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RESEARCH ARTICLE

Growth performance and survival rate of *Portunus pelagicus* (Linnaeus, 1758) broodstock females fed varying doses of amaranth extracts [version 1; peer review: awaiting peer review]

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Abstract

Background: Formulated diets made from food waste enriched with amaranth extract largely determine the level of softshell crab production. The study was carried out to analyze the growth performance and survival rate of blue swimming crab *Portunus pelagicus* (Linnaeus, 1758) broodstock females fed formulated diets with different doses of amaranth extracts.
Methods: The female crab samples were collected from the coastal region of Padang, West Sumatra. The method used in this study was a completely randomized design, with four treatments and five replications of amaranth extract-enriched diets (0, 250, 500, and 750 ng/g crab).
Results: The results show that the enrichment of the formulated diet with amaranth extracts significantly affected ($P < 0.05$) the absolute weight gain (AWG), carapace length (ACL), and carapace width (ACW).
Conclusions: A quadratic relationship existed between the amaranth extract dose in the formulated diet and the AWG, ACL and ACW.

Keywords

Blue swimming crab, broodstock, formulated diets, growth performance, amaranth extracts

Open Peer Review

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Introduction

The waters of the province of West Sumatra provide a water marine area that includes the Indonesian Exclusive Economic Zone, which encompasses approximately 186,580 km^2, with a long coastline of 2420.387 km, and provides habitat for many biological natural resources[1]. This marine area, when used wisely, from planning to the administration, implementation, and supervision, will support the welfare of people along the coast in general and the coast of Indonesia in particular. The crab (*Portunus pelagicus*) belongs to the Portunidae family, is a marine biota resource. It has great potential to become an important fishery export commodity[2–6], since in recent years, domestic and international demand has increased from year to year[7,8]. According to data from the last ten years for the period 1993–2002, the export volume of crabs increased by an average of 16.72% per year, from 6,081 tons in 1993 to 11,246 tons in 2002[1], whereas in the period 2007–2009 reports on the Indonesian market share of crab decreased from 17.6% to 16.3%[9]. The intensive harvest of crabs could cause a decline in natural populations of crabs[10]. Because little control exists in crab harvesting, populations of crabs are rare in Indonesian waters[11]. Until now the demand for crabs, for domestic consumption and for export, has still relied on the catch from the sea, so this concern will affect the crab population in nature[12].

An increase in *P. pelagicus* production can be achieved in an intensive aquaculture system. Aquaculture is one of the ways to address the threat of crab population decline from overexploitation[13–16]. The business of crab farming is still in its early phase in Indonesia and in many countries in the world[17]. Some researchers have reported that a low growth performance and the survival rate remain as problems in culture of the blue swimming crab[17], which is caused by various factors, such as disease[18–20], molting syndrome[21], cannibalism[22], and feed[3,7,8]. Feed is the main component needed by the crab to survive and grow. The completeness of nutrients in the feed is absolutely necessary to ensure the normal growth of the crabs. Nutritional requirements for crab growth, including proteins, fats, carbohydrates, vitamins, and minerals, differ by crab type and size[3,8,23], with advantages being provided from formulated feeds over the live feeds (fresh feed)[24].

One important breakthrough in the cultivation of softshell crabs, which has been developed by Fujaya[25], is the discovery of a molting stimulant derived from amaranth plant extract (*Amaranthacea tricolor*) called vitomolt. Amaranth extract containing ecdysteroids and application through injection proved to accelerate molting and did not cause death in the crabs; furthermore, the growth of crabs that received the application of amaranth extract was greater than that of the crabs that did not. Based on the description given above, an in-depth assessment of the effects of the feeding of amaranth extracts in varying doses on the quality of formulated diets for growth performance and survival rate of *P. pelagicus* (Linnaeus, 1758) was needed. Therefore, this study was conducted.

Methods

The study was conducted from January until June 2019 at *Balai Benih Ikan Pantai* (BBIP) *Teluk Buo*, *Balai Benih Ikan* (BBI) Bungus, in the city of Padang and at the Laboratory of Animal Physiology Department of Biology, Padang, West Sumatra.

Ethical considerations

In recent years, the production of crabs has decreased both in number and size. While Blue Swimming crab (*Portunus pelagicus*) is classified as vulnerable to endangered in the Indonesian marine waters, the Government of the Republic of Indonesia does not require licenses to be obtain to capture and rear this species (Regulation of the Minister of Environment and Forestry of the Republic of Indonesia No: P.92/MENLHK/SETJEN/KUM.1/8/2018; https://www.jalaksuren.net/wp-content/uploads/2018/10/Permen-LHK-P.92-2018.pdf), hence no licenses are applicable to this study. No animals suffered as a result of the activities of this study. *P. pelagicus* was transported to the hatchery for rearing and at the end of the experiment *P. pelagicus* remained in good condition.

Crab collection and husbandry

In total 70 female crab samples were collected on January 2019; 20 were females at stage II of ovarian maturity, which were selected for this study[2]. The female crab samples were collected from the coastal region of Padang (100° 22'12" BT, 1° 0' 54" LS dan 100° 25'58,8" BT, 1° 4' 40,8" LS), West Sumatra, and the crabs were placed randomly in four concrete tanks (200 cm × 100 cm × 100 cm), each divided into five units, i.e., plastic boxes (45.5 cm × 32.5 cm × 16.5 cm), where the crabs were held at a maximum density of one crab per box. Tanks were provided with approximately a 15-cm-thick layer of sand for substrate and with adequate aeration[2,7,8,26–28]. The crabs were maintained in monitored water with a water depth of 25 to 30 cm, salinity of 30 to 32 ppt, pH 7.77 to 7.96, temperature 26 to 27°C, and dissolved oxygen (DO) 7.00 to 7.30 ppm. Each crab was provided with a shelter made of PVC pipe, 13 cm in diameter and 40 cm in length, to serve as a refuge during molting. Dietary Vitamin E was fed daily at 3% biomass (17.00 to 18.00 hours), and uneaten food was removed every morning.

Feed intervention

The method used in this study was a completely randomized design (CRD) with four treatments and five replications of amaranth extract-enriched diets, with treatments as follows: Fdiet 1, formulated diet without amaranth extract 0 ng/g crab; Fdiet 2, formulated diet enriched with amaranth extract at 250 ng/g crab; Fdiet 3, formulated diet enriched with amaranth extract at 500 ng/g crab; and Fdiet 4, formulated diet enriched with amaranth extract at 750 ng/g crab. The formulated diet[3,7,29] was a modified formulation of one used for the broodstock of mud crab *Scylla serrata*[16]. Amaranth extract was dissolved in 80% ethanol in a ratio of 1:1 and then homogenized. Then, the 80% ethanol solution was added at a rate of 20 mL/kg of feed by even spraying of the solution onto the test feed, and the feed was then left to dry. The test feed was stored until it was ready for use[30].

Prior to receiving artificial feed, the broodstock was first given a natural feed (fresh bivalve molluska + sardinella fish; 1:1) and gradually acclimatized with artificial feed for 10 days. Feed was given at a dose of 8–10% of biomass per day for

natural feed and 3–4% for artificial feed. The feed was given three times a day at 08.00, 13.00 and 17.00, with a percentage of 40% in the morning and the remainder being divided into two periods: the afternoon and evening. The remaining food was discarded every morning, and the amount of food was adjusted to the weight of the crab mother at the time of observation. The number of dead crabs were observed and recorded daily.

Variables assessed

Measured parameters (absolute weight gain, absolute carapace length, absolute carapace width and survival rate) and water quality refer to Efrizal *et al.*[7]. The absolute weight gain was calculated as follows: AWG = $WG_f - WG_o$, where AWG is weight gain (g), WG_f is the weight of the crab at the end of experiment (g), and *WGo* is the weight of crab at the start of experiment (g). The absolute carapace length was calculated as follows: ACL = $CL_f - CL_o$, where ACL is carapace length gain (mm), CL_f is the carapace length of the crab at the end of experiment (mm), and CL_o is the carapace length of crab at the start of experiment (mm). The absolute carapace width was calculated as follows: ACW = $CW_f - CW_o$, where ACW is carapace width gain (mm), CW_f is the carapace width of the crab at the end of experiment (mm), and CW_o is the carapace width of crab at the start of experiment (mm). The carapace length, carapace width, and survival rates were measured as described previously[2,3,31]. Weights were measured to 0.01 g on the electronic balances (BL3200H-SHIMADZU).

The water quality parameters that were monitored daily were temperature (°C), salinity (ppt), pH, and water depth (cm) while dissolved oxygen (ppm) and CO_2 (ppm) thrice weekly using a maximum-minimum thermometer, hand-held Atago refractometer model 8808, Thermo Orion Benctop pH meter models 410 A plus, weighted line, YSI oxygen meter model 57, and APHA[32], respectively.

Statistical analysis

The data for growth performance (absolute weight gain, absolute carapace length, and absolute carapace width) and survival rate[7] were analyzed using one-way ANOVA and Duncan's Multiple Range test to compare the differences among the means of the different treatments[33] were performed using SPSS software (version 19.0 for Windows; SPSS Inc., Chicago, II. Arcsine transformation was performed before the data, in percentages, was analyzed.

Results

Absolute weight gain

The growth measure of absolute weight is a measure of the weight difference observed in a female crab for a certain time when weighed at the beginning and end of the period. The observation of the average weight and absolute weight gain in the female parent crab *P. pelagicus* (Linnaeus, 1758) with different dietary treatments is presented in Table 1. Table 1 shows that the growth tends to increase the absolute weight of the maintenance period for 0 to 40 days. From the weighing results (Table 1), the highest absolute value of the average weight obtained in the administration was achieved with Fdiet 3 (55.03 g), followed by Fdiet 4 (18.05 g), Fdiet 1 (32.49 g), and Fdiet 2 (42.49 g); analysis of variance showed significant differences ($P < 0.05$). Duncan's test showed further significant differences ($P < 0.05$), which can be observed between the treatments Fdiet 3 and Fdiet 1 and between Fdiet 3 and Fdiet 4, whereas no significant differences ($P > 0.05$) were observed between Fdiet 3 and Fdiet 2, and between Fdiet 1 and Fdiet 4. The relationship between the amaranth-extract doses in the formulated diet and the absolute weight gain is presented in Figure 1. The regression equation is AWG = -0.0002DAE + 0.1274DAE + 29.816 ($R^2 = 0.4155$; $P < 0.05$).

Absolute carapace length

Absolute carapace length is calculated from the difference in the size of the parent crab carapace length achieved within a certain time, when the carapace length is measured at the beginning and end of the period. Treatment with the different diets caused relatively large changes in the growth of the absolute carapace length during the maintenance period for the 40 days, ranging from 1.22 to 7.09 mm (Table 2), with analysis of variance showing significant differences ($P < 0.05$). Based on the data in Table 2, the accretion of the greatest absolute carapace length was obtained with the treatment Fdiet 3 (7.09 mm) in comparison with the treatment Fdiet 4 (1.22 mm), Fdiet 1 (3.60 mm) and Fdiet 2 (4.61 mm). Similarly, the results of a further test for significant differences ($P < 0.05$) with Duncan's Test showing that treatment Fdiet 3 differs from Fdiet 4, whereas the treatment Fdiet 3 does differ significantly from Fdiet 1 and Fdiet 2 ($P < 0.05$), and Fdiet 4 is not significantly different from Fdiet 1 and Fdiet 2 ($P > 0.05$). The relationship between the dose of the amaranth extracts in the formulated diet and absolute carapace length (mm) of the blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock was quadratic (ACL = -2 x 10^{-5}DAE^2+ 0.0166DAE+3.1695; $R^2 = 0.2212$; $P < 0.05$; Figure 2).

Absolute carapace width

The growth of the absolute carapace width is also calculated from the difference in the size of the parent crab carapace width achieved in a specific time, when the carapace width is measured at the beginning and end of the period. From the measurement results (Table 3), artificial feeding at Fdiet 3, a dose of 500 ng of amaranth extracts/g crab provides relatively high added value to the mean carapace width (13.14 mm) compared to artificial feeding at Fdiet 1, an amaranth extract dose of 0 ng/g crab (6.25 mm); Fdiet 2, 250 ng/g crab (8.98 mm); and Fdiet 3, 750 ng/g crab (1.92 mm), with analysis of variance showing a significant difference ($P < 0.05$). Similarly, Duncan's test results showed significant differences ($P < 0.05$), as seen between the treatment Fdiet 3 with Fdiet 1 and Fdiet 4, whereas the treatment Fdiet 2 with Fdiet 3 and the treatment Fdiet 1 with Fdiet 4 did not show significant differences ($P > 0.05$). The relationship between the amaranth-extract dose in the formulated diet and absolute carapace width was found to be quadratic (ACW = -5 x 10^{-5} DAE^2- 0.0349DAE+5.502; $R^2 = 0.1724$; $P > 0.05$; Figure 3).

Table 1. Average weight (g) and absolute weight gain (g) of blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock fed a formulated diet containing amaranth extracts at different doses.

Treatment	Replications	Weight (g)					AWG (g)
		0 days	10 days	20 days	30 days	40 days	
	1	120.89	122.95	159.77	161.22	170.52	49.63
	2	119.10	120.35	154.25	155.95	166.05	46.95
Fdiet 1	3	227.85	228.90	231.82	233.02	234.15	6.30
	4	200.83	201.94	205.09	206.89	216.35	15.52
	5	125.77	126.84	156.57	158.13	169.83	44.06
	Total	**794.44**	**800.98**	**907.50**	**915.21**	**956.90**	**162.46**
	Avarage	**158.89**	**160.20**	**181.50**	**183.04**	**191.38**	**32.49**
	SE	**25.79**	**25.68**	**17.54**	**17.50**	**15.80**	**10.03**
	1	144.91	146.34	185.12	186.97	197.38	52.47
	2	243.69	244.99	250.99	252.55	264.03	20.34
Fdiet 2	3	136.22	137.65	172.62	174.52	185.08	48.86
	4	149.64	150.85	156.2	191.75	193.48	43.84
	5	121.88	123.21	153.43	155.21	166.41	44.53
	Total	**796.34**	**803.04**	**918.36**	**961.00**	**1006.38**	**210.04**
	Avarage	**159.27**	**160.61**	**183.67**	**192.20**	**201.28**	**42.01**
	SE	**24.18**	**24.17**	**19.88**	**18.29**	**18.53**	**6.30**
	1	133.55	134.87	169.53	171.48	187.13	53.58
	2	119.13	120.49	157.88	159.98	174.76	55.63
Fdiet 3	3	116.38	117.88	157.1	158.95	175.45	59.07
	4	176.4	177.98	217.9	219.74	234.54	58.14
	5	249.5	250.87	260.65	296.35	298.24	48.74
	Total	**794.96**	**802.09**	**963.06**	**1006.5**	**1070.12**	**275.16**
	Avarage	**158.99**	**160.42**	**192.61**	**201.30**	**214.02**	**55.03**
	SE	**28.00**	**28.00**	**22.73**	**29.33**	**26.54**	**2.06**
	1	116.46	116.99	129.51	160.21	161.61	45.15
	2	185.09	185.95	189.23	190.63	207.78	22.69
Fdiet 4	3	127.5	128.64	132.75	134.1	135.35	7.85
	4	117.37	117.37	120.15	122.01	123.51	6.14
	5	251.84	252.87	256.77	258.66	260.27	8.43
	Total	**798.26**	**801.82**	**828.41**	**865.61**	**888.52**	**90.26**
	Avarage	**159.65**	**160.36**	**165.68**	**173.12**	**177.70**	**18.05**
	SE	**29.40**	**29.53**	**28.85**	**27.30**	**28.19**	**8.27**

Values are means ± standard errors (SE). AWG, absolute weight gain; Fdiet 1, formulated diet without amaranth extract 0 ng/g crab; Fdiet 2, formulated diet enriched with amaranth extract 250 ng/g crab; Fdiet 3, formulated diet enriched with amaranth extract 500 ng/g crab; and Fdiet 4, formulated diet enriched with amaranth extract 750 ng/g crab.

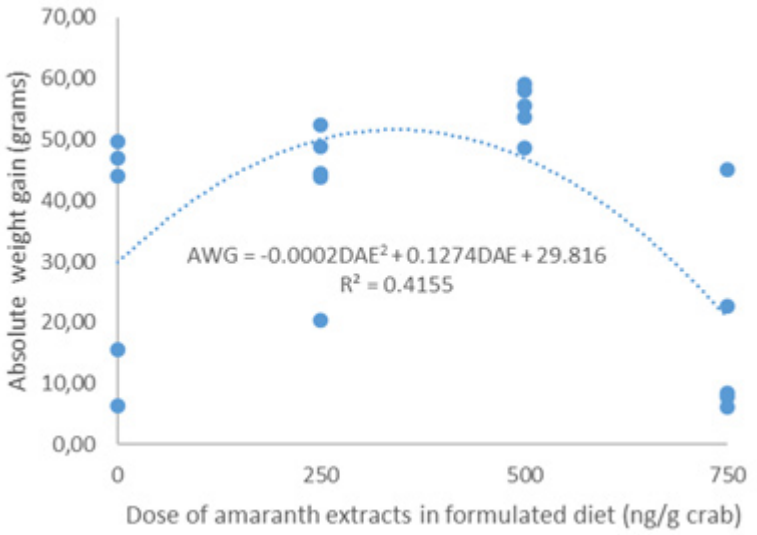

Figure 1. The relationship between the dose of amaranth extracts (DAE) in a formulated diet and the absolute weight gain (g) of blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock.

Table 2. Average carapace length (mm) and absolute carapace length (mm) of blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock fed a formulated diet with amaranth extracts at different doses.

Treatment	Replications	carapace length (mm)					ACL (mm)
		0 days	10 days	20 days	30 days	40 days	
	1	53.86	53.86	59.97	59.97	59.97	6.11
	2	53.40	53.40	59.28	59.28	59.28	5.88
Fdiet 1	3	64.68	64.68	64.68	64.68	64.68	0.00
	4	59.03	59.03	59.03	59.03	59.03	0.00
	5	54.28	54.28	60.28	60.28	60.28	6.00
	Total	**285.25**	**285.25**	**303.24**	**303.24**	**303.24**	**17.99**
	Avarage	**57.05**	**57.05**	**60.65**	**60.65**	**60.65**	**3.60**
	SE	**2.41**	**2.41**	**1.15**	**1.15**	**1.15**	**1.64**
	1	55.19	55.19	62.17	62.17	62.17	6.98
	2	64.95	64.95	64.95	64.95	64.95	0.00
Fdiet 2	3	52.17	52.17	58.87	58.87	58.87	6.70
	4	59.08	59.08	59.08	65.18	65.18	6.10
	5	54.88	54.88	58.17	58.17	58.17	3.29
	Total	**286.27**	**286.27**	**303.24**	**309.34**	**309.34**	**23.07**
	Avarage	**57.25**	**57.25**	**60.65**	**61.87**	**61.87**	**4.61**
	SE	**2.48**	**2.48**	**1.43**	**1.64**	**1.64**	**1.48**
	1	52.18	52.18	59.36	59.36	59.36	7.18
	2	53.63	53.63	60.44	60.44	60.44	6.81
Fdiet 3	3	51.88	51.88	58.8	58.8	58.8	6.92
	4	59.04	59.04	66.46	66.46	66.46	7.42
	5	65.18	65.18	65.18	72.28	72.28	7.10
	Total	**281.91**	**281.91**	**310.24**	**317.34**	**317.34**	**35.43**
	Avarage	**56.38**	**56.38**	**62.05**	**63.47**	**63.47**	**7.09**
	SE	**2.85**	**2.85**	**1.76**	**2.90**	**2.90**	**0.12**

Treatment	Replications	carapace length (mm)					ACL (mm)
		0 days	10 days	20 days	30 days	40 days	
	1	52.10	52.10	52.10	58.21	58.21	6.11
	2	61.29	61.29	61.29	61.29	61.29	0.00
Fdiet 4	3	51.91	51.91	51.91	51.91	51.91	0.00
	4	51.77	51.77	51.77	51.77	51.77	0.00
	5	64.95	64.95	64.95	64.95	64.95	0.00
	Total	**282.02**	**282.02**	**282.02**	**288.13**	**288.13**	**6.11**
	Avarage	**56.40**	**56.40**	**56.40**	**57.63**	**57.63**	**1.22**
	SE	**3.13**	**3.13**	**3.13**	**2.90**	**2.90**	**1.37**

Values are means ± standard errors (SE). ACL, absolute carapace length; Fdiet 1, formulated diet without amaranth extract 0 ng/g crab; Fdiet 2, formulated diet enriched with amaranth extract 250 ng/g crab; Fdiet 3, formulated diet enriched with amaranth extract 500 ng/g crab; and Fdiet 4, formulated diet enriched with amaranth extract 750 ng/g crab.

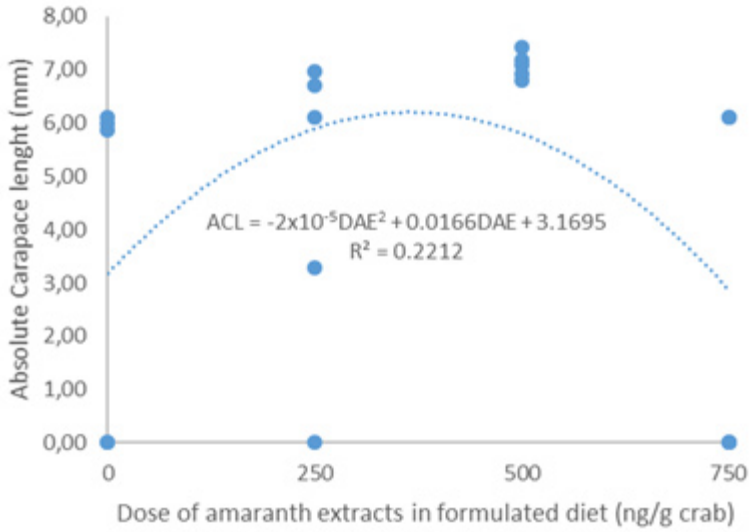

Figure 2. The relationship between the dose of amaranth extracts (DAE) in a formulated diet and the absolute carapace length (mm) of blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock.

Survival rate

The results show that the different dietary treatments for the female parent crabs during the maintenance period of 40 days in controlled cultivation containers provided a high survival rate (100%) for all treatments (Table 4). The high survival rate value is due to the maintenance in a controlled container cultivation, where no deaths occurred in the female parent crabs. This happens because the water quality (physical and chemical factors) during the study remained in the range conducive for crabs (Table 5).

Discussion

The growth responses of the crab to the four formulated diets with amaranth extract occurred because of the varied composition of the raw materials used in formulations. Our results demonstrate that the different nutrient levels in the feed, which are especially caused by the levels of amaranth extract. The blue swimming crab needs to maintain the existence of life and its growth, and it will grow well if the available feed contains all the nutrients needed at optimal levels. According to Gutierrez-Yurrita and Montes[34], the nutrient composition of essential feed will determine the growth and efficiency of the organisms being fed.

In this experiment (Table 1), the formulated diet that included a dose of amaranth extract influenced the physiological processes of the experimental crab known as absolute weight gain. The highest weight gain was shown by the test crabs receiving the Fdiet 3 treatment (55.03 ± 2.06 g) compared to other feed

Table 3. Average carapace width (mm) and absolute carapace width (mm) of blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock fed a formulated diet with amaranth extracts at different doses.

Treatment	Replications	Carapace width (mm)					ACW (mm)
		0 days	10 days	20 days	30 days	40 days	
	1	117.65	117.65	129.20	129.20	129.20	11.55
	2	116.79	116.79	126.92	126.92	126.92	10.13
Fdiet 1	3	139.54	139.54	139.54	139.54	139.54	0.00
	4	135.11	135.11	135.11	135.11	135.11	0.00
	5	109.65	109.65	119.2	119.20	119.20	9.55
	Total	**618.74**	**618.74**	**649.97**	**649.97**	**649.97**	**31.23**
	Avarage	**123.75**	**123.75**	**129.99**	**129.99**	**129.99**	**6.25**
	SE	**6.44**	**6.44**	**3.90**	**3.90**	**3.90**	**2.87**
	1	119.77	119.77	132.07	132.07	132.07	12.30
	2	140.5	140.5	140.5	140.5	140.5	0.00
Fdiet 2	3	117.4	117.4	128.28	128.28	128.28	10.88
	4	120.52	120.52	120.52	131.12	131.12	10.60
	5	117.89	117.89	129.02	129.02	129.02	11.13
	Total	**616.08**	**616.08**	**650.39**	**660.99**	**660.99**	**44.91**
	Avarage	**123.22**	**123.22**	**130.08**	**132.20**	**132.20**	**8.98**
	SE	**4.87**	**4.87**	**3.61**	**2.44**	**2.44**	**2.53**
	1	116.11	116.11	131.31	131.31	131.31	15.20
	2	116.84	116.84	130.24	130.24	130.24	13.40
Fdiet 3	3	111.92	111.92	123.87	123.87	123.87	11.95
	4	125.5	125.5	138.5	138.5	138.5	13.00
	5	138.87	138.87	138.87	151.02	151.02	12.15
	Total	**609.24**	**609.24**	**662.79**	**674.94**	**674.94**	**65.70**
	Avarage	**121.85**	**121.85**	**132.56**	**134.99**	**134.99**	**13.14**
	SE	**5.36**	**5.36**	**3.14**	**5.18**	**5.18**	**0.65**
	1	114.58	114.58	114.58	124.18	124.18	9.60
	2	132.79	132.79	132.79	132.79	132.79	0.00
Fdiet 4	3	106.45	106.45	106.45	106.45	106.45	0.00
	4	111.88	111.88	111.88	111.88	111.88	0.00
	5	140.78	140.78	140.78	140.78	140.78	0.00
	Total	**606.48**	**606.48**	**606.48**	**616.08**	**616.08**	**9.60**
	Avarage	**121.30**	**121.30**	**121.30**	**123.22**	**123.22**	**1.92**
	SE	**7.36**	**7.36**	**7.36**	**7.12**	**7.12**	**2.15**

Values are means ± standard errors (SE). ACW, absolute carapace width; Fdiet 1, formulated diet without amaranth extract 0 ng/g crab; Fdiet 2, formulated diet enriched with amaranth extract 250 ng/g crab; Fdiet 3, formulated diet enriched with amaranth extract 500 ng/g crab; and Fdiet 4, formulated diet enriched with amaranth extract 750 ng/g crab.

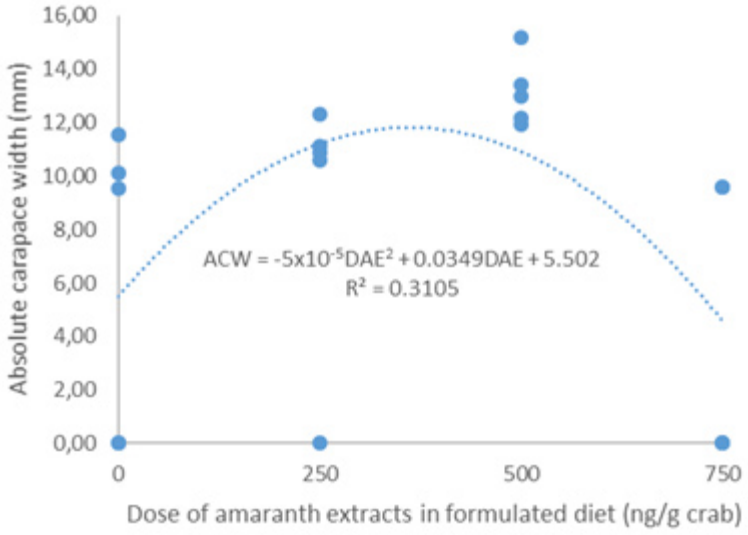

Figure 3. The relationship between the dose of amaranth extracts (DAE) in a formulated diet and the absolute carapace width (mm) of blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock.

Table 4. Percentage of average survival rate (%) of blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock fed a formulated diet with amaranth extracts at different doses.

Treatment	Replications	Weight (g)					H (%)
		0 days	10 days	20 days	30 days	40 days	
	1	1.00	1.00	1.00	1.00	1.00	100
	2	1.00	1.00	1.00	1.00	1.00	100
Fdiet 1	3	1.00	1.00	1.00	1.00	1.00	100
	4	1.00	1.00	1.00	1.00	1.00	100
	5	1.00	1.00	1.00	1.00	1.00	100
	Total	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**500**
	Avarage	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**100**
	SE	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	1	1.00	1.00	1.00	1.00	1.00	100
	2	1.00	1.00	1.00	1.00	1.00	100
Fdiet 2	3	1.00	1.00	1.00	1.00	1.00	100
	4	1.00	1.00	1.00	1.00	1.00	100
	5	1.00	1.00	1.00	1.00	1.00	100
	Total	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**500**
	Avarage	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**100**
	SE	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
	1	1.00	1.00	1.00	1.00	1.00	100
	2	1.00	1.00	1.00	1.00	1.00	100
Fdiet 3	3	1.00	1.00	1.00	1.00	1.00	100
	4	1.00	1.00	1.00	1.00	1.00	100
	5	1.00	1.00	1.00	1.00	1.00	100
	Total	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**500**
	Avarage	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**100**
	SE	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Treatment	Replications	Weight (g)					H (%)
		0 days	10 days	20 days	30 days	40 days	
	1	1.00	1.00	1.00	1.00	1.00	100
	2	1.00	1.00	1.00	1.00	1.00	100
Fdiet 4	3	1.00	1.00	1.00	1.00	1.00	100
	4	1.00	1.00	1.00	1.00	1.00	100
	5	1.00	1.00	1.00	1.00	1.00	100
	Total	**5.00**	**5.00**	**5.00**	**5.00**	**5.00**	**500**
	Avarage	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**100**
	SE	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Values are means ± standard errors (SE). H, survival rate; Fdiet 1, formulated diet without amaranth extract 0 ng/g crab; Fdiet 2, formulated diet enriched with amaranth extract 250 ng/g crab; Fdiet 3, formulated diet enriched with amaranth extract 500 ng/g crab; and Fdiet 4, formulated diet enriched with amaranth extract 750 ng/g crab.

Table 5. The water quality of maintenance media for blue swimming crab *P. pelagicus* (Linnaeus, 1758) female broodstock fed a formulated diet with amaranth extracts at different doses.

Water quality parameter	Range
Temperature (°C)	26.0 – 27.0
pH	7.77 – 7.96
O_2 (ppm)	7.00 – 7.30
CO_2 (ppm)	3.85 – 6.30
Water depth (cm)	25.0 – 30.0

treatments. The absolute weight gain decreased dramatically with an increase in the amaranth extract dose of 750 ng/g crab (18.05 ± 8.27 g). This dose (Fdiet 3, 500 ng/g crab) performed well, giving good results. In contrast to the findings of the Fdiet 3 treatment, a decrease occurred in the absolute weight of crabs receiving the Fdiet 4 treatment, apparently because of a treatment overdose effect, which causes hormonal imbalances in the body, thereby affecting the absolute weight gain.

Amaranth extract is a molting stimulant containing molting hormone (phytoecdysteroid). Ecdysteroids are the main steroid hormone in arthropods, which functions as a molting hormone and regulate physiological functions, such as growth, metamorphosis and reproduction[35]. This hormone is secreted by Y-organs in the form of ecdysone. The hemolymph then converts ecdysone into a 20-hydroxyecdysone (20E) active hormone, which is the enzyme 20-hydroxylase found in the epidermis of other organs and tissues. The titers of 20-hydroxyecdysone (20E) in circulation vary throughout the molting phase. Shortly after the ecdysis (molting), the titers are very low, where they remain throughout the intermolt phase.

Our results cast a new light on the administration of amaranth extract at 0-500 ng/g crab, which caused an increase in absolute carapace length (3.60 ± 1.64 - 7.09 ± 0.12 mm) and contributed to relatively large absolute carapace widths (6.25 ± 2.97- 13.14 ± 0.65 mm); at higher doses (750 mg/g crab), a drastic decrease occurred, with successive values of 1.22 ± 1.37 and 1.92 ± 2.15 mm. The increase in absolute carapace length and absolute carapace width was apparently attributable to the synergistic cooperation of the hormones contained in amaranth extract with natural hormones in the body of the crab. This is supported by Fujaya *et al.*[17], who reported that amaranth plants (*Amaranthaceae tricolor*) contain phytoecdysteroid, which acts as a stimulant for molting and the production of softshell crabs. Kuballa *et al.*[36] noted that molting is physiologically controlled by the molting hormone. Wahyuningsih[37] reported an increase in molting percentage by 54% in crabs that received vitomolt supplementation (phytoecdysteroid) at 250 ng/g crabs compared to controls that only showed a 15% increase. Meanwhile, in the study by Susanti[38], a molting percentage of 90% was obtained after the vitomolt treatment in formulated diet (933 ng/g feed) compared to only 20%. A dramatic decrease at the higher doses in this study (Fdiet 4) is attributed to the threshold of phytoecdysteroid hormone levels in the blood that affects the molting process in the crab. In addition, a decrease in protein synthesis as a result of the disruption of the physiology of the molting hormone produced was also suspected. Techa and Chung[39] suggested the most prominent metabolic action of steroids is activated protein metabolism. Preston and Dinan[40] suggest that ecdysteroids also play a role in increasing protein formation by increasing the mRNA synthesis. According to Lafont and Dinan[41], ecdysteroid also stimulates carbohydrate metabolism and lipid biosynthesis and acts as an immunostimulant and antioxidant.

It is important to note that the water quality is critical to the survival, health and growth of crabs, especially in semi-intensive and intensive culture. The physical and chemical properties of water should be kept within certain levels. According to Habashy and Hassan[42], the required water quality for the maintenance of crustaceans includes salinity, temperature and pH[43]. Moreover, salinity affects other physiological processes of crustaceans, and various indices exist to evaluate their physiological responses and metabolism, which include glucose, superoxide dismutase and acid phosphatase[44,45]. Table 5 shows

that water salinity between 30.0 to 32.0 ppt was observed in the present study. Salinity is therefore within the range that is highly favorable to the survival of the crab[46–48]. Mud crabs are highly tolerant to varying salinity conditions ranging between salinity of 10 ppt to 34 ppt[49]. Water temperature and changes in water temperatures have considerable influence on the rate of growth and survival of aquatic organisms. In this study, temperature during the observation ranged from 26.0 to 27.0°C; therefore, the temperature was in the range of support for the activities of life, growth and reproduction of crab[50,51]. The pH is a variable that is known to affect survival and development in many brachyuran species. In this study, the range of pH for survival was 7.77–7.96. The optimal pH for growth is from 8–8.5[52]. Low pH can stress crustaceans and cause soft shell and poor survival. At a pH between 7.26 and 8.00, mortality is not usually observed[7].

Many studies have been conducted on the oxygen requirements of crustaceans[52–54]. Liao and Murai[53] reported that the oxygen respiration of *P. monodon* remained constant at dissolved oxygen levels above 3.00 to 4.00 ppm at salinity 5–45 ppt and temperature 20-30°C. In the present study, dissolved oxygen was relatively high, ranging from 7.00–7.30 ppm. In heavily stocked crab ponds, the carbon dioxide can become high as a result of respiration. Boyd and Tucker[55] explain that free carbon dioxide is good for the crustacean at no more than 12 ppm and must not be less than 2 ppm. In the present experiments, carbon dioxide levels ranged from 3.85–6.30 ppm (Table 5).

Conclusion

The present study demonstrated that the enrichment of formulated diet with amaranth extracts had a significant effect on the absolute weight gain (AWG), carapace length (ACL), and carapace width (ACW) of *Portunus pelagicus* (Linnaeus, 1758) broodstock females. The enrichment of amaranth extracts in formulated diets causes an increase in the AWG (18.05-55.03 g), ACL (1.22-7.09 mm) and ACW (1.92-13.14 mm) and yielded a 100% survival rate for all treatments during the 40-day maintenance period. Based on regression analysis, a quadratic relationship was observed between the dose of amaranth extracts found in the formulated diets and the AWG, ACL and ACW.

Data availability

All data underlying the results are available as part of the article (Table 1–Table 5) and no additional source data are required.

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