



Research Article

Utilization of Paku Uban (*Nephrolepis biserrata*) Extract as a Molting Stimulant of Mud Crabs (*Scylla* spp.) in Traditional Ponds

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Abstract

Mud crab (*Scylla* spp.) is a fishery commodity with high economic value. For this reason, efforts to increase production are required with a more effective applicative technology. This study aims to determine the molting response and survival rate of mud crabs injected with paku uban leaf extract (*Nephrolepis biserrata*) at different doses. The study was conducted for 30 days, located in Tibi Island, Tanah Lia District, Tanah Tidung. The design used in this study was a completely randomized design (CRD) with five treatments and eight replicates. Mud crabs weighing 80 - 150 g were tested in K1 (without injection), K2 (controlled injection), P1 (100 ppm extract), P2 (125 ppm extract) and P3 (150 ppm extract). The effectivity of paku uban (*N. biserrata*) extract with the highest percentage of molting of 50% took place at P3, 37.5% at P2, and 25% at P1. The lowest levels of molting percentage were at K1 and K2 by 0%. The fastest molting period was ten days at P2, and the longest was 29 days at P1. The highest weight growth reached 33.75 g at P3, followed by P2 and P1 with 31 and 18.75 g, respectively. The survival rate of mud crabs for all treatments reached 100%. The results of the analysis of variance indicated a significant bet effect ($P < 0.05$) on the molting and weight growth percentage. The BNT test suggested that a dose of 150 ppm was the optimum treatment.

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1. Introduction

Aquaculture production in Indonesia began increasingly in 2011-2016 (BPS, 2017). One commodity that contributes to the increased production of farmed, namely mud crabs. Mud crab (*Scylla* sp) is one commodity of brackish water with high economic and ecological value. The demand for these commodities increased rapidly, spreading from local markets, regionally, and internationally. Based on the data from BPS (Indonesian Bureau of Statistics) in 2017, the export value of crabs in the period of 2016-2017 increased by 27.81%, which indicated a significant bet increase. Based on the data from BPS in 2016, the production of mud crab (*S. serrata* Forsskål, 1775) significant betly improved from 2003 to 2015, i.e., from 30,537.9 tons per year to 109,624.4 tons per years. To meet consumer needs for mud crabs, sustainable stock in a sufficient number, and by market demand becomes a necessity (Hamasaki et al., 2002). The executable steps to keep increasing the production of mud crabs and to provide sustainable stocks are by improving the cultivation performance. Also, to meet market needs and maintain the population of mud crabs in nature, it is necessary to use methods that could be accelerate growth in mud crabs.

The growth of mud crabs improves through a process of shell shedding known as molting. Molting is a phenomenon where old cuticles accumulate and produce new cuticles, which are mostly found in crustaceans and animals from other invertebrates. The changes in the physical criteria determine the molting stages, then the deposition of collagen fibers, carbohydrates, lipids, and calcium salts in the histological aspect of the whole molting cycle (Promwikorn et al., 2004). The molting cycle consists of molt, postmolt, intermolt, and pre-molt (Kuballa and Elizur, 2007). The hormones that play a role in the process of molting of crustaceans such as crabs are the molt stimulating hormone (MSH) and molt inhibiting hormone (MIH) (Huberman, 2000). MSH are produced by an organ called ecdysteroids. These hormones affect the process of growing and molting, while MIH is produced by the hormone inhibits organ growth, it plays a role in the reproductive process. Growth acceleration requires the proper use of technology.

Technology for mud crab cultivation continues to experience significant bet development, one of which is cultivation technology for producing soft shell crab. The export volume of crab, according to the data from the Directorate General of P2HP KKP in 2017, indicated 27,367.452 tons and tended to experience an increase annually. The technology used in soft shell crabs culti-

vation is autotomy or mutilation technology, by detaching crab's claw and foot organs, except for the swimming legs.

Although autotomy method practice is very common, and easily implemented and crab experience molting in a relatively shorter period, the survival rate, as well as the crab's molting percentage, is very low of approximately 18% (Djunaedi, 2016). The low survival rate happens because mutilated crabs experienced interference of physiological balance in their bodies. If the crabs are mutilated, their immune system weakens, and become very stressed. Providing an opportunity for parasites, virus, and bacteria to enter and damage the physiological functions on crabs eventually leading to death. Inaccurate treatments and techniques applied in the amputation process also affect the percentage of molting and, survival, and the growth degree (Moon et al., 2016).

The use of plant extracts in mud crab aquaculture has been widely used, such as the use of karamunting (*Melastoma malabathricum*) for stimulation of female crab maturation (Farizah et al., 2017), increase in the growth and molting rate (Ridwan et al., 2016). spinach extract, mulberry leave extract (*Morus* spp), and cycads extract (*Cycas revolute*) are known to contain ecdysteroid hormone that could be used to speed up the molting process (Fujaya et al., 2010; Herlinah et al., 2014; Suryati et al., 2013). The use of plant extracts is very promising as a technological innovation in the production of crabs, especially soft shell crabs, and is proven to be capable of replacing the mutilation method (Karim, 2007).

Paku uban (*N. biserrata*) is a plant that has no economic value. The results of the phytochemical analysis suggest that *paku uban* (*N. biserrata*) is one of the plants that contain steroid/ecdysteroid compounds (Astuti et al., 2013). Information regarding the use of *paku uban* extract for mud crab molting is not yet available. This study aims to determine the effectiveness of using *paku uban* extract as a molting stimulant to mud crab, as it is expected to replace or substitute crustacean ecdysteroid, which is relatively expensive.

2. Material and Method

2.1 *Paku Uban* leaves Extraction

The gathered *paku uban* plant were dried at room temperature for two weeks. Its dry leaves were then put in a blender and weighed as much as ± 200 g, and then macerated for 3 days using methanol and chloroform to 80% (w/v 1:4). After three days of maceration, the extract was put in a shaker for ± 3 hours. The solution

was filtered and evaporated to obtain the concentrated extract. The concentrated extract was diluted using distilled water by a predetermined dose.

2.2 Paku Uban Administration

The study was conducted in the Tibi Island aquaculture area, Bulungan Regency. The test animals used for experiments were mud crabs from Tibi island, weighing \pm 80-110 g. The injections made after the crab acclimatization for 2-3 days. The crabs were weighed and measured to determine the initial body weight and size. Injection in crabs was done on the segments of the swimming feet on the soft membrane. The fluid was injected from the base of the foot, circulating throughout the body's tissues through blood vessels (Fujaya *et al.*, 2010). Injected crabs were then placed in a crab box container containing 40 crabs with a dimension of 15 x 18.5 x 20 cm. Each box contained one treatment batch, arranged randomly into the pond.

2.3 Experimental Design

The method used is the experimental method. The study used a completely randomized design with five treatment dosages of *paku uban* extract, consisting of 0 ppm/ as control or without injection (K1); 0 ppm/ aquadest (K2) injection control; 100 ppm (P1); 125 ppm (P2) and 150 ppm (P3) with eight crabs in each treatment as replicates.

2.5 Breeding

The breeding of mud crabs was conducted for 30 days. During breeding, the crab was fed with fresh feed (rough fish) as much as 5% per day of body weight (Fujaya, 2008) with the feeding frequency once a day. Every five days, samples were taken, including the measurement of weight, width, and length of the carapace. Also, the state of the crabs' body parts was observed.

2.6 Measured Parameters

The data collection covered the frequency and molting period, the percentage and the latency period of molting, survival rate of mud crabs, the growth rate of mud crabs, and water quality.

2.6.1 Molting frequency and period

The molting frequency was a value that represents the separate levels of molting in mud crabs during the breeding process. In contrast, the molting period shows the average length required for the molting process. Molting is a mechanism to replace the crustaceans' shell for the growth process, both increasing

mass and body length. The growth rate will increase if the crabs molt frequently. Molting observation was conducted every day. Molting frequency and period could be calculated by the following formula (Farizah *et al.*, 2017):

$$\text{Frequency} : \frac{(n_1 + n_2 + \dots + n_i)}{(i)}$$

$$\text{Period} : \frac{30}{\text{Frequency}}$$

Where:

- n : The number of molting per animal during breeding
- i : The number of animals

2.6.2 Molting Percentage and Latency Period

The molting percentage molting was calculated based on the ratio of crab numbers that molted during the breeding period with the initial number of crabs being treated by 100 times (Arifin, 2010). Molting latency was observed by calculated the number of days required for the crabs to molt after the injection. The molting latency mass data was reported during the research period.

2.6.3 The Survival Rate (SR) the Mud Crabs

The observations of the crabs' survival rate were conducted every day started from the beginning until the end of the research. The total amount of survival could be calculated utilizing the following formula (Efendi, 2002):

$$\text{SR} = (\text{Nt} / \text{No}) \times 100\%$$

Where:

- SR : Survival rate (%)
- Nt : The number of crabs that live at the end of the breeding period (head)
- No : The number of crabs that live at the beginning of the breeding period (head)

2.6.4 Absolute Weight Growth (GR)

Absolute weight growth (GR) is the increasing biomass weight of the crabs at the end of the breeding period. The absolute weight growth was calculated according to the different ratios of crabs' weight in the final (Wt) and the initial (Wo) weight of the breeding process. The growth rate could be calculated with the

following formula (Effendi, 2002):

$$GW = (Wt - Wo) / t$$

Where:

- GW : Absolute growth (grams/head/day)
 Wt : The average of crabs' weight in the final of the breeding process (g)
 Wo : The average of crabs' weight in the initial of the breeding process (g)
 t : The duration of the breeding process (days)

2.7 Water Quality Measurement

Water quality parameters observed in the study were pH, temperature, salinity, and ammonia. The pH, temperature, and salinity were measured every day on-site, while the ammonia parameters were measured on day 1, 10, 20, and 30 of the breeding process in the water quality laboratory, Faculty of Fisheries and Marine Sciences, Universitas Borneo Tarakan.

2.8 Data Analysis

The data obtained were analyzed statistically by analysis of variance (One-Way ANOVA) to determine the difference between the treatment and control groups and to determine the significant effect of the average difference at a 95% confidence level ($p < 0.05$).

3. Results and Discussions

3.1 Molting Frequency and Period

Molting frequency is a value that represented the mud crabs molting rate during the breeding process, while the molting period was the average time required for the molting process. Molting is a mechanism to replace the crustaceans' shell for the growth process, both increasing mass and body length. Growth rate would increase in accordance to the crabs molting frequency. The molting process was affected by steroid hormones or specifically by ecdysteroid hormones. Ecdysteroid hormones in hemolymph would decrease during the intermolting phase, increase during the promoting phase, reach their peak during the molting process, and then decrease again during the oxidization and post molting process (Skinner, 1985). Higher amount of ecdysteroid hormone produced will result in more frequent molting. In general, the frequency of molting will go in parallel with age, in young crabs will molt every 10 days, after adult 4-5 times per year, and when they become a parent and have spawned the crabs experience molting

1-2 times per year (Chang and Mykles, 2011). In this study, the crabs used were adult crabs which generally experience molting every 60 days; the use of paku uban extract aims to increase the frequency of molting and shorten the molting period.

In-vivo test results showed that the administration of *paku uban* extract could increase the frequency and shorten the molting period compared to the control group, where the crabs were not injected with the extract and did not experience molting during the 30-day maintenance period (Table 1).

The highest molting frequency and the shortest molting period occurred at P3, i.e., the injection of *paku uban* extract at a concentration of 150 mg/L. This indicates that the extract concentration influenced the molting process. Based on the results of the phytochemical test, *paku uban* extract positively contained steroids, although the GC-MS test did not indicate any ecdysteroids in the extract (data not attached). Presumably, there are compounds in *paku uban* extract with a mechanism similar to the ecdysteroid hormone, which accelerate the molting process. In the qualitative phytochemical testing of *paku uban* extract, steroid compounds were positively detected (Maulianawati et al., 2019). Steroids in the form of ecdysteroids are known to influence the process of reproduction, metabolism, and acceleration of crab molting (Mykles, 2011).

3.2 Molting Percentage and Latency Period

The percentage of molting indicates the number of crabs that experienced molting during the study, while the molting latency was observed by looking at the number of days the crab required for molting after injection. The retrieval of molting latency data was carried out during the study period.

The research results indicated that there is a positive response to the administration of *paku uban* extracts against mud crab molting for 30 days of research. In-vivo test results revealed the highest frequency and percentage of molting was at P3, namely the administration of *paku uban* extract at a concentration of 150 ppm with 50% molting percentage, followed by treatments P2 and P1 with molting percentage of 37.5% and 25%, respectively. Uninjected crabs did not experience any molting (K-1 and K-2), meaning the molting percentage was 0%. Analysis of variance suggested a significant difference between treatments on the molting percentage ($p < 0.05$). The best result was on P3 treatment with a concentration of 150 mg/L. This treatment indicated that the extract concentration affected the molting percentage, where the greater the extract concentration was, the greater the percentage of molting (Table 1 and Figure 1). It is suspected that the greater the concentration

Table 1. The frequency, period, and percentage of Mud crab molting during 30 days of breeding

Treatment	Number of Crabs	Number of Molting Crabs	Molting Frequency	Molting Period	Molting Percentage (%)
K-1	8	0	0.0 ± 0.00 ^a		0.0 ± 0.00 ^a
K-2	8	0	0.0 ± 0.00 ^a		0.0 ± 0.00 ^a
P1	8	2	0.25 ± 0.005 ^{ab}	120	25.0 ± 0.46 ^{ab}
P2	8	3	0.375 ± 0.005 ^{ab}	80	37.5 ± 0.52 ^{ab}
P3	8	4	0.5 ± 0.005 ^b	60	50.0 ± 0.53 ^b

The data are presented as mean ± standard deviation (n = 8). Data with the same lowercase indicates no significant bet difference (p <0.05).

Table 2. The latency period of Mud crab molting for 30 days of research

Treatment	Time range (days)						
	0	5	10	15	20	25	30
K1							
K2							
P1						←————→	
P2		←————→					
P3				←————→			

Table 3. Mud crab survival for 30 days of breeding

Treatment	Number of Crabs	Number of Dead Crabs	SR (%)
K1	8	8	100
K2	8	8	100
P1	8	8	100
P2	8	8	100
P3	8	8	100

Table 4. Results of water quality measurement for 30 days of breeding

Parameter	Range	Quality standards	Unit
Salinity	13-16	15-30 *	Ppt
pH	7.8 - 9.1	6.8 - 8.2 *	-
Temperature	27-38	25 - 35 *	°C
Ammonia	0.03 - 0.05	<1.0 **	mg/L

The quality standard used is water quality for the cultivation of Mud crabs (*Scylla* spp).

Note: * Fujaya *et al.*, 2012, ** Taruni, 2007

used, the greater the concentration of compounds that played an important role in the molting mechanism.

The administration of vitomolt with different doses is known to be capable of increasing the percentage of mud crab molting, where the higher the dose used, the greater the percentage of molting (Fujaya *et al.*, 2011). The mechanism of molting occurrence in crabs is influenced by the availability of ecdysteroid hormones. The total ecdysteroids present in hemolymph vary during each molting period and depend on the type of crustaceans. This hormone is secreted by the organ Y in the form of ecdysone.

In hemolymph, this hormone is converted to the active hormone 20-hydroxyecdysone by the enzyme 20-hydroxylase found in the epidermis of organs and other body tissues. Decreased ecdysteroids in the hemolymph could be trigger the release of the exoskeleton. Ablation of certain parts of the eye or body parts of crustaceans could be eliminate the main source of inhibiting neuropeptides, activating organ Ys and increasing levels of ecdysteroid hemolymph (Mykles, 2011). However, ablation treatment to increase the percentage of molting is considered ineffective because of the high mortality rate (Djunaedi, 2016). Increased hormone-steroid could be done with the use of plant extracts containing ecdysteroid compounds, although it is not certain that there are ecdysteroid compounds in paku uban extract, results of this study suggests that there are other steroid compounds that could be accelerate the process of molting or triggers in the exoskeleton release.

Male crabs weighing over 80-100 grams would experience molting for 38 and 53 days for female crabs (Promwikorn *et al.*, 2004). In this study, there was no injection conducted on the control and the crabs did not experience molting. This proves that the administration of *paku uban* extract could be accelerate the molting process, and the higher the concentration, the higher the percentage of crabs molting.

The fastest latency period in this study was 3-15 days (P2) with the extract concentration of 125 ppm, then P3 with a molting latency period of 13-24 days, and P1 with a molting latency period of 21-30 days. The length of time required by mud crabs was influenced by several factors, including size (weight), sex, species, and hormones. Periodically, members of the Crustacea family who underwent molting process would grow larger, but the amount of ecdysteroid hormones in the body of mud crabs was small, which was at around 500 nanograms per kg of body weight. Therefore, the molting process took a long time, which was more than 30 days. Molting is a phenomenon of the accumulation of

old cuticles and produces new cuticles that are found in many crustaceans and animals from other invertebrates (Promwikorn *et al.*, 2004).

Changes in physical criteria first determine the molting stage, then deposition of collagen fibers, carbohydrates, lipids, and calcium salts in histology throughout the molting cycle (Promwikorn *et al.*, 2004). The molting cycle consists of molt, postmolt, intermolt, and premolt (Kuballa and Elizur, 2007). Hormones that play a role in the process of molting crustaceans such as shrimp are molting stimulating hormone (MSH) and molt inhibiting hormone (MIH) (Huberman, 2000). MSH is produced by organ Y and is called an ecdysteroid hormone that functions for growth and molting, while MIH is produced by organ X, which inhibits growth but plays a role in the reproductive process.

In the development of the shrimp cycle at a time when hormones are activated between the hormones MIH and MSH. The difference in latency molting in this study could be due to the size and weight, sex, and species of mud crabs used that were not identical. Also, the difference in the concentration of *paku uban* extract used. This result has been confirmed by Fujaya *et al.* (2011), which states that the higher the concentration or dose of Vito molt used, it could be linearly increase growth but does not occur in the molting process, where lower doses accelerate the molting process.

3.3 The Survival of Mud Crabs

Observation of crab shrimp survival was carried out every day from the beginning until the end of the study by observing the mortality of mud crabs. The survival rate of mud crabs during the study period was 100%, and no mortality has occurred for all treatments (Table 2). The dose of *paku uban* extract used is the preliminary LD50 test results. LD50 in *paku uban* extract has low toxic power with a concentration of 2704 mg/L (Maulianawati *et al.*, 2019) The results of the analysis of variant showed no significant bet difference between treatments for mud crab survival ($p > 0.05$). This result also did not show a significant bet difference with the stimulation of molting using autotomy induction with SR of 80-100% (Purnama *et al.*, 2016).

The survival rate obtained in this study showed that the injection process and administration of the extract did not affect the condition of mud crabs and tended to increase SR and in accordance with the results of the toxicity test that the gray nail extract was safe to be used for in-vivo testing (Maulianawati *et al.*, 2019). Acceleration of molting by giving gray nail extracts also gave better results in the survival value compared to the

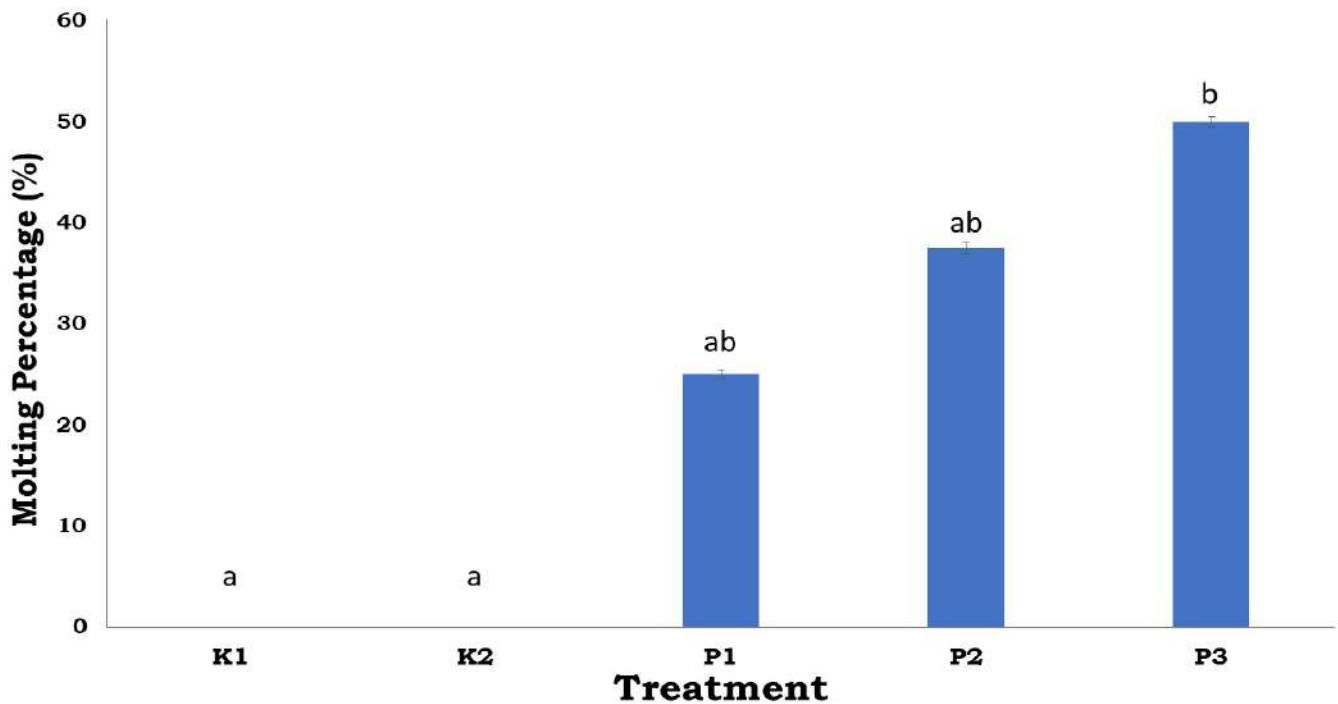


Figure 1. The percentage of crab molting during 30 days of breeding ($p < 0.05$).

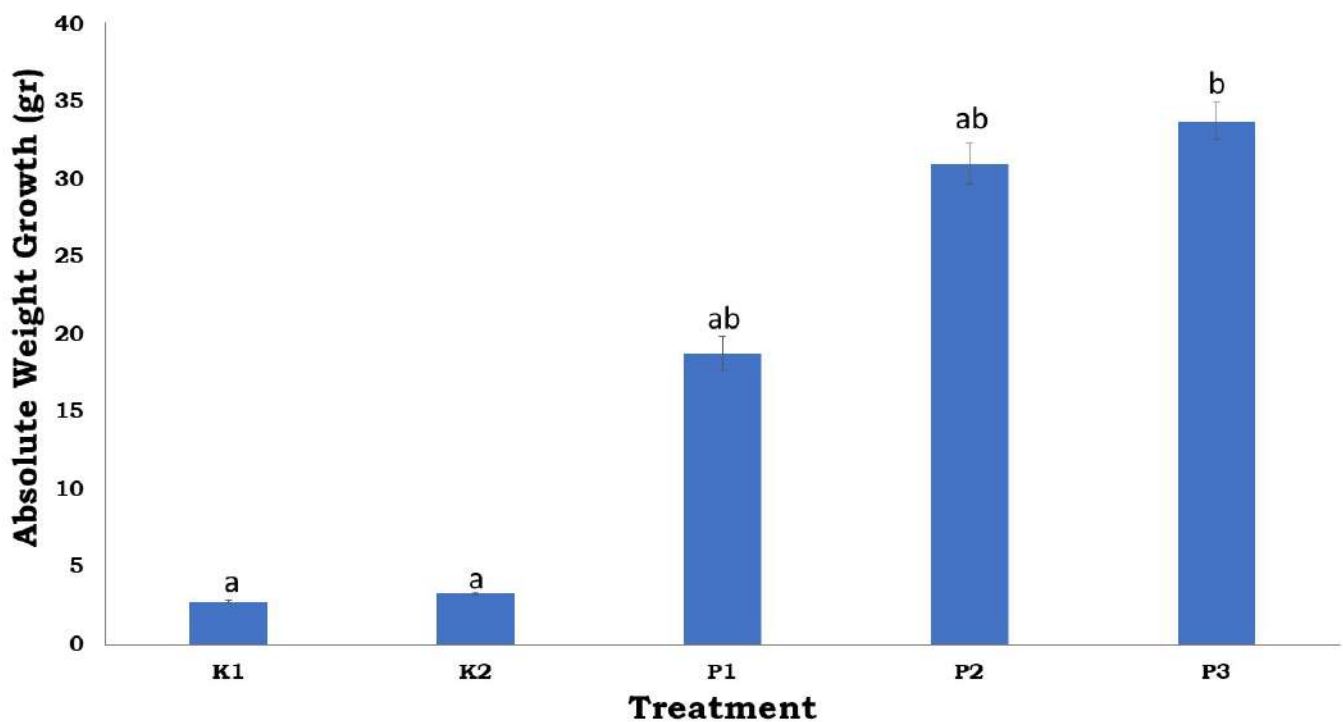


Figure 2. Crabs' absolute weight growth during 30 days of the breeding period ($p < 0.05$)

method of cutting the foot of the road with a survival rate of 50% (Samidjan and Rachmawati, 2015). The use of plant extracts to accelerate molting is known to have a high survival rate, which is around 91.37% for the use of spinach extract (Herlina *et al.*, 2014).

3.4 Growth in Absolute Weights

Absolute growth (GR) is the weight gain of crab biomass at the end of maintenance, where the difference between the weight of crabs at the end of maintenance

(Wt) and the weight at the beginning of maintenance (Wo) is compared to the time of maintenance (Effendi, 2002).

The results showed the best absolute weight growth in the P3 treatment with a growth of 33.75 ± 3.58 g and the lowest absolute weight growth in the K1 treatment of 2.75 g (Figure 2). There is a difference in absolute weight growth between treatments because the percentage of molting in P3 treatment is the highest when compared to other treatments. These results indicate differences with previous studies that used mulberry leaf extract (*Morus* spp) with the same dose experiencing growth only of 25.44 g (Herlina et al., 2014), with the autotomy induction method of 65.87 g (Fajar et al., 2016).

In the treatment of K1 and K2, the absolute weight growth of mud crabs was low. It occurred since the crabs did not experience molting, which means that there was no growth on the crabs even though the same amount of feed was given to the treatments. This condition could be caused by several factors. The feed that was given only for survival purposes, the crabs entering the stadium where the growth was no longer maximal, and the breeding period that was performed relatively shorter. The analysis of variance resulted that there were significant differences among treatments on the absolute weight growth of the mud crabs. The growth in the mud crabs was the increase in the body weight and carapace width that occurred periodically after the molting (Catacutan, 2002). Molting for crustaceans is a critical period that describes the physiological conditions of the old skin turnover process (exoskeleton) (Gimenez et al., 2001). Molting is influenced by external factors such as salinity, temperature, and internal factors, including nutritional status and ablation of the eye (Koo et al., 2005). These results indicated that the administration of *paku uban* extract could be affect the growth rate of mud crabs.

4. Conclusion

This study uses *paku uban* extract to mud crabs as stimulant molting. *Paku uban* extract could be increase the molting percentage, survival rate, and accelerate the latent molting period. These conditions are caused by the metabolite content in *paku uban*. Further research related to the availability of ecdysteroid hormone in crabs that are injected with *paku uban* extract, as well as the effects of *paku uban* extract used to increasing ecdysteroid hormones on mud crabs are required.

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Authors' Contributions

DM collected the data, drafted the manuscript and designed the figures. RUK and AWAL devised the main conceptual ideas and critical revision of the article. MI collected the data and field monitoring. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests

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