.* (2014). Furthermore, lobsters were reared for 60 days.

Media and Culture system

Containers used for lobster rearing in this study were fiber tanks with dimensions of 1.2 × 0.95 × 1 m^3 and filled with 800 L of filtered seawater. Lobsters' seeds were reared in a recirculation system which equipped with two water treatment units (protein skimmer and filter). The filter media applied in this system were sand, activated carbon, and biofoam. The oxygen supply in the recirculation system used micro bubble aeration.

Testing materials, adaptation, and lobster rearing

P. Homarus lobsters were caught from Muncar beach, Banyuwangi Regency, East Java. Lobsters were adapted first for ten days until they looked active and had a good appetite, and then they were treated with different concentrations of alkalinity. Lobsters were fed with chopped trash fish 2-3 cm in size. Trash fish used for lobster feeding were *Sardinella* sp., which their digestive tract has been cleaned. Lobsters were fed once per day at 5 p.m., with a feeding rate (FR) of 3% of the body weight, and their growth data were recorded every ten days.

Water quality parameters

Water quality parameters were recorded daily during the study, consisting of pH, temperature, and dissolved oxygen (DO) using a DO meter and YSI 550A. Alkalinity and salinity were measured every three days. Nitrate (NO_3^-), Nitrite (NO_2^-), and Ammonia (NH_3) were measured every ten days and analyzed with a spectrophotometer. Water quality measurement refers to APHA standard method (1999).

Biochemical responses

Biochemical response parameters of lobsters measured during the research were Total Hemocyte Count (THC) refers to Blaxhall and Daishley (1973) method, and glucose

hemolymph refers to Li *et al.* (2008). Total protein refers to Lowry *et al.* (1951), and the level of calcium hemolymph refers to Wilder *et al.* (2009). Hemolymph pH was measured using with pH meter (pH meter, LAQUAtwin). Hemolymph samples were analyzed on days 0, 3, 10, 20, and every ten days until the end of the research. We took them from three lobsters (0.1 ml per lobster) per replication. The hemolymph samples were mixed until homogeneous for biochemical analysis.

Production performance

Production performance parameters measured during the study were body weight (g), total body length (mm), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR). Lobster body weight and total body length were measured every ten days. The SGR value can be calculated with the following formula:

Specific Growth Rate (SGR; %/day) = [(ln final weight of lobster-ln initial weight of lobster)/duration of experiment] ×100%.

The survival rate value can be calculated using the following formula:
Survival Rate (SR; %) = [(final number or surviving lobster/initial number of lobster) × 100%]

The value of the Feed Conversion Ratio (FCR) can be calculated with the following formula:
Feed Conversion Ratio (FCR) = (total feed intake/total wet weight gain)

Statistical Analysis

Statistics data were analyzed with Microsoft Excel 2013 and Minitab 16. Water quality parameters were analyzed descriptively. The effects of treatments on experimental parameters were analyzed using analysis of variance (ANOVA) with the F test at 95% of confidence interval. Tukey's test was used to determine if the treatment had a significant effect.

Results

Water quality parameter

Water pH value during the research increased as the alkalinity of the water increased (**Figures 1** and **2**). Water quality parameters data during the study are presented in **Table 1**. Ammonia, nitrite, and nitrate concentration values during the study were still within lobster tolerance limits. While the data on water temperature, DO, and salinity during the study fluctuated, but these conditions still supported lobsters' growth.

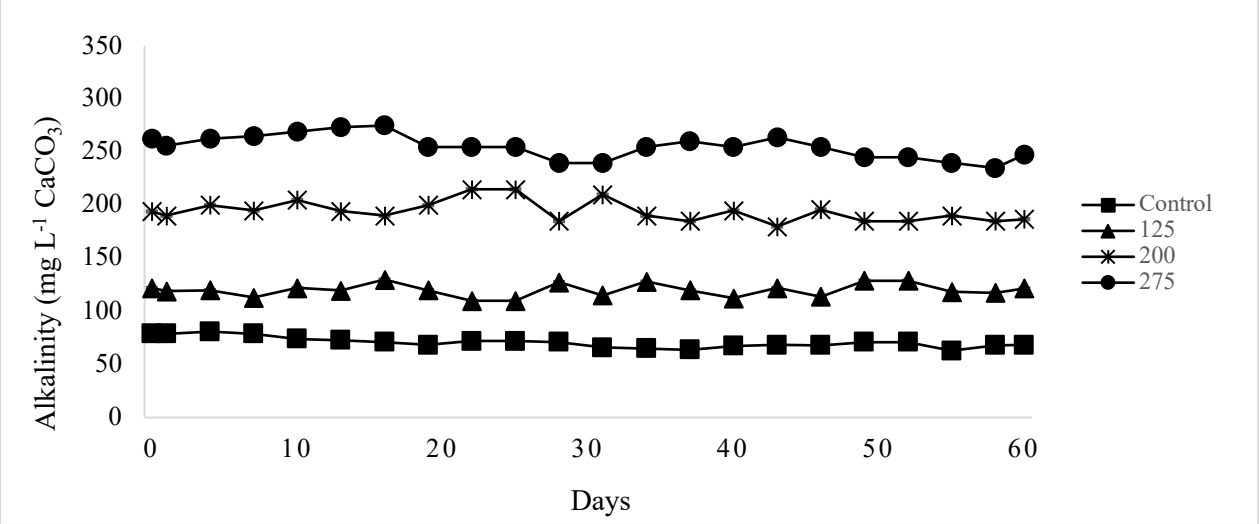

Figure 1 Water alkalinity in each treatment tank.

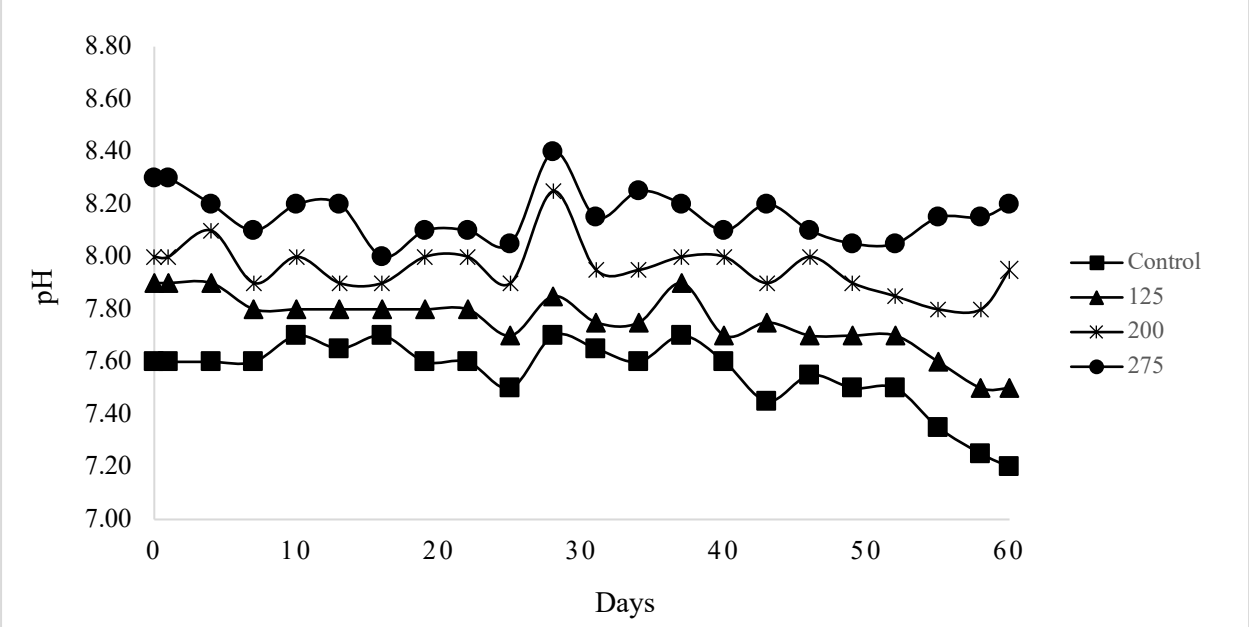

Figure 2 Water pH in each treatment tank.

Table 1 Water quality parameters in all treatment tanks.

Water quality parameter	Alkalinity (mg L^{-1} as $CaCO_3$)			
	Control	125	200	275
NH_3 (mg L^{-1})	0.01-0.05	0.01-0.04	0.01-0.04	0.01-0.04
NO_2^- (mg L^{-1})	0.34-1.15	0.66-1.37	0.55-1.03	0.58-1.19
NO_3^- (mg L^{-1})	0.65-3.54	0.68-3.42	0.67-3.68	0.69-3.78
Temperature (°C)	27.1-27.8	26.9-27.9	27.1-27.9	26.9-27.9
DO (mg L^{-1})	4.95-6.20	4.43-5.87	4.53-6.08	4.53-6.23
Salinity (g L^{-1})	27.5-31.5	25.0-30.0	27.0-31.5	26.5-30.5

Total Hemocyte Count (THC)

Observation of Total Hemocyte Count (THC) value in this study showed that THC value increased on day 10th, especially in control and 275 L^{-1} $CaCO_3$ alkalinity treatment. Still, after the 20th day, its value declined until the end of the study period (**Figure 3**). On the 10th day, THC values at the control and 275 alkalinity treatments were significantly different from 200 and 125 mg L^{-1} $CaCO_3$ alkalinity treatment.

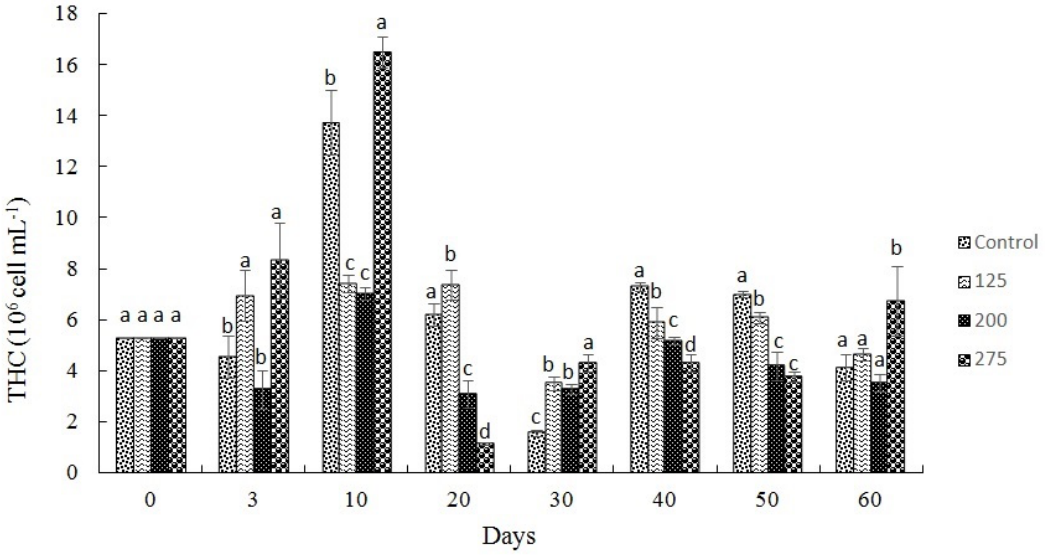

Figure 3 Effect of alkalinity on Lobster THC. Data with different letters are significant different ($p < 0.05$).

Hemolymph glucose

Lobster hemolymph glucose level during the study fluctuated, and its value tended to increase in each treatment until the end of the study period (**Figure 4**). The highest hemolymph glucose levels were found in the control treatment on day 30th. However, from day 40th until the end of the study period, 125 L^{-1} $CaCO_3$ alkalinity treatment reaches slightly high glucose levels than the other treatments.

Hemolymph protein

At the beginning of the study, especially in the control treatment and 125 mg L^{-1} $CaCO_3$, there was an increase in hemolymph protein levels, but its value decreased on day 20th. Then protein hemolymph value started to increase on day 30th until the end of the study (**Figure 4**). The highest total protein was found in 125 mg L^{-1} $CaCO_3$ treatment on day 50th.

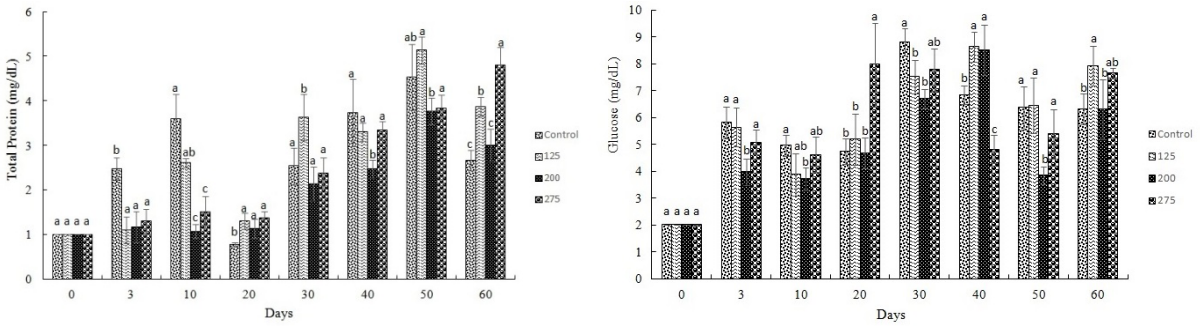

Figure 4 Effect of alkalinity on Lobster hemolymph's Glucose and Total Protein. Data with different letters are significant different ($p < 0.05$).

Calcium hemolymph

The value of calcium hemolymph tends to increase from day 3rd and reach its peak on day 20th, then their level slowly decreases until the end of the study period (**Figure 5**). The highest value was found in 275 mg L^{-1} $CaCO_3$ alkalinity treatment on day 20th and this treatment also resulting a higher calcium value than other treatments. Calcium hemolymph value at alkalinity 275 mg L^{-1} $CaCO_3$ significantly differed from other treatments on days 20th, 30th, and 60th.

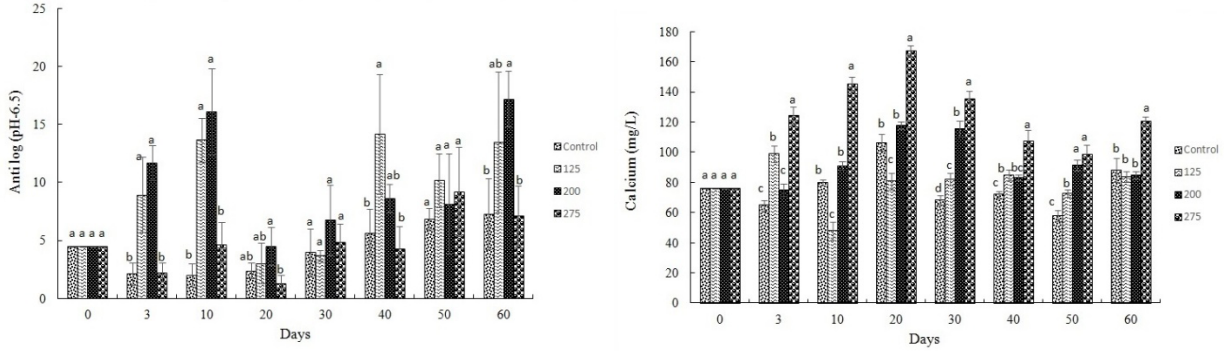

Figure 5 Effect of alkalinity on Lobster Hemolymph's pH and calcium. Data with different letters are significant different ($p < 0.05$).

Hemolymph pH

Hemolymph pH value during the study fluctuated, and the results ranged from 1.29-17.22. The value tends to increase on day 3rd until day 10th and then decrease on day 20th. Hemolymph pH value slowly increased from day 30th until day 60th. The highest Hemolymph pH value was found in 200 mg L^{-1} $CaCO_3$ alkalinity treatment on day 60th (**Figure 5**).

Production Performance

The results of the production performance parameters assessment during the study are presented in **Table 2**. At the end of the study period, the highest average lobster weight was found in the alkalinity treatment of 125 mg L^{-1} $CaCO_3$, which their weight reached 84.14±0.18 g. However, this value was not significantly different from the 200 and 275 mg L^{-1} $CaCO_3$ alkalinity treatment results. The best survival rate (SR) was achieved by 200 mg L^{-1} $CaCO_3$ alkalinity treatment, and its value was not significantly different from 275 mg L^{-1} $CaCO_3$. Still, this value differed significantly from the control and 125 mg L-1 CaCO3 alkalinity treatment. Meanwhile, the best FCR values were found in the 125 mg L^{-1} $CaCO_3$ alkalinity treatment, and these values were significantly different from the control treatment.

Table 2 Effect of alkalinity on lobster production performance. Data with different letters are significant different ($p < 0.05$).

Production performance	Alkalinity (mg L^{-1} as $CaCO_3$)			
	Control	125	200	275
Final weight (g)	76.53±4.49^{b}	84.14±0.18^{a}	83.17±0.56^{a}	81.41±0.59^{ab}
Final length (mm)	128.69±0.28^{a}	132.57±0.05^{a}	130.28±0.21^{a}	130.36±0.23^{a}
Survival Rate (%)	60.00±0.00^{b}	64.44±3.85^{b}	86.67±0.00^{a}	80.00±6.67^{a}
SGR (% day^{-1})	0.46±0.10^{b}	0.62±0.00^{a}	0.60±0.01^{a}	0.57±0.01^{ab}
FCR	10.21±1.93^{a}	6.74±0.04^{b}	7.78±0.46^{ab}	7.25±0.40^{b}

Discussion

The increase in water alkalinity levels correlates with water pH value during the study (**Figures 1** and **2**). Linear correlation between pH and alkalinity is following Boyd et al. (2016) research, which stated that H^+ ions would bind to carbonate ion (CO_3), resulting in the increase of pH value at higher alkalinity. It has been reported that maintaining an adequate level of alkalinity is critical for nitrification maintenance, the wastewater literature reports that 40-80 mg/l (as $CaCO_3$) is the minimum alkalinity required to support nitrification (Paz, 1984; Biesterfeld et al., 2003). RAS operates at suboptimal alkalinity and may experience greater pH fluctuations, higher concentrations of TAN and NO_2-N, lower nitrification efficiency, and microbial community instability (Mydland et al., 2010). These conditions may be harmful to the lobsters.

Water quality parameters, such as ammonia, are still within tolerance limits for lobsters (**Table 1**). According to Mojjada et al. (2012), the ammonia tolerance limit for lobsters is less than 0.1 mg L^{-1}. Nitrite (NO_2^-) level during the study period fluctuated while nitrate (NO_3^-) concentration tended to increase until the end of the study. This indicates the occurrence of the nitrification process by *Nitrosomonas* bacteria which converts ammonia to nitrite, and *Nitrobacter* bacteria which converts nitrite to nitrate. The results of this study show that nitrite and nitrate levels in the recirculation system still met nitrite and nitrate tolerance limits for lobster culture. Estrella (2002) stated that nitrite level, which can be tolerated by lobsters, is less than 5 mg L^{-1}, and nitrate level is less than 100 mg L^{-1}. Temperature, DO, and salinity parameters met with lobster culture requirements. The optimal temperature for lobster rearing is 25-31°C (Jones, 2009), DO level is more than 3.5 mg L^{-1} (Mojjada et al., 2012), and optimal salinity level is ranged from 25 to 35 g L^{-1} (Vidya and Joseph, 2012).

Total hemocyte count (THC) is a physiological parameter that can be used to determine crustacean health (Johansson et al. 2000). Stress conditions describe steady-state disturbance

beyond the normal range and their repair and recovery processes. Stress also affects the immune system of fish through metabolic pathways (Adiyana et al., 2014; Leland et al., 2013 Yeh et al., 2010). THC plays an important role in the immune system of crustaceans. Harrington et al. (2019) found that changes in water temperature outside their normal habitat resulting in elevated THC levels in *Homarus americanus,* indicated their stress response to environmental changes. The different alkalinity treatments in this study affected the THC response of the lobster hemolymph. The THC data after ten days showed the highest value, especially in the control treatment and 275 mg L^{-1}, but at a concentration of 200 mg L^{-1} the THC value tended to be lower and stable. The lower and stable THC value in the 200 mg L^{-1} treatment indicated that at this concentration, the stress level was lower when compared to other treatments. Lobster seeds experienced stress because they did not adapt to previous handling, on-land holding time, and human touch when they were being transferred from the acclimatization pond to the treatment pool. Verghese et al. (2007) found that environmental changes cause stress and affect the immune response of *P. homarus*, resulting in changes in THC concentration, phenoloxidase activity, and phagocyte activity. A short-term increase in THC at the start of this study also occurred in Leland et al. (2013), which stated that this indicates stress response due to changes in environmental conditions. In *Panulirus Cygnus*, THC levels may increase during handling and transport due to stress. The THC value in all treatments decreased on the 20th day, and it tended to be stable until the end of the study. THC concentration of 200 mg L^{-1} CaCO3 treatment had the lowest and most stable tendency compared to the other treatments. The results of this study indicate that an alkalinity concentration of 200 mg L^{-1} can potentially reduce stress on lobster seeds.

Glucose levels in lobster hemolymph can be used as an indicator to determine the stress on lobsters. When lobsters are stressed, their glucose levels will increase or achieve hyperglycemia. Hyperglycemia is a major biomarker of adaptive stress in crustaceans, causing CHH to release glucose from stored glycogen and use it as a substrate during anaerobic respiration (Powel et al., 2017). Glucose is produced through the process of glycogenolysis and gluconeogenesis to satisfy an energy source for improved hemostasis during stress (Ocampo et al., 2003; Hastuti et al., 2004). Factors that can lead to increased glucose levels in *Hommarus Americans* are exposed to air, handling, and other environmental stressors (Lorenzon 2005). This study found that hemolymph glucose levels in all treatments were increased at the beginning of the rearing period, but their level slowly decreased on the 10th day. On the 20th day, the increase in glucose levels occurred because lobsters were experiencing stress due to their adaption to a new tank. On the 30th day, hemolymph glucose levels increased again until the end of the study period, which occurred in all treatments. According to Lorenzon et al*.* (2007), escalation of glucose levels can also be an indicator of an increase in energy demand in line with body weight gain in crustaceans. The lobsters' hemolymph glucose concentration treated with 200 mg L^{-1} $CaCO_3$ was relatively lower and more stable than other treatments, which tended to show high and unstable glucose values. This result indicated that 200 mg L^{-1} $CaCO_3$ treatment was more successful than other treatments in reducing stress in lobsters.

The fluctuation of hemolymph protein levels in crustaceans is influenced by environmental changes. Hemolymph protein also plays a role in many physiological functions of crustaceans, ranging from oxygen distribution to stress response (Lorenzon et al., 2011). Hemolymph protein concentration is also considered as an indicator of physical endurance in lobsters (Bolton et al., 2009). Lorenzon *et al*. 2011 stated that the quantity of total protein in crustacean hemolymph varies widely in each species, which is the highest number found in penaeid shrimp. Mercier et al. 2006 stated that metabolic parameters such as total protein, glucose, triglycerides, and hemocyanin could be used to observe the physiological changes of stressed crustaceans. Hemocyanin is the major type of protein in the total protein of crustacean hemolymph, and its quantity can reach 60% to more than 93% (Sladkova and kholodkevich, 2011). Hemocyanin has the main function as an oxygen carrier and distributes

it throughout the crustacean body. During the study, hemolymph total protein level increased at the start of the study and declined on the 20th day. This was due to lobster adaptation to changes in environmental conditions. On the 30th day, the hemolymph total protein level increased again until the end of the study period. Overall, the trend of total protein data in 200 mg L^{-1} $CaCO_3$ treatment looked as the lowest and most stable when compared to the other treatments.

The concentration of hemolymph calcium during the study fluctuated, and the highest concentration was found in 275 mg L^{-1} $CaCO_3$ alkalinity treatment on the 20th day. The value was significantly different from other treatments. Wheatly (1999) stated that the calcium concentration in hemolymph could be absorbed by carapace during molting. Crustaceans can obtain calcium from the water and their feed; calcium can be absorbed through gills and intestines and then stored in hemolymph and other tissues such as hepatopancreas (Li dan Cheng, 2012). Boyd (2016) stated that calcium carbonate ($CaCO_3$) has chemical equation $Ca^{2+} + CO_3^{2-}$ and if dissolved in water its equation will become $CaCO_3 + CO_2 + H_2O = Ca^{2+} + 2HCO_3^-$. This equation showed that if more quantity of $CaCO_3$ is dissolved in water, then it will increase calcium level. This study also found an increase in the concentration of hemolymph calcium at 275 mg L^{-1} $CaCO_3$ of alkalinity treatment; this may be influenced by adding $CaCO_3$ to water.

The pH response of hemolymph can be used to analyze stress levels against viral infections in *Litopenaeus vannamei* (Chakraborty, Ghosh 2014), ocean acidification in *Crassostrea gigas* (Lannig et al. 2010), transport in *H. americanus* (Lorenzon et al. 2007) and *C. pagurus* (Lorenzon et al. 2008). Hemolymph pH value fluctuated during the study, which increased on the 10th day. The highest Hemolymph pH value was found in 200 mg L^{-1} $CaCO_3$ of alkalinity treatment, which reached 7.70, while the hemolymph pH value in the control treatment only reached 6.7. The pH value of normal crustacean hemolymph in *C. pagurus* was 7.76 (Lorenzon *et al.* 2008) and in *Crassostrea gigas* was 7.60 (Lannig *et al.* 2010). After ten days, hemolymph pH decreased subsequently and remained stable for all treatments until the end of the study. The increase in hemolymph pH was associated with a high concentration of ammonia in lobster tissue, as it could not be excreted into the environment. On the other hand, a decrease in hemolymph pH is related to fluctuations in glucose levels, which are associated with increased lactate levels as an indicator of stress (Lorenzon et al., 2007). Overall, hemolymph pH conditions in control, 125 mg L^{-1} $CaCO_3$ and 275 mg L^{-1} $CaCO_3$ treatments tended to be more acidic than the 200 mg L^{-1} $CaCO_3$ treatment. Lobster hemolymph pH condition treated with 200 mg L^{-1} $CaCO_3$, resulting in lower stress level compared to other treatments.

Growth is the increase in body length and weight due to the conversion of forage energy into body biomass. Solanki *et al.* (2012) stated that lobster growth was influenced by internal and external factors. Internal factors affecting growth are physiological conditions or genetic characteristics, while examples of external factors are aquatic environment and feed. Furthermore, growth in crustaceans is characterized by periodic molting.

Final body weight and specific growth rate of lobsters in 125 and 200 mg L^{-1} $CaCO_3$ of alkalinity treatments were significantly different from control but not significantly different from those treated with 275 mg L^{-1} $CaCO_3$. The specific growth rate of 125 mg L^{-1} $CaCO_3$ treatment was the best, reaching 0.62 ± 0.00% day^{-1}. Lobster body length also increased during the study period, but there was no significant difference between treatments. The 125 mg L^{-1} $CaCO_3$ treatment had the highest increase in body length, reaching 132.57 ± 0.05 mm.

The highest survival rate at the end of the study was found in 200 mg L^{-1} $CaCO_3$ of alkalinity treatment which reached 86.67 ± 0.00%. Control treatment experienced the most lobster mortality during the study. Mortality was caused by molting and post molting failure, presumably due to lower alkalinity, stress, and pH values than other treatments. Since lobsters were rearing in a recirculation system in which its nitrification process needed bicarbonate ions (HCO_3^-), hence control treatment (without $CaCO_3$ addition) must have lower alkalinity than other treatments. According to Agnalt et al. (2013), low pH values will interfere calcification process during molting and disturb the swimming ability of *Homarus gammarus*

lobster juveniles. Meanwhile, *L. vannamei* shrimp will have the best survival rate (92.12 ± 5.30%) when reared in 225 mg L^{-1} $CaCO_3$ of water alkalinity level (Furtado et al. 2014).

Feed conversion ratio measures animal body capability to convert their feed into body mass. The results of this study showed that the FCR values of control were not significantly different from 200 mg L^{-1} $CaCO_3$ but significantly different from 125 and 275 mg L^{-1} $CaCO_3$ treatments. However, a better FCR value was achieved in 125 mg L^{-1} $CaCO_3$ treatment which reached 6.74 ± 0.04. In general, lobsters that are fed with fresh feed (trash fish or clam) tend to have a higher FCR value than the FCR of fish or shrimp because lobster wet feed contains more water than industrial pellet feed. According to Phillips and Kittaka (2000), the use of wet feed in juvenile lobsters resulted in a feed conversion ratio of 3-9, and even in other studies,> 22 was achieved. The factors affecting feed conversion efficiency include feed type, lobster age, body size, feeding level, salinity, and temperature.

The results of this study indicate that water parameters in the system still meet water quality for lobster cultivation. Lobster biochemical responses fluctuated at the start of the study, but the values returned to normal until the end. Whereas body weight, body length, specific growth rate, and feed conversion ratio showed better results in treatment with 125 mg L^{-1} $CaCO_3$ alkalinity, the results did not differ significantly. Alkalinity treatment with 200 mg L^{-1} $CaCO_3$ achieved the highest survival rate, which reached 86.67±0.00%, and the results were significantly different from the other treatments.

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