



# Enhancing the growth and survival rate of broodstock female *Portunus pelagicus* with formulated feed enriched with amaranth (*Amaranthus hybridus*) extract and vitamin E

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**Abstract.** Phytoecdysteroids have significant potential in *Portunus pelagicus* aquaculture, particularly in protecting polyunsaturated fatty acids in membrane phospholipids and plasma lipoproteins, enhancing the quality of processed foods supplemented with spinach extract and vitamin E. The formulated feed in this study is a substitute medium that uses food additives and amaranth extracts, which are known to include phytoecdysteroids, a minimal form of hormone that controls a crustacean's molt. The objective was to assess the nutritional value of formulated feed supplemented with vitamin E and amaranth extract for broodstock female *P. pelagicus* based on biological assays. Four different treatments of amaranth extract and vitamin E (0 ng g<sup>-1</sup> crab + 0 IU kg<sup>-1</sup>; 250 ng g<sup>-1</sup> crab + 100 IU kg<sup>-1</sup>; 500 ng g<sup>-1</sup> crab + 200 IU kg<sup>-1</sup>; 750 ng g<sup>-1</sup> crab + 300 IU kg<sup>-1</sup>) were used in this study. A total of 4 concrete tanks (200×100×100 cm<sup>3</sup>) were randomly filled with female crab samples that were gathered from the coastal region of Padang, West Sumatera, and cultured individually in crab boxes measuring 45.5×32.5×16.5 cm. The result showed that formulated feed enrichment with amaranth extract and vitamin E had a significant effect ( $P < 0.05$ ) on the absolute weight gain (AWG), carapace length (ACL), and carapace width (ACW). Enrichment with amaranth extract and vitamin E of the formulated feed led to an increase in AWG (from 13.14 ± 1.54 to 58.89 ± 4.33 g), ACL (from 1.29 ± 0.09 to 8.04 ± 0.12 mm) and ACW (from 1.78 ± 0.12 to 15.98 ± 0.73 mm) and yields a 100% survival rate over the 40 days maintenance period. The feed enriched with spinach extract and vitamin E with a concentration of 500 ng g<sup>-1</sup> crab + 200 IU kg<sup>-1</sup> and 750 ng g<sup>-1</sup> crab + 300 IU kg<sup>-1</sup> proved to be the optimal result for *P. pelagicus* biology testing.

**Key Words:** blue swimming crab, broodstock female, Vitamin E, amaranth extracts.

**Introduction.** The Blue Swimming Crab (BSC), *Portunus pelagicus*, known for its high commercial value, is in great demand, particularly from the United States, Japan, and Singapore. This robust demand has translated into millions of dollars in annual revenue for Indonesia, as documented in various studies (Xiao & Kumar 2004; Efrizal et al 2015; Romano et al 2016; Efrizal et al 2018; Efrizal et al 2019; Efrizal et al 2020). According to the World Bank (2012), Indonesia stands as the leading exporter of central BSC, accounting for 31% of the market, followed by China at 24.7%, Thailand at 13%, Vietnam at 11%, and the Philippines at 7.3%. Regrettably, the burgeoning global demand and environmental pressures on marine resources have led to an annual decline in Indonesia's crab stocks by 20-30%, as observed in a study by Madduppa et al (2021).

Hence, it is imperative to prioritize the sustainability of the natural blue swimming crab population through measures such as stock enhancement, effective management, and the promotion of intensive cultivation. Cultivating *P. pelagicus* offers a dual benefit: it can provide hatchery-raised seeds to bolster wild populations and enhance the economic stability of farmers engaged in commercial crab culture. The molting process in crustaceans is regulated by molting hormones and spinach extract, which contains phytoecdysteroids having the potential to stimulate the molting cycle, leading intermolt

crabs into a premolt stage, as Skinner (1985) has elucidated. According to Lafont & Dinan (2003), phytoecdysteroids are also known to have a number of other characteristics, such as the stimulation of protein synthesis in humans and animals as well as adaptogenic, antimutagenic, hypocholesterolemic, immunostimulating, nutritional, and tonic qualities.

Phytoecdysteroids have been shown in numerous studies to have potential importance in aquaculture (Hutacharoen et al 1989; Putschakarn 1991). However, even though the effects of exogenous ecdysteroids have been well studied in a variety of species, *P. pelagicus* has not yet been subjected to such research. Vitamins are essential organic compounds required in the feed for the normal growth and well-being of crabs (Lubis et al 2023a; Lubis et al 2023b). Since crabs often cannot synthesize these vitamins themselves, they must be provided through their feed. Specifically,  $\alpha$ -tocopherol functions as a non-specific, lipid-soluble antioxidant that breaks chains and prevents the spread of free radical reactions. Studies by Wang & Quinn (1999), Traber & Atkinson (2007), and Niki (2014) have demonstrated the critical function this vitamin plays in protecting polyunsaturated fatty acids (PUFAs) inside membrane phospholipids and plasma lipoproteins by acting as a scavenger for peroxy radicals. In this study, biological tests on female broodstock of *P. pelagicus* (Linnaeus, 1758) were used to evaluate the quality of prepared meal supplemented with amaranth extract and vitamin E.

## Material and Method

**Time and site.** The experiments were carried out at two hatcheries, namely the Teluk Buo Hatchery and the Bungus Hatchery, which are under the Department of Marine and Fisheries of Padang City. This research was conducted from August to October 2023. The analysis of samples was conducted utilizing the animal physiology laboratory facilities located within the Department of Biology at Andalas University.

**Experimental design and formulated feed.** Following Steel & Torrie's (1990) methodology, this study used a completely randomized design (CRD) with five replications of food formulations and four unique treatments. The treatments employed involved varying levels of amaranth extract and vitamin E. Specifically, they were as follows:

- FdietPEE 1: Formulated diet without amaranth extract and vitamin E ( $0 \text{ ng g}^{-1}$  crab +  $0 \text{ IU kg}^{-1}$ ).
- FdietPEE 2: Formulated diet enriched with amaranth extract ( $250 \text{ ng g}^{-1}$  crab) and vitamin E ( $100 \text{ IU kg}^{-1}$ ).
- FdietPEE 3: Formulated diet enriched with amaranth extract ( $500 \text{ ng g}^{-1}$  crab) and vitamin E ( $200 \text{ IU kg}^{-1}$ ).
- FdietPEE 4: Formulated diet enriched with amaranth extract ( $750 \text{ ng g}^{-1}$  crab) and vitamin E ( $300 \text{ IU kg}^{-1}$ ).

The diet used in this study is based on a modified formulation (previously described by Efrizal 2017a; Efrizal 2017b; Efrizal et al 2018; Efrizal et al 2019; Efrizal et al 2020) created for the mud crab (*Scylla serrata*) broodstock. The amaranth extract was made by dissolving it in 80% ethanol at a ratio of 1:1 and then homogenizing it. After that, the resultant solution was uniformly sprayed onto the test feed and added at a rate of 20 mL per kilogram of feed. The feed was then dried and kept until the experiments took place.

**Source of experimental crabs and broodstock rearing.** The broodstock female crabs used in the study were captured from the wild and were of the *P. pelagicus* species. They were collected using crab traps commonly employed by small-scale fishermen in the coastal region of Padang, West Sumatera. The crabs were randomly distributed into four concrete tanks, each measuring  $200 \times 100 \times 100 \text{ cm}^3$ . Within these tanks, the crabs were housed individually in plastic boxes, each box measuring  $45.5 \times 32.5 \times 16.5 \text{ cm}$ , with a maximum stocking density of one crab per box. The tanks were equipped with a substrate consisting of approximately 15 cm of sand and provided with sufficient aeration, following the methods detailed in Efrizal et al (2018; 2019; 2020). To ensure

appropriate conditions for the crabs, the water parameters were monitored and maintained within specific ranges. The crabs were kept in water with a depth of 27 to 28 cm, salinity levels between 30 to 32 ppt, a pH range of 7.77 to 7.96, a temperature of 26 to 28°C, and dissolved oxygen (DO) levels of 6.50 to 7.50 ppm. Each crab was provided with a shelter in the form of a PVC pipe, measuring 13 cm in diameter and 40 cm in length. This shelter served as a refuge for the crabs during molting. The daily diet for the crabs consisted of formulated feeds made from food waste and enriched with amaranth extracts. These feeds were provided at a rate of 3% of the crab biomass, typically between 17:00 and 18:00 hours. Any uneaten food was removed the following morning.

**Variables assessed and statistical analysis.** The data from the biological tests, which included parameters such as absolute weight gain (AWG), absolute carapace length (ACL), absolute carapace width (ACW), and survival rate, were subjected to statistical analysis. The analysis involved using one-way analysis of variance (ANOVA), as well as Duncan's test, to assess the differences among the treatments, following the methodology described by Steel & Torrie (1990). Standard deviations for each parameter and treatment were calculated and presented as Mean  $\pm$  SE (Standard Error). The significance level for treatments' effects was set at  $P < 0.05$ , indicating that differences between treatments were considered significant if the p-value was less than 0.05. An arcsine transformation was applied to the data in percentage form, in order to ensure that the assumptions of ANOVA, such as normal distribution and homogeneity of variances, were met before performing the statistical analysis.

**Results.** The results of the study demonstrate a statistically significant difference in the growth of female broodstock crabs fed with a formulated feed containing amaranth extract (PE) and vitamin E at different doses (Table 1 and Figure 1) ( $P < 0.05$ ). Table 1 and Figure 1 depict a noticeable increase in the absolute weight of the female crabs over the 40-day maintenance period.

Table 1  
The average weight (g) and absolute weight (g) of broodstock female crabs *Portunus pelagicus* fed a formulated feed with amaranth extract (PE) and vitamin E at different doses

Sampling (days)	Treatment (n=5)			
	FdietPEE 1	FdietPEE 2	FdietPEE 3	FdietPEE 4
0	156.61 $\pm$ 37.48	156.94 $\pm$ 21.80	157.19 $\pm$ 22.81	157.22 $\pm$ 23.54
10	157.86 $\pm$ 37.47	159.53 $\pm$ 21.87	158.70 $\pm$ 22.83	158.74 $\pm$ 23.59
20	160.20 $\pm$ 37.60	169.22 $\pm$ 26.12	162.13 $\pm$ 22.79	162.35 $\pm$ 23.10
30	164.89 $\pm$ 38.03	174.19 $\pm$ 26.22	174.67 $\pm$ 23.98	185.88 $\pm$ 21.64
40	169.75 $\pm$ 37.54	193.63 $\pm$ 28.87	212.69 $\pm$ 21.94	216.11 $\pm$ 26.44
AWG	13.14 $\pm$ 1.54 <sup>a</sup>	36.68 $\pm$ 8.76 <sup>b</sup>	55.50 $\pm$ 5.05 <sup>c</sup>	58.89 $\pm$ 4.33 <sup>c</sup>

Means within a given column with different superscripts are significantly different ( $P < 0.05$ ). Values are means  $\pm$  standard errors (SE). n = replication; AWG, absolute weight gain; FdietPEE 1: Formulated diet without amaranth extract and vitamin E (0 ng g<sup>-1</sup> crab + 0 IU kg<sup>-1</sup>); FdietPEE 2: Formulated diet enriched with amaranth extract (250 ng g<sup>-1</sup> crab) and vitamin E (100 IU kg<sup>-1</sup>); FdietPEE 3: Formulated diet enriched with amaranth extract (500 ng g<sup>-1</sup> crab) and vitamin E (200 IU kg<sup>-1</sup>); FdietPEE 4: Formulated diet enriched with amaranth extract (750 ng g<sup>-1</sup> crab) and vitamin E (300 IU kg<sup>-1</sup>).

In Table 1, the recorded weights show that the highest average absolute weight was achieved with the administration of FdietPEE 4 (58.89 g), followed by FdietPEE 3 (55.50 g), FdietPEE 2 (36.68 g), and FdietPEE 1 (13.24 g). The analysis of variance revealed significant differences among these treatments ( $P < 0.05$ ). Duncan's test confirmed these significant differences ( $P < 0.05$ ) and showed distinctions between FdietPEE 4 and FdietPEE 3, as well as between FdietPEE 2 and FdietPEE 1. However, there were no significant differences ( $P > 0.05$ ) observed between FdietPEE 4 and FdietPEE 1.

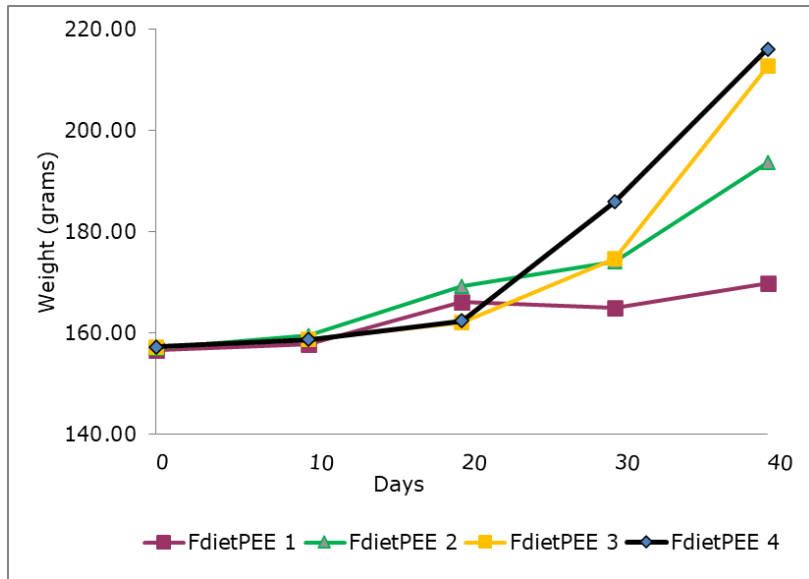


Figure 1. Growth weight (g) graph of individual broodstock female crabs *Portunus pelagicus* fed with a formulated feed with amaranth extract (PE) and vitamin E at different doses.

The relationship between the doses of amaranth extract and vitamin E in the formulated feed and the absolute weight gain is presented in Figure 2. The regression equation,  $AWG = -0.0003^2 + 0.224 + 14.485$ , is provided with an  $R^2$  value of 0.7639, indicating a statistically significant relationship ( $P < 0.05$ ; Figure 2). This equation describes the relationship between the doses of amaranth extract and vitamin E and the absolute weight gain of the female crabs.

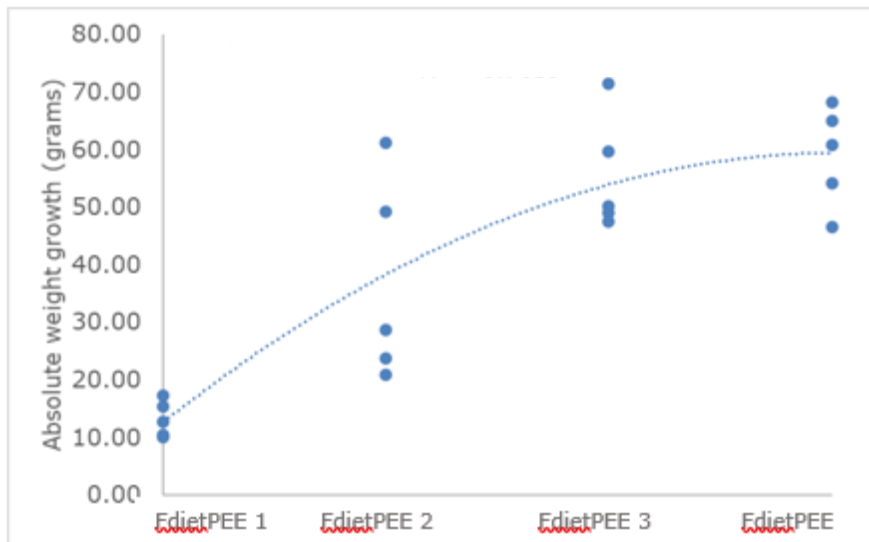


Figure 2. The relationship between the dose of FdietPEE and the absolute weight growth (g) of broodstock female crabs *Portunus pelagicus*.

The study reveals that the dosage of amaranth extract (PE) and vitamin E in the formulated feed had a substantial impact on the growth of the absolute carapace length during the 40-day maintenance period, with measurements ranging from 1.29 to 8.04 mm (Table 2 and Figure 3). The analysis of variance confirmed significant differences among the treatments ( $P < 0.05$ ).

Table 2

Average carapace length (mm) and absolute carapace length (mm) of broodstock female crabs *Portunus pelagicus* fed with a formulated feed with amaranth extract (PE) and vitamin E at different doses

Sampling (days)	Treatment (n=5)			
	FdietPEE 1	FdietPEE 2	FdietPEE 3	FdietPEE 4
0	58.93 ± 4.30	58.82 ± 3.87	58.75 ± 2.81	58.59 ± 2.80
10	58.93 ± 4.30	58.82 ± 3.87	58.75 ± 2.81	58.59 ± 2.80
20	59.35 ± 4.29	59.20 ± 3.88	59.38 ± 2.78	58.98 ± 2.79
30	59.76 ± 4.31	59.86 ± 3.85	61.16 ± 3.30	62.07 ± 2.69
40	60.22 ± 4.37	61.57 ± 4.73	66.20 ± 2.80	66.63 ± 2.70
ACL	1.29 ± 0.09 <sup>a</sup>	2.75 ± 1.18 <sup>a</sup>	7.45 ± 0.30 <sup>b</sup>	8.04 ± 0.12 <sup>b</sup>

Means within a given column with different superscripts are significantly different ( $P < 0.05$ ). Values are means ± standard errors (SE). n = replication; AWG, absolute carapace length; FdietPEE 1: Formulated diet without amaranth extract and vitamin E ( $0 \text{ ng g}^{-1} \text{ crab} + 0 \text{ IU kg}^{-1}$ ); FdietPEE 2: Formulated diet enriched with amaranth extract ( $250 \text{ ng g}^{-1} \text{ crab}$ ) and vitamin E ( $100 \text{ IU kg}^{-1}$ ); FdietPEE 3: Formulated diet enriched with amaranth extract ( $500 \text{ ng g}^{-1} \text{ crab}$ ) and vitamin E ( $200 \text{ IU kg}^{-1}$ ); FdietPEE 4: Formulated diet enriched with amaranth extract ( $750 \text{ ng g}^{-1} \text{ crab}$ ) and vitamin E ( $300 \text{ IU kg}^{-1}$ ).

As per the data in Table 2, the most significant increase in absolute carapace length was observed with the treatment FdietPEE 4 (8.04 mm), followed by FdietPEE 3 (7.45 mm), FdietPEE 2 (2.75 mm), and FdietPEE 1 (1.29 mm). Further examination using Duncan's Test verified these significant differences ( $P < 0.05$ ). Specifically, treatment FdietPEE 4 differed significantly from both FdietPEE 1 and FdietPEE 2, while treatment FdietPEE 3 also differed significantly from FdietPEE 1 and FdietPEE 2 ( $P < 0.05$ ). However, FdietPEE 4 did not exhibit a significant difference when compared to FdietPEE 3 ( $P > 0.05$ ).

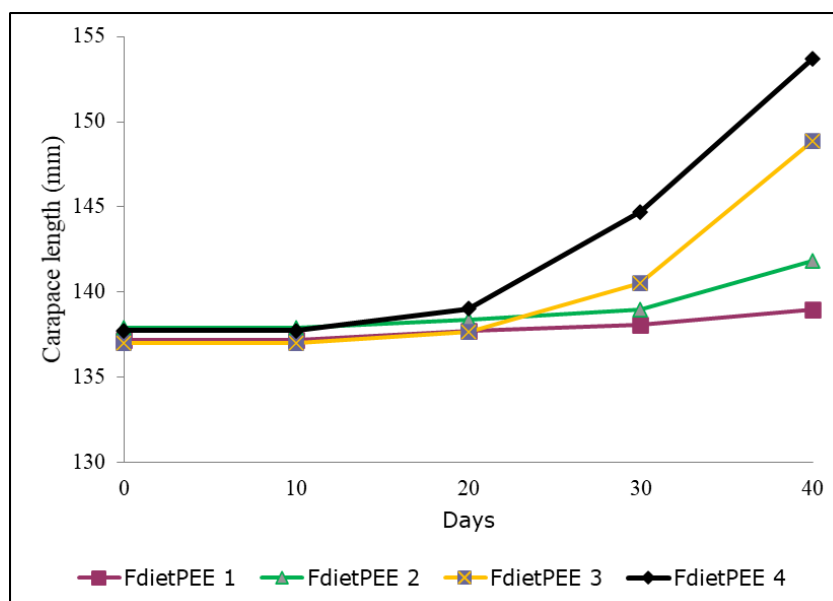


Figure 3. Growth of the carapace length (mm) of individual broodstock female crabs *Portunus pelagicus* fed a formulated feed with amaranth extract (PE) and vitamin E at different doses.

The relationship between the dose of amaranth extract in the formulated feed and the absolute carapace length (mm) of the female broodstock of the blue swimming crab *P. pelagicus* (Linnaeus, 1758) was found to be quadratic. The regression equation provided is  $ACL = -3 \times 10^{-5} DPEE^2 + 0.0287 DPEE + 0.8974$ , with an  $R^2$  value of 0.8517, signifying a statistically significant relationship ( $P < 0.05$ ; Figure 4). This equation describes how the doses of amaranth extract and vitamin E in the formulated feed correlate with the absolute carapace length of the crabs.

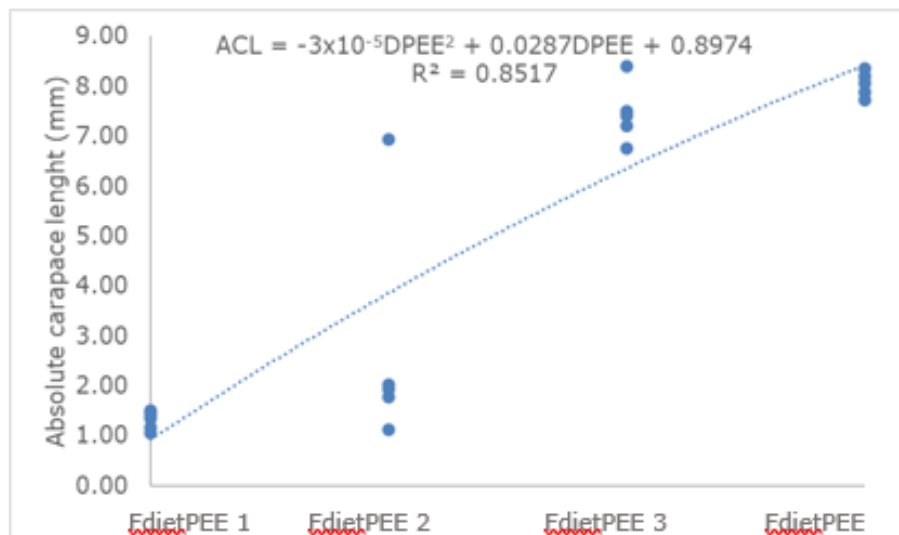


Figure 4. The relationship between the dose of FdietPEE and the absolute carapace length (mm) of broodstock female crabs *Portunus pelagicus*.

The measurement results (Table 3 and Figure 5) indicate that the formulated feed with a dosage of 750 ng g<sup>-1</sup> crab of amaranth extract and 300 IU kg<sup>-1</sup> of vitamin E led to a significantly higher mean carapace width (15.98 mm) compared to the formulated feed with 500 ng g<sup>-1</sup> crab of amaranth extract and 200 IU kg<sup>-1</sup> of vitamin E (11.81 mm), 250 ng g<sup>-1</sup> crab of amaranth extract and 100 IU kg<sup>-1</sup> of vitamin E (3.91 mm), and the control feed with 0 ng g<sup>-1</sup> crab of amaranth extract and 0 IU kg<sup>-1</sup> of vitamin E (1.78 mm). The analysis of variance confirmed these significant differences (P<0.05).

Table 3  
Average carapace width (mm) and absolute carapace width (mm) of broodstock female crabs *Portunus pelagicus* fed a formulated feed with amaranth extract (PE) and vitamin E at different doses

Sampling (days)	Treatment (n=5)			
	FdietPEE 1	FdietPEE 2	FdietPEE 3	FdietPEE 4
0	137.20 ± 16.51	137.92 ± 7.83	137.04 ± 6.16	137.75 ± 5.23
10	137.20 ± 16.51	137.92 ± 7.83	137.04 ± 6.16	137.75 ± 5.23
20	137.72 ± 16.61	138.38 ± 7.81	137.68 ± 6.04	139.02 ± 5.31
30	138.11 ± 16.68	138.97 ± 7.93	140.52 ± 6.90	144.73 ± 6.09
40	138.98 ± 16.56	141.84 ± 9.33	148.85 ± 8.69	153.72 ± 4.84
ACW	1.78 ± 0.12 <sup>a</sup>	3.91 ± 1.94 <sup>a</sup>	11.81 ± 2.86 <sup>b</sup>	15.98 ± 0.73 <sup>b</sup>

Means within a given column with different superscripts are significantly different (P<0.05). Values are means ± standard errors (SE). n = replication; AWG, absolute carapace length; FdietPEE 1: Formulated diet without amaranth extract and vitamin E (0 ng g<sup>-1</sup> crab + 0 IU kg<sup>-1</sup>); FdietPEE 2: Formulated diet enriched with amaranth extract (250 ng g<sup>-1</sup> crab) and vitamin E (100 IU kg<sup>-1</sup>); FdietPEE 3: Formulated diet enriched with amaranth extract (500 ng g<sup>-1</sup> crab) and vitamin E (200 IU kg<sup>-1</sup>); FdietPEE 4: Formulated diet enriched with amaranth extract (750 ng g<sup>-1</sup> crab) and vitamin E (300 IU kg<sup>-1</sup>).

Furthermore, Duncan's test results also demonstrated significant differences (P <0.05), particularly between treatments FdietPEE 4 and 3 in comparison to treatments FdietPEE 1 and 2. However, treatment FdietPEE 4 and treatment FdietPEE 3 did not exhibit a significant difference (P>0.05).

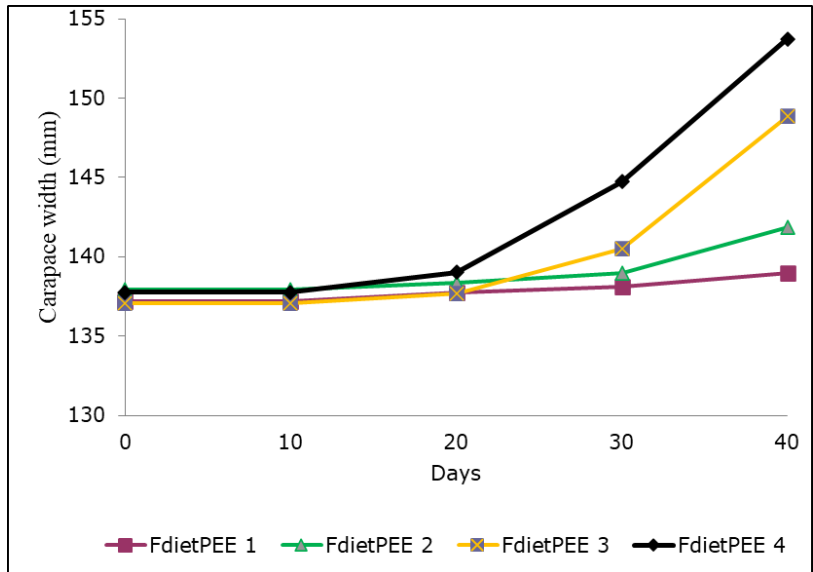


Figure 5. Growth carapace width (mm) of individual female broodstock *Portunus pelagicus* fed a formulated feed with amaranth extract (PE) and vitamin E at different doses.

The relationship between the doses of amaranth extract and vitamin E in the formulated feed and the absolute carapace width was found to be quadratic. The regression equation provided is  $Y = -2 \times 10^{-5} X^2 + 0.0378 X + 1.2647$ , with an  $R^2$  value of 0.7589, indicating a statistically significant relationship ( $P < 0.05$ ; Figure 6). This equation describes how the doses of amaranth extract and vitamin E in the feed correlate with the absolute carapace width of the crabs.

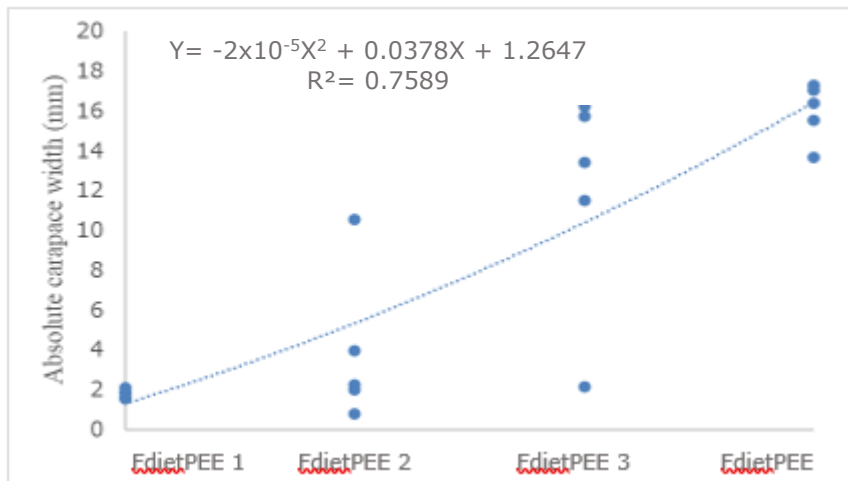


Figure 6. The relationship between the dose of FdietPEE and the absolute carapace width (mm) of broodstock female crabs *Portunus pelagicus*.

The survival rate serves as an important indicator of the well-being of organisms, reflecting the outcomes of interactions between the environment and the provided feed. In this particular study, the results indicate that the various dietary treatments, involving a formulated feed enriched with amaranth extract (PE) and vitamin E for female broodstock crabs, over a 40-day maintenance period in controlled cultivation containers yielded a remarkably high survival rate of 100% across all treatments (Table 4).

Table 4

Percentage of average survival rate (%) of broodstock female crabs *Portunus pelagicus* fed a formulated feed with amaranth extract (PE) and vitamin E at different doses

Sampling (days)	Treatment (n=5)			
	FdietPEE 1	FdietPEE 2	FdietPEE 3	FdietPEE 4
0	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
10	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
20	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
30	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
40	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
H	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>	100 ± 0.00 <sup>a</sup>

Means within a given column with different superscripts are significantly different ( $P < 0.05$ ). Values are means ± standard errors (SE). n = replication; AWG, absolute carapace length; FdietPEE 1: Formulated diet without amaranth extract and vitamin E (0 ng g<sup>-1</sup> crab + 0 IU kg<sup>-1</sup>); FdietPEE 2: Formulated diet enriched with amaranth extract (250 ng g<sup>-1</sup> crab) and vitamin E (100 IU kg<sup>-1</sup>); FdietPEE 3: Formulated diet enriched with amaranth extract (500 ng g<sup>-1</sup> crab) and vitamin E (200 IU kg<sup>-1</sup>); FdietPEE 4: Formulated diet enriched with amaranth extract (750 ng g<sup>-1</sup> crab) and vitamin E (300 IU kg<sup>-1</sup>).

This high survival rate can be attributed to the controlled conditions in which the crabs were cultivated. Notably, no crab fatalities occurred during the maintenance period. This positive outcome can be primarily attributed to the maintenance of water quality, including both physical and chemical factors, which remained within a favorable range for the crabs throughout the study, as indicated in Table 5. These optimal conditions contributed to the high survival rate observed in the study.

Table 5

The water quality of maintenance media for broodstock female crabs *Portunus pelagicus* fed a formulated feed with amaranth extract (PE) and vitamin E at different doses

Water quality parameter	Range
Salinity (ppt)	29.0 – 32.5
Temperature (°C)	26.0 – 28.0
pH	7.58 – 8.00
O <sub>2</sub> (ppm)	6.50 – 7.50
CO <sub>2</sub> (ppm)	3.65 – 6.40
Water depth (cm)	27.0 – 28.0

**Discussion.** The current practice of feeding blue swimming crabs with low-cost feeds such as trash fish, mollusks, and fresh or boiled slaughterhouse waste comes with several challenges. Using these feeds necessitates diligent management to prevent water quality deterioration. Moreover, these feeds often need to be frozen for storage, adding complexity to the feeding process. Additionally, the availability of fresh feed can be inconsistent, limiting its suitability as a regular feed for blue swimming crabs. To address these issues, it's crucial to have access to data regarding the nutrient requirements of candidate species like blue swimming crabs. According to Tacon (2000), this information is necessary for the creation of commercial feeds that are both reasonably priced and nutritionally balanced. Millamena & Qunitio (2000) further emphasize the significance of essential dietary fatty acids in natural feed by showing that their lack can have a detrimental effect on broodstock reproductive performance when compared to carefully formulated feed or a mix of formulated and natural feed. Therefore, having formulated feed data specific to blue swimming crab broodstock is vital in developing commercial feeds that are both nutritionally balanced and cost-effective, ultimately enhancing the sustainability and productivity of blue swimming crab aquaculture. The importance of creating specially designed feed in the future to supplement natural feed in order to improve broodstock spawning and the quality of eggs and larval production is highlighted in the study conducted by Millamena & Bangcaya (2001). The present investigation revealed that the addition of vitamin E and amaranth extract to the formulated feed



significantly affected the biological test results ( $P < 0.05$ ) in terms of absolute weight gain, absolute carapace length, and absolute carapace width. Significant differences were found between the various dietary treatments in terms of absolute weight gain, absolute carapace length, and absolute carapace width. Specifically, the results for FdietPEE 1, FdietPEE 2, FdietPEE 3, and FdietPEE 4 were as follows: FdietPEE 1: 13.14 g, 1.29 mm, and 1.78 mm; FdietPEE 2: 36.68 g, 2.75 mm, and 3.91 mm; FdietPEE 3: 55.50 g, 7.45 mm, and 11.81 mm; FdietPEE 4: 58.89 g, 8.04 mm, and 15.98 mm. Regression analysis indicated that the relationship between the doses of amaranth extract and vitamin E in the feed and the biological parameters (AWG, ACL, and ACW) was quadratic in nature. The values of  $R^2$  for AWG, ACL, and ACW were 0.7639, 0.8517, and 0.7589, respectively, signifying statistically significant relationships ( $P < 0.05$ ). These results highlight the effectiveness of the formulated feed enriched with amaranth extract and vitamin E in promoting growth and development in broodstock female crabs. The results of the study also demonstrate the positive impact of the amaranth extract and vitamin E treatments on the survival and molting process of the broodstock female crabs, *P. pelagicus*. The feed enriched with these additives provides a valuable source of energy, steroids, and sterols that are integral to the metamorphic and growth processes. The presence of phytoecdysteroid hormones in the feed consumed by female crabs contributes to higher energy availability within their bodies, thereby supporting various physiological functions and growth metabolism. These findings align with the perspective presented by Gunamalai et al (2004), who emphasized that phytoecdysteroid (ecdysone) serves as the primary steroid hormone in arthropods and plays a pivotal role in regulating critical physiological functions, including growth, metamorphosis, and reproduction. This emphasizes the significance of the role of phytoecdysteroids and the nutritional quality of the feed in the overall well-being and development of broodstock female crabs. According to Frankel (2014), lipid oxidation can be reduced by using antioxidants to prevent lipid oxidation from starting and from spreading further. This context highlights the importance of tocopherol, a well-known lipid-soluble antioxidant and free radical scavenger; its most prevalent and physiologically active form is  $\alpha$ -tocopherol (Herrera & Barbas 2001). Wang et al (2015) provided evidence that  $\alpha$ -tocopherol can prevent color changes in meat products and increase the shelf life of animal fats. Furthermore, as a necessary fat-soluble vitamin, vitamin E stabilizes membranes, interrupts the cyclic propagation of lipid peroxidation, and supports obscure processes in gene transcription, cellular transport, and cell signaling (Herrera & Barbas, 2001; Galli & Azzi 2010). There are two types of vitamin E: unsaturated tocotrienols and saturated tocopherols. The most biologically active isoform of vitamin E is alpha-tocopherol ( $\alpha$ -TP). In synthetic or highly bioavailable natural formulations, it is frequently given as a dietary supplement (Herrera & Barbas 2001; Siciliano et al 1997; Pagan 2005).

According to the study's findings, crab larvae in the zoea-2 and zoea-3 stages had the greatest survival rate and the quickest rate of metamorphosis at a dose of 2 mg per 100 g of prepared feed. Furthermore, for the megalopa and crablet stages, a dose of 4 mg per 100 g of formulated feed worked well (Nikhilani & Sukarti 2017). These results emphasize how crucial it is to use phytoecdysteroids at the right dosages in order to maximize the growth and survival of crab larvae. Higher animals and humans have long used exogenous hormones to raise hormone levels in their bodies, as studies like Techa & Chung (2015), Bakrim et al (2008) and Fujaya et al (2008) have shown. Crabs haven't had this kind of hormone supplementation thoroughly studied, though. Twenty-Hydroxyecdysone (20-HE) was used by Lubis et al (2023a) to induce molting in crabs. As noted in studies by Aslamyah & Fujaya (2010), Fujaya et al (2011), Fujaya et al (2014), and Herlinah et al (2015), phytoecdysteroids from the Amaranthaceae family have been used in different contexts to stimulate molting in the production of mangrove crabs, *Scylla serrata*. According to Fujaya et al (2014) and Ahmad et al (2015), phytoecdysteroids made from mulberry leaf extract have also been utilized to aid in the development of *P. pelagicus* larvae. The effects of mulberry leaf extract from *Morus alba* on crab development and growth were examined in these studies using a range of doses, from 1 to 4 mg per 100 g of feed to 4 to 400 mg per 100 g of feed. The study reported a remarkably high survival rate of 100% for all dietary treatments at the end of the 40-day

period. This exceptional survival rate can be attributed to the overall favorable environmental conditions within the maintenance medium, which played a crucial role in supporting the growth and metamorphosis processes of the female crabs. Additionally, the treatments involving amaranth extract, which contains the hormone ecdysone, had a positive impact on the survival of the broodstock female crabs. Like with many other species, the nutritional value of the feed has a big impact on how quickly a crustacean grows. As a result, formulated feed is largely relied upon in the research on the nutritional requirements for broodstock maturation, particularly in commercial applications, where it is preferred (Djunaidah et al 2003). A fascinating physiological process in blue swimming crabs is the molting cycle, which involves changing out their old skin for a new skin. This cycle, which is shared by all arthropods, including insects and crustaceans, is crucial for development because it involves the atrophy of the skeletal muscles in the claws, the regeneration of lost appendages, and the breakdown of the old exoskeleton. It is also essential for both metamorphosis and reproduction. As noted by Fujaya et al (2008), a better understanding of the mechanisms underlying reproduction and growth in cultured animals can result in enhanced productivity in processes such as the soft shell crab production, growth, and seeding. The water quality parameters observed during the captivity periods, including salinity (ranging from 29.0 to 32.5 ppt), temperature (between 26.0°C and 28.0°C), dissolved oxygen (DO) levels (ranging from 6.50 to 7.50 ppm), pH (within the range of 7.58 to 8.00), carbon dioxide (CO<sub>2</sub>) concentration (ranging from 3.65 to 6.40), and water depth (between 27.0 and 28.0 cm), were found to have no significant impact on the growth and survival rates of broodstock female crabs, *P. pelagicus*. This conclusion is supported by various studies, including those conducted by Arshad et al (2006), Oniam et al (2012), and Efrizal and colleagues (Efrizal et al 2017b; Efrizal et al 2018; Efrizal et al 2019; Efrizal et al 2020). These findings suggest that the specified water quality parameters remained within acceptable ranges for the well-being of the crabs, allowing for consistent growth and high survival rates. In crustaceans, growth occurs through a process that involves shedding the old exoskeleton and forming a new one. Immediately after the ecdysis, which is the molting process, the newly synthesized cuticle absorbs water to expand the new exoskeleton, leading to an increase in size (Hosamani et al 2017). The molt cycle in crustaceans is intricately regulated by a combination of several hormones, both internal and external factors. Two of the key hormones involved in molt regulation are molt-inhibiting hormone (MIH) and ecdysteroids, which often act in a counteractive manner to each other. Additionally, methyl farnesoate (MF) plays a role in inducing molting by promoting the synthesis and release of ecdysteroids from Y-organs. Beyond these hormones, various internal molecules like opioids (as noted by Hosamani et al 2017) and external environmental factors such as light, temperature, and food availability are also influential in regulating the molting process in crustaceans. These complex interactions highlight the multifaceted nature of the molt cycle in these animals (Fujaya et al 2008; Kuballa et al 2011).

**Conclusions.** In conclusion, the study findings reveal that feed enriched with amaranth extract and vitamin E at specific levels at concentrations of 500 ng/g crab + 200 IU/kg and 750 ng/g crab + 300 IU/kg, respectively, yield the following values for the studied biological parameters: an absolute weight gain (AWG) of  $55.50 \pm 5.05$  g and  $58.89 \pm 4.33$  g, respectively, an absolute carapace length (ACL) of  $7.45 \pm 0.30$  mm and  $8.04 \pm 0.12$  mm, respectively, and an absolute carapace width (ACW) of  $11.81 \pm 2.86$  mm and  $15.98 \pm 0.73$  mm, respectively. Furthermore, the regression analysis demonstrates a quadratic relationship between the formulated feed enriched with amaranth extract and vitamin E and the AWG ( $R^2=0.7639$ ), ACL ( $R^2=0.8517$ ), and ACW ( $R^2=0.7589$ ), indicating their significant impact on the growth and development of broodstock female crabs. Importantly, all crab groups maintained a remarkable 100% survival rate over the 40-day study period.

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