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Growth performance and survival rate of spiny lobster *Panulirus homarus* (Linnaeus, 1758) with formulated feeding enriched by spinach extract

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Abstract. *Lubis AS, Efrizal, Syaifullah, Rusnam, Nurmiati, Puari AT. 2023. Growth performance and survival rate of spiny lobster* Panulirus homarus (*Linnaeus, 1758*) *with formulated feeding enriched by spinach extract*. *Biodiversitas 24: 6010-6016.* In the context of spiny lobster *Panulirus homarus* (Linnaeus, 1758) aquaculture and marine conservation, assessing factors that influence growth and survival is critical. Spinach extract can be added as a feed additive to enhance the growth and survival rate of lobsters. This study aimed to analyze the effect of formulated feed enriched with spinach extract on spiny lobsters' growth and survival performance. A completely randomized design with five treatments and five replications: formulated feed with spinach extract 0 mg (Feed A1), 0.4 mg (Feed A2), 0.5 mg (Feed A3), 0.6 mg (Feed A4), and 0.7 mg (Feed A5) were used. The result showed that the formulated feed enriched with spinach extract had a significant effect on absolute weight, specific growth rate, length, and width of the carapace in growth performance ($P<0.05$) but had no significant on the survival rate and the molting frequency ($P>0.05$). The best result was treatment A3, adding 0.5 g of spinach extract to lobster feed with absolute weight 39.67±6.20 g, length of carapace 0.46±0.06 cm, width of carapace 0.28±0.05 cm, specific growth rate 0.38±0.06% day^{-1}, molting frequency 1.00±0.00 times $lobster^{-1}$, and survival rate 100±0.00%. The Spinach extract of 0.5 mg was the best for the survival and growth of the lobster (Duncan's test). The chemical of the feed showed that the tested feed met the nutritional needs of lobsters with a protein content of 39.74-42.60%. However, the number of amino acids in the best test feeds was still low quantity compared to several other studies, so the growth of lobsters was still slow. Based on research results, enriched 0.5 g of spinach extract to formulated feed is the best result for increasing the growth and survival rate of lobsters.

Keywords: Growth performance, lobster, steroid hormone, survival rate

INTRODUCTION

Based on the Marine and Fisheries Ministry's statistical data (2021), lobster catches have fluctuated from 2016 to 2020. Increasing fishing activities will affect the balance of populations and the availability of lobster stocks in nature, which will result in species extinction. Lobster enlargement activities are carried out to reduce continuous fishing. Efforts to cultivate lobsters are being made to meet the demand for seawater lobsters, which continues to increase from year to year. The main problem that occurs in lobster cultivation is its slow growth (Liliyanti et al. 2016). Larasati et al. (2018) stated that to prevent population decline due to high fishing intensity, information is needed about lobster resources that support its preservation and development in terms of population parameters and spawning potential. One aspect of population parameters is the growth rate and survival of the species. The values of growth rate and asymptotic carapace length are used to determine the maximum age that can be achieved by a lobster population, while the survival rate determines the lobster's adaptation to its environment (Damora et al. 2018).

Aquaculture is one way to overcome the threat of declining populations that are overexploited (Priyambodo et al. 2020). There are several criteria that are required, both technically and biologically, to be able to develop a potential species through cultivation. The success and sustainability of the marine aquaculture business, apart from depending on environmental conditions, must also be supported by the availability of sustainable seeds (Erlania et al. 2014). Feed is the main component, so complete nutrition in feed is very necessary for the survival and growth of a species, while the type and size of feed differ based on the age of the lobster (Zakaria et al. 2022; Efrizal et al. 2023). Feeding in lobster cultivation is one of the factors that can affect and if it is not in accordance with the quantity and quality, it will affect the growth and survival of lobsters (Priyambodo et al. 2020; Tirtadanu et al. 2022). Communities that cultivate lobsters generally use trash fish and fresh squid as their main feed. But it is not continuously available, is nutritionally incomplete, and is expensive. In addition, lobsters are arthropods that shed their skin, so they need molting hormones to help the lobsters molt (Efrizal et al. 2019).

Trash fish and fresh squid do not have this hormone, so the feed is modified by making formulated feed that is adjusted to the nutritional needs, and eating habits of lobsters and contains molting hormones. Several researchers have tried to make feed for lobsters, but it still needs to be developed to increase the growth of these lobsters. The nutritional needs of lobster feed in general are protein, carbohydrates, fats, vitamins, and minerals (Poore et al. 2017; Ridwanudin et al. 2018; Syafrizal et al. 2018). The objective of this research was to analyze the growth and survival rates of lobsters by utilizing formulated feed enriched with spinach extract. According to Aslamyah and Fujaya (2011) and Efrizal et al. (2019), spinach contains saponins, which are used to synthesize steroid hormones. Spinach extract is a molting stimulant containing ecdysteroid, which is the main steroid hormone in arthropods that regulates physiological functions. The use of spinach extract in optimal concentrations can improve several crustaceans with various applications. Indeed, understanding the correct concentration is essential to enhance both the growth and survival of lobsters.

MATERIALS AND METHODS

Research location and materials

This research was conducted at the Integrated Laboratory of Bung Hatta University, Padang, West Sumatra, from July to December 2021. Spiny lobsters were obtained from Padang, West Sumatra, with a weight of ±92.55 g, length of ±10.27 cm, and a density of 3 lobsters per box. The lobsters were adapted by giving trash fish and the combination of the test feed with the composition of the test feed used was a mixture of 37% of fish meal, 8% of *Batissa* sp. meat, 2% of squid, 11% of wheat, 5% shrimp heads, 0.5% beef liver, 10% of soybeans, 1% fish oil, 1% of vitamins and a minerals mix, and 0.5% of vitamin C. The feed is sprayed with spinach extract in different doses. The preparation of spinach extract refers to the research of Efrizal et al. (2019).

Spiny lobster rearing

Lobsters were reared for 90 days and weight and length were measured every 14 days. This study used a completely randomized design with five treatments and five replications (formulated feed with spinach extract 0 mg (feed A1); 0.4 mg kg feed^{-1}(feed A2); 0.5 mg kg feed^{-1} (feed A3); 0.6 mg kg feed^{-1} (feed A4), and 0.7 mg kg feed^{-1} (feed A5)). Frequency of feeding twice a day with 3% of the biomass. Lobsters used for research are shown in Figure 1.

Observed parameters

The parameters that have been assessed in this research primarily encompass lobster growth and survival rate data, with water quality data serving as supplementary information. The observed parameters refer to the research conducted by Syafrizal et al. (2018).

(i) Survival rate is the percentage of lobsters that are alive at the end of the study. The survival rate of lobsters was measured by SR = (Nt / No) x 100%.
(ii) The absolute weight growth of lobsters is obtained from the difference between the final and initial weights. The absolute weight growth was measured by AWG = WGt-WGo.
(iii) The carapace length of lobsters is determined by the difference between the final and initial carapace lengths. Absolute carapace length was measured by ACL = CLt-Clo.
(iv) The carapace width of lobsters is derived from the difference between the final and initial carapace widths. Absolute carapace width was measured by ACW = CWt-CWo.
(v) The specific growth rate is expressed as a percentage of the difference between the final and initial weights divided by the duration of the rearing period. The specific growth rate was measured by SGR = (ln WGt-WGo/t) x 100%.
(vi) Molting frequency is the count of occurrences of lobsters undergoing molting during the rearing period. Molting frequency was calculated by MFq = Xmolt / Ntot.

The feed tested was fed with the best results or the addition of 0.5 mg of spinach extract to find out whether the feed used could meet the lobster amino acid requirements. The feed sample to be tested was 100 g and analyzed using the Ultra Performance Liquid Chromatography (UPLC) method and was carried out in two replications. Water quality controlled during the study was salinity (ppt), temperature (°C), pH, dissolved oxygen (ppm), ammonia (ppm), nitrite (ppm), and alkalinity (ppm).

Data analysis

The average survival rate, absolute weight, absolute carapace length, absolute carapace width, specific growth rate, and molting frequency were analyzed using parametric statistics one-way ANOVA with a significance level of 0.05 and Duncan's Multiple Range tests were carried out to analyze the differences between treatments. Water quality, chemical feed, and feed amino acids were measured by displaying tables and analyzed descriptively.

Figure 1. Lobster *Panulirus homarus*

RESULTS AND DISCUSSION

Absolute weight growth and specific growth rate

The addition of spinach extract to artificial feed significantly influenced the growth of both the absolute and specific weight of lobsters ($P<0.05$). The highest absolute weight in Table 1 was Feed A3 and followed by Feed A2, Feed A1, Feed A4, and Feed A5. The highest specific growth rate was in Feed A3, and followed by Feed A2, Feed A1, Feed A4, and Feed A5 (Table 1). The relationship between artificial feed enriched with spinach extract and absolute weight is presented in Figure 2.

The results of Duncan test analysis (Table 1) showed that the Absolute weight of Lobster in feed A4 is not significantly different from that of feed A5, while the other treatments are significantly different. Table 1 further indicates that the specific growth rate of lobsters in feed A2 is not significantly different from that in feed A1 and A3, while the specific growth rate in feed A4 is not significantly different from that in feed A5.

Based on Figure 2, the growth trend showed an upward curve at 0 to 0.5 concentration of spinach extract in the lobster feed formulation. Meanwhile, 0.6 to 0.7 mg concentration in the lobster feed formulation showed a decreasing curve. Figure 2 shows that the value 0.5 mg is the optimal concentration of spinach extract for lobster weight growth. The relationship between formulated feed enriched with different doses of spinach extract and the absolute weight of the lobster reaches 60.03% with R^2 = 0.6003.

Length and width of carapace

The average data for the length and width of the carapace are seen in Table 1. The results of the one-way ANOVA test on the absolute carapace length and width parameters showed a significant effect ($P<0.05$). The highest growth of carapace length and width was in Feed A3 followed by Feed A2, Feed A1, Feed A4 and Feed A5. The relationship between the research treatment with the absolute length and width of the carapace is presented in Figure 3 and Figure 4.

According to Figure 3, the growth of the carapace width trend displayed an upward curve when the concentration of spinach extract in the lobster feed formulation ranged from 0 to 0.5. Conversely, a declining trend was observed with a concentration of 0.6 to 0.7 mg in the lobster feed formulation. The figure demonstrates that the optimum concentration for lobster weight growth is 0.5 mg of spinach extract. The correlation between carapace width and formulated feed enriched with spinach extract reaches 70,99% with an R-squared value of 0.7099.

Table 1. The average value of spiny lobsters in the test parameters

Parameters	Treatments				
	Feed A1	**Feed A2**	**Feed A3**	**Feed A4**	**Feed A5**
Survival rate (%)	100±0.00[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]
Absolute weight (g)	25.30±3.13[c]	30.64±2.52[b]	39.67±6.20[a]	14.21±2.07[d]	12.76±0.63[d]
Length of carapace (cm)	0.24±0.04[b]	0.31±0.10[b]	0.46±0.06[a]	0.15±0.02[c]	0.08±0.008[c]
Width of carapace (cm)	0.19±0.02[c]	0.23±0.03[b]	0.28±0.05[a]	0.10±0.02[d]	0.06±0.01[e]
Specific growth rate (% day^{-1})	0.28±0.06[a]	0.32±0.02[ab]	0.38±0.06[b]	0.17±0.03[c]	0.14±0.005[c]
Molting frequency (times lobster^{-1})	0.67±0.00[b]	0.67±0.00[b]	1.00±0.00[a]	1.00±0.00[a]	0.47±0.19[c]

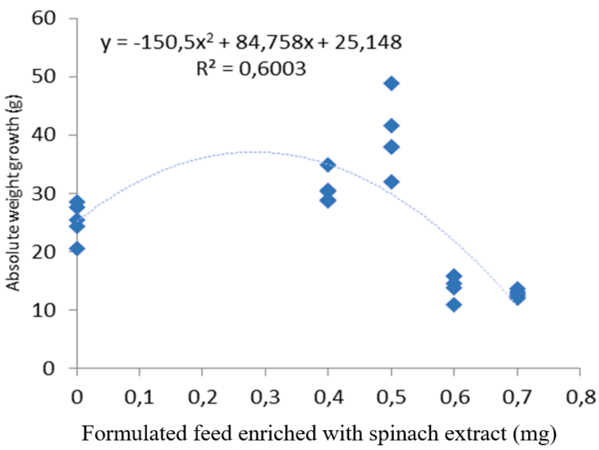

Figure 2. The relationship between formulated feed enriched with different doses of spinach extract and absolute weight spiny lobster

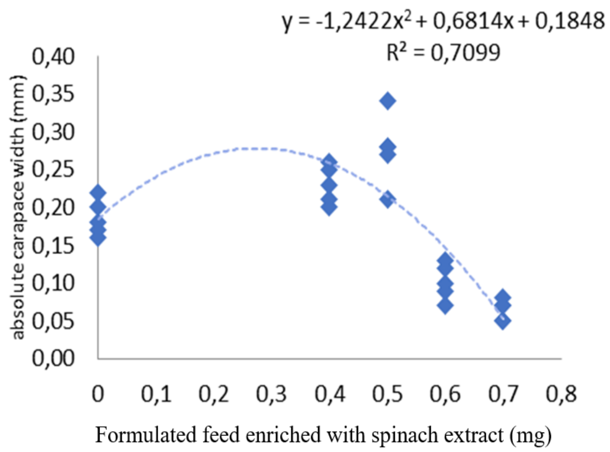

Figure 3. The relationship between formulated feed enriched with different doses of spinach extract and absolute carapace width spiny lobster

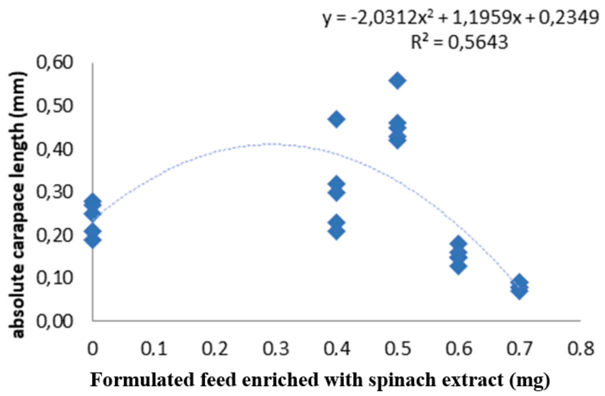

Figure 4. The relationship between formulated feed enriched with different doses of spinach extract and absolute carapace lenght spiny lobster

Figure 4 shows that the trend in carapace length growth exhibited an upward curve when the concentration of spinach extract in the lobster feed formulation ranged from 0 to 0.5. Conversely, a declining trend was observed with a concentration of 0.6 to 0.7 mg in the formulated feed of lobster. The figure illustrates that the optimal concentration for carapace length growth in lobsters is 0.5 mg of spinach extract. The correlation between carapace length and formulated feed enriched with spinach extract reaches 56.43% with an R-squared value of 0.5643.

Survival rate and frequency molting

The survival rate of lobsters was observed by counting the number of lobsters at the beginning and end of the rearing. The frequency of molting is observed every day, the molting process usually occurs from evening to morning. Based on maintenance for 90 days, the lobster survival rate was 100% for all treatments (Table 1). The highest molting frequency in Table 1 was found in treatments C and D, followed by treatments A and B, while the lowest was in treatment E (Table 1). Based on Table 1, the results of one-way ANOVA analysis showed that the treatment in this study had no significant effect on lobster survival ($P>0.05$) but had a significant effect on lobster molting frequency ($P<0.05$).

Amino acid

Amino acid analysis in this study was fed A3 formulated feed with spinach extract 0.5 mg. Based on the results of the amino acid analysis in Table 2, the values of essential amino acids were considered not to meet the needs of lobster amino acids because the protein in the feed is not balanced with essential amino acids.

The nutritional requirements of lobsters, including protein, carbohydrates, fat, crude fiber, water content, and ash content, are consistent with those of other aquatic organisms. The values presented in Table 2 for feed chemical composition encompass the nutritional range necessary to meet the dietary needs of lobsters. Table 2 also presents the amino acid content of the feed that produced the best growth results. This particular feed contains a total of 17 amino acids. However, the amount of each individual amino acid is insufficient to fulfill the nutritional needs of lobsters.

Water quality

In this study, there were several water quality parameters measured, namely temperature, pH, DO, ammonia, nitrite, alkalinity, and salinity. Water quality data during lobster rearing is shown in Table 3. Based on the data obtained, only the ammonia parameter has exceeded the lobster's tolerance limit, the other parameters are still within the tolerance limit based on relevant references.

Table 3 presented various water quality parameters in the lobster-rearing environment throughout the research. These parameters remain within the acceptable range for lobster survival, and they do not disrupt the research procedures. This is achieved through consistent control of the container and maintenance media.

Table 2. Chemical and amino acid content of formulated feed enriched with different doses of spinach spiny lobster

Parameters	Value (%)
Chemical	
Water	11.94-14.20
Ash	14.15-14.85
Crude protein	39.74-42.60
Fat	3.18-4.63
Crude fiber	2.06-3.73
Carbohydrate	24.77-29.29
Amino acids	
L-Serine	0.54
L-Glutamic acid	2.22
L-Phenylalanine	0.48
L-Isoleucine	0.30
L-Valine	0.39
L-Alanine	0.44
L-Arginine	0.47
Glycine	0.47
L-Lysine	0.33
L-Aspartic acid	0.77
L-Leucine	0.64
L-Tyrosine	0.23
L-Proline	0.71
L-Threonine	0.41
L-Histidine	0.21
L-Cystine	0.26
L-Methionine	0.01

Table 3. Water quality of rearing spiny lobster

Parameters	Value
Temperature (°C)	26-30
pH	7-8
DO (ppm)	3.26-6.06
Ammonia (ppm)	0.094-0.195
Nitrite (ppm)	0-0,088
Alkalinity (ppm)	254.68-275.56
Salinity (ppt)	33-35

Discussion

Formulated feed enriched with spinach extract showed a good response to lobster growth performance. The growth of lobster weight is followed by the growth of carapace length and width. Good metabolic processes in the body and energy to be recovered for the growth process (Menu-Courey et al. 2019; Wang et al. 2021). Most of the energy needs of lobsters have been met by non-protein nutrients. Therefore, the protein in the feed can be used for growth. Feed A3 showed the best results compared to other feeds. Spinach extract used in feed, apart from being a molting hormone, also contains essential and non-essential amino acids (Rono et al. 2018; Yousif et al. 2019).

The findings from this investigation indicate that incorporating spinach extract into the feed has the potential to enhance both the growth and survival rates of lobsters. These results gain additional support from various prior research endeavors that have employed spinach extract with several crustacean species. Ridwan et al. (2021) observed that the application of red spinach ethanol extract at a concentration of 40 ppm had a notable effect on increasing body length and reducing molting duration in vaname shrimp (*Litopenaeus vannamei*). Hasnidar et al. (2021) administered spinach extract at a dose of 42 µg mL-1 and noted improvements in molting duration, molting frequency, weight gain, and the survival rate of orange mud crabs (*Scylla olivacea*). Other research was conducted by Suyono et al. (2021) 100% feed given spinach extract at a dose of 5% of the biomass weight using the popeye method for molting acceleration on mud crab (*Scylla Serrata*).

The quality of protein in the feed is determined by the chemical value and the number of amino acids. Protein functions as a building material for new tissues to regulate the body's metabolic processes and as fuel when energy needs are not met by fats and carbohydrates. Protein is a macro-nutrient found in biological systems that has the form of structural elements, enzymes, hormones, antibodies, receptors, signaling molecules, and certain biological functions. Protein is needed by the body and provides essential amino acids for the development and maintenance of muscles (Saputra and Fotedar 2022).

The test feed has the best chemical and amino acid content, as shown in Table 2. Based on this, the chemical and amino acid content in the feed does not meet the needs of the lobster body. Amino acids are biomolecules that function as building blocks of proteins and pathways of substance metabolism. They serve as precursors for the synthesis of various biological substances. Amino acids are obtained from protein in feed, and the quality of feed protein is seen from essential and non-essential amino acids. High-quality protein feeds are easily digested and contain essential amino acids in amounts that are needed by the fish (Mohanty et al. 2014; Nunes et al. 2014).

The feed amino acid analysis results in this study were lower than feed amino acids in Boghen and Castell (1981) and Kropielnicka-Kruk et al. (2022). Amino acids occupy a central position in cell metabolism because almost all biochemical reactions catalyzed by enzymes consist of amino acid residues. Amino acids are essential for the metabolism of carbohydrates and fats to make protein tissues and important compounds and nicotine acids. In addition, amino acids also serve as a source of metabolic energy (Nunes et al. 2014). This amino acid value was considered not to meet the needs of lobster amino acids because the protein in the feed is not balanced with essential amino acids. Essential amino acids cannot be synthesized in the lobster's body, or the amount that can be synthesized is insufficient to meet the physiological needs of the crustaceans to grow and develop. The feed supports its existence; if the meal is inadequate, growth is low, and the molting cycle is more extended (Abo-Taleb 2019; Wang et al. 2021).

Feed formulations that are poor in amino acids are thought to be the disproportionate use of feed protein, resulting in a lack of natural essential amino acids and heat treatment, which destroys protein during the feed manufacturing process. Amino acids present in the feed are also more soluble in water at the time of administration if the soaking time is longer. Crustaceans take a long time to chew their food, so the nutrients in the feed dissolve too much in the water (Efrizal et al. 2023). Multi-amino acids in the maintenance of crustaceans can maintain the immune system and can eliminate toxic substances. Valine can help in sending other amino acids, lysine helps in calcium absorption, muscle protein production, hormone production, antibody production, and enzymes (Mohanty et al. 2014; Nunes et al. 2014). Lysine is one of the essential amino acids. Lysine has a role in forming carnitine, which functions as a growth promoter, protects against ammonia poisoning, and increases the body's defense against extreme temperature changes. The addition of lysine in feed can increase protein synthesis in the fish body so that the protein content in fish meat will increase and affect the growth process of fish and their survival as well. Lysine also functions as a basic material for blood antibodies, strengthening the circulatory system and maintaining the growth of normal cells (Francis et al. 2014; Gorelick et al. 2020).

Figures 1, 2, and 3 show that formulated feed enriched with spinach extract will experience a decrease in growth with a dose of more than 0.5 mg. Spinach contains anti-nutrients, so excessive administration can affect the immune system. The tannin in spinach is known to inhibit the bioavailability of proteins and minerals (Lo et al. 2018). Crustaceans that will increase their growth are marked by molting. Spinach extract is a molting stimulant containing ecdysteroids, which are the main steroid hormone in arthropods, which function for molting and regulating physiological functions (Bakrim et al. 2008). Ecdysteroids will stimulate carbohydrate metabolism and lipid biosynthesis as immunostimulants and antioxidants. The molting process occurs because the Y organ secretes ecdysteroid hormones in the form of ecdysone. The hemolymph then converts ecdysone into 20-hydroxyecdysone (20E), the active hormone in the form of molting hormone. After ecdysis (molting), the titer will decrease during the intermolt phase. Lobsters that have finished molting need shelter because of their weak physical condition (Kuballa et al. 2011; Shyamal et al. 2018).

Ecdysteroid hormones that have met the lobster's need for molting will be supported by increased weight and carapace length and width. Ecdysone titer secreted before the premolt phase will cause premature molting, so it has a negative effect on the molting response. The molting process occurs when the lobster's body is not accommodated in the carapace, so it stimulates the lobster to change the carapace (Sorach et al. 2013; Tavares et al. 2021). The survival rate is one of the important parameters in aquaculture activities. Biotic and abiotic factors greatly affect the survival of a species (Zakaria and Annisa 2020). Biotic factors consist of age and adaptability, abiotic factors include feed and environmental quality. The age and adaptability of lobsters were considered very good, with 100% survival in all treatments (Kilada et al. 2012; Reindl et al. 2018).

The temperature in the study ranged from 26-30°C and the optimal temperature for lobster is 23-32°C, temperature affects the metabolic activity of lobsters during rearing (Kulmiye and Mavuti 2005; Ooi et al. 2019). The pH during lobster rearing is between 7 and 8. pH value is considered to still be able to support the survival rate and growth of lobsters (Sorach et al. 2013; Ridwanudin et al. 2018). The dissolved oxygen (DO) of the waters during the study ranged from 3.26 to 6.06 ppm, which is a suitable dissolved oxygen for lobster aquaculture. Hidayat et al. (2016) explained that during the maintenance period, lobsters need clear water, rich in Dissolved Oxygen (Dissolved Oxygen) and free from toxins such as sulfide acid (H2S) and ammonia (NH3). Ammonia (NH3) water levels during maintenance ranged from 0.094-0.195 ppm. Ammonia levels have passed the requirements for lobster. The ideal ammonia value for lobster cultivation is no more than 0.1 mg/L. Water ammonia levels during rearing increase due to the accumulation of feed residues and the rest of the lobster's metabolism (Simon 2009; Powell et al. 2017).

Nitrite conditions during the study ranged from 0 to 0.088 ppm. Overall, the nitrite concentration during the study was still suitable for lobster life. Nitrite during the study increased due to the accumulation of leftover feed and lobster feces, while bacteria that convert nitrite to nitrate were not well-formed, causing the concentration of nitrite to increase (Francis et al. 2014). Alkalinity conditions ranged from 254.68-275.56 ppm. High alkalinity correlates with a high hardness, which indicates the mineral content in the water, such as calcium, magnesium, and phosphorus, which lobsters need to support molting activity (Middlemiss et al. 2016). Salinity ranges from 33-35 ppt and is suitable for sand lobster cultivation. Lobsters have a salinity tolerance of 25-45 ppt. Seawater input is added from the reservoir, which is flowed through a pipe using a water pump (Vidya and Joseph 2012; Ross and Behringer 2019).

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