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Research Article
Evaluation of Formulated Diets Enriched by Spinach Extracts for the Broodstock Females, *Portunus pelagicus* (Linnaeus, 1758)

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Abstract

Background and Objective: Spinach extract (*Amaranthacea tricolor*) is a very prospective feed raw materials to stimulate the molting and growth of crab female broodstock. The purpose of this study was to evaluate the quality of broodstock females, *Portunus pelagicus* (Linnaeus, 1758) at different levels of spinach extract based on physical, organoleptic and chemical tests. **Materials and Methods:** Four different treatments of spinach extract (0, 250, 500 and 750 ng g^{-1} crab, respectively) were used in this study. The female crab samples were collected from coastal region of Padang, West Sumatera and placed randomly in four concrete tanks (200×100×100 cm). Each concrete tank consist of five units of plastic box (45.5×32.5×16.5 cm) with the maximum density was one crab per box. **Results:** The organoleptic test showed that formulated diets enriched with spinach extract (0, 250 and 500 ng g^{-1} crab) had a smooth texture, pungent aroma and brown. Whereas from the physics test results obtained good water stability (rupture velocity ranged from 89.20-105.40 min and solids dispersion ranged from 4.97-7.17%), hardness (92.66-98.07%) and sinking velocity (3.88-5.88 cm sec^{-1}) ($p<0.05$). The results also showed that formulated diet enriched with spinach extract doses of 250-750 ng g^{-1} crab gave a value of delicacy of feed (0.195-0.386 g $crab^{-1}$ weight/day) which was significantly different ($p<0.05$) with 0 ng g^{-1} crab (0.445 g $crab^{-1}$ weight/day). The chemical test shows the moisture content were 11.60%, ash 9.31%, protein 44.38%, fat 7.64% and carbohydrate 14.46%. **Conclusion:** It was concluded that there was a linear relationship between dose of spinach extracts in formulated diet and rupture velocity and a quadratic relationship between dose of spinach extracts in formulated diet and solid disperse, hardness and sinking velocity and delicacy of feed.

Key words: *Amaranthacea tricolor*, crab, formulated diet, *Portunus pelagicus*, spinach extract, broodstock females, rupture velocity, Linnaeus, 1758

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Commodity demand for crab is increases every year, while the supply of this commodity is still depending on nature fishing[1-4]. The efforts are needed towards controlled cultivation, but the constraints of this effort are still difficult to obtain the right amount and time, because seeding technology is needed. To anticipate this problem, it need a technique to ripen the gonads of the mother crab which is the initial determinant of hatchery success. To stimulate the process of gonadal maturation, it is usually carried out by hormonal manipulation, feed and environmental manipulation[5,6]. Improvement of nutrients in artificial feed for the gonadal maturation process has been carried out on various types of fish[7]. In mangrove crabs, *Scylla serrata* has also been carried out by several researchers[8,9]. According to Millamena and Quinito[8], for the maturation process of gonadal crabs, the balance diet of Omega-3 and Omega-6 is needed. Feed tested on crustaceans (*Penaeus japonicus*) containing a mixture of soybean oil (Omega-6 source) and squid liver oil (Omega-3 source) with a ratio of 3:4% is better for the gonad maturation process compared to feed containing soybean oil or squid liver oil only[10]. Likewise, the conclusions of several other researchers stated that feed which is quality is feed with a protein content, fat, carbohydrates, minerals and vitamins that are balanced[8,9,11].

The development of the next crab cultivation technology is quite promising with the emergence of soft shell production, with selling prices reaching twice as high as hard-shelled ones[12]. The introduction of soft shell production technology to induce molting using spinach extract (*Amaranthus tricolor*) was discovered by Aslamyah and Fujaya[13]. The artificial feed enriched spinach extract (*vitomolt*) with protein content of 30.62 and 41.72% carbohydrate effectively stimulated the molting of mangrove crabs, with a percentage of molting[14] (100%).

Recently, there is no research which is related to the application of spinach extract (*A. tricolor*) to improve the nutritional quality of formulated diets for molting and growth of crab female broodstock. Therefore, this study was aimed to investigate the formulated diets enriched with spinach extract to find the best formula for molting and growth of crab female broodstock.

MATERIALS AND METHODS

Time and site: The study was conducted at Balai Benih Ikan Pantai (BBIP) Teluk Buo, Balai Benih Ikan (BBI) Bungus, Padang and the Laboratory of Animal Physiology, Department of Biology, Padang, West Sumatera from April-July, 2018.

Experimental design and formulated diet: The method used in this study is completely randomized design (CRD) with four treatments and five replications of dietary with spinach extracts. They were treatment with Fdiet 1, formulated diet without spinach extract 0 ng g^{-1} crab; Fdiet 2, formulated diet enriched with spinach extract 250 ng g^{-1} crab; Fdiet 3, formulated diet enriched with spinach extract 500 ng g^{-1} crab; and Fdiet 4, formulated diet enriched with spinach extract 750 ng g^{-1} crab. Formulated diet[15-17] is a modified formulation (Table 1) for the broodstock of mud crab, *Scylla serrata*[8]. Spinach extract was dissolved with 80% ethanol with the ratio 1:1 then homogenized. The solution was added 80% ethanol to 20 mL kg^{-1} of feed and sprayed evenly into the test feed, then the feed was dried. The test feed is stored until it is ready for use[13].

Organoleptic test: Organoleptic testing includes texture, odour and color of feed. The texture of the feed can be seen from the surface of the feed is smooth, fibrous or perforated. The odour of feed determines the quality of the feed as it is closely related to the recipient or the allure of the crab to the feed. The odour of the feed is determined by making the category not pungent, pungent and quite pungent. Feed color depends on the type of raw materials used[18].

Physical test: Physical tests include the stability of the feed in water, the level of hardness, the sinking velocity and the delicacy of feed[18].

Stability of feed in water: The stability test in water is a test of the endurance level of feed in the water or how long it takes until the feed is mushy and crumble. The stability of

Table 1: Modified composition of broodstock formulated diet for females blue swimming crab, *P. pelagicus* in g/100 g dry diet

Ingredients	Diet (g/100 g)
Fish flour	20
Clam flour	20
squid flour	20
wheat flour	17
Seaweed	4
Cod liver oil	5
Lesitin (lecithin)	3
Cholesterol	1
Vitamin mix	3
Mineral mix	4
Dicalcium phosphate	3
Vit. E (IU kg^{-1} feed)	600

feed in water including rupture velocity and dispersion of solids. The crash test measures how long it takes until the feed is destroyed in the water. This rupture test was observed visually. Included feed 10 bars into a beaker filled with 1 L of water, observations made every 5 min to know the feed was soft or not. Observation continues until the feed is broken/destroyed. The solid dispersion is carried out by feeding 10 g inserted into a 6×6×6 cm gauze box with pores of about 1 mm, then immersed in the aquarium for 2 h then lifted and dried under the sun. After that, it is weighed to a constant weight. The solid dispersion is calculated using the formula:

$$\text{Solid dispersion (\%)} = \frac{\text{Dry weight of initial feed-Dry weight of final feed}}{\text{Dry weight of initial feed}} \times 100$$

Hardness level test: The feed hardness rate test was measured by feeding 5 g of feed into a paralon pipe with a height of 1 m. Then the feed is loaded with a weight of a weighing 500 g. Feed that has been loaded then sieved using a sieve of 0.5 mm. The level of hardness is calculated in the percentage of non-destructive feed using a sieve.

Sinking velocity test: The sinking velocity test is performed by measuring the length of time it takes for feed to move from the water surface to the bottom of the maintenance medium. Feed five pellets put into a beaker glass with a bottom height of the container 20 cm from the surface of the water. The stopwatch is executed just as the feed is dropped into the water surface. The sinking speed is the distance in the feeding time until it is based on the measuring cup.

Delicacy feed test: The delicacy feed test was measured from the amount of feed consumed per crab body weight[19]. The female crab samples were collected from coastal region of Padang, West Sumatera and placed randomly in four concrete tanks (200×100×100 cm) which each put five units in a plastic box (45.5×32.5×6.5 cm) at maximum density of one crabs per box. Tanks were provided with substrate of around 15 cm thick layer of sand and adequate aeration[2,11,20]. The crabs were maintained in monitored water depth of 25-30 cm with the salinity of 30-32 ppt, pH 7.77-7.96, temperature 26-27°C and DO 7.00-7.30 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. Formulated diets made from food wastes enriched by spinach extracts were fed daily at 3% biomass (1700-1800 h) and uneaten food was removed every morning.

Chemical test: Chemical test of feed includes determination of quantity and quality of nutrition in feed which is determination of proximate composition of protein, fat, ash, water, coarse fiber and NFE content in test feed[21]. This method refers to the test method of BSN[22].

Data analysis: The Organoleptic data were analyzed descriptively by comparing the results obtained between treatments with supporting literature. While the data physical and chemical tests were analyzed by using one way ANOVA and then Duncan's test to know the difference between treatment[23]. The standard deviation of each parameter and treatment was determined and expressed as the Mean±SE. The treatment effects were considered to be significant at $p<0.05$. Arcine transformation is performed before analyzing data in percentage form. The relationship between dose of spinach extracts in formulated diet and physical tests were analysed using statistical computer software (version 19.0 for Windows, SPSS Inc., Chicago, IL), for significant correlation between them.

RESULTS

Organoleptic test: Based on the organoleptic test, the results have showed that different texture of diet treatment. The texture treatment of Fdiet 1 has a smooth texture, while the treatment of Fdiet 2, 3 and 4 has a fibrous texture. Organoleptic observations of feed odour also showed differences. The odour of treatment Fdiet 1 has a strong odour, Fdiet 2 and 3 smell quite stinging, while Fdiet 4 treatment has a non-stinging odour. Furthermore, the 3rd organoleptic test is the color of the feeds test. The results showed that the same result for all samples which the color is brown (Table 2).

Physical test: Observations of physical tests include: (a) The stabilization of feed in the water (rupture velocity and solid dispersion), (b) Level of feed hardness, (c) Velocity of feed sinking and (d) Delicacy of feed.

Stabilization of feed in the water

Rupture velocity: The rapture velocity at different doses of spinach extract was generally longest ranging from 89.20±3.63 to 105.40±3.55 min. The rapture velocity (min) decreased with increasing dose of spinach extract ($p<0.05$). The longest rapture velocity was achieved at 0 and 250 ng g^{-1} crab, whereas the lowest ($p<0.05$) rapture velocity occurred at 750 ng g^{-1} crab with 89.20±3.63 min (Table 2). The relationship between dose of spinach extracts in

Table 2: Organoleptic and physical tests of formulated diets made from food wastes enriched by spinach extracts for the broodstock females blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758)

	Treatments (n = 5)					
Parameters of the test	Fdiet 1	Fdiet 2	Fdiet 3	Fdiet 4	Regression equation	R^2
Organoleptic test						
Texture	Smooth	Fibrous	Fibrous	Fibrous		
Odour	Pungent	Quite pungent	Quite pungent	No pungent		
Color	Light brown	Light brown	Light brown	Light brown		
Physical test						
Stabilization of feed in water						
Rupture velocity (min)	105.40±3.55^{a}	100.20±2.95^{ab}	94.20±1.64^{b}	89.20±3.63^{c}	RV = -0.0218DSE+105.44 (Linear)	0.5563
Solids disperse (%)	6.69±0.37^{ac}	6.01±0.40^{ab}	4.97±0.22^{b}	7.17±0.52^{c}	DS = 1×10^{-5} DSX^2-0.0085DSX+6.8732 (Quadratic)	0.4431
Hardness (%)	92.66±1.25^{a}	95.54±0.57^{b}	98.07±0.36^{c}	93.04±1.06^{a}	H = -3×10^{5} DSE^2+0.0252DSE+92.299 (Quadratic)	0.5636
Sinking velocity (cm sec^{-1})	3.88±0.36^{a}	5.20±0.19^{b}	5.88±0.16^{c}	4.97±0.17^{b}	SV = -9×10^{-6} DSE^2+0.0083DSE+3.8288 (Quadratic)	0.7313
Delicacy of feed (g $crab^{-1}$ weight/days)	0.445±0.273^{b}	0.195±0.075^{a}	0.129±0.024^{a}	0.386±0.141^{a}	DF = 2×10^{-6} DSE^2-0.0016DSE+0.4518 (Quadratic)	0.1724

Means within a given column with different superscripts were significantly different (p<0.05). Values are means±standard errors (SE). n = Replication, Fdiet 1: Formulated diet without spinach extract 0 ng g^{-1} crab, Fdiet 2: Formulated diet enriched with spinach extract 250 ng g^{-1} crab, Fdiet 3: Formulated diet enriched with spinach extract 500 ng g^{-1} crab and Fdiet 4: Formulated diet enriched with spinach extract 750 ng g^{-1} crab

Table 3: Proximate composition of formulated broodstock diet for females blue swimming crab, *P. pelagicus* without spinach extract on a dry matter basis

Proximate composition (%)	Formulated diet (%)
Moisture	11.60
Ash	9.31
Crude protein	44.38
Fat	7.64
Carbohydrate	14.46

formulated diet and rupture velocity was linear with an equation of RV = -0.0218DSE+105.44. This relationship was found to be highly significant (R^2 = 0.5563; p<0.05).

Solids disperse: The doses of spinach extract (0, 250 and 500 ng g^{-1} crab) significantly influenced (p<0.05) the percentage of solid dispersion in formulated diet. The highest percentage of solid dispersion was achieved at spinach extract levels of 0, 250 and 750 ng g^{-1} crab (6.01±0.40 to 7.17±0.52%), whereas the lowest percentage of solid dispersion is found at 500 ng g^{-1} crab (4.97±0.22%). The relationship between dose of spinach extracts in formulated diet and solid disperse was found to be quadratic (DS = 1×10^{-5} DSX^2-0.0085DSX+6.8732; R^2 = 0.4431; p<0.05) (Table 2).

Hardness level: Treatment of different doses of spinach extract (0, 250, 500 and 750 ng g^{-1} crab) showed different effects on hardness level in formulated diet (Table 2). The highest (p<0.05) percentage of hardness level was achieved by spinach extracts in formulated diet at 500 ng g^{-1} crab (98.07±0.36%) compared to hardness level at 0 ng g^{-1} crab (92.66±1.25%), 250 ng g^{-1} crab (95.54±0.57%) and 750 ng g^{-1} crab (93.04±1.06%). A quadratic relationship of H = -3×10^{5} DSE^2+0.0252DSE+92.299; R^2 = 0.5636; p<0.05) was found between dose of spinach extracts in formulated diet and hardness.

Sinking velocity: The sinking velocity of Fdiet 3 (5.88±0.16 cm sec^{-1}) revealed the increase (p<0.05) at doses of spinach extract 500 ng g^{-1} crab, whereas the lowest (p<0.05) sinking velocity occurred at 0 ng g^{-1} crab with 3.88±0.36 cm sec^{-1} (Table 2). The relationship between dose of spinach extracts in formulated diet and sinking velocity was found to be quadratic (SV= -9×10^{-6} DSE^2+0.0083DSE+3.8288; R^2 = 0.7313; p<0.05).

Delicacy of feed: Data (Table 2) showed that doses of spinach extract in formulated diets (p<0.05) affected delicacy of feed. The lowest dose of spinach extract (0 ng g^{-1} crab) resulted in the highest feed delicacy (0.445±0.273 g $crab^{-1}$ weight/days). Regression analysis showed the relationship to be quadratic (DF = 2×10^{-6} DSE^2-0.0016DSE+0.4518; R^2 = 0.1724; p>0.05).

Chemical test: Based on the results of chemical testing with the proximate analysis of the test feed in Table 3. The results showed that the nutritional composition of the test feed were containing 11.60% moisture content, 9.31% ash, 44.38% protein, 7.64% fat, 14.46% carbohydrate and which is a fairly balanced percentage with the needs of blue swimming crabs.

DISCUSSION

The application of spinach extract on formulated diets have an effect on the textures and odour of diet. Then, the composition of the raw materials and appearance also have an effect on the texture of feed. The surface texture of feed showed smooth, fibrous or perforated[24]. Furthermore, the fineness of the raw material, the amount of fiber and the binder used in the composition have an effect on the texture of formulated diet. The texture of feed and the level of fineness of the raw material have affected the compactness and flatness[22]. The different of feed odour has related to the composition of raw materials such as; spinach extract doses. According to previous report, the type and amount of spinach extract added to the process making of feed has effect to odour[24]. Furthermore, the good formulated diet has natural scent odour and taste[7,25]. The colour level of the formulated diets (Table 2) was within the acceptable limits (light brown). There was no different colour in each diet, because, the different of spinach doses extracts has no effect on feed composition. Similar results with previous study, the colour of formulated diet has related or depends on the type of raw material[24].

The rupture velocity of formulated diet is strongly affected by the fineness of raw materials[26]. Furthermore, the rupture velocity of pellets can be greatly improved by proper selection of feed ingredients, processing techniques and the use of proper processing equipment[27]. The lowest value of rupture velocity (Fdiet 4) is found at doses spinach extract 750 ng g^{-1} crab. The lower rapture velocity at high doses spinach extract may be due to the feed raw material to be less bound or less compact which results in accounting for the difference in water stability. The rapture velocity of feed is influenced by several factors, such as the amount and type of raw material[28]. The results from this study showed spinach extract to be water stable pellet than those by Solomon *et al.*[29] studies who produced a water stability of around 56-57 min on feed made from local carbohydrate sources and added with *Saccharomyces cerevisae* yeast. However, it is still lower than the general requirements of crustacean feed. According to a report, the stability of feed in water ranges[28] from 3-5 h.

The high doses of spinach extract 750 ng g^{-1} crab provided the highest percentage of solid dispersion (7.17±0.52%), while the lowest value (4.97±0.22%) was observed at doses of spinach extract 500 ng g^{-1} crab. The level of solid dispersion should be no more than 10%, because it greatly affected the quality and quantity of nutrients contained in the feed[30]. In general, all the formulated diets in this study already have good solid dispersion values (4.97±0.22-7.17±0.52%) compared to the Aslamyah and Karim[24] studies who produced solid dispersions above 10% (11.14-11.87%).

In the present study, the results indicated that the highest level of hardness was found in the Fdiet 3, which was 98.07% because its caused of the fineness of the feed and the composition used were balanced. The level of hardness of feed is influenced by the fineness of the particles and the level of density of particles as often as the constituent ingredients of feed[19]. The lowest percentage level of hardness in the Fdiet 1 was 92.66%. The difference may be due to feeding of spinach added which made the feed a little more compact. Therefore, it can be concluded that low percentage was included in the level of good hardness as the level of hardness of treatment above 80% was definitely high[24]. Meanwhile, other studies showed that good pellets are harder than usual pellets[28]. The range of levels of feed hardness obtained in this study was almost same as that obtained from tiger shrimp feed containing several types of seaweed flour as adhesive[19], namely 92.27-94.67%.

Throughout the monitoring period, the treatment of Fdiet 3 with the addition of spinach extract as much as 500 ng g^{-1} crab had the highest and best sinking velocity of 5.88 cm sec^{-1}, followed by Fdiet 2 and 4, respectively. Spinach extract may directly inclusion increased the specific gravity of the feed so that the feed will sink faster. Specific gravity of feed has been reported by Mudjiman[28], feed buoyancy has to do with it, the greater specific gravity of the feed compared to the specific gravity of water the faster it will sink. It was also reported that shrimp, catfish and demersal fish pellets should sink faster than usual. The Fdiet 1 treatment has the longest sinking velocity of 3.88 cm sec^{-1}. Therefore, it can be concluded that effect of spinach are increase in volume of feed composition[28].

In the short term study reported that the delicacy test of feed is increased by attractants and odours by it contains[19]. The findings of current study were also supported by previous reports that good quality food has a distinctive odour that is favored by culture. The low tasty power in the Fdiet 1 treatment with a value of 0.445 g was thought to be without additives which spinach extract had a pungent odour not liked by the crab, so that it had low tasty power[25, 26].

Current study showed the result of proximate composition of the formulated diet. The result showed that the nutritional composition of the test feed contains a fairly balanced percentage with the needs of crabs. The formulated diet has lower moisture content. Moisture content is within the recommended value (<12%)[18,31]. Moisture content of formulated diet is an important parameters of their

susceptibility to microbial spoilage. When the moisture content is on the high side, it encourages the growth of micro-organisms[7]. Ash content of the formulated diet was relatively low (9.31%). The BSN recommended (≤13%) ash contents for the formulated diet[18]. In addition, Piedecausa *et al.*[32] and Ayuba and Iorkohol[33] recommended ash content of fish formulated diets are lower than recommended value by BSN[18]. This might be due to the ash effect on digestibility and growth[34-36].

High protein content of diet is thought to be due to the high protein content of the raw materials for the formulated diet used, such as fish meal, squid and shellfish[8,37]. Protein content of diet is within the recommended (40-50% in dry form for crab growth)[2,8,17,38]. The fat content of the diet was obtained 7.64%, this contents are within the recommended value[18] of BSN of ≥5%. Low fat content of formulated diet may be necessary to prolong its shelf life also by avoiding lipids peroxidation. Furthermore, the high fat content will cause side effects that decrease food consumption, growth and liver degeneration[10]. Thus, the optimal fat requirement of omnivorous cultivation group[39] is 6-8%. Carbohydrates, just like fats are needed for energy. Carbohydrates content of the formulated diet was relatively lower than recommended value by Alava and Pascual[40] (25-30%). The carnivorous group cultivation needs around 10-20% carbohydrates[39].

CONCLUSION

Based on organoleptic, physical and chemical tests on evaluated feed, it was showed that the enrichment of spinach extract in different formulated diets have produced different feed quality. The proximate analysis showed that the nutrients of feed tests were within the range requirement blue swimming crab. There was a linear relationship between dose of spinach extracts in formulated diet and rupture velocity and a quadratic relationship between dose of spinach extracts in formulated diet and solid disperse, hardness, sinking velocity and delicacy of feed.

SIGNIFICANCE STATEMENT

Formulated diet is an alternative media application of spinach extract (SE) which is known containing molting stimulant. However, the utilization of the formulated diet is an expensive fed with a very high protein concentration and it's mainly produced from fish based materials. Therefore, it is needed further study regarding formulated diet which substitute with vegetable materials that could be used for stimulated the molting and growth of blue swimming crabs.

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