Organoleptic, physical and chemical tests of formulated feed for *Panulirus homarus*, enriched with spinach extract

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Abstract. Spinach extract contains moulting hormone (phytoecdysteroid) which functions as a skin moulting hormone and regulates physiological functions, such as: growth, metamorphosis and reproduction in arthropods. The purpose of this study was to evaluate the quality of formulated feed for *Panulirus homarus* enriched with spinach extract based on organoleptic, physical and chemical tests. Five treatments with different doses of spinach extract (0; 0.4; 0.5; 0.6 and 0.7 mg) were used in this study. The parameters observed were organoleptic, physical and chemical. Data analysis used the Kruskal Wallis test for organoleptic parameters, the ANOVA test for physical parameters and the descriptive test for chemical parameters. Kruskal Wallis's analysis showed that spinach extract had a significant effect on the aroma of the test feed ($P<0.05$) and had no significant effect on the color and texture of the test feed ($P>0.05$). The one-way ANOVA results showed that adding spinach extract to the artificial feed had a significant effect on breaking speed, solids disperse, hardness level, sinking speed, attractiveness and deliciousness ($P<0.05$). Physical testing of each parameter showed a breaking speed of 293.35-419.28 min, a density dispersion of 7.56-9.40%, a hardness level of 78.80-99.58%, a sinking speed of 4.94-6.99 cm sec^{-1}, an attractiveness of 3.27-5.21 cm sec^{-1} and the feed deliciousness of 0.15-0.21 g $lobster^{-1}$ day^{-1}. The chemical analysis of the feed showed a water content between 11.94 and 14.87%, an ash content between 14.15 and 14.85%, a crude protein between 39.74 and 42.6%, a crude fat between 3.18 and 4.63%, carbohydrates between 24.77 and 29.29% and crude fiber between 2.06 and 3.73%. Based on the organoleptic, physical, and chemical tests, it was shown that the artificial feed enriched with spinach extract at different concentrations resulted in different feed quality.
Keyword: artificial feed quality, organoleptic, physical, chemical parameters.

Introduction. Lobster is a high-value fishery commodity that is always in high demand on the world's commercial fish markets. Demand and consumption of lobster continues to increase from 2012, exports in Indonesia reached 2,428 tons with a value of around US $ 28 million, while exports of frozen lobster reached 584 tons with a value of US $ 5.5 million (Supriyono et al 2017; Wahyudin et al 2017). Lobster has a high nutritional value which causes a high demand in the global market, addressing the needs of the tourism and food service industries (Larasati et al 2018; Subhan et al 2018). The increasing activity of catching spiny lobsters risks to affect the balance of the population. Unsustainable exploitation of a species for trade will result in its population decline (Zakaria et al 2019; Zakaria et al 2020), eventually leading to the extinction of species or to an imbalance in the males to females ratio. The community of lobster cultivation still faces various obstacles, including the availability of feed, diseases and relatively long maintenance times. The increasing activity of catching and exporting spiny lobsters can lead to a decrease in the stock if the management is less focused and controlled. Some researchers have reported slow growth and low survival as issues in cultivation, caused by several factors such as: disease, molting syndrome, cannibalism and feed (Efrizal et al 2019; Efrizal et al 2020).

Efrizal et al (2019) stated that feed is the main component in cultivation, so that the completeness of nutrition in the feed is necessary for the survival and growth of a species. The nutritional requirements for lobster growth are protein, fat, carbohydrates, vitamins and minerals, while the feed type and size differ based on the age of the lobster.

The development of artificial feed in lobster cultivation activities needs to be carried out in order to reduce the trash fish. Spinach extract phytoecdysteroid functions as a skin molting hormone and regulates physiological functions such as: growth, metamorphosis and reproduction in arthropods (Aslamyah & Fujaya 2011; Efrizal et al 2019). Efrizal et al (2019) stated that 500 ng g^{-1} of spinach extract in artificial feed can increase growth with no mortality during the maintenance of *Portunus*. According to Aslamyah & Fujaya (2011), spinach extract contains ecdysteroid and its application by injection at a concentration of 700 ng g^{-1} can accelerate molting and does not cause death in *Scylla serrata*.

There is no research on feed development in order to improve the survival and growth of *Panulirus homarus*. Therefore, it is necessary to evaluate the quality of *P. homarus* feed enriched with spinach extract based on organoleptic, physical and chemical tests.

Material and Method

Time and sites. Organoleptic and physical tests were carried at the research, practice and training laboratory, while chemical tests were performed at the chemistry laboratory of Bung Hatta University, Padang, West Sumatera, Indonesia. This research was conducted from July to October 2020.

Experimental design and formulated feed. The research method used was experimental. with a completely randomized design (CRD) using 5 treatments with 5 replications. The treatments in this research were feed A, formulated without spinach extract 0 mg; feed B, a formulated feed with 0.4 mg spinach extract; feed C, a formulated feed with 0.5 mg spinach extract; feed D, a formulated feed with 0.6 mg spinach extract and feed E, a formulated feed with 0.7 mg spinach extract. The feed formulation was obtained by the trial and error method, by mixing fish flour, mangrove shell, squid, flour, wheat, shrimp heads, beef liver, soybeans, fish oil, vitamin and mineral mix, vitamin C and tapioca into a dough, then pelleted. Spinach powder for each treatment was put into a spray bottle and dissolved with 80% ethanol in a ratio of 1:1, until homogeneous. To the homogeneous solution, 20 mL of 80% ethanol were added and sprayed evenly onto the artificial feed, then the feed was dried and air-dried. Once dry, the feed is ready to be given to the sand lobsters. The test feed was stored until used (Aslamyah & Fujaya 2011; Efrizal et al 2019).

Variables assessed. Organoleptic testing included the texture, aroma, and color of feeds. The texture of the feeds can be seen from the surface of the feedsand it can be smooth, fibrous or hollow. The aroma of feeds determines the quality of the feed because it relates to the lobster recipients of the feed. The aroma of the feeds coorespond to the categories: not pungent, pungent and quite pungent. The color of the feed depends on the type of the raw material used. Physical parameters carried out on the test feed are the breaking speed, solids disperse (products containing at least two distinct components), hardness level, sinking speed, allure and tasty power. The physical testing technique for the test feed refers to the tests carried out by Efrizal et al (2019). Feed chemical tests were carried out to determine the quality of the given test feed. The parameters tested were moisture, ash, crude protein, fat, carbohydrates and crude fiber.

Statistical analysis. Organoleptic data were analyzed using the Kruskal Wallis test and the physical data were analyzed using the ANOVA test. To determine the differences between the treatments the Duncan test was used. Chemical data were analysed descriptively, by comparison with the results of other literature studies.

Results

Organoleptic test. Based on the results of the Kruskal Wallis analysis, spinach extract had a significant effect on the aroma of the test feed ($P<0.05$), but had no significant effect on the color of the test feed and on the texture of the test feed ($P>0.05$). The organoleptic test results are shown in Table 1.

Table 1
Organoleptic formulated feed enriched with spinach extract for *Panulirus homarus*

Test parameters	*A*	*B*	*C*	*D*	*E*
Texture	Fibrous	Fibrous	Fibrous	Fibrous	Fibrous
Aroma	Quite pungent^{a}	Quite pungent^{a}	Pungent^{b}	Pungent^{b}	Pungent^{b}
Color	Light brown	Light brown	Light brown	Light brown	Light brown

Different superscript letters on the same line indicate a marked difference A-artificial feed without spinach extract (control); B-0.4 mg of spinach extract in 1 kg of artificial feed; C-0.5 mg of spinach extract in 1 kg of artificial feed; D-0.6 mg of spinach extract in 1 kg of artificial feed; E-0.7 mg of spinach extract in 1 kg of artificial feed.

Physical test. One way ANOVA results showed that adding spinach extract to the artificial feed had a significant effect on breaking speed, solids disperse, hardness level, sinking speed, attractiveness and deliciousness ($P<0.05$). The longest average test feed breaking rate was noticed for the treatment D (419.28±12.12 min) and the fastest with the treatment E (293.35±18.56 min). The highest average solids disperse was measured in the treatment E (9.40±0.37%) and the lowest in the treatment C (7.56±0.38%). The highest average level of hardness was recorded in the treatment D (99.58±0.13%) and the lowest in the treatment A (78.80±1.47%). The average fastest sinking speed of feed was observed in treatment D (4.94±0.16 cm s^{-1}) and the longest in treatment E (6.99±0.19 cm s^{-1}). The average of the fastest feed attractiveness was determined in treatment A (3.27±0.16 cm s^{-1}) and the longest in treatment E (5.21±0.15 cm s^{-1}). The highest average of feed tastefulness power was observed in treatment A (0.21±0.001 g $lobster^{-1}$ day^{-1}) and the lowest in treatment E (0.15±0.002 g $lobster^{-1}$ day^{-1}). The physical test results are presented in Table 2 and the aspect of the test feed given to *P. homarus* can be seen in Figure 1.

Figure 1. The test feed given to *Panulirus homarus* (original).

Chemical test. The water content of the test feed had levels of 11.94-14.87%. The ash content in the test feed ranged from 14.15 to 14.85%. Crude protein content ranged from 39.74 to 42.60%. The range of crude fat in this study was of 3.18-4.63%. The crude fiber in the test feed ranged from 2.06 to 3.73%. The carbohydrate value content ranged from 24.77 to 29.29%. The chemical test results are seen in Table 2.

Table 2
Physical and chemical test results for the formulated feed enriched with spinach extract for *Panulirus homarus*

Test parameters	A	B	C	D	E
			Physical tests		
Breaking rate (min)	322.96±18.94^{b}	382.47±16.99^{c}	395.74±11.35^{c}	419.28±12.12^{d}	293.35±18.56^{a}
Solids disperse (%)	9.20±0.37^{c}	8.36±0.26^{b}	7.56±0.38^{a}	9.20±0.37^{c}	9.40±0.37^{c}
Hardness (%)	78.80±1.47^{b}	81.98±0.85^{b}	99.18±0.42^{c}	99.58±0.13^{d}	88.44±1.29^{d}
Sinking speed (cm s^{-1})	5.35±0.16^{b}	6.56±0.20^{c}	5.48±0.15^{b}	4.94±0.16^{a}	6.99±0.19^{d}
Attractiveness (cm s^{-1})	3.27±0.16^{a}	3.42±0.11^{a}	4.71±0.18^{c}	3.88±0.10^{b}	5.21±0.15^{d}
Deliciousness (g lobster^{-1} day^{-1})	0.21±0.001^{d}	0.16±0.005^{a}	0.20±0.001^{c}	0.17±0.005^{b}	0.15±0.002^{a}
			Chemical tests		
Water (%)	11.94	12.56	14.87	12.25	14.20
Ash (%)	14.15	14.48	14.50	14.73	14.85
Crude protein (%)	39.74	41.08	42.12	42.60	42.10
Fat (%)	4.63	3.24	3.21	3.45	3.18
Crude fiber (%)	3.73	3.15	2.43	2.09	2.06
Carbohydrate (%)	29.29	28.69	24.77	26.48	25.19

Different superscript letters on the same line indicate a marked difference A-artificial feed without spinach extract (control); B-0.4 mg of spinach extract in 1 kg of artificial feed; C-0.5 mg of spinach extract in 1 kg of artificial feed; D-0.6 mg of spinach extract in 1 kg of artificial feed; E-0.7 mg of spinach extract in 1 kg of artificial feed.

Discussion. Spinach extract is applied by spraying it after the feed is pelleted, so that it does not change the texture and color of the feed. Ethanol is a volatile, colorless, organic solvent commonly used in extracting natural dyes from various plants (Chemat et al 2019; Masturi et al 2017). The texture quality of the artificial feed is influenced by the ingredients, especially by the crude fiber content and by the adhesive used, which in this research is the tapioca flour. The tapioca flour contains amylose and amylopectin in high concentrations; when heated it becomes a substance that can glue particles together and the resulting artificial feed will be solid. The resistance to the feed-in water is influenced by the type of adhesive used. Feed that can last a long time in the water is used optimally by organisms, increasing the feed consumption, growth and productivity in cultivation businesses. On the other hand, low feed resistance causes waste of feed and contaminates the culture media (Marchese et al 2019; Saleela et al 2015).

Chemat et al (2019) stated that the ethanol used as a solvent in the extraction process is non-toxic, besides it has a high polarity so that it is easy to dissolve resin compounds, fats, oils, fatty acids, carbohydrates and other organic compounds present in spinach. The spinach extract, when applied to the test feed in different concentrations determines differences in the generated aroma. If the concentration of spinach extract given is higher, it reduces thereby the distinctive aroma of the test feed. However, the feed enriched spinach extract did not cause significant color changes. The color of all test feeds was light brown. That caused the test feeds contains high protein. High protein levels will produce a brown material called melanoidin, through the Maillard reaction or the non-enzymatic browning, the lipid oxidation and the interaction between amino acids and lipid oxidation products (Verma et al 2020). The accumulation of brown pigments is an indication that shows the Maillard reaction in foods containing protein and carbohydrates (Verma et al 2020; Yu et al 2018).

An artificial feed with low water stability causes the feed to be easily crushed and not eaten by *P. homarus*. The test feed in this study has a good breaking speed value.

Efrizal et al (2019) stated that, in general, the durability of shrimp feed in water ranges from 3-5 hours. The longer it takes to destroy the feed, the higher the cohesiveness of the artificial feed and the less soluble the feed nutrients. One of the factors damaging the pellets is a bad texture. Good feed texture is related to the feed speed of breaking. The adhesive material is needed to bind the feed ingredients so that they have a compact structure (Marchese et al 2019; Saleela et al 2015).

Solids disperse greatly affects the quantity and quality of the test feed. An artificial feed with low water stability causes the feed to be easily crushed and dispersed so that it cannot be eaten. High water stability (not easily destroyed) allows feed to last longer in water so that when it is eaten, no nutritional content is lost due to the destruction of the pellets (Johnston & Johnston 2007). The artificial feed dispersion value of more than 10% prevents its consumption by the aquatic biota. The factors that affect the feed stability in the water are the fine granularity of the feed raw materials and the process of mixing the ingredients when making the feed (Efrizal et al 2019).

The granularity of the feed ingredients used is quite good. These are smooth and will be better mixed to produce more compact and stable pellets (Francis et al 2014). Smooth raw materials form a homogeneous mixture. The compact feed texture will withstand the effects of strong pressure during the transportation and storage and will not break easily in water (Aslamyah & Fujaya 2011). The results of this study indicate that the hardness level is 78.70-99.58%, higher than in the research conducted by Efrizal et al (2019) where it ranged between 75-89%. Smooth raw materials provide an advantage in increasing the stability of feed raw materials during the storage, also facilitating the processes of mixing the ingredients and of feed molding. Fine raw materials form a homogeneous mixture so that the feed does not break easily (Aslamyah & Fujaya 2011).

The size of the test feed is longer than the size of the commercial feed, influencing the sinking speed, which explains the differences between treatments. The sinking rate depends on the size, shape and density. The size of the test feed is used to adjust the eating habits of the sand lobsters at the bottom of the waters, so that the lobsters can use the feed more quickly. In lobsters, the process of eating begins by tearing or cutting the food into smaller pieces using the large claws, then the pieces are inserted into the mouth using the walking leg the closest to the lobster mouth. Saleela et al (2015) stated that crustacean feed faster when the feed reaches faster the bottom of the maintenance container. The sinking speed is related to the rate of the water absorption by the feed, which is determined by the characteristics of the binder used (Marchese et al 2019).

The spinach extract aroma slightly eliminates the distinctive odor of the test feed itself, so that each treatment has a different rate of attraction, depending on the concentration of the spraying quantity of spinach leaf extract. According to Haryono et al (2015), one of the factors that motivates the cultivates to approach or consume the feed is the presence of a strong aroma or a substance that functions as an attractant. The more stinging the test feed aroma, the faster the test fish response. The ingredients with a strong aroma in this study were the shrimp head meal, fish meal and fish oil, containing the amino acid glycine, which is an attractive ingredient stimulating the lobsters to approach and consume the test feed (Efrizal et al 2019; Marchese et al 2019; Saleela et al 2015).

The highest level of delicacy obtained by treatment A was followed by the *P. homarus* response during feeding. *P. homarus* will immediately eat the A given feed. Because in treatment A the spinach extract was not applied on the test feed, the distinctive aroma of the feed was not reduced, so that the *P. homarus* quickly approached the feed to consume it and produced the intense perception of deliciousness (Nisa et al 2013). The hard texture of the feed and the difficulty to eat also reduces the level of deliciousness. Nelson et al (2006) stated that one indicator of feed deliciousness perception was the response of lobsters during feeding. If *P. homarus* reduced its response, it means that it was full. *P. homarus* use the feed faster before the physical and chemical quality of the feed decreases as a consequence of the influence of water as a solvent, making feed utilization suboptimal. Spinach extract affects the deliciousness intensity and appetite of the test *P. homarus*. The nutritional quality and the quantity of spinach extract in all test feed samples provided different appetite stimuli to the test *P. homarus*. When feed resides longer in the water, in addition to reducing the quality and quantity of nutrients, it also reduces the attractiveness of the pellets (Cox & Johnston 2003).

The difference of water content in each feed is due to the provision of spinach extract, which is attached to the test feed particles, adding wet ingredients. Simon (2009) stated that excessive water content can reduce feed resistance so that feed is easily damaged due to the mold growth and to a decreased nutritional content. Unused feed must be stored in suitable containers to prevent nutrient degradation and feed damage caused by environmental factors in the storage area and by the condition of the feed itself. The appropriate water content will prevent feed being overgrown with fungi, so that the shelf life of the feed can be maximized (Ruscoe et al 2005).

The ash content in the feed is an indicator of the amount of mineral content in the feed (Powell et al 2017). The higher the value of the ash content, the higher the inorganic content in the product. The components of inorganic materials in a material vary greatly in both type and quantity. The content of inorganic substances contained in a material includes calcium, potassium, phosphorus, iron, magnesium and others (Castell & Budson 2011; Efrizal et al 2019). Protein is an important element for aquatic biota, which in the artificial feed is provided by the fish meal (Castell & Budson 2011). The protein content of the test feed came from the raw materials of the feed and was influenced by the applied quantity of spinach extract. Materials that have high protein in this study are fish meal and shrimp head meal. The protein content of the test feed is classified as high and appropriate to *P. homarus* feeding requirements, ranging between 25-55% (Aslamyah & Fujaya 2011; Efrizal et al 2019).

Fat is a high energy storage used for the growth by water biota because it tends to have a low carbohydrase enzyme activity in the digestive tract. Fat is also a source of essential fatty acids and a solvent for several fat-soluble micronutrients such as vitamins A, D, E and K. The need for fat content in the feed is around 9% for seawater biota (Cox & Johnston 2003; Marchese et al 2019; Nelson et al 2006). Crude fiber is an organic material, insoluble in weak acids and bases, consisting of cellulose, hemicellulose and lignin. The source of crude fiber comes from plants that are resistant to breakdown by enzymes in the digestive tract so that it cannot be absorbed. Crude fiber functions to maintain the function of the digestive tract and improve nutrient absorption (Francis et al 2014; Haryono et al 2015; Verma et al 2020). Crude fiber contains carbohydrates that cannot be digested and is not an essential nutrient for the marine fish. Crude fiber will cause contamination in the culture container, but it is still needed to facilitate feces removal. Too much crude fiber (>10%) will result in a decreased digestibility and absorption, and in an increased amount of metabolic waste, altering the quality of culture water (Efrizal et al 2019).

In the shrimp body, carbohydrates function as a Krebs cycle, glycogen storage, formation of chitin substances, formation of steroids, and fatty acids (Aslamyah & Fujaya 2011; Efrizal et al 2019). Based on the statement of Rahman et al (2018), aquatic animals with omnivorous eating habits need about 10-50% carbohydrates in their feed for growth. This means that the test feed has met the carbohydrate needs of *P. homarus*. Efrizal et al (2019) stated that the carbohydrates' recommended level in the feed range from 25-30%.

Conclusions. Based on the organoleptic, physical and chemical tests, it was shown that the artificial feed enriched with spinach extract at different concentrations resulted in feed quality variability. The organoleptic test showed that the spinach extract applied to artificial feed had a significant effect on the aroma, but had no significant effect on the texture and color of the feed. There was a significant effect in the artificial feed enriched with different concentrations of spinach extract on the breaking speed, spreading density, hardness level, sinking speed, attractiveness and deliciousness of the feed. The proximate analysis showed that the test feed nutrition was within the range of nutrient needs for *P. homarus*.

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