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The Doses Effect of Red Spinach Leaves Extract (*Amaranthus tricolor*) On The Molting Success of Mandac Crab (*Scylla serrata*)

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ABSTRACT

Mud crab (*Scylla* sp.) is a fishery commodity that has high economic value and is one of the superior fishery products. The demand for mud crabs continues to increase, so intensive cultivation efforts are needed. Mangrove crabs have high economic value. However, farmers often face problems in cultivating mud crabs, namely molting, which takes a long time, resulting in non-uniform growth. Therefore, this research aims to optimize the speed of skin turnover and uniform growth with an alternative technology for dosing spinach leaf extract, which can be applied via injection. The research method used was a Completely Randomized Design (CRD) method with 4 treatments and 3 replications. The treatments to be carried out are: P0 = (control), P1 (spinach leaf extract 3 µl/g), P2 (spinach leaf extract 5 µl/g), and P3 (spinach leaf extract 7 µl/g). Parameters observed: Daily growth rate, feed conversion ratio, molting percentage, survival rate (SR), and water quality observations. The results of the research were that the effect of giving red spinach leaf extract (*Amaranthus tricolor*) on the molting success of mud crabs (*Scylla serrata*) had a very significant effect on Daily Growth Rate (LPH), Feed Conversion Ratio (FCR), and Molting Percentage. However, it did not have a real effect on the Survival Rate (SR) of mud crabs (*Scylla serrata*).

Keywords: Spinach doses, molting, *Scylla*, fish, Aceh

INTRODUCTION

Mangrove crabs (*Scylla* sp) is one of the coastal fisheries commodity, especially in mangrove forest in Aceh (Kanna, 2002). Mangrove crabs are a fishery commodity with significat economic value and have become one of the leading fisheries product recently. The substantial market potential offer an opportunity for more serious, commercial development of mangrove cultivation. One of the increasing export deman dis for soft-shell crabs.

The production of soft-shell crabs has bright propects as an alternative to other fisheries. Various efforthave been made to overcome obstacle, including food manupulation, environmental adjustment, and limb autotomy techniques (Karim *et al*, 2007). When using mutilation technology, crabs molt faster-within approximately 1 month-but growth is minimal or non exixtent, and mortality is high (Only around 50%) dueto stress and infection during the rearing periods.

Additionally, marketing issues have emerged, such as rejection from several crab-importing countries that are un willing to accept them. One important breakthrough made by Aslamyah & Fujaya (2010) is the discovery of a molting stimulant derived from spinach extract.

Based of research conducted, spinach extract can be administered via artifical feed andi s affective in accelerating molting and increasing growth (Aslamyah & Fijaya, 2010). Therefore, the dosage of spinach extract in feed to accelerate the molting duration of mangrove crabs need futher study.

Based in the description above, the author is interested in exploring the effect of spinach extract. Based on the daily growth rate, molting success, and survival rate of angrove crabs (Scylla serrata). This study aims to examine the effect of spinach leaf extract dosage on molting succes in mangrove crabs.

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MATERIAL AND METHOD

Time and Location

The research will be conducted from January to February 2024, located at Fename Jaya, Bendahara District, Seunebok Aceh Village, Kerani Hasyim Hamlet.

Research Tools and Materials

The tools used in this research include crab box rearing containers, syringes, scoop nets, stationery, blenders, cameras, digital scales, and water quality testing equipment such as Thermometers, DO Meters, pH Meters, and Refractometers. Meanwhile, the materials used are mangrove crabs, trash fish as feed, spinach leaves, 70% ethanol, and distilled water.

Research Design

The research design used laboratorium experiment using controlled habitat in aquarium. The treatment and design research using complet random design (RAL) with 4 treatment and 3 repeatation. The crabs molting process monitoring in each 24 hours. The treatment for each aquarium are: 1) P0 as contorol without spinach extract (0µl/g); 2) P1 the spinach extract contain 3µl/g; 3) P2 the spinach extract contain 5µl/g; 4) the spinach extract contain 7µl/g.

Research Activity

Species Test

The test animals were prospective broodstock of mangrove crabs *(Scylla serrata*), with weights ranging from 80 to 100 g/individual. The total number of crabs tested was 36 individuals. The crabs were in good condition, with intact body parts and an active response to external stimuli. Furthermore, the test crabs were acclimatized for 5 days in the rearing containers (Sihombing *et al*., 2020).

Aquarium

The aquarium used in this research are "Crab boxes" measuring 15 x 18.5 x 20 cm. Each rearing aquarium is filled with 3 individual mangrove crabs, for a total of 36 crabs. This is to prevent cannibalism during rearing, especially after molting. The aquarium are then floated in a pond with a depth of approximately 1 meter.

Preparation of Red Spinach Leaf Extract

The material is 1000 g of ground red spinach leaves were macerated with 1500 ml of 70% ethanol for 5 days, protected from light, and stirred frequently. Then, the macerate was filtered and evaporated using a rotary evaporator at 50°C, followed by concentration using a water bath until a thick extract of red spinach leaves was obtained (Nabila, 2020).

Hormone Application and Rearing

Crabs were weighed and measured to determine their initial weight and size. This was followed by injecting red spinach leaf extract into the base of the swimming legs. The injection procedure was performed once with an injection volume of 0.1 ml according to the method of Fujaya *et al*. (2011), with the dosage given according to the treatment. The feed provided to the mangrove crabs during rearing consisted of fresh trash fish, with a feeding rate of 5% of body weight per day (Fujaya, 2008). Feeding frequency was twice a day, namely in the morning at 08.00 WIB and in the late afternoon at 17.00 WIB. Afternoon feeding is due to the nocturnal nature of crabs.

Test Parameters

Molting Percentage

Observations of molting were conducted daily by counting the number of crabs that molted (Sadrianto, 2020). The molting percentage is calculated using the following formula:

$$\text{PM} = \frac{\text{jym}}{\text{jk}} \text{ x } 100$$

Notation:
PM = Molting percentage
JYM = Number of molting
JK = Number of crabs

Daily Growth Rate

The specific daily growth rate can be determined using the formula (Changbo *et al*., 2004):

$$LPH(\%) = \frac{LnWt - LnWo}{t} \; x \; 100$$

Notation:
LPH = Specific daily weight growth rate (%/day)
Wo = Average weight of crabs at the first (g)
Wt = Average weight of crabs at time t (g)
t = Rearing period (days)

Feed Conversion Ratio (FCR)

The feed conversion ratio is the conversion value of the amount of feed given during the study, calculated according to Gusrina (2008) as follows:

$$FCR = \frac{F}{(Wt + D) - Wo}$$

Notation:
F = amount of feed given (gr)

Wt = weight at time (t) (gr)
Wo = weight at first (gr)
D = amount of dead fish (gr)

Survival Rate (SR)

The formula for calculating the survival rate according to Effendie (1997) is:

$$SR = \frac{Nt}{No} x100$$

Notation:
SR = Survival rate (%)
No = Number of fish at the first (ind)
Nt = Number of fish at the end of the study (ind)

Water Quality Monitoring

The water quality monitoring collected in the study are temperature, salinity, and dissolved oxygen. This parameter also record at same time with

RESULT AND DISCUSSION

Molting Portion

Molting percentage is a representation of the number of crabs that undergo molting during the rearing period compared to the initial number of treated crabs (Maulianawati et al., 2020). The following molting percentages during the study can be seen in Table 1 below:

Table 1. The average molting percentage of mangrove crabs during the study.

Treatment	Molting (%)
P0	11.11 ± 19.24^{a}
P1	44.44 ± 19.26^{ab}
P2	77.78 ± 19.22^{bc}
P3	88.89 ± 19.22^{c}

Notes: Different letters in the same column indicate significantly different treatment effects (P<0.05). The data listed are mean values ± standard deviation.

Based on the ANOVA results, the administration of red spinach leaf extract (*Amaranthus tricolor* L.) on the molting percentage of mangrove crabs (*Scylla serrata*) has a significant effect (P<0.05). Based on the Duncan test, the P0 treatment is not significantly different from P1 but is significantly different from P2 and P3. Meanwhile, P1 is not significantly different from P0 and P2 but is significantly different from P3. The Duncan test for P2 shows that it is not significantly different from P1 or P3, but is significantly different from P0. For P3, it is not significantly different from P2 but is significantly different from P1 and P0. The results show that the best treatment is P3, the injection of crabs with an extract dose of 7 µl/g, with a molting percentage of 88.89%, while the lowest molting percentage was observed in P0 (control), with 11.11%.

In the P3 treatment, the red spinach leaf extract dose of 7 µl/g is the best treatment because, in P3, the ecdysteroid content in the red spinach leaf extract dose is higher than in treatments P1 and P2. Ecdysteroids are hormones that regulate molting in arthropods and crustaceans (Bakrim *et al*., 2012). The presence of ecdysteroid hormones can increase protein metabolism in cells, thereby promoting crab growth. This triggers the release of the shell and the formation of a new shell. Ecdysteroids originate from spinach extract (*Amaranthus tricolor*). This hormone is converted in the hemolymph by the 20-hydroxylase enzyme, found in the epidermis and other body tissues, into the active hormone 20-OH-ecdysone, thereby triggering molting in the crab (Fujaya *et al*., 2012).

Molting time

The results of the study on the effects of red spinach leaf extract (*Amaranthus tricolor* L.) on the molting time of mangrove crabs (*Scylla serrata*) during the research are shown in Table 2.

Table 2. Average duration of mangrove crab molting during the study.

Treatment	Molting average (day)
P0	0.33 ± 0.33^{a}
P1	1.33 ± 1.333^{ab}
P2	2.33 ± 2.333^{bc}
P3	2.67 ± 2.6733^{c}

Notes: Different letters in the same column indicate significantly different treatment effects (P<0.05). The data listed are mean values ± standard deviation.

Based on the ANOVA results, the administration of red spinach leaf extract (*Amaranthus tricolor* L.) on the molting time of mangrove crabs (*Scylla serrata*) has a significant effect (P<0.05). The results obtained for molting time show that the best treatment is P3, which involves injecting crabs with an extract dose of 7 µl/individual, with an average of 2.67/day. Meanwhile, the lowest molting percentage was observed in P0 (control), with a molting time of 0.33/day. In the P3 treatment, with a spinach leaf extract dose of 7 µl/individual being the best, it is known that spinach extract contains compounds, such as phytohormones, that can stimulate the production of ecdysteroids, the main hormones that regulate molting. Through the method of direct injection into the crab's body, these compounds

directly affect the Y-organ (gland) that controls molting (Harahap et al., 2016). On the other hand, the lowest treatment was P0, where the crabs did not receive any injection of red spinach leaf extract (*Amaranthus tricolor* L.).

Laju Petumbuhan Harian

The results of the study on the effects of red spinach leaf extract (*Amaranthus tricolor* L.) on the molting success of mangrove crabs (*Scylla serrata*) and daily growth rate during the study are shown in Table 3.

Table 3. Average daily growth rate of mangrove crabs during the study.

Treatment	LPH(%)
P0	0.42 ± 0.21^{a}
P1	1.57 ± 0.12^{b}
P2	1.82 ± 0.22^{b}
P3	2.23 ± 0.02^{c}

Notes: Different letters in the same column indicate significantly different treatment effects (P<0.05). The data listed are mean values ± standard deviation.

Based on the ANOVA results, the administration of red spinach leaf extract (*Amaranthus tricolor* L.) on the growth rate of mangrove crabs (*Scylla serrata*) has a significant effect (P<0.05). The results showed that the highest daily growth rate was observed in treatment P3 (7 µl/g) at 2.23%, followed by P2 (5 µl/g) at 1.82%, P1 (3 µl/g) at 1.57%, while the lowest was observed in treatment P0 (control) at 0.42%. P3, with the highest extract dose, produced a high growth rate and also showed accelerated molting due to the high dose of spinach leaf extract applied to the crabs.

This is suspected to be because the addition of spinach extract via injection increases the daily growth rate of crabs compared to those without injection. This can be understood as the role of phytoecdysteroid hormones in spinach extract is very significant for growth, namely by increasing protein synthesis via mRNA translation; additionally, phytoecdysteroids will stimulate carbohydrate metabolism and lipid biosynthesis. The increase in protein metabolism induced by steroid hormones improves feed protein utilization efficiency. With ecdysteroid supplementation, body protein synthesis remains high even when feed protein levels are low. Research results by Fujaya *et al*. (2007) show that spinach extract at 700 ng EB/g in crabs can accelerate and synchronize molting, does not cause death, and the growth of crabs receiving spinach extract application is greater than that of those without spinach extract application.

Food Conversion Rate (FCR)

Feed conversion ratio is the ratio of the total amount of feed provided during the study to the total amount of gain. The effect of red spinach leaf extract (*Amaranthus tricolor* L.) administration on the molting success of mangrove crabs (*Scylla serrata*) in terms of feed conversion during the research can be seen in Table 4.

Table 4. The average feed conversion ratio of mangrove crabs during the study.

Treatment	FCR%)
P0	5.01 ± 1.67^{b}
P1	1.75 ± 0.24^{a}
P2	1.65 ± 0.28^{a}
P3	1.17 ± 0.22^{a}

Notes: Different letters in the same column indicate significantly different treatment effects (P<0.05). The data listed are mean values ± standard deviation.

Based on the ANOVA results, the injection of red spinach leaf extract (*Amaranthus tricolor* L.) on the feed conversion of mangrove crabs (*Scylla serrata*) has a very significant effect (P<0.05). Based on the Duncan test, the P0 treatment is significantly different from P1, P2, and P3, while P1, P2, and P3 are not significantly different from each other. The best feed conversion value is seen in P3 (7 µl/g) at 1.17%, followed by P2 (5 µl/g) at 1.65%, P1 (3 µl/g) at 1.75%, and the lowest is in P0 (Control) at 5.01%. P3 shows the best feed conversion ratio, indicating that the consumed feed is of good quality (Agustono *et al*., 2013).

This is consistent with the opinion of Mathieu *et al.* (2007) that red spinach leaf extract contains phytoecdysteroids that can increase the efficiency of protein and carbohydrate metabolism in the crab's body. This makes the crabs receiving the highest extract dose more efficient at utilizing nutrients from the same feed, producing more energy than those receiving doses P2 and P1. The 5% of their body weight given to the crabs is sufficient to meet their metabolic needs. Insufficient feeding levels result in inhibited growth and molting, while excessive feeding can cause water pollution from the accumulation of residual feed on the bottom.

Survival Rate (SR)

Survival rate, or survival level, is the ratio of individuals alive at the end of the study to those at the beginning (Djunaidah *et al*., 2004). The

Survival Rate (SR) during the study is shown in Table 5.

Table 5. Average survival rate of mangrove crabs during the study.

Treatment	SR(%)
P0	88.92±19.22[a]
P1	77.85±19.22[a]
P2	100 ± 0.00[a]
P3	88.92±19.22[a]

Notes: Figures followed by the same letter indicate no significant difference ($p < 0.05$). The values listed are the means of the standard deviations.

Based on the ANOVA results, the injection of red spinach leaf extract (*Amaranthus tricolor* L.) on the survival rate of mangrove crabs (*Scylla serrata*) did not have a very significant effect ($P<0.05$). This indicates that the injection of red spinach leaf extract does not affect mortality in mangrove crabs. This is in accordance with the statement of Fujaya *et al*. (2009) that spinach leaf extract does not cause death in mangrove crabs. The Duncan test results for Survival Rate showed that none of the treatments were significantly different. The Survival Rate of mangrove crabs over the 40 days of the study showed that the highest treatment was P2 (5 µl/g), at 100%, and the lowest was P1 (3 µl/g), at 77.85%. The mortality rate at P1 during the study was caused by molting failure. According to Muhammad (2016), death due to molting (Molting Syndrome) or imperfect skin shedding can be caused by internal factors of the organism where during the long shedding process, the old skin or carapace does not completely detach from the body, leaving part of the body trapped in the old skin, while during molting, the body's condition is weak because all energy is focused on the shedding process. The lack of energy to support the molting process causes the crab to run out of energy and eventually die.

Meanwhile, at P0, mortality occurred because the crabs did not molt, but the deaths were caused by several factors, including stress from temperature changes and bacteria from residual feed. Malik (2009) states that once a crab is stressed, its physiological balance is disturbed, leading to decreased body resistance; this provides an opportunity for parasites, viruses, and water-quality fluctuations to enter and disrupt the crab's physiological functions. According to Agus et al. (2010), a well-controlled rearing environment and an adequate amount of feed can also support a high survival rate of mangrove crabs during rearing.

Water Quality

An external factor besides feed that supports the life and growth of cultivated organisms is water quality. Organisms can survive and grow if the water quality matches their natural habitat. The results of the water quality analysis in this mangrove crab research show that the water quality parameters are in normal conditions and suitable for mangrove crab cultivation. The water quality measurement results in the study, namely salinity, temperature, pH, and dissolved oxygen, are presented in Table 6.

Table 6. Mangrove crab water quality parameters during the study and range on normal status

Treatment	Result	Range parameter
Sainitas (ppt)	26 ppt–28	27–34 ppt (Tulangow *et al.,* 2019)
Suhu ˚C	26.3 ˚C–29	29 ˚C (Tulangow *et al.,* 2019)
pH	6.4-7	7,2-8,8 (Tulangow*et al.* ,2019)
Oksigen terlarut (Mg/l)	5-7	>5 mg/L (FAO, 2011)

Water plays a very important role as a living medium; therefore, in aquaculture, the water quality of the living medium must be maintained (Herlina, 2016). Temperature measurements during the study ranged from 26.3°C to 29°C. According to Tulangow *et al*. (2019), the temperature tolerance limit for mangrove crabs is 23–32°C. Furthermore, Cholik (2005), as cited in Agus (2008), states that the acceptable temperature range for mangrove crab life is 18–35°C. The pH range during the study was 6.4–7; according to Tulangow *et al.* (2019), a good pH range for mangrove crab growth is 7.2–8.8. Dissolved oxygen content during the study ranged from 5 to 7 mg/L. Meanwhile, the ideal dissolved oxygen concentration for mangrove crabs is 5 mg/L (FAO, 2011). The salinity levels measured during this study ranged from 26 to 28 ppt. Tulangow (2019) states that mangrove crabs can survive and grow well in the range of 27–34 ppt.

CONCLUSION

Based on the results of this study, it can be concluded that the effect of red spinach leaf extract (*Amaranthus tricolor* L.) administration on the molting success of mangrove crabs (*Scylla serrata*) has a very significant effect on the Molting Percentage, Daily Growth Rate (DGR), and Feed Conversion Ratio (FCR). However, it does not significantly affect the Survival Rate (SR) of mangrove crabs (*Scylla serrata*). The effect of red

spinach leaf extract (*Amaranthus tricolor* L.) administration on the molting success of mangrove crabs (*Scylla serrata*) via injection showed that the P3 treatment at 7 μg/g was the best dose.

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