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A preliminary study of the effect of alkalinity level on the survival rate and growth of the *Panulirus homarus* lobster

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Abstract
The present study was aimed to discover the effects of alkalinity on the cultivation of the scalloped spiny lobsters *Panulirus homarus* which were kept for 30 days. The lobsters used in the present study had an average initial weight of 51.22±1.87 g. The complete randomized design consisted of a treatment without the addition of $CaCO_3$ or the control alkalinity, 160, and 240 mg L^{-1} as $CaCO_3$. The lobsters were kept in fiberglass tanks sized 1.2 x 0.95 x 1 m^3 filled with 800 L of seawater with a stocking density of 15 lobsters. The results of this study demonstrated that the increased alkalinity was proportionate to the pH. During the course of the study, the NH_3 content was 0.02-0.05 mg L^{-1}, NO_2^- 0.26-1.39 mg L^{-1}, NO_3^- 0.14-2.47 mg L^{-1}, temperature 26.9-27.4 °C, dissolved oxygen 5.44-6.23 mg L^{-1}, and salinity 27.5-31.5 g L^{-1}. The lobsters' physiological response during the maintenance period did not exhibit any signs of stress; however, the hemolymph glucose level in the 240 mg L^{-1} as $CaCO_3$ alkalinity treatment demonstrated an increase at the end of the maintenance period. The survival rate in the treatment with 160 mg L^{-1} as $CaCO_3$ was 96.67±6.67 with a final weight of 62.12±1.66, better than the other treatments but not significantly different.

Keywords: Alkalinity, *P. homarus*, physiological response, survival rate

1. Introduction

The scalloped spiny lobster *Panulirus homarus* has a lot of potentials to be cultivated in Indonesia because it has a high economic value. The price of scalloped spiny lobsters in Indonesia for the domestic market ranges between IDR 250,000 and 350,000/kg, while the export price ranges between IDR 490,000 and 500,000/kg (Wahyudin *et al*. 2017) [1]. The main export destination for Indonesian lobsters is the countries in the South East Asian region, Hong Kong, China, and Japan (ACIAR 2007) [2].

The cultivation of scalloped spiny lobsters in Indonesia is generally conducted in floating net cages. The cultivation of lobsters in floating net cages faces a number of obstacles such as a low growth rate, a low survival rate, cannibalism, inability to manage feed detritus, inability to manage water quality, and a high production cost. Studies that have been conducted on the cultivation of the lobster *Panulirus homarus* in increasing the production performance include the application of shelter and optimizing the stocking density in a controlled vessel (Supriyono *et al*. 2017; Subhan *et al*. 2018) [3, 4].

Lobster production could be increased by improving the quality of the aquatic environment. Decreased water quality such as the pH is commonly caused by feces and organic materials from the feed that accumulates on the seabed. Drastic changes in pH could disrupt physiological functions and interfere with the growth of aquatic organisms. Alkalinity functions as a buffer for the decrease in the water pH. The alkalinity value could be increased by adding $CaCO_3$ lime. The total alkalinity is the concentration of titrated base in the water. Bases will react to neutralize hydrogen ions (H^+). A number of common substances that react with hydrogen ions are hydroxides, carbonates, ammonia, phosphates, borates, silicates, and organic acids (Boyd *et al*. 2016) [5].

A previous study pertaining to alkalinity conducted on *L. vanamei* shrimp kept at an alkalinity of 225 mg L^{-1} as $CaCO_3$ resulted in a survival rate of 92.12 % (Furtado *et al*. 2014) [6]. *M. rosenbergii* lobsters kept at an alkalinity of 250 mg L^{-1} as $CaCO_3$ or higher would experience death (Gonzales-Vera and Brown 2017) [7]. *P. homarus* lobsters raised with a modified shelter with an individual compartment system could grow at an alkalinity range of 32.1-241.3 mg L^{-1} as $CaCO_3$ (Pratiwi 2016) [8]. The increase in alkalinity is directly proportionate to the increase in pH. An exceedingly low pH (acidic) at 5 or high pH (base) at 9.5 disrupted the physiological functions and production performance in the *P. homarus* lobster (Verghese *et al*. 2007) [9]. The effects of alkalinity on the lobster-raising medium and the effects on the physiological response and production performance need to be studied. The purpose of the present study was to discover the effect of the cultivation alkalinity on the *P. homarus* lobster.

2. Materials and Methods

2.1 Time and location

The present study was conducted from January 2018 to March 2018. It was conducted in the Laboratory of Marine Sciences, Bogor Agricultural University, Jalan Pasir Putih 2 Ancol Timur, North Jakarta. The analysis of physiological responses was conducted at the Laboratory of Primate Research Center (Pusat Studi Satwa and Primata (PSSP)) Bogor Agricultural University, while the analysis of water quality parameters was conducted at the Laboratory of Aquaculture Environment, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University.

2.2 Research design

The present study used $CaCO_3$ lime to increase alkalinity. The treatments used were the treatment without the addition of $CaCO_3$ or the control alkalinity, the 160 mg L^{-1} as $CaCO_3$ alkalinity, and the 240 mg L^{-1} as $CaCO_3$ alkalinity. The present study applied a complete randomized design. The alkalinity was increased in three different treatments and they were repeated twice. The lobsters were kept for 30 days.

2.3 Preparation of experiment materials and maintenance media

The present study used *P. homarus* lobsters with an average weight of 51.22±1.87 g. The lobsters were adapted for ten days prior to the treatments until the lobsters appeared to be active and had good appetites. The feed given was rough fish from the *Sardinella sp*. species which were chopped to a size of 2–3 cm. During the course of the study, the lobsters were fed once daily at 17.00 Western Indonesia Time with a Feeding Rate (FR) of 3%. The lobsters were kept in fiberglass tanks sized 1.2 x 0.95 x 1 m^3 which were filled with 800 L of seawater at a stocking density of 15 lobsters. The PVC shelters provided for 5:4 of the lobster population (Djai *et al.* 2017) [10].

2.4 Study parameters

The parameters observed in the present study were water quality, physiological response, and production performance. The water quality was measured daily which included the temperature, pH, and Dissolved Oxygen (DO). The alkalinity and salinity were measured every three days. The ammonia, nitrite, and nitrate were measured every ten days, referring to APHA (1999) [11]. The physiological responses observed were the Total Hemocyte Count (THC) which referred to Blaxal and Daishley (1973) [12] and hemolymph glucose which referred to Li *et al.* (2008) [13]. The THC and hemolymph glucose were calculated every ten days from the hemolymph samples. The hemolymph samples were collected using a syringe that had been rinsed with an anticoagulant. The hemolymph samples were taken from the hind walking leg near the abdomen. The production performance parameters observed were the body weight, survival rate (SR), and specific growth rate (SGR).

2.5 Data analysis

The data that had been collected were tabulated using Microsoft Excel 2013 and analyzed by ANOVA using the Minitab 16 program. To see the difference between treatments, the follow-up Tukey test was conducted at a confidence rate of 95%. The water quality data collected were analyzed descriptively.

3. Results and Discussion

3.1 Water quality

The results of the water quality observations during the study included the alkalinity, pH, ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-), temperature, Dissolved Oxygen (DO), and salinity (Table 1). The increased pH in each treatment was due to the addition of $CaCO_3$. The ammonia, nitrite, and nitrate contents were within the optimum condition for lobster maintenance. The increased nitrite content was believed to be due to the nitrification process by *Nitrosomonas* bacteria which oxidize NH_3 into NO_2^-, whereas the *Nitrobacter* bacteria oxidize NO_2^- into NO_3^-. The temperature, DO, and salinity were still supportive of maintenance.

Table 1: Water quality during the course of the maintenance period

Water quality parameter	Treatment (mg L^{-1} as $CaCO_3$)			Optimum value
	Control	160	240	
Alkalinity	68-89	160-176	240-258	
pH	7.40-7.51	7.92-8.02	8.09-8.30	7.8-8.2 (Mojjada *et al*. 2012) [14]
NH_3 (mg L^{-1})	0.02-0.05	0.03-0.04	0.03-0.04	<0.1 (Mojjada *et al*. 2012) [14]
NO_2 (mg L^{-1})	0.26-0.79	0.64-1.24	0.66-1.39	<5 (Drengstig and Bergheim 2013) [15]
NO_3 (mg L^{-1})	0.14-2.35	0.14-2.47	0.14-2.30	<5 (Drengstig and Bergheim 2013) [15]
Temp (˚C)	27.1-27.4	27.1-27.4	26.9-27.3	22-31 (Jones 2009) [16]
DO (mg L^{-1})	5.44-6.20	5.46-6.08	5.60-6.23	>3.5 (Mojjada *et al*. 2012) [14]
Salinity (g L^{-1})	28.5-31.5	28.5-31.5	27.5-30.5	25-35 (Vidya and Joseph 2012) [17]

3.2 Physiological response

The Total Hemocyte Count (THC) in the control alkalinity treatment was $6.29 \pm 0.45 \times 10^6$ cells mL^{-1}, the 160 mg L^{-1} as $CaCO_3$ alkalinity $7.86 \pm 1.25 \times 10^6$ cells mL^{-1}, and the 240 mg L^{-1} as $CaCO_3$ alkalinity $7.69 \pm 0.95 \times 10^6$ cells mL^{-1} (Figure 1). The THC plays an important role pertaining to the health of crustaceans such as the phagocytosis process, encapsulation, cytotoxicity, melanization, and communication among cells (Johansson *et al*. 2000) [18]. The THC increases when exposed to environmental changes (Smith and Johnston 1992) [19]. The results of the current study revealed an increase in the THC in all of the treatments on day 20. The greatest increase was in the 160 mg L^{-1} as $CaCO_3$ alkalinity treatment. This indicated that the increase in THC during the maintenance period was the lobsters' response to changes in the alkalinity and pH and also an indication of infection by pathogens. The increased THC on day 20 was believed to be due to the increased phagocytosis capability. According to Jiravanichpaisal *et al*. (2006) [20], an increase in THC also causes an increase in granular cells which activate the release of prophenoloxidase (proPO) which functions as a trigger during microbial activity by identifying the peptidoglycan of bacteria or β-1,3 glucan of fungi as a defensive measure against pathogens. At the end of the maintenance period, the THC decreased again in all of the treatments. This indicated that the lobsters were again in a normal condition.

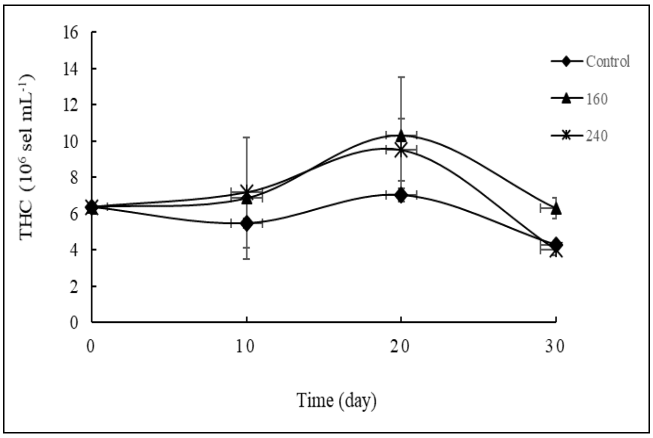

Fig 1: The lobsters' THC during the maintenance period

The glucose level in the hemolymph during the maintenance period in the alkalinity control treatment was 3.50±0.74 mg dL^{-1}, the 160 mg L^{-1} as $CaCO_3$ alkalinity 3.38±0.52 mg dL^{-1}, and the 240 mg L^{-1} as $CaCO_3$ alkalinity 4.25±1.30 mg dL^{-1} (Figure 2). The experiment revealed that the glucose level fluctuated with the greatest increase in the 240 mg L^{-1} as $CaCO_3$ alkalinity treatment at the end of the experiment. The increase in the glucose level was believed to be the lobsters' energy utilization as an immediate response to the environmental condition in the 240 mg L^{-1} as $CaCO_3$ alkalinity treatment. On the other hand, the glucose level in the 160 mg L^{-1} as $CaCO_3$ alkalinity treatment was relatively stable. This was probably because the lobsters felt more comfortable in this alkalinity. The increased glucose level or hyperglycemia could indicate that the lobsters were under stress (Telford 1968) [21]. The mechanism in play is glycogenesis, which is the breakdown of glycogen molecules in the liver and muscles followed by gluconeogenesis. Gluconeogenesis is the breakdown of non-carbohydrate molecules, protein, and lipid, into glucose which is triggered by cortisol hormones. Cortisol hormones regulate the release of insulin, causing a high glucose level. An insulin-like hormone in crustaceans is produced by the hepatopancreas and is also known as the insulin-like growth factor (IGF-I). The working mechanism of this hormone is influenced by changes in the environment such as the pH and salinity. This hormone regulates the supply of glucose in crustaceans during moments of stress and has a direct effect on the osmoregulatory process (Gutierrez *et al*. 2007) [22].

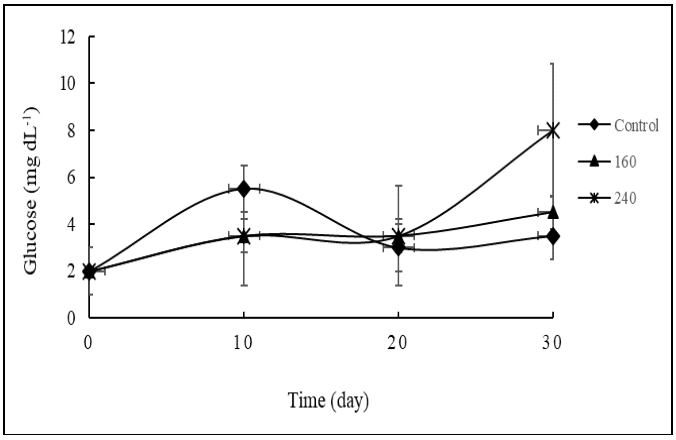

Fig 2: The hemolymph glucose during the maintenance period

3.3 Production performance

The results of the body weight, survival rate (SR), and specific growth rate (SGR) measurements are presented in Table 2. Weight gain in crustaceans occurs periodically post molting and is accompanied by an increase in length. The body weight increased in the 160 mg L^{-1} as $CaCO_3$ alkalinity treatment to 62.12±1.66 g. In the treatment with 160 mg L^{-1} as $CaCO_3$ alkalinity, it is suggested that the lobsters felt more comfortable with the environmental conditions, indicated by the relatively stable glucose level compared to the other treatments. The specific growth rate (SGR) increased in proportion to the body weight; however, this was not statistically significant in each of the treatments. In the present study, the lobsters were kept in an optimum density. The results of a study by Subhan *et al*. (2018) [4] revealed that *P. homarus* lobsters kept at a density of 10-26 individuals/m^3 had an SGR of 0.22-0.38%/day. The survival rate (SR) during the study was not significantly different. The SR in the control alkalinity treatment was lower due to mortality and cannibalism post molting.

Table 2: The production performance during the maintenance period

Production performance	Treatment (mg L^{-1} as $CaCO_3$)		
	Control	160	240
Initial weight (g)	51.22±1.87	51.22±1.87	51.22±1.87
Final weight (g)	61.10±1.95^a	62.12±1.66^a	58.24±3.71^a
Spesific Growth Rate (%/day)	0.33±0.06^a	0.36±0.06^a	0.23±0.12^a
Survival Rate (%)	86.67±0.00^a	96.67±6.67^a	93.33±3.33^a

4. Conclusion

Alkalinity influenced changes in pH but did not influence the other water quality parameters. In the 160 mg L^{-1} as $CaCO_3$ alkalinity treatment, the physiological response of the lobsters was better with a survival rate of 96.67±6.67 and final body weight of 62.12±1.66; however, these findings were not significantly different. There needs to be further study to discover the optimum alkalinity for the cultivation of scalloped spiny lobsters with different alkalinity levels and a longer maintenance period.

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