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The frequency of calcium and magnesium differences in recirculation systems for increasing production of mudcrab *Scylla serrata* seed

Frekuensi penambahan kalsium dan magnesium yang berbeda pada sistem resirkulasi untuk meningkatkan produksi budidaya benih kepiting bakau *Scylla serrata*

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ABSTRACT

Molting phase is one of many factors that can inhibit mudcrab growth. Recirculation system in culturing mudcrab has a weakness which is the decreasing of ions. Calcium and magnesium in the water can affect the molting phase. The aim of this study was to evaluate the best additional frequency of calcium and magnesium in recirculation system. This research used mudcrab seeds that have weight of 54.856±2.195 gram. This research used completely randomized design with four treatments and three replicates. The treatments were additional frequency of Ca and Mg, comprised of four levels, without additional Ca and Mg (A), additional 30 mg/L Ca and 30 mg/L Mg in every five days (B), additional 30 mg/L Ca, and 30 mg/L Mg in every 10 days (C), and additional 30 mg/L Ca and 30 mg/L Mg in every 15 days (D). The result showed that total of biomass in every treatments were A (379.99±86.16 gram), B (517.65±103.94 gram), C (808.68±59.29 gram), and D (1,054.41±73.54 gram). The highest final biomass was the D treatment (1,054.41±73.54), which was significantly different to others (P<0.05).

Keywords: mudcrab, resirculation, calcium, magnesium, molting, production

ABSTRAK

Salah satu faktor penghambat pertumbuhan kepiting bakau adalah fase molting. Sistem resirkulasi budidaya kepiting bakau memiliki kelemahan yaitu berkurangnya ion-ion. Fase moting pada kepiting bakau sangat dipengaruhi oleh keberadaan ion kalsium dan magnesium dalam air. Penelitian ini bertujuan untuk menentukan frekuensi waktu penambahan kalsium dan magnesium terbaik dalam sistem resirkulasi. Penelitian ini menggunakan benih kepiting bakau dengan berat rata-rata 54,856±2,195 gram. Penelitian ini menggunakan rancangan acak lengkap (RAL) dengan empat perlakuan dan tiga ulangan. Perlakuan penambahan Ca dan Mg sebanyak 30 mg/L terdiri atas empat macam frekuensi, yaitu tanpa penambahan Ca dan Mg (A), frekuensi lima hari sekali (B), frekuensi 10 hari sekali (C), dan frekuensi 15 hari sekali (D). Hasil penelitian menunjukkan jumlah biomassa masing-masing perlakuan adalah A (379,99±86,16 gram), B (517,65±103,94 gram), C (808,68±59,29 gram), dan D (1.054,41±73,54 gram). Perlakuan terbaik diperoleh pada perlakuan D dengan jumlah biomassa sebesar (1.054,41±73,54 gram) ini berbeda nyata (P<0,05) dengan perlakuan lainnya.

Kata kunci: kepiting bakau, resirkulasi, kalsium, magnesium, molting, produksi

INTRODUCTION

The consumer of mud crab *Scylla serrata* is still dominated by the upper middle class society. The culture process of mud crab currently is only located in the area that is near with the source of sea water. Recirculation system is an altenative technology in aquaculture that can provide opportunities for urban people to conduct the culture of mud crab due to its potential to minimize the use of sea water that is costly to be supplied to urban areas. This technology can also applied by the farmers in coastal areas to reduce the water replacement during the farming process. Recirculation system is a circulation system of water, draining water from rearing containers to filter (treatment) containers, then drained back to rearing containers (Timmons & Losordo, 1994). This system is applied in the fish farming under the controlled environmental conditions. Recirculation system is a method in aquaculture that does not depend on the time and seasons (Shannon *et al.*, 2008). However, recirculation system also had a disadvantage that is the reduction of ions contained in the water during the rearing process. This is caused by the absorption of ions by organisms that live in the water to support their growth.

One of ions that is reduced in large quantities in recirculation system is calcium. This occurs because calcium is used by crabs for the formation of carapace. Carapace is the largest part of the crab body. The high requirement of calcium in crabs cultured in recirculation system cannot be fulfilled only from the environment where crabs live and the feed. It needs an addition from an external source to add the amount of calcium contained in the environment. Calcium is important for the formation of bones and exoskeleton of crustacea. Calcium is an essential mineral that is requred in a high amount (Teruaki *et al.*, 2009). In crustacea experiencing postmolt phase, the shell hardening occurs through the deposition of calcium in the shell. Calcium derived from the environment is very dominant in the hardening process of the shell of crustacea (Granado, 2010).

Calcium is required by crustacea in the shell formation phase. In normal condition, the calcium content of crab is 10.75 g/100 g while in the shell formation phase the calcium content increases to be 29.14 g/100 g (Marzuki *et al.,* 2013). Magnesium contained in the body is able to increase the absorption of calcium (Fabritius *et al.*, 2012). Due to this relationship between calcium and magnesium, so magnesium is also used to increase the absorption of calcium during the mud crab shell formation phase. The molting period in the cultured crab juveniles has not been known, and the molting times are also not simultaneous. In this study, the different frequencies of the addition of calcium and magnesium were applied, so when crabs molted, calcium contained in the water is on an adequate amount.

This study aimed to determine the optimum frequency of the addition of calcium and magnesium in recirculation system to increase the production and the shell formation of mud crab.

MATERIALS AND METHODS

Animals and experimental design

Animals used in this study were 120 mud crabs with an average weight of 54.856±2.195 g derived from a collecting farmer in Demak, Central Java. Each experimental tank was stocked with 10 mud crabs reared in recirculation system (Figure 1). The sources of calcium and magnesium used in this study were origined from CaO (calcium oxyde) and $MgSO_4$ (magnesium sulfate).

This study was conducted through completely randomized design (CDR) consisting of four treatments and three replicates for each treatment. Treatments applied consisted of: A (without the addition of Ca and Mg), B (30 mg/L Ca and 30 mg/L Mg added every five days), C (30 mg/L Ca and 30 mg/L Mg added every 10 days), and D (30 mg/L Ca and 30 mg/L Mg added every 15 days).

Experimental parameters

In this study, several parameters were tested including the amount of calcium and magnesium, dynamics of water quality, physiological response, and production parameters.

Parameters indicating the amount of calcium and magnesium consisted of: the calcium content in the water, the calcium content in the crab body, the magnesium content in the water, the magnesium content in the crab body, the amount of calcium in the feed, and the amount

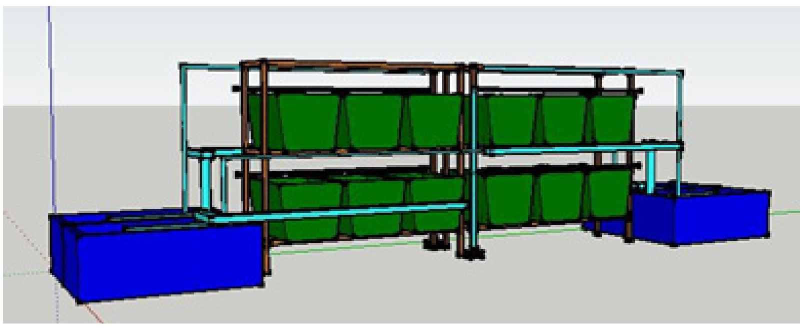

Figure 1. Recirculation system applied in this study.

of magnesium in the feed. The measurements of these parameters were performed at the beginning and the end of the rearing period. The measurement technique used atomic absorption spectrophotometer (AAS) (Marzuki *et al.*, 2013).

Water quality parameters: daily and weekly water quality parameters. Daily water quality parameters included: temperature, dissolved oxygen and water pH, the measurement used tools which could directly measured those parameters *in-situ* (APHA, 2012). Weekly water quality parameters included: ammonia, nitrite, nitrate, alkalinity, and salinity. The measurement used titration method or used spectrophotometer (APHA, 2012).

Osmotic gradient measured using osmometer (The formula of osmotic gradient = [the crab hemolymph osmolarity (mOsm/L H_2O) – the medium osmolarity (mOsm/L H_2O)].

Production parameters: feed conversion ratio, daily growth rate, the number of molting, survival, and total biomass (NRC, 1977). The calculation was conducted at the end of the study based on the results of sampling conducted every seven days.

Statistical analysis

Data obtained were tabulated and were analyzed using Microsoft Excel 2013 and SPSS 17.0. Data obtained were presented in table, chart, or figure. Data were analyzed through analysis of variance (ANOVA) with F test at a confidence level of 95%. If there were significant differences, then Duncan's test was performed to determine differences among treatments. Quantitative description analysis was used to explain the feasibilty of water quality in the rearing mediums for the life of mud crabs during the study.

RESULTS AND DISCUSSION

Results

The content of calcium and magnesium

The results of the analysis of the calcium content in the water, the crab and the feed (Table 1) showed that the highest value of the calcium content in the water at the end of the study was found in treatment A that was 649.86±2.63 mg/L, and it was significantly different ($P<0.05$) to other treatments. On the other hand, the lowest value was found in treatment B that was the addition of 30 mg/L Ca and 30 mg/L Mg every five days that resulted a value of 466.19±2.99 mg/L. The highest amount of calcium contained in the crab was obtained in treatment C that was 49,244.52±206.08 mg/L and it was significantly different ($P<0.05$) to other treatments. On the other hand, the lowest value was obtained in treatment A that was a treatment without the addition of Ca and Mg that resulted a value of 16,154.43±134.27 mg/L. The calcium content contained in the feed was 19,685.05±47.21 mg/L.

Calcium and magnesium have a relationship in the formation of carapace in crustacea. Based on the results of the measurements of the magnesium content in the water, the crab and the feed (Table 2), it could be known that the highest value of Mg in the water was found in treatment C that was 1,022.99±8.60 mg/L, and it was significantly different ($P<0.05$) to treatment B and C. On the other hand, the lowest value of Mg in the water was found in treatment B that was 924.41±2.84 mg/L. The highest amount of Mg in the crab at the end of the study was obtained in treatment A that was 6,800.67±200.38 mg/L, and it was significantly different ($P<0.05$) to other treatments. The lowest amount of Mg contained in the crab was obtained in treatment C that was 3,251.12±10.41 mg/L. The amount of Mg contained in the feed was 864.75±13.35 mg/L.

Water quality

The addition of Ca and Mg to the rearing water influenced the content of Ca and Mg in the water and the crab, it also influenced the water quality of the rearing water. The most influenced water quality parameters because of the addition of Ca and Mg were water pH and alkalinity. The results of the measurements of water quality parameters were presented in Table 3 and Figure 2.

Based on the observations of water quality parameters during the rearing period, so data of temperature, dissolved oxygen, ammonia, nitrite, nitrate, salinity, and alkalinity were obtained (Table 3). The values of water quality indicated that all treatments had water quality ranges that could be tolerated by crabs to support their lives.

Osmotic gradient

The result of ANOVA indicated that treatments applied in this study significantly affected ($P<0.05$) to osmotic gradient of crab. The result of the measurement of osmotic gradient (Figure 3) during the rearing period gave an information that the lowest value was obtained in treatment D that was 0.263±0.008 mOsm/L H_2O, while the highest value was obtained in treatment A that was 0.398±0.012 mOsm/L H_2O.

Table 1. The calcium content in the water, the crab, and the feed

Treatments	Calcium in the water (mg/L)	
	Initial	Final
(A) Without any addition	381.72±2.21	649.86±2.63a
(B) Every 5 days	381.72±2.22	466.19±2.99d
(C) Every 10 days	381.72±2.23	613.16±0.18b
(D) Every 15 days	381.72±2.24	602.81±4.46c
Treatments	Calcium in the crab (mg/L)	
	Initial	Final
(A) Without any addition	47,792.29±98.41	16,154.43±134.27d
(B) Every 5 days	47,792.29±98.42	44,611.03±151.93b
(C) Every 10 days	47,792.29±98.43	49,244.52±206.08a
(D) Every 15 days	47,792.29±98.44	35,201.51±99.4c
In the feed (ppm) = 19,685.05±47.21		

Note: Different letters after numbers indicate significant differences (P<0.05).

Table 2. The magnesium content in the water, the crab, and the feed

Treatments	Magnesium in the water (mg/L)	
	Initial	Final
(A) Without any addition	903.35±6.37	1,010.72±12.2a
(B) Every 5 days	903.35±6.38	958.51±2.73b
(C) Every 10 days	903.35±6.39	1,022.99±8.61a
(D) Every 15 days	903.35±6.40	924.41±2.84c
Treatments	Magnesium in the crab (mg/L)	
	Initial	Final
(A) Without any addition	4,909.38±15.21	6,800.67±200.38a
(B) Every 5 days	4,909.38±15.22	4,579.43±34.56b
(C) Every 10 days	4,909.38±15.23	3,251.12±10.41c
(D) Every 15 days	4,909.38±15.24	4,506.50±64.34b
In the feed (mg/L) = 864.75±13.35		

Note: Different letters after numbers indicate significant differences (P<0.05).

Oxygen consumption rate (OCR)

The result of ANOVA indicated that treatments given in this study significantly affected (P<0.05) to oxygen consumption rate of mud crab. The lowest oxygen consumption rate was obtained in treatment D that was 0.0065±0.0004 mgO_2/g/hour, while the highest of that was found in treatment A that was 0.0113±0.0002 mgO_2/g/hour. The result of the measurement of oxygen consumption rate was presented in Figure 4.

Feed conversion ratio and daily growth rate (DGR)

The result of ANOVA indicated that treatments in this study significantly affected (P<0.05) to feed conversion of crab. The result of the calculation of feed conversion ratio (Figure 5) during the rearing period revealed an information that the lowest value was obtained in treatment C that was 8.18±0.18, while the highest value was obtained in treatment A that was 10.65±0.85.

The result of ANOVA indicated that treatments in this study significantly affected (P<0.05) to daily growth rate of crab. The result of the calculation of daily growth rate (Figure 6) during the rearing period demonstrated an information that the lowest value was obtained in treatment A that was 1.116±0.04%/day, while the highest value was obtained in treatment C that was 1.733±0.06%/day.

Table 3. The water quality ranges during the rearing period

Composition	Treatments			
	A) Without any addition	(B) Every 5 days	(C) Every 10 days	(D) Every 15 days
Temperature (°C)	27.35±0.16	27.44±0.14	27.54±0.14	27.54±0.15
Dissolved oxygen (mg/L)	6.56±0.09	6.73±0.12	6.66±0.09	6.64±0.07
Ammonia (mg/L)	0.0343±0.003	0.591±0.59	0.315±0.06	0.213±0.02
Nitrite (mg/L)	1.138±0.034	0.595±0.036	0.651±0.039	0.632±0.039
Nitrate (mg/L)	0.382±0.018	0.339±0.019	0.343±0.023	0.416±0.018
Salinity (g/L)	23.25±0.11	22.86±0.22	22.72±0.12	23.49±0.09
Alkalinity (mg/L $CaCO_3$)	108.69±14.14	143.19±17.93	139.74±20.92	169.07±14.94

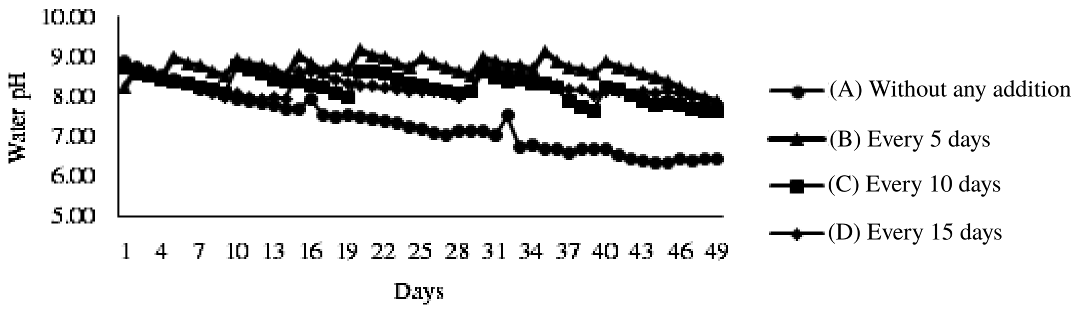

Figure 2. The water pH values during the rearing period

The number of molting, survival, and total biomass

Based on the result of ANOVA, it could be known that treatments applied in this study significantly affected ($P<0.05$) to the number of molting of crab. The result of the calculation of the number of molting (Figure 7) during the rearing period demonstrated that the highest value was obtained in treatment D that was 18.67±1.15 shells while the lowest value was obtained in treatment A that was 10±1.73 shells.

Based on the result of ANOVA, it could be known that treatments given in this study significantly affected ($P<0.05$) to survival of crab. The result of the calculation of survival (Figure 8) during the rearing period showed that the highest value was obtained in treatment D that was 86.67±5.77%, while the lowest value was obtained in treatment A that was 40±5%.

Based on the result of ANOVA, it could be known that treatments in this study significantly affected ($P<0.05$) to total biomass. The result of the calculation of total biomass (Figure 9) during the rearing period indicated that the highest value was obtained in treatment D that was 1,054.41±73.54 g, while the lowest value was obtained in treatment A that was 376.99±86.16 g.

Discussion

The calcium content in the water at the beginning and the end of the rearing period experienced an increase in its solubility. Treatments through the addition of calcium and magnesium demonstrated the increasing of the calcium content. This was caused by the addition of calcium to rearing mediums. In the treatment without the addition of calcium and magnesium, the calcium content increased because crabs took magnesium to replace calcium in their bodies. This treatment also had the lower calcium content in the body than that on other treatments. This was indicated by the calcium content at the end of the rearing period (Table 1) and the magnesium content in the crab (Table 2). According to Bogart *et al.* (2016) crab needs calcium for the formation of carapace but its absorption is impaired with a low amount of free calcium contained in the medium. Calcium in sea water binds to CO_2 forming $CaCO_3$. According to Calhoun and Zou (2016) calcium and magnesium are the largest components of exoskeleton (carapace) that are absorbed before molting.

The most influenced water quality parameters because of the addition of Ca and Mg were water pH and alkalinity. Alkalinity values among

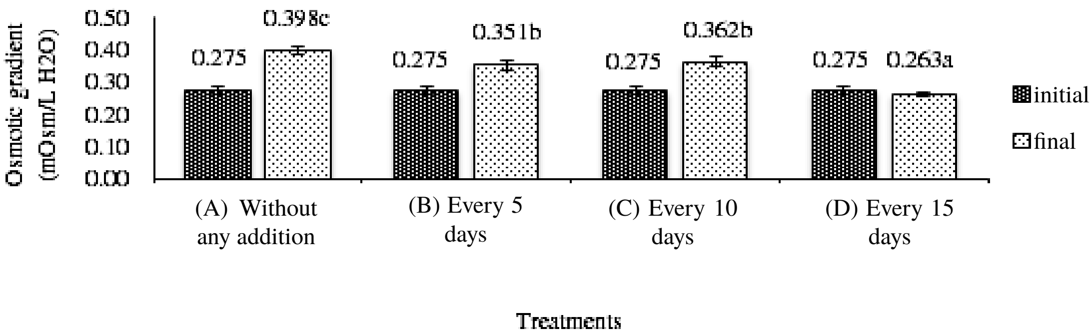

Figure 3. Osmotic gradient of crab. Different letters on bars indicate significant differences at a significance level of 5% (Duncan's test).

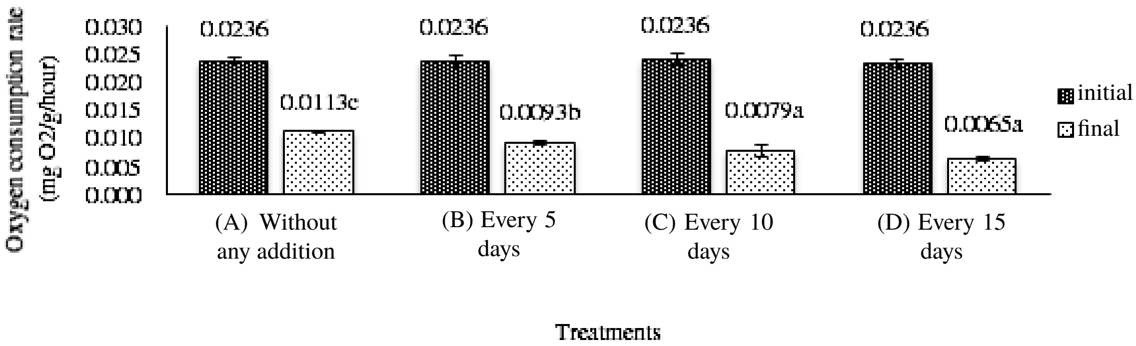

Figure 4. Oxygen consumption rate of mud crab. Different letters on bars indicate siginificant differences at a significance level of 5% (Duncan's test).

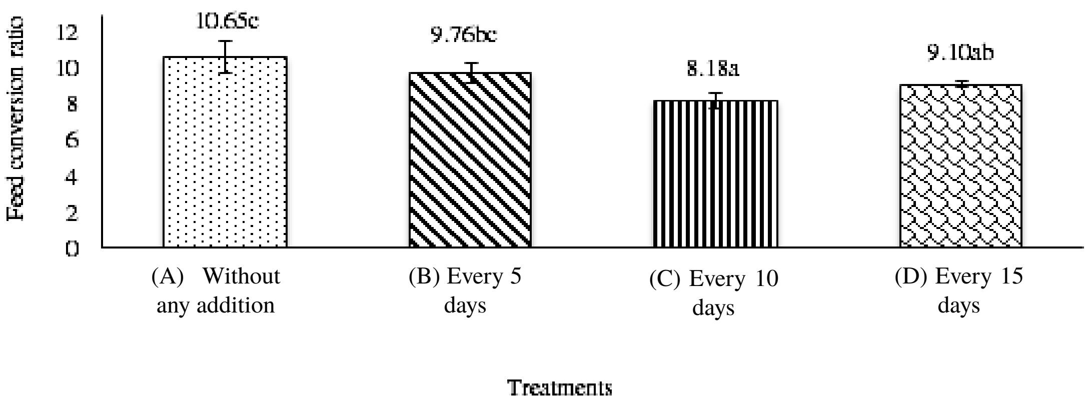

Figure 5. Feed conversion during the rearing period of mud crab. Different letters on bars indicate significant differences at a significance level of 5% (Duncan's test).

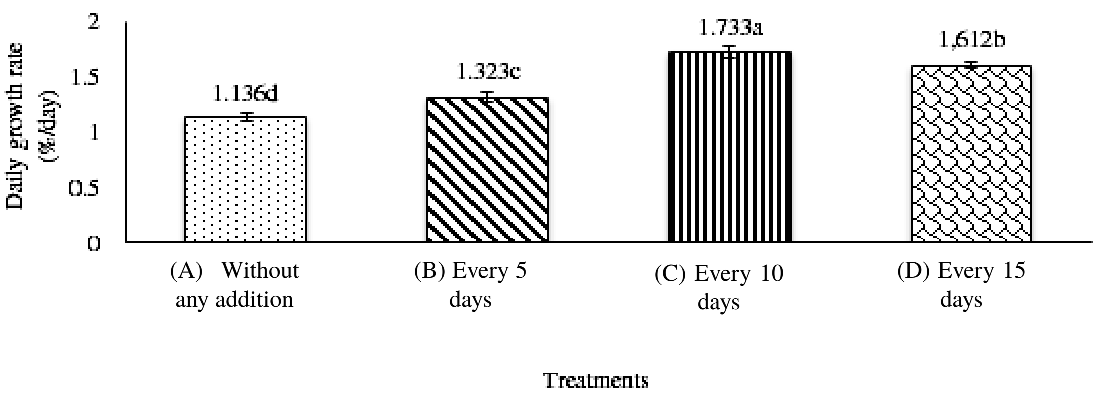

Figure 6. Daily growth rate of mud crab. Different letters on bars indicate significant differences at a significance level of 5% (Duncan's test).

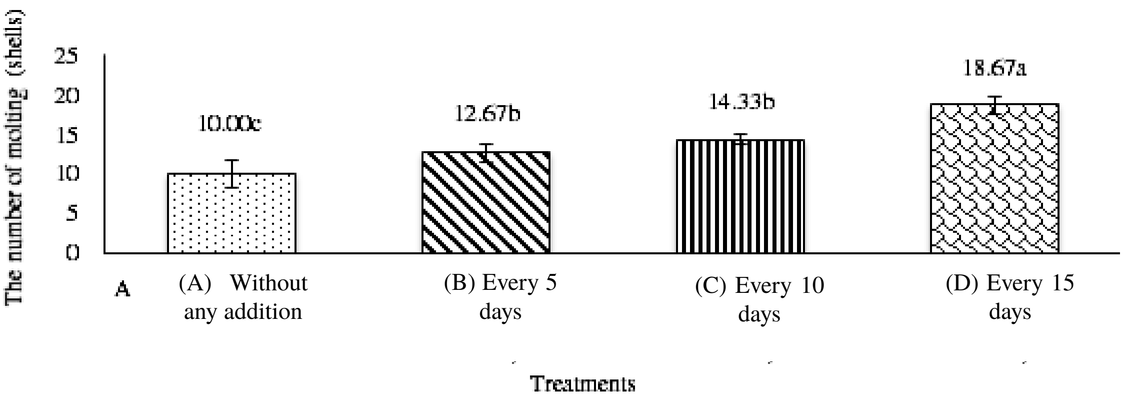

Figure 7. The number of molting of mud crab. Different letters on bars indicate significant differences at a significance level of 5% (Duncan's test).

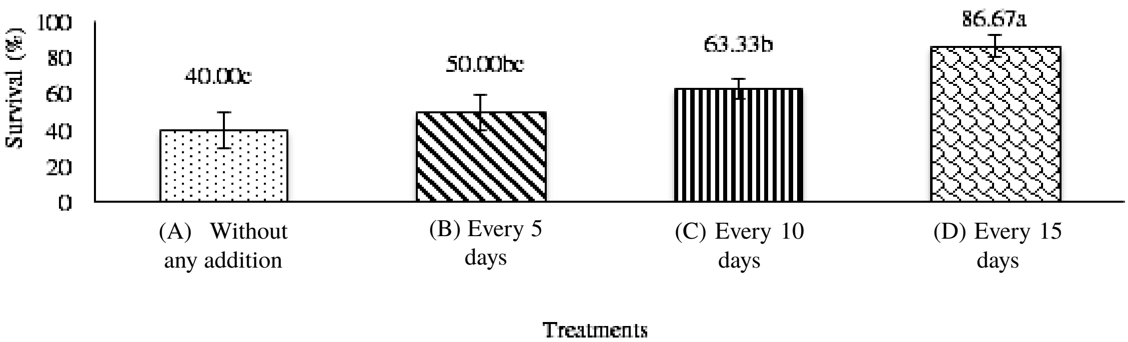

Figure 8. Survival of mud crab. Different letters on bars indicate significant differences at a significance level of 5% (Duncan's test).

treatments indicated that treatment A (without the addition of Ca and Mg) had the lowest value compared to other treatments. This occurred because the composer of alkalinity was $CaCO_3$. According to Bogart *et al.* (2016) alkalinity is composed by many minerals that can influence alkalinity and hardness. So that when calcium is absorbed by crabs, it causes a decrease in alkalinity. On the other hand, treatments with the addition of Ca and Mg (B, C, and D) had stable alkalinity values.

In addition to alkalinity, pH also becomes the most influenced parameter because of the addition of Ca and Mg. The pH values during the rearing period showed that pH in treatment A decreased compared to other treatments. On the other hand, treatments with the addition of Ca and Mg had dynamics that are almost same among treatments (Figure 1). According to Glandon and Miller (2016) the molting process in crabs can cause a drop in water pH. Low pH can be risen up by the addition of Ca and Mg.

Osmotic gradient in treatment D (the addition of 30 mg/L Ca and 30 mg/L Mg every 15 days) showed that in this treatment, body osmotic fluid with medium osmotic fluid tended on a balanced condition or nearly isoosmotic. Thus, the physiological function of crabs would run normally because the energy used for osmoregulation is not too high. In this treatment, the working process of osmoregulation caused by hyperosmotic conditions in crabs to the environment will be reduced by the addition of 30 mg/L Ca and Mg every 15 days. Therefore, body osmotic fluid with medium osmotic fluid tended to be more isoosmotic and caused osmotic gradient value in this treatment becoming lower than that on other treatments. Calcium plays a role to keep the body condition of organisms against changes in osmotic pressure (Sakamoto *et al.* 2013). High osmotic gradient values in other treatments indicated that body osmotic fluid with medium osmotic fluid tended to be hyperosmotic or hypoosmotic. This caused a high amount of energy required for osmoregulation to prevent the loss of minerals in the body. According to Hong *et al.* (2013) osmolarity can influence the use of energy by organisms in the rearing process. Isoosmotic condition is the optimum condition for the growth of crabs.

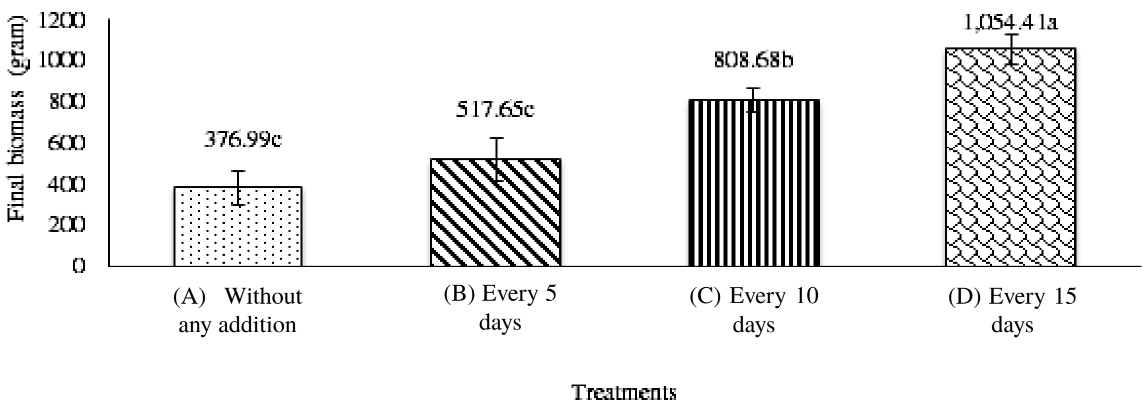

Figure 9. Final biomass of mud crab. Different letters on bars indicated significant differences at a significance level of 5% (Duncan's test).

The lowest oxygen consumption rate was obtained in treatment D (the addition of 30 mg/L Ca and 30 mg/L Mg every 15 days) that was 0.0065±0.004 mgO_2/g/hour. A low oxygen consumption rate in this treatment closely related to the lowest osmotic gradient that was also obtained in the same treatment. When crabs require energy for osmoregulation process, crabs will utilize energy sources existing in their bodies that are glucose and oxygen for oxidation. Thus, a low value in osmotic gradient will save more energy, as well as the consumption of oxygen that is used as a material for the oxidation of energy source materials derived from the consumed feed. The highest oxygen consumption rate in the treatment without the addition of Ca and Mg was in accordance with the highest osmotic gradient that was also obtained in this treatment, so crabs will perform osmoregulation process to maintain their homeostasis states. According to Li *et al.* (2007) the fish will perform more moving and swimming activities, so it will have a high respiratory activity. This condition was expressed by the highest oxygen consumption rate in the treatment without the addition of Ca and Mg than that on other treatments.

Oxygen consumption rate can be used as a parameter to determine the metabolism rate of aquatic organisms. Oxygen consumption rate influences the use of energy when the metabolism process occurs in the crab body (Silikavuopio & James, 2015). The lower the oxygen consumption rate the less energy is used for metabolism and energy is expected to be available more for the growth.

Feed conversion is a ratio between weight gain of crabs and the amount of the consumed feed. The results of this study showed that treatment C and D had insignificant different (P>0.05) feed conversion values. This led to insignificant growth rates. A high growth is characterized by a faster molting process. The transfer process of calcium and magnesium from hemolymph to carapace is performed through active transport mechanism that requires an energy. Transfer of Ca and Mg to carapace that runs faster is characterized by a high deposition rate of calcium and magnesium in carapace that also requires a high amount of energy. This large energy requirement is obtained from the consumed feed, as Tavabe *et al.* (2013) stated that a deficiency of Ca ion in crab could inhibit its growth that led to an increase in feed conversion.

The highest feed conversion was on treatment A that was 10.65±0.85 (Figure 4). On the other hand, the lowest growth rate was 1.136±0.04%. A high feed conversion indicated that there was a high amount of energy lost for osmoregulation process on the reared crabs. If the environmental condition is on optimum condition that is characterized by low osmotic gradient and oxygen consumption, so the energy obtained from the feed will be utilized maximally for the growth. Hypreosmotic and hypoosmotic conditions cause the feed given becoming not used for the growth of crabs. But the energy will be spent to equalize osmotic conditions both outside and inside the body. Tavabe *et al.* (2015) stated that Ca ion when interacted with magnesium could increase the growth of crustacea. According to Ling *et al.* (2013), calcium plays a role in the formation of body tissues especially bones and exoskeleton.

Frequencies in the additon of Ca and Mg resulted differences in the number of molting in mud crabs. Treatment D had the highest number of molting (18.67±1.15 shells) and it was significantly different ($P<0.05$) to other treatments. Molting is the shell replacement process in crustacean group. The Ca^{2+} plays a role as a trigger in ecdysteroidogenesis or ecdysteroid synthesis (Teruaki *et al.*, 2009). The Ca^{2+} also plays a role in gastrolisation process that is the absorption of Ca^{2+} contained in crustacean body for the hardening process of the new shell after sucessfully removing the old shell. This occurs when the crab flesh size becomes larger while exoskeleton does not become larger because exoskeleton is a rigid part, so to adapt in that condition this animal will remove the old exoskeleton and forms the new exoskeleton with an aid derived from calcium (Soundarapandian *et al.*, 2013). A young crab with a high growth rate has a molting frequency that is more often than an adult crab. Molting is also influenced by sex, physiological conditions, temperature, water chemical factors, quality and quantity of food. Molting cycle is influenced by hormonal system, organism and feed, it is also influenced by environmental factors of the water (Zaidy *et al.*, 2008).

CONCLUSION

The optimum frequency of the addition of calcium and magnesium in recirculation system to increase the production and the formation of the mud crab shell is every 15 days with a concentration of 30 mg/L.

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