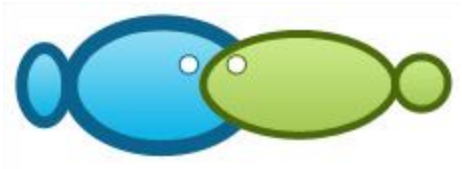

Potential of red spinach (*Amaranthus tricolor*) extract in shortening the molting duration of vannamei shrimp (*Litopenaeus vannamei*)

[1]Ahmad Ridwan, [2]Awaludin, [1]Lilis Lisnawati

[1] School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia; [2] Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Borneo Tarakan, Tarakan, Indonesia. Corresponding author: A. Ridwan, ridwan@sith.itb.ac.id

Abstract. Molting is one indicator of shrimp growth. The production of vannamei shrimp (*Litopenaeus vannamei*) can be increased by shortening the duration of molting. Red spinach (*Amaranthus tricolor*) extract has been studied due to its potential in shortening the duration of mangrove crab molting via the injection method. However, the injection method of red spinach extract is not effective if applied to encourage shrimp enlargement. The dipping method is thought to be a solution for applying red spinach extract in vannamei shrimp enlargement. This research aims to determine the optimum concentration of red spinach extract in shortening the molting duration of vannamei shrimp via the dipping method. This research method uses experimental observations with a total of seven treatments (control, 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 60 ppm), and six replications were observed for 28 days. The series of methods carried out include extraction of leaves and stems of red spinach, drying, maceration, extract emulsification, conducting Liebermann–Burchard test and gas chromatography/mass spectrometry (GCMS) test, dipping of vannamei shrimp from PL 17, molting observation, measurement of shrimp length and body mass, and measurement of water quality. The results showed a fluctuation in the percentage of molting during observation. Moreover, statistically, a significant difference was observed in the rate of molting in the treatment between controls with concentrations of 40 ppm and 60 ppm and between concentrations of 10 ppm with 40 ppm and 60 ppm ($p < 0.05$). The increase in average body length and the highest average body mass increase at the extract concentration of 40 ppm were 1.13±0.14 cm ($p < 0.05$) and 0.074±0.043 g ($p \geq 0.05$), respectively. Based on GCMS results, red spinach extract contains stigmast-5-en-3-ol, which is strongly suspected to be a building block of molting hormone and contains carbohydrates and fatty acids that play a role in shrimp metabolism. Based on the obtained data, it can be concluded that red spinach extract can shorten the molting duration of vannamei shrimp via the dipping method at an optimum concentration of 40 ppm.

Key Words: red spinach, dipping, phytosteroid, molting, *Litopenaeus vannamei*.

Introduction. The wealth of the sea is utilized by coastal communities to carry out aquaculture. The vast land in Indonesia has a great opportunity to cultivate only the commodity types. Shrimp is one type of fishery commodity most in demand and is a mainstay of Indonesian exports (Suhana 2017). From 2000 to 2015, shrimp farming production in Southeast Asia (Indonesia, Malaysia, Thailand, Vietnam, Bangladesh, the Philippines, Myanmar, and Taiwan) has increased, while China experienced a very drastic increase. In 2016-2019, shrimp aquaculture production in the world only increased by 4.8%, where China had the highest shrimp production compared with other countries (Subasinghe 2017).

One type of shrimp being farmed in Indonesia is vannamei shrimp (*Litopenaeus vannamei*), which contributes to the increasing amount of shrimp production globally. This poses a challenge for vannamei shrimp farmers in Indonesia to increase their shrimp production. Shrimp growth influences the level of shrimp production. Growth is an increase in body mass and body volume (Suhana 2017). Vannamei shrimp belongs to the crustacean subphylum with an outer frame or exoskeleton composed of hard chitin. When the shrimp grows, it experiences molting by releasing the old exoskeleton and then

synthesizing a new exoskeleton to compensate for its growing body size (Hosamani et al 2017).

Molting is an indicator of shrimp growth. The more often the shrimp experiences molting, the faster it grows. However, molting events vary depending on the type, season, and internal factors, such as hormones (Hosamani et al 2017). In one molting, a shrimp needs to prepare large amounts of energy reserves and increase its molting hormone (ecdysteroid) (Suryati & Tenriulo 2013). The ecdysteroid hormone in hemolymph regulates the duration of molting (Suryati & Tenriulo 2013). Ecdysteroid functions as a regulator of the skin replacement process and controls new exoskeleton formation to replace the old exoskeleton. According to Shrivastava & Princy (2013), the raw material for ecdysteroid production is cholesterol (steroid derivatives); however, a shrimp cannot produce their cholesterol, so a shrimp needs to take it from outside the body.

One type of plant that is thought to be rich in steroids is red spinach (*Amaranthus tricolor*). Red spinach has a short life cycle and is easy to cultivate. It contains carbohydrates, lipids, proteins, and minerals (Yang & Keding 2009). Fujaya & Trijuno (2007) studied spinach by extracting spinach metabolite compounds and then injecting it into mangrove crabs (*Scylla* sp.) and found that it can shorten the molting duration of *Scylla* sp. Other plants that contain steroids such as karamunting (*Melastoma malabatrhicum*) extract can accelerate the growth of tiger prawns (Ridwan & Awaludin 2020a). Spinach extraction aims to extract metabolites in spinach via the solvent diffusion principle (Saptarini et al 2017).

The injection method is thought to be less effective if it is applied to the rearing vannamei culture. Awaludin & Ridwan (2016) conducted a research using the dipping method, which proved to have a positive effect on shrimp growth; thus it is thought that it can be applied to shrimp rearing. The immersion method requires an accurate concentration of the extract to encourage optimum shrimp growth and shorten the molting duration.

Therefore, it is necessary to carry out research to determine the optimum concentration of spinach extract, which can shorten the molting duration of vannamei shrimp via the dipping method. It is hoped that the soaking technique of red spinach extract can shorten the duration of shrimp molting, thereby increasing shrimp production for Indonesian vannamei farmers.

Material and Method. The study was conducted from February until September 2019 at Animal Behavior Laboratory, Research Building and ITB Innovation and Behavior Analysis Laboratory, Laboratory of Animal Physiology, Labtek XI SITH ITB.

Red spinach maceration. Red spinach was obtained from Randu Kurung, Cilame Village, Ngamprah District, Bandung Regency. The parts of red spinach that were taken were the leaves and stems. Before drying, red spinach stalks were cut to speed up the drying process. The drying method was carried out with a dry wind, making sure the sample was not exposed to direct sunlight, for 7-8 days (Luliana et al 2016). The dried red spinach was then blended. Red spinach simplicia was macerated with a ratio of 100 g of red spinach simplicia in hexane in a 400-mL ethanol (82:18) (Saptarini et al 2017). Furthermore, the solution was left to stand for 24 hours and two repetitions were carried out. The result of maceration was evaporated with a rotary evaporator at a temperature of 50-70^{o}C. A thick extract was then obtained, and the yield was calculated using the following formula (Saptarini et al 2017):

$$\text{Yield} = (\text{mass of thick extract} / \text{total dry simplicia mass}) \times 100$$

Identification of compounds in extracts. The viscous extract was dissolved in the solvent and then dropped onto a drop plate. A Liebermann-Burchard reagent (acetic acid anhydrous/concentrated sulfuric acid 10:1) was added (Saptarini et al 2017). The change in color was observed. A bluish-green discoloration indicated that the thick red spinach extract contained steroids/triterpenoids (Saptarini et al 2017). Furthermore, the gas chromatography/mass spectrometry (GCMS) test was continued. Before the sample was

injected into the GCMS device, the sample was derivatized with BSTFA and TMCS 1%, vortexed, and then heated at 60-70^{o}C for 20 min. After cooling, the samples were injected into the GCMS-QP2010 Ultra device with RTX5 column type, Helium carrier gas, 280^{o}C injector temperature, 250^{o}C ion source temperature, and 300^{o}C interface temperature (Hanwar et al 2015).

Soaking vannamei shrimp in extract. The extract was emulsified using emulator Tween 80 and span 80 (Izadi et al 2012) and was then diluted in seawater. A preliminary test was carried out to determine the treatment extract's concentration by inserting PL 14 shrimp into several concentrations, namely, 5 ppm, 10 ppm, 50 ppm, 100 ppm, 500 ppm, 1000 ppm, and control with three replications each.

The number of shrimps that were still alive and the percentage of mortality after 24 h of each treatment were calculated using Abbott's formula (Sumihe et al 2014):

Mortality (%) = {(% deaths in test – % deaths in controls) / (100% – % deaths in controls)} × 100

The concentration that has been determined is based on the calculation of the cholesterol requirement of vannamei shrimp (Morris et al 2011) and the preliminary test results, namely, 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm, 60 ppm, and control with six replications each, emulsified and diluted in seawater. The soaking water extract was put into a 14 × 14 × 5 cm container that has been installed with an aerator. Into each container were put 15 vannamei shrimp (PL17) with a density of 765 individus/m.s, which previously weighed the initial total body mass and measured the initial body length (from the rostrum to telson). The number of molting shells was calculated every day and the number of live shrimp per day for 28 days (Awaludin & Ridwan 2016). Shrimp feed was given as much as 5% of the total weight of shrimp with a frequency of three times a day at 08.00, 14.00 and 20 00 WIB. At the end of the observation, the final total body mass and the final body length were measured. Measurement of water quality, which includes measuring water temperature using a thermometer, water salinity using a refractometer, and water pH using a pH meter (pH is maintained in the range of 7-8), and changing the immersion water by 20% every 2 days were carried out. Moreover, changing the immersion water was also carried out if the pH value of the water was above 8 or less than 7.

The data on the amount of molting skin was processed in the form of molting percentage per day with the formula (Herlinah et al 2014):

Molting (%) = (number of shells / number of shrimp) x 100

Calculation of the increase in body length and body mass of shrimp are as follows:

The amount of increase in body length = Lt: Nt - (L0: N0)
where: Lt = total final body length;
Nt = total number of final shrimp at the end of observation;
L0 = total length of the initial body;
N0 = total number of shrimp at the beginning of observation.

The amount of increase in body mass = Mt: Nt - (M0:N0)
where: Mt = total final body mass at the end of experiment;
Nt = total number of shrimp at the end of experiment;
M0 = total mass of initial body;
N0 = total number of shrimp at the beginning of experiment.

Calculation of survival rate (SR) or shrimp at the end of observation after 28 days was made using the formula:

SR% = (NA:NT) x 100

where: SR = survival rate;
NA = number of shrimp that died;
NT = initial number of shrimp.

Statistical analysis. Data were analyzed using SPSS ver.21.0. All the data were presented as means±standard error of the mean calculated from three replicates. One-way ANOVA was performed at a significance level of 0.05 following confirmation of normality and homogeneity of variance. Tukey's procedure was used for multiple comparisons.

Results

Identification of phytochemical compounds in ethanol extract of red spinach. The qualitative identification of the chemical compounds in the extract in red spinach shows that the color changes in the extracted sample to a bluish-green, indicating that the red spinach extract is positive for steroids/triterpenoids (Xiong et al 2007). Figure 1 shows the results of the color change. Color change occurs because the steroids/triterpenoids in the extract react with anhydrous acetic acid and concentrated sulfuric acid to form chromophore groups (Xiong et al 2007).

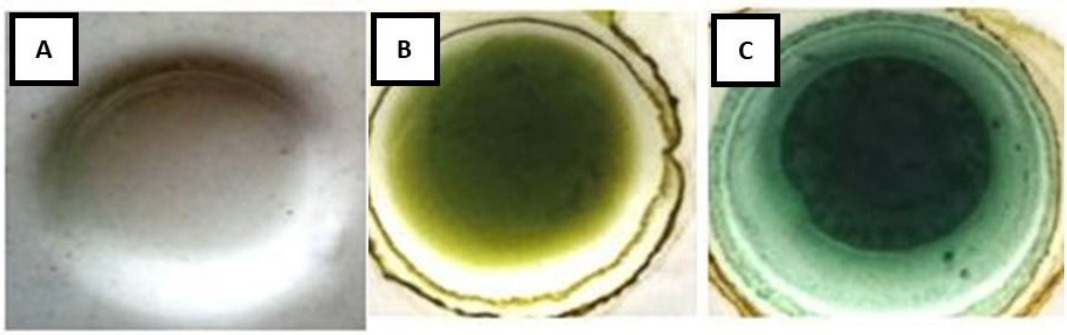

Figure 1. Negative control (A), before adding reagent LB (B), positive result (C).

Table 1 presents the results of GCMS test of red spinach extract. GCMS allows to identify the compounds contained in a plant extract based on the volatility level of each compound and its interaction with its stationary phase, as well as mass fragmentation (Hanwar et al 2015). Derivatization makes it easier for compounds with large molecular weights such as steroid compounds or compounds with hydroxyl groups by breaking their hydrogen bonds or hydroxyl groups, which are then replaced by trimethylsilyls to cause nonvolatile compounds to become more volatile compounds (Hanwar et al 2015).

Table 1

Contents of red spinach compound from GCMS test results

Retention time (s)	*Relative area (%)*	*Height*	*Name constituent*
12.15	2.76%	15818	Butanedioic acid, TMS
14.65	1.08%	9123	Butanedioic acid, TMS
18.40	2.70%	16.819	D-fructose, 1,3,4,5,6-pentakis-0-TMS
18.48	6.28%	42713	D-fructose, 1,3,4,5,6-pentakis-0-TMS
18.55	4.79%	31805	D-fructose, 1,3,4,5,6-pentakis-0-TMS
18.82	0.78%	6442	beta-D-galactofuranose, 1,2,3,4,6-pentakis-O-TMS
19.32	21.43%	153732	Galactopyranose, 1,2,3,4,6-pentakis-O-TMS
20.20	31.20%	247751	Galactopyranose, 1,2,3,4,6-pentakis-O-TMS
20.58	3.74%	20245	Hexadecanoic acid, TMS
24.47	6.29%	17029	Stigmast-5-en-3-ol
25.98	10.255	62149	Sucrose-Octa, TMS

Based on the GCMS results, red spinach extract contains carbohydrates, fatty acids, and steroids. The carbohydrate compounds identified in the red spinach extract are fructose,

galactofuranose, galactopyranose, and sucrose. Carbohydrates include polar compounds so that they dissolve in the shrimp medium. The fatty acid compounds identified were butanedioic acid and hexadecanoic acid. The steroid compounds identified were stigmast-5-en-3-ol, including phytosteroid compounds. Fatty acids and stigmast-5-en-3-ol are nonpolar compounds, requiring an emulsification process to dissolve them in water media (Adams et al 1998).

The extract's emulsification aims to dissolve the nonpolar compounds in the medium water extract when the shrimp are immersed. The emulsification of nonpolar compounds in red spinach extract (stigmast-5-en-3-ol and fatty acids) is included in the oil in water (o/w) emulsion type (Hisprastin & Nuwarda 2018). The extract/emulator ratio (1:2) produces a stable emulsion. This is strongly supported by Rahate & Nagarkar (2007), who conducted an emulsion study using nonionic surfactants (tween and span) to emulsify sunflower oil and sesame oil to produce a stable emulsion for 6 months. Once every 2 days and when the pH is above 8 or below 7, the media water was replaced with clean water as much as 20%, preventing high levels of ammonia in the water and renewing the compound content of the red spinach extract in the soaking water. Metabolite compounds in the thick extract of red spinach in medium water can change. Steroid compounds may be degraded. Several factors can cause steroids to degrade, including aeration, ambient temperature, and the presence of microbial biomass. According to Shi et al (2004), ammonia-oxidizing bacteria in water can degrade estradiol-type steroids. Steroid concentration in the environment can affect the degradation of steroid compounds; it is suspected that heterotrophic microbes cannot use low concentrations of cholesterol as a carbon source. Microorganisms in water can excrete cholesterol oxidase enzymes that oxidize cholesterol in water, but this enzyme has low activity on the stigmast-5-en-3-ol (phytosteroid) substrate (MacLachlan et al 2000). In addition, microorganisms in water secrete the enzyme cholesterol dehydrogenase, which can oxidize cholesterol but is only active at alkaline pH (Yang et al 2007).

Deksissa (2008) found that steroid compounds are found in groundwater, sewage, agricultural waste, soil, and sediment; even a small part is found in drinking water. Payus et al (2017) examined the presence of stable steroid compounds in water, not even damaged or denatured in conventional wastewater treatment. Wastewater, including household waste containing natural or synthetic steroids, is treated. The treated water is then tested, and it is stated that it still contains leftovers of steroid compounds. These studies strongly support the assumption that stigmast-5-en-3-ol has stability in water and strong resistance in the environment and does not readily react with other compounds, such as ammonia. This assumption is evidenced by the nature of stigmast-5-en-3-ol, including stable compounds with a melting point of 136^{o}C (Jati et al 2019); thus, they are not easily denatured at values below the room temperature. Johnsson et al (2003) stated that stigmast-5-en-3-ol was oxidized at 120^{o}C which was heated for 72 h. Carbohydrates and fatty acids in water extracts cannot undergo hydrolysis since they are simple compounds or in monosaccharide forms, except sucrose. Sucrose is a disaccharide that can be hydrolyzed to form glucose and fructose; however, its hydrolysis is very slow, except in acidic pH. Carbohydrates and fatty acids can be oxidized when heated at high temperatures. According to BeMiller & Whistler (1996), the temperature that can oxidize carbohydrates and fatty acids is at above 110^{o}C for more than 1 h with D-glucose boiling point at 146^{o}C. Acidic pH conditions or pH below 7 can lead to the degradation of carbohydrates and fatty acids (Woo et al 2015). However, during the extract immersion, the water temperature was below 110^{o}C and the pH of the water was not in an acidic condition. Therefore, it was assumed that carbohydrates and fatty acids were not oxidized when the extract was immersed.

Primary test. The primary test for 24 h showed no difference in the percentage of mortality (p-value ≥ 0.05). The percentages of average mortality at concentrations of spinach extract at 100 ppm, 500 ppm, and 1000 ppm were 62±10%, 87±08%, and 87±14%, respectively. After 24 h, the mean percentage mortality at a concentration of 100 ppm caused more than 50% mortality. Therefore, the treatment concentration was determined to be below the 100-ppm concentration. Many factors can cause the mortality

of vannamei shrimp during the premolar test, even though it has been acclimatized under laboratory conditions for 5 days. Several factors can cause death, including the content of extract compounds (secondary metabolites) (Sumihe et al 2014). The higher the extract concentration, the higher the mortality (Awaludin et al 2020).

The number of molting shrimp. Observation of the number of molting vanamae shrimp treated with red spinach extract for 28 days is shown in Figure 2.

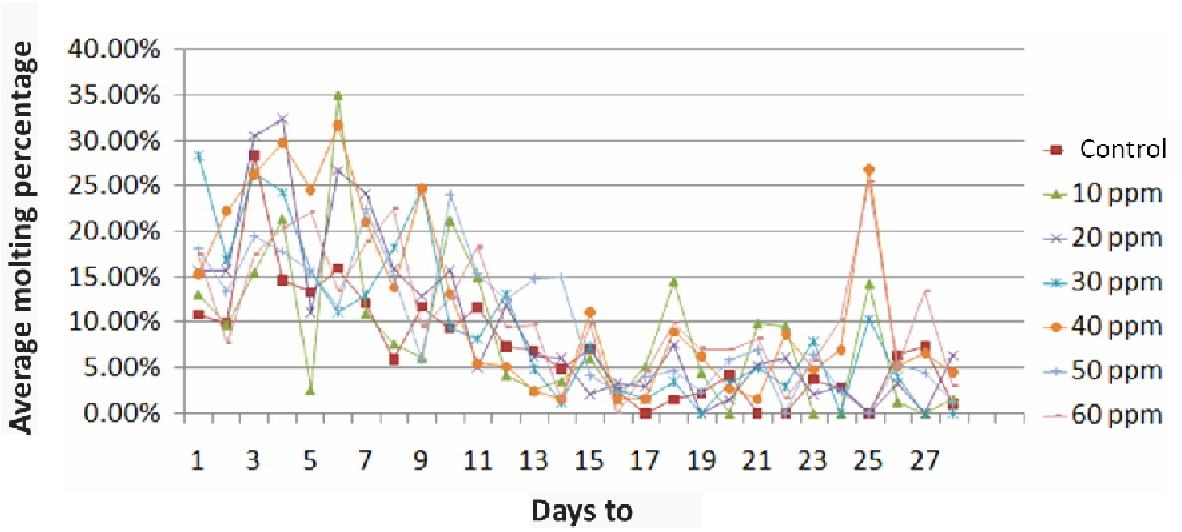

Figure 2. Percentage of vannamei shrimp molting per day for 28 days.

Every day during the observation, a fluctuation in the average molting percentage of shrimp was observed. At a concentration of 20 ppm, the highest percentage of molting during the observation occurred on days 3 and 4. At a concentration of 10 ppm, the highest percentage of molting occurred on day 6 and the second highest on day 6 at a concentration of 40 ppm. The concentration of 50 ppm had the highest percentage of molting on day 10. Concentrations of 40 ppm and 60 ppm experienced the highest spike in the percentage of molting on day 25. Based on statistical tests, there was an effect of differences in the percentage of molting between control treatment and treatment with concentrations of 40 ppm and 60 ppm and between treatment concentrations of 10 ppm with a concentration of 40 ppm and 60 ppm (p-value < 0.05).

Various factors, both internal and external, caused the fluctuation in the percentage of shrimp molting observed every day. Internal factors are influenced by shrimp's hormonal conditions, availability of energy, levels of molting hormone, shrimp's physiological processes, and shrimp's developmental factors (Kumar et al 2018). The molting hormone (ecdysteroid) regulates the molting period of shrimp. Cholesterol as raw material for molting hormone synthesis can be met in red spinach extract, which contains stigmast-5-en-3-ol, a steroid derivative found in plants (phytosterols). The shape of stigmast-5-en-3-ol, which is similar to cholesterol, is thought to replace cholesterol as raw material for the synthesis of the ecdysone hormone (hormone that regulates molting) in crustaceans (Cheng & Hardy 2004; Kumar et al 2018). In addition, stigmast-5-en-3-ol is thought to be a component of lipoprotein, which functions as transport of lipids throughout the shrimp body and is a means for lipid absorption (Kumar et al 2018). Apart from red spinach extract, karamunting plant also containS cholesterol (Ridwan et al 2015). Karamunting extract containing cholesterol can accelerate molting of tiger prawns (*Penaeus monodon*) (Ridwan & Awaludin 2020a).

A shrimp also needs a lot of energy to do one molting. The larger the shrimp's size postlarvae, the longer the intermolt period, which is the time it takes from one molting to the next. The energy source needed for molting can be met from the carbohydrate and fatty acid content found in red spinach extract. Carbohydrate compounds in the red spinach extract have a higher percentage area in the Table 1

compared with stigmast-5-en-3-ol and fatty acids. This indicates that the carbohydrate content or abundance is higher than stigmast-5-en-3-ol and fatty acids. Carbohydrates can be used as raw material for the cellular respiration of shrimp. Carbohydrates are converted into cellular respiration to produce energy in the form of ATP. Cheng et al (2002) show that the shrimp uses this energy to carry out several physiological processes, such as the active transport of ions or minerals required for the formation of a new exoskeleton. Energy is also used to balance the shrimp's osmotic pressure so that its body's osmoregulation remains balanced. Fatty acids are used as energy reserves. If the energy obtained from cellular respiration with carbohydrate as a raw material is insufficient for cellular respiration needs, then fatty acids will be directly used to produce energy. Fatty acids will undergo β-oxidation, are converted to acetyl CoA, and enter the citric acid cycle and produce ATP. However, if the need for ATP has been met from cellular carbohydrate respiration, the fatty acids will be carried and stored in the hepatopancreas as energy reserves (Kumar et al 2018).

Red spinach extract containing carbohydrates, stigmast-5-en-3-ol, and fatty acids is emulsified so that it dissolves in the water of the shrimp immersion medium. These compounds dissolve and can enter the shrimp body when the shrimp absorbs water through the oral medium along with the incoming food. Water and food that enter the middle intestine of shrimp containing fatty acids, sterol esters, phospholipids, triacylglycerols (TAG), or other types of fat will be broken down by the lipase enzyme (specifically TAG lipase, phospholipase, and cholesterol esterase) (Kumar et al 2018).

Free fatty acids that have been absorbed by midgut cells are converted into diacylglycerol (DAG) and then converted into triacylglycerol (TAG) by diacylglycerol acyltransferase (DGAT). Likewise, cholesterol will be esterified into ester cholesterol by acyl-CoA: cholesterol acyltransferase (ACAT). Cholesterol and fatty acids that form lipoproteins with TAG and cholesterol esters in the middle of the lipoproteins are covered with phospholipids with a polar or hydrophilic outer and a nonpolar or hydrophobic interior. Lipoproteins are transported throughout the shrimp body through the hemolymph until they reach specific receptors on the target cell membrane (Kumar et al 2018). These lipoproteins can be directed to the hepatopancreas to be stored or directly utilized to synthesize ecdysteroid hormones in Y organ. Ecdysteroid hormone biosynthesis occurs in organ Y in two stages: the first stage, stigmast-5-en-3-ol is converted to 5β-diketol, then converted to 3-dehydroechysone (3DE). The next stage, 3DE is converted to ecdysone, then converted to 20-hydroxyecdysone (20-E) which is the main active ecdysteroid circulating in the hemolymph and has an effect on crustacean molting (Kumar et al. 2018). Table 2 presents the increase in average body length and body mass gain in vannamei shrimp for 28 days.

Table 2

Growth of vannamei shrimp with red spinach extract

Concentration (PPM)	*Body length (cm)*	*Body mass (gram)*
0	0.64 ± 0.30^{a}	0.048 ± 0.024^{a}
10	0.92 ± 0.34^{ab}	0.056 ± 0.027^{a}
20	1.08 ± 0.14^{b}	0.060 ± 0.021^{a}
30	1.03 ± 0.04^{b}	0.055 ± 0.026^{a}
40	1.13 ± 0.14^{b}	0.074 ± 0.043^{a}
50	1.00 ± 0.08^{ab}	0.043 ± 0.017^{a}
60	0.91 ± 0.27^{ab}	0.057 ± 0.038^{a}

Note: Means followed by the same capital letter in the line do not differ significantly ($p < 0.05$).

Based on the one-way ANOVA statistical test, there is a significant difference in the shrimp's average length (p-value < 0.05). The difference in the significant increase in body length was found in the shrimp immersion in the concentrations of 20 ppm, 30 ppm, and 40 ppm compared to the control treatment, which was not significant at concentrations of 10 ppm, 50 ppm, and 60 ppm. Based on these data, the increase in body length varies with each treatment. The higher the extract concentration, the greater

the body length. The amount of increase in body length in all treatments of soaking red spinach extract was higher than the control. The highest increase in body length is at a treatment concentration of 40 ppm. The increase in body length at a concentration of 40 ppm was significantly different from the increase in body length in the control treatment. The shorter the duration of molting, the more frequently the shrimp experiences molting. The more often the shrimp experiences molting, the more often the shrimp sheds their skin, and the greater the increase in body length. The old exoskeleton that is released will be replaced by a new exoskeleton, which is now longer is size. However, shrimp that are frequently molted do not necessarily show a longer body length. Shrimp may often shed their skin/exoskeleton; however, due to insufficient mineral availability in the water, the new exoskeleton that is formed is not optimal (Aisyah et al 2017). This is thought to cause the length of the body to be different for each treatment.

Although the amount of increase in the shrimp body mass was not significant, the amount of body mass gain in all treatments was higher when compared to the control. The highest body mass gain at a concentration of 40 ppm was in line with the highest increase in body length. During the intermolt period, the shrimp must prepare various things, which include preparing the availability of molting hormone and energy reserves for use during molting and energy for the growth process. When the energy reserves and the ecdysteroid hormone are met in a relatively short time, it can shorten the molting period; thus molting can occur more quickly. The weight gain was not significantly different among treatments, but on the other hand, the body length tended to show an increase in results for all treatments compared to the control. This shows that the increase in size is not always in line with the increase in shrimp body mass. From these results, it can be assumed that molting causes an increase in body length or body size, due to the release of the old exoskeleton, which is synthesized by a new exoskeleton. In theory, the more frequently the molting, the larger the shrimp will grow, both in terms of body size or length and body mass (Zufadhillah et al 2018). The results showed that only a concentration of 40 ppm produced a significant different molting percentage accompanied by the highest increase in body length and body mass gain. However, other treatments showed that the increase in body mass was not always in line with the increase in body length and molting. One reason may be the presence of shrimp with a not well-pigmented hepatopancreas during morphology observation. The hepatopancreas is a gland that produces digestive enzymes. It is suspected that a not perfectly pigmented hepatopancreas causes the production of digestive enzymes, for example protein-breaking enzymes, with slow activity or the production enzymes in small quantities, causing the breakdown of protein in the feed to be imperfect. This imperfect protein breakdown results in less amino acids that are needed for development of new body cells, causing stunted growth (Kumar et al 2018).

Water quality. The results of water quality measurements during maintenance can be seen in Table 3.

Table 3

Water quality

No	*Parameter*	*Kisaran*
1	Temperature (^{o}C)	21.7-23.2
2	Salinity (ppt)	21.3-26.1
3	pH	7-8
4	DO (mg L^{-1})	5-6

Dissolved oxygen (DO) is always sufficient in the presence of an aerator and is an important factor for the survival of vannamei shrimp. When they are deprived of oxygen, shrimp can die immediately. The results of measuring the water quality parameters of vannamei shrimp rearing are still classified as optimum (Ridwan & Awaludin 2020b).

Conclusions. Red spinach ethanol extract has secondary metabolic compounds of carbohydrates, fatty acids, and steroids, where these compounds have the potential to stimulate molting in shrimp. Based on the molting percentage obtained, it shows there is a tendency to increase in body length for all treatments compared to the control. Meanwhile, the weight gain for all treatments was also greater than control, although it was not statistically significant. So, it can be concluded that spinach extract can shorten the duration of molting for vannamei shrimp by immersing/dipping at an optimum concentration of 40 ppm.

Acknowledgements. Thanks to PT. Paragon Technology and Innovation for providing research funding assistance, PT. Suri Tani Pemuka for providing vannamei shrimp fry, and LIPI Cibinong Natural Material Laboratory and SITH-ITB laboratory for helping in the realization of this research.

References

Adams F., Walstra P., Brooks B. W., Richmond H. N., et al, 1998 Modern aspects of emulsion science. Royal Society of Chemistry, 442 pp.

Aisyah N., Agus M., Mardiana T. Y., 2017 [Analysis of dolomite utilization in feed on molting period of vaname shrimp (*Litopenaeus vannamei*) in Unikal Pond]. Pena Akuatika 16(1):94-102. [in Indonesian]

Awaludin A., Ridwan A., 2016 [Survival rate improvement of Windu shrimp seed (*Penaeus monodon*) by souring ethanol extract karamunting (*Melastoma malabahricum*)]. Jurnal Harpodon Borneo 9(1):32-35. [in Indonesian]

Awaludin A., Kartina K., Maulianawati D., Manalu W., Andriyanto A., Septiana R., Arfandi A., Lalang Y., 2020 Phytochemical screening and toxicity of ethanol extract of *Sauropus androginus*. Biodiversitas Journal of Biological Diversity 21(7):2966-2970.

BeMiller J. N., Whistler R. L., 1996 Dietary fiber and carbohydrate digestibility. In: Food chemistry. Fennema O. R. (ed), 3rd edition, Marcel Dekker INC, New York, pp. 218-223.

Cheng W., Liu C. H., Yan D. F., Chen J. C., 2002 Hemolymph oxyhemocyanin, protein, osmolality and electrolyte levels of whiteleg shrimp *Litopenaeus vannamei* in relation to size and molt stage. Aquaculture 211(1-4):325-339.

Cheng Z. J., Hardy R. W., 2004 Protein and lipid sources affect cholesterol concentrations of juvenile Pacific white shrimp, *Litopenaeus vannamei* (Boone). Journal of Animal Science 82(4):1136-1145.

Deksissa T., 2008 Fate and transport of steroid hormones in the environment. UCOWR Conference, Paper 17, 11 pp.

Fujaya Y., Trijuno D. D., 2007 [Haemolymph ecdysteroid profile of mud crab during molt and reproductive cycles]. Torani 17(5):415-421. [in Indonesian]

Hanwar D., Suhendi A., Trisharyanti I., Santoso B., Safitri M., Haryoto, 2015 [Secondary metabolite profile analysis of emprit flower extract with gas chromatography-mass spextroscope]. The 1st University Research Colloquium, pp. 158-166. [in Indonesian]

Herlinah H., Tenriulo A., Septiningsih E., Suwoyo H. S., 2014 [Moulting response and review of Bakau crab (*Scylla olivacea*) injected with murbei leaves extract (*Morus* sp.)]. Prosiding Forum Inovasi Teknologi Akuakultur, pp. 173-183. [in Indonesian]

Hisprastin Y., Nuwarda R. F., 2018 [The difference of emulsion and microemulation in vegetable oil]. Farmaka 16(1):133-140. [in Indonesian]

Hosamani N., Reddy B. S., Reddy P. R., 2017 Crustacean molting: regulation and effects of environmental toxicants. Journal of Marine Science: Research & Development 07(05):236.

Izadi Z., Nasirpour A., Garousi G., 2012 Optimization of phytosterols dispersion in an oil/water emulsion using mixture design approach. Journal of Dispersion Science and Technology 33(12):1715-1722.

Jati B. N., Yunilawati R., Nuraeni C., Oktarina E., Aviandharie S. A., Rahmi D., 2019 [Extraction and identification of phytosterol in microalgae *Nannochloropsis occulata*]. Jurnal Kimia dan Kemasan 41(1):31-36. [in Indonesian]
Johnsson L., Andersson R. E., Dutta P. C., 2003 Side-chain autoxidation of stigmasterol and analysis of a mixture of phytosterol oxidation products by chromatographic and spectroscopic methods. Journal of the American Oil Chemists' Society 80(8):777-783.
Kumar V., Sinha A. K., Romano N., Allen K. M., Bowman B. A., Thompson K. R., Tidwell J. H., 2018 Metabolism and nutritive role of cholesterol in the growth, gonadal development, and reproduction of crustaceans. Reviews in Fisheries Science and Aquaculture 26(2):254-273.
Luliana S., Purwanti N. U., Manihuruk K. N., 2016 [The effect of drying method of senggani (*Melastoma malabathricum* L.) leaf simplicia on antioxidant activity using the DPPH method (2,2-diphenyl-1-pikrilhidrazil)]. Pharmaceutical Sciences and Research 3(3):120-129. [in Indonesian]
MacLachlan J., Wotherspoon A. T. L., Ansell R. O., Brooks C. J. W., 2000 Cholesterol oxidase: sources, physical properties and analytical applications. The Journal of Steroid Biochemistry and Molecular Biology 72(5):169-195.
Morris T. C., Samocha T. M., Davis D. A., Fox J. M., 2011 Cholesterol supplements for *Litopenaeus vannamei* reared on plant based diets in the presence of natural productivity. Aquaculture 314(1-4):140-144.
Payus C., Roger J., Ping S. Y., 2017 Steroid sex hormone ethinylestradiol in recycled wastewater at recreational park. Journal of Pharmacology and Toxicology 12(3): 148-153.
Rahate A. R., Nagarkar J. M., 2007 Emulsification of vegetable oils using a blend of nonionic surfactants for cosmetic applications. Journal of Dispersion Science and Technology 28(7):1077-1080.
Ridwan A., Awaludin, 2020a Growth response larvae of giant tiger (*Penaues monodon*) on ethanol extract of karamunting (*Melastoma malabhatricum*) that mixed in feed. IOP Conference Series: Earth and Environmental Science 564:012080.
Ridwan A., Awaludin A., 2020b Measurement hormone progesterone and gonadal maturity on white shrimp (*Litopenaeus vannamei*) after the administration of the karamunting (*Melastoma malabhtricum*) ethanol extracts. BIOTROPIA - The Southeast Asian Journal of Tropical Biology, pp. 1-11 (in press).
Ridwan A., Awaludin A., Anggraeni T., 2015 Gonadal maturity induction using karamunting (*Melastoma malabatrhicum*) ethanol extract on white shrimp female (*Litopenaeus vannamei*). Proceeding of 6th International Conference on Global Resouce Conservation, pp. 69-74.
Saptarini N. M., Herawati I. E., Hernawati N. F., 2017 Total phytosterol content in roselle (*Hibiscus sabdariffa*) calyx from Subang and Bandung. Research Journal of Pharmaceutical Biological and Chemical Sciences 8(1):174-178.
Shi J., Fujisawa S., Nakai S., Hosomi M., 2004 Biodegradation of natural and synthetic estrogens by nitrifying activated sludge and ammonia-oxidizing bacterium *Nitrosomonas europaea*. Water Research 38(9):2322-2329.
Shrivastava S., Princy A., 2013 Moult inhibiting hormone: a new approach to the discovery and design of growth promoters in crustaceans? Reviews in Aquaculture 5(2):111-120.
Subasinghe R., 2017 World aquaculture 2015: a brief overview. FAO, Rome, Fisheries and Aquaculture Report No. 1140, 47 pp.
Suhana, 2017 Jokowi dan Ekonomi Udang Indonesia. Artikel harian Kompas online yang terbit pada 7 November 2017. [in Indonesian]
Sumihe G., Runtuwene M. R. J., Rorong J. A., 2014 [Phytochemical analysis and determining Lc50 value of liwas leave methanol extract]. Jurnal Ilmiah Sains 14(2):125-128. [in Indonesian]
Suryati E., Tenriulo A., 2013 [Potential of plant extracts as moulting stimulants on Windu shrimp parent (*Penaeus monodon* Fab.)]. Prosiding Forum Inovasi Teknologi Akuakultur, pp. 249-255. [in Indonesian]

Woo K. S., Kim H. Y., Hwang I. G., Lee S. H., Jeong H. S., 2015 Characteristics of the thermal degradation of glucose and maltose solutions. Preventive Nutrition and Food Science 20(2):102-109.
Xiong Q., Wilson W. K., Pang J., 2007 The Liebermann-Burchard reaction: sulfonation, desaturation, and rearrangment of cholesterol in acid. Lipids 42(1):87-96.
Yang R. Y., Keding G. B., 2009 Nutritional contributions of important African indigenous vegetables. In: African indigenous vegetables in urban agriculture. Shackleton C. M., Pasquini M. W., Drescher A. (eds), Earthscan, London, UK, pp. 105-143.
Yang X., Dubnau E., Smith I., Sampson N. S., 2007 *Rv1106c* from *Mycobacterium tuberculosis* is a 3β-hydroxysteroid dehydrogenase. Biochemistry 46(31):9058-9067.
Zufadhillah S., Thaib A., Handayani L., 2018 [Effectiveness of nano CaO addition from mangrove crabs (*Scylla serrata*) shells into commercial feed to boost frequency of growth and molting in prawn (*Macrobrachium rosenbergii*)]. Acta Aquatica: Aquatic Sciences Journal 5(2):69-74. [in Indonesian]

Received: 15 February 2021. Accepted: 10 April 2021. Published online: 16 May 2021.
Authors:
Ahmad Ridwan, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia, e-mail: ridwan@sith.itb.ac.id
Awaludin, Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Borneo Tarakan, Jl. Amal lama No. 1 Tarakan, Indonesia, e-mail: awaludin@borneo.ac.id
Lilis Lisnawati, School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia, e-mail: lilisnawati11@students.itb.ac.id

How to cite this article:
Ridwan A., Awaludin, Lisnawati L., 2021 Potential of red spinach (*Amaranthus tricolor*) extract in shortening the molting duration of vannamei shrimp (*Litopenaeus vannamei*). AACL Bioflux 14(3):1271-1281.