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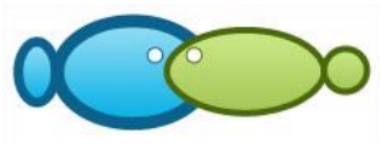

Effects of formulated diets supplemented with vitamin E on the egg quality and ovi somatic index of female *Portunus pelagicus* broodstock

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Abstract. The study of the blue swimming crab (*Portunus pelagicus*) broodstock nutrition is significant for achieving the reproductive success, which implicitly increases the larval quantity and quality in the mass production of cultured species. Therefore, the objective of this work was to determine the effects of the dosage of vitamin E in the diets on the egg quality and ovi somatic index of female *P. pelagicus* broodstock. Four dosage levels of vitamin E were tested: 0 IU kg^{-1} (control), 300 IU kg^{-1}, 600 IU kg^{-1}, and 900 IU kg^{-1}, in a formulated diet (44.38% crude protein). *P. pelagicus* was fed once a day between 17.00 and 18.00 hours, at the ration of 3% of the body weight, for 40 days. The results showed that the fertilization rate, the egg hatching rate and the ovi somatic index increased with an increasing vitamin E dosage in the diet, from control (0 IU kg^{-1}) to 300 IU kg^{-1}, then it decreased when the dosage was increased up to 900 IU kg^{-1} formulated diet. A quadratic relationship existed between the dose of vitamin E (IU kg^{-}) in the formulated diet and the egg quality incubation period (days) and ovi somatic index of the female *P. pelagicus* broodstock. The modification of the formulated diet by supplementation with different doses of vitamin E can reduce the incubation period of female *P. pelagicus* broodstock and increase the egg quality and ovi somatic index value of the female parent.
Key Words: fertilization rate, fecundity, hatching rate, incubation period, nutrition.

Introduction. *Portunus pelagicus* represents a new commodity in aquaculture, with excellent development prospects. However, the breeding technology of the *P. pelagicus* cultivation is still in progress. In order to take advantage of the opportunity of an increasing market value and demand, most crab cultivators bring the seed from the wild to the culture ponds (Efrizal et al 2019a). The blue swimming crab, particularly the genus *Portunus*, is a commercially valuable species cultivated in several Asian countries, such as India, Indonesia, Philippines, Malaysia, Taiwan, China, Vietnam, and Sri Lanka. The worldwide export of the pasteurized *P. pelagicus* has a positive trend due to the main markets: the United States, Japan and Singapore, delivering a multi-million dollars annual income to Indonesia (Kangas 2000; Fahmi et al 2015; Efrizal 2017).

Currently, in Indonesia and Thailand, the culture methods for breeding, nursing and rearing the *P. pelagicus* have attained significantly higher productivity and survival rates. Although the necessary information for the development of the mass production of crab seeds is available (Soundarapandian et al 2007; Oniam et al 2012; Efrizal & Rusnam 2017; Efrizal et al 2019b) a systematic research on the optimal nutrition for the crab production systems is essential for the future breeding or culturing programs. In this study, the effects of formulated diets were examined, by evaluating the impact of different doses of vitamin E on the egg quality and ovi somatic index (OSI) of female *P. pelagicus* broodstock.

Material and Method

Time and site. The experiments were located at the Fish Seed Center Beaches (BBIP) Teluk Buo, Bungus Hatchery of Padang City. The sample analysis was carried out using

the facilities of the animal physiology laboratory, Department of Biology, Andalas University, Padang, West Sumatera, Indonesia.

Crab collection and husbandry. *P. pelagicus* in the study were obtained on January 2018. Twenty parental crabs were at the ovarian maturation stage II, with a bodyweight ranging between 170.81 and 203.53 g. The *P. pelagicus* sample specimens were captured from the wild and kept at the experimental site location. They were distributed randomly into 200 x 100 x 100 cm (four concrete tanks), holding five units per tank, in a plastic box (45.5 x 32.5 x 16.5 cm), at the density of one crab per box. Tanks were equipped with hides (PVC pipes with a diameter of 13 cm and a length of 40 cm), providing a substrate of around 15 cm thick layer and an appropriate aeration (Efrizal 2015; Efrizal et al 2015; Efrizal et al 2019c). The crabs were held a pH reading of 7.26 to 8.00, an oxygen reading of 6.15 to 7.45 mg L^{-1}, a salinity reading of 29 to 32 ng L^{-1}, a temperature reading of 26-28°C and a water depth reading of 25-30 cm.

Supplementation. *P. pelagicus* was fed once a day, between 17.00 and 18.00 hours, at the ration of 3% of the body weight, for 40 days. Uneaten food was removed every morning. The experiment used a completely randomized design (CRD) consisting in 4 treatments with dietary vitamin E and 5 replications: formulated diet Fdiet 1, with 0 IU kg^{-1}; formulated diet Fdiet 2, with 300 IU kg^{-1}; formulated diet Fdiet 3, with 600 IU kg^{-1}; and formulated diet Fdiet 4, with 900 IU kg^{-1}. The diet is a moderated formulation for *Scylla serrata* broodstock (Millamena & Quinitio 2000) and *P. pelagicus* broodstock (Efrizal & Rusnam 2017; Efrizal et al 2019a; Efrizal et al 2019c). *P. pelagicus* broodstock specimens were initially fed with fresh feed, before gradually switching to the artificial experimental diet. The first ten days they were fed to satiation.

Variables assessed. Measured parameters of the egg quality (incubation period, egg diameter, fertilization rate, hatching rate, fecundity) and ovi somatic index were determined according to Efrizal et al (2006). The egg incubation period was the number of days from spawning to hatching (Efrizal et al 2006). The fertilization rate (Cerda et al 1994; Millamena & Quinitio 2000) was calculated as follows:

$$FR=FE/(FE+UFE)x100\%$$

Where:
FR - the fertilization rate (%);
FE - the fertilized eggs number;
UFE - the unfertilized eggs number.

The hatching rate (Millamena & Quinitio 2000; Adebayo & Popoola 2008) and fecundity (Cerda et al 1994; Oniam & Taparhudee 2010) were calculated as follows:

$$HR=HZ/(HZ+UFE)x100\%$$
$$F=HZ+UFE$$

Where:
HR - the hatching rate (%);
F - fecundity;
HZ - the hatched zoea number;
UFE - the unfertilized eggs number.

The ovi somatic index was calculated as follows (modified from Aryani & Suharman 2015):

$$OSI=OEW/WWCx100\%,$$

Where:
OSI - the ovi somatic index (%);
OEW - the ovulated egg weight (g);
WWC - the wet weight of the crab (g).

The ovulated egg weight was obtained from differences between the weight of the berried female and the weight of the non-berried female (post-spawning). Weights were measured with a precision of 0.01 g on the electronic balances (BL3200H-SHIMADZU).

The water quality parameters were determined according to Rice et al (2012) and Efrizal et al (2019c).

Statistical analysis. The data for the egg quality (incubation period, egg diameter, fertilization rate, hatching rate, and fecundity) and ovi somatic index were subjected to the analysis of variance (ANOVA), followed by Duncan's Multiple Range test to compare the mean differences among treatments (Steel & Torrie 1990). Arcsine transformation was done in the analysis of the data in percentages. The relationships among the dose of vitamin E (IU kg^{-1}) in a formulated diet and the egg quality and ovi somatic index were analyzed using the statistical software, SPSS version 19.0, to identify significant correlations between them.

Results and Discussion

Egg quality

Incubation period. The data (Table 1) show that the incubation period was not significantly (P>0.05) affected by the formulated diets of vitamin E supplementation at different doses. The doses of vitamin E of 300 IU kg^{-1} in the formulated diet resulted in the shortest incubation period (6.80±0.42 days). The longest incubation period was achieved at Fdiet 1 (vitamin E 0 IU kg^{-1}), Fdiet 3 (vitamin E 600 IU kg^{-1}) and Fdiet 4 (vitamin E 900 IU kg^{-1}) with 7.60±0.27, 7.20±0.27 and 7.20±0.27 days, respectively. The relationship between the doses of vitamin E (DVE) in the formulated diet and the incubation period (IP) is shown in Figure 1. The regression equation is $IP=3x10^{-6}\ DVE^2-0.0026\ DVE+7.53$ ($R^2=0.1626$; P>0.05).

Table 1

The incubation period (days) of female *Portunus pelagicus* broodstock at a different dosage of supplemented vitamin E in the formulated diet

Treatment (n=5)	*The incubation period (days)*
Fdiet 1	7.60±0.27[a]
Fdiet 2	6.80±0.42[a]
Fdiet 3	7.20±0.22[a]
Fdiet 4	7.40±0.27[a]

Means (±SE) within a given column with different superscripts are significantly different (P<0.05); n-replication; Fdiet 1-vitamin E 0 IU kg^{-1} formulated diet (control); Fdiet 2-vitamin E 300 IU kg^{-1} formulated diet; Fdiet 3-vitamin E 600 IU kg^{-1} formulated diet; Fdiet 4-vitamin E 900 IU kg^{-1} formulated diet.

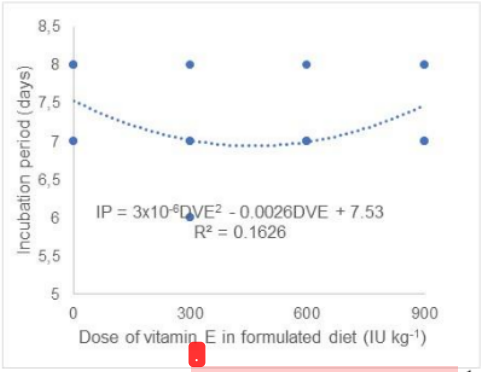

Figure 1. The relationship between the dose of vitamin E (IU kg^{-1}) in a formulated diet and the incubation period (days) of female *Portunus pelagicus*) broodstock.

Egg diameter. The data on the egg diameter are summarized in Table 2 and Figure 2. Viable eggs of berried female *P. pelagicus* were spherical, yellowish-orange, and

somewhat translucent, and ranged from 0.334-0.357 mm in diameter. The greatest egg diameter (0.357 mm) of the berried female was observed in the treatment with 300 IU kg^{-1} vitamin E in the formulated diet, and the lowest egg diameter was found in the treatment of 0 IU kg^{-1} vitamin E in the formulated diet (Table 2). The ANOVA showed that the treatment significantly ($P<0.05$) affected the egg diameter of the berried female blue swimming crab, and then, Duncan's test showed that the Fdiet 2 effect was significantly different ($P<0.05$) compared to that of Fdiet 1 on the egg diameter. The relationship between the doses of vitamin E in the formulated diet and the egg diameter (ED) is shown in Figure 2. The regression equation was found to be quadratic and was calculated as follows: $ED=-8x10^{-8}\ DVE^2+9x10^{-5}\ DVE+0.3347$. The $R^2=0.5772$ ($P>0.05$) indicated an increase of the egg diameter of approximately 42% with the addition of vitamin E to the formulated diet (IU kg^{-1}).

Table 2

Mean egg diameter (mm) of female *Portunus pelagicus* broodstock, at a different dosage of supplemented vitamin E in formulated diet

Replication (n=5)	*Treatment*			
	Fdiet 1	*Fdiet 2*	*Fdiet 3*	*Fdiet 4*
1	0.333±0.031	0.360±0.026	0.354±0.020	0.342±0.019
2	0.327±0.024	0.350±0.029	0.350±0.021	0.355±0.020
3	0.338±0.026	0.353±0.027	0.355±0.023	0.350±0.025
4	0.335±0.029	0.369±0.030	0.346±0.028	0.351±0.026
5	0.335±0.029	0.355±0.034	0.377±0.023	0.365±0.020
ED (mm)	0.334±0.002[a]	0.357±0.004[b]	0.356±0.006[b]	0.353±0.004[b]

Means (±SE) within a given column with different superscripts are significantly different ($P<0.05$); n - replication; ED-egg diameter; Fdiet 1-vitamin E 0 IU kg^{-1} formulated diet (control); Fdiet 2-vitamin E 300 IU kg^{-1} formulated diet; Fdiet 3-vitamin E 600 IU kg^{-1} formulated diet; Fdiet 4-vitamin E 900 IU kg^{-1} formulated diet.

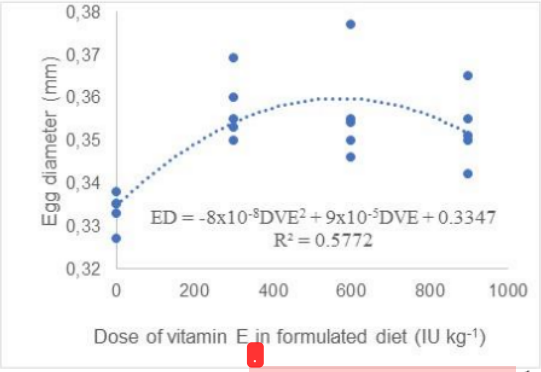

Figure 2. The relationship between the dose of vitamin E (IU kg^{-1}) in a formulated diet and the egg diameter (mm) of female *Portunus pelagicus* broodstock.

Fertilization rate. The success rate for egg fertilization with the different diet treatments was generally high and ranged from 80.53 to 96.42% (Table 3). The fertilization rate (%) decreased with the increasing dose of vitamin E in the formulated diet ($P<0.05$). The highest fertilization rate (96.42±0.98%) was achieved at the dose of vitamin E of 300 IU kg^{-1} in the formulated diet, whereas the lowest ($P<0.05$) fertilization rate occurred at the dose of vitamin E of 0 IU kg^{-1} in the formulated diet with 80.53±0.73% (Table 3). The relationship between the dose of vitamin E in the formulated diet and the egg fertilization rate (FR) was quadratic, with an equation of $FR=-5\ x10^{-5}\ DVE^2+0.0505\ DVE+81.81$ (Figure 3). This relationship was found to be highly significant ($R^2=0.6584$; $P<0.05$).

Table 3

Mean egg fertilization rate (%) of female *Portunus pelagicus* broodstock, at a different dosage of supplemented vitamin E in formulated diet

Treatment (n=5)	*Number of eggs sampled*		*Fertilization rate (%)*
	Fertilized	*Total*	
Fdiet 1	534.40±2.02	688.60±6.34	80.53±0.73[a]
Fdiet 2	656.40±11.82	680.00±10.23	96.42±0.98[b]
Fdiet 3	619.20±12.58	682.20±10.09	90.75±0.94[c]
Fdiet 4	607.20±15.68	681.00±6.79	89.14±1.76[c]

Means (±SE) within a given column with different superscripts are significantly different (P<0.05); n-replication; Fdiet 1-vitamin E 0 IU kg^{-1} formulated diet (control); Fdiet 2-vitamin E 300 IU kg^{-1} formulated diet; Fdiet 3-vitamin E 600 IU kg^{-1} formulated diet; Fdiet 4-vitamin E 900 IU kg^{-1} formulated diet.

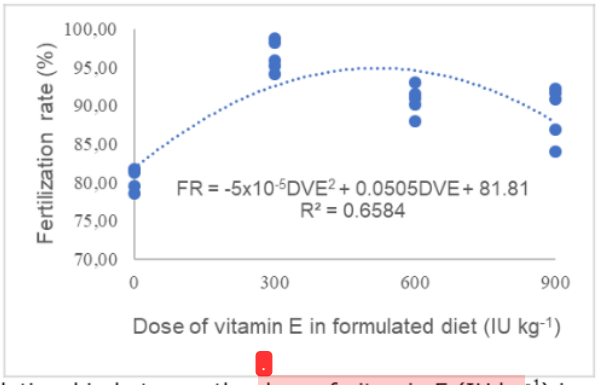

Figure 3. The relationship between the dose of vitamin E (IU kg^{-1}) in a formulated diet and the fertilization rate (%) of female *Portunus pelagicus* broodstock.

Hatching rate. The formulated diet with vitamin E supplementation (0, 300, 600, and 900 IU kg^{-1} formulated diet) significantly influenced (P<0.05) the hatching rate (Table 4).

Table 4

Mean zoeal hatching rate (%) of female *Portunus pelagicus* broodstock at a different dosages of supplemented vitamin E in formulated diet

Treatment (n=5)	*Number of eggs*			*Hatching rate (%)*
	Hatching	*Unfertilized*	*Total*	
Fdiet 1	464,560.42± 118,909.36	128,527.20± 33,654.05	593,087.62± 152,108.72	78.29±0.83[a]
Fdiet 2	668,294.51± 136,861.03	86,448.06± 17,363.87	754,742.57± 151,107.22	88.46±1.08[b]
Fdiet 3	577,812.58± 119,869.02	281,853.10± 111,083.11	688,895.68± 146,080.61	84.05±0.92[c]
Fdiet 4	546,864.36± 118,617.25	116,287.65± 26,609.22	663,152.01± 144,602.41	82.48±0.94[c]

Means (±SE) within a given column with different superscripts are significantly different (P<0.05); n-replication; Fdiet 1-vitamin E 0 IU kg^{-1} formulated diet (control); Fdiet 2-vitamin E 300 IU kg^{-1} formulated diet; Fdiet 3-vitamin E 600 IU kg^{-1} formulated diet; Fdiet 4-vitamin E 900 IU kg^{-1} formulated diet.

Similar to the fertilization rate, the highest zoeal hatching rate of *P. pelagicus* eggs was achieved in the groups of crab fed on dietary treatments at 300 IU kg^{-1} vitamin E in formulated diet (88.46±1.08%), whereas the lowest hatching rate of eggs was observed at 0 IU kg^{-1} vitamin E in formulated diet (78.29±0.83%), compared to Fdiet 3 (300 IU kg^{-1} vitamin E in formulated diet; 84.05±0.92%) and Fdiet 4 (300 IUkg^{-1} vitamin E in formulated diet; 82.48±0.94%). Duncan's test demonstrated that the zoeal hatching rate

reared at the 0 IU vitamin E kg^{-1} formulated diet was significantly lower than those reared with the other formulated diets.

The relationship between the zoeal hatching rate and dose of vitamin E in the formulated diet was found to be quadratic ($HR=-3x10^{-5}DVE^2+0.0321DVE+79.157$, $R^2=0.5864$, $P<0.05$) (Figure 4).

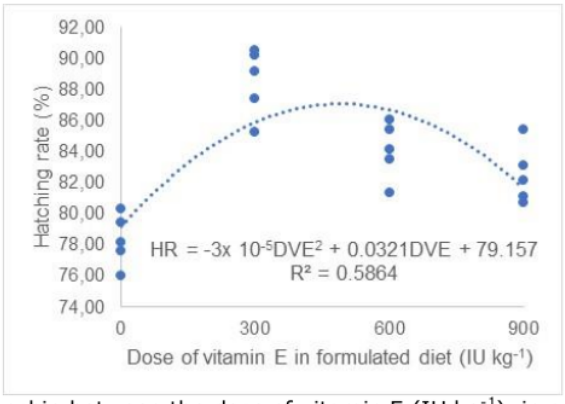

Figure 4. The relationship between the dose of vitamin E (IU kg^{-1}) in a formulated diet and the hatching rate (%) of female *Portunus pelagicus* broodstock.

Fecundity. The estimated mean number of eggs per batches produced by female crabs with the different formulated diets after hatching under laboratory conditions ranged from 593,087.62 to 754,742.57 eggs $batch^{-1}$ (Table 5). The lowest fecundity (593,087.62±152,108.72 eggs $batch^{-1}$) was found in a berried female *P. pelagicus* that received the formulated diet supplementation of vitamin E 0 IU kg^{-1} (Fdiet 1), whereas the highest fecundity (754,742.57±151,107.22 eggs $batch^{-1}$) was found in a berried female *P. pelagicus* receiving the formulated diet supplementation of vitamin E 300 IU kg^{-1} (Fdiet 2). On the other hand, females fed with Fdiet 3 (600 IU kg^{-1} formulated diet) and Fdiet 4 (900 IU kg^{-1} formulated diet) showed a decreased fecundity, 688,895.68±146,080.61 and 663,152.01±144,602.41 eggs $batch^{-1}$, compared to those fed with Fdiet 2 (300 IU kg^{-1} formulated diet), but the results of the variance tests (ANOVA) for this difference did not reveal a significant difference ($P>0.05$). The relationship between the fecundity and dose of vitamin E in the formulated diet was quadratic with an equation of $F=-0.5206DVE^2+516.61DVE+606468$ ($R^2=0.0332$, $P>0.05$) (Figure 5).

Table 5

Mean fecundity (egg $batch^{-1}$) of female *Portunus pelagicus* broodstock, at a different dosage of supplemented vitamin E in formulated diet

Replication (n=5)	*Treatment*			
	Fdiet 1	*Fdiet 2*	*Fdiet 3*	*Fdiet 4*
1	358,389.72	606,186.79	499,408.48	504,048.09
2	1,068.355.99	500,062.22	917,012.05	1,178.255.90
3	384,044.25	559,902.79	568,548.58	569,488.84
4	727,380.36	878,290.59	1,073.024.15	551,069.52
5	427,267.77	1,229.270.45	386,485.15	512,897.71
F	593,087.62± 152,108.72[a]	754,742.57± 151,107.22[a]	688,895.68± 146,080.61[a]	663,152.01± 144,602.41[a]

Means (±SE) within a given column with different superscripts are significantly different ($P<0.05$); n-replication; F-fecundity; Fdiet 1-vitamin E 0 IU kg^{-1} formulated diet (control); Fdiet 2-vitamin E 300 IU kg^{-1} formulated diet; Fdiet 3-vitamin E 600 IU kg^{-1} formulated diet; Fdiet 4-vitamin E 900 IU kg^{-1} formulated diet.

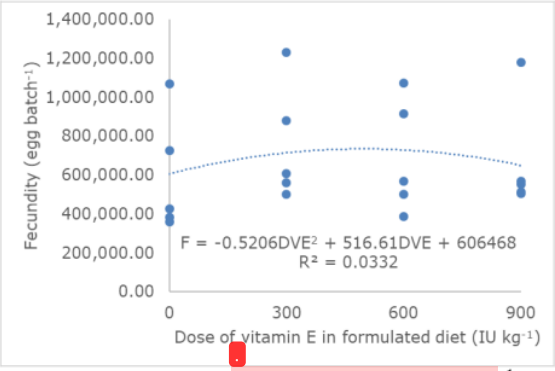

Figure 5. The relationship between the dose of vitamin E (IU kg^{-1}) in a formulated diet and the fecundity (egg $batch^{-1}$) of female *Portunus pelagicus* broodstock.

Ovi somatic index. The final percentage for the ovi somatic index (OSI) differed significantly ($P<0.05$) between treatments (Table 6). This index ranged from 7.67-22.94% in all the experiments. The highest OSI value of broodstock females in this study was obtained from females receiving the Fdiet 2 and was 22.94% (Table 6 and Figure 5), followed by Fdiet 3 (17.09%), Fdiet 4 (14.77%) and Fdiet 1 (7.67%). When further tested with the Duncan's test, a significant difference ($P<0.05$) was also observed among Fdiet 1, Fdiet 2 and Fdiet 3, no significant difference ($P>0.05$) was observed between Fdiet 1 and Fdiet 4 (Table 6). The R^2, which was of 0.3884 (OSI=-5Ex10^{-5} DVE2+0.0491 DVE+8.9086, $P>0.05$) (Figure 6), suggested that approximately 62% of *P. pelagicus* showed an effect in the OSI with the addition of vitamin E in the formulated diet (IU kg^{-1}).

Table 6

Mean ovi somatic index (%) of female *Portunus pelagicus* broodstock, at a different dosage of supplemented vitamin E in the formulated diet

Treatment (n=5)	*Body weight before berried (g)*	*Body weight after berried (g)*	*Egg batch weight (g)*	*Ovi somatic index (%)*
Fdiet 1	170.81±30.02	158.73±30.19	12.08±1.42	$7.67±1.27^{a}$
Fdiet 2	203.53±27.87	158.77±26.93	44.76±3.19	$22.94±2.75^{b}$
Fdiet 3	191.15±28.36	158.75±25.62	32.40±6.50	$17.09±3.65^{b}$
Fdiet 4	186.68±26.98	159.32±24.37	27.35±6.43	$14.77±3.45^{ab}$

Means (±SE) within a given column with different superscripts are significantly different ($P<0.05$); n-replication; Fdiet 1-vitamin E 0 IU kg^{-1} formulated diet (control); Fdiet 2-vitamin E 300 IU kg^{-1} formulated diet; Fdiet 3-vitamin E 600 IU kg^{-1} formulated diet; Fdiet 4-vitamin E 900 IU kg^{-1} formulated diet.

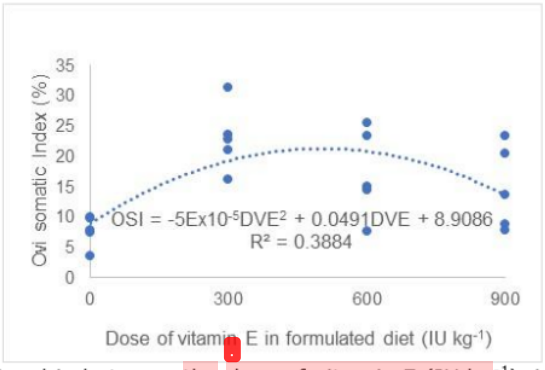

Figure 6. The relationship between the dose of vitamin E (IU kg^{-1}) in a formulated diet and the ovi somatic index (%) of female *Portunus pelagicus* broodstock.

Discussion. The egg incubation period of *P. pelagicus* decreased with an increasing dose of vitamin E in the formulated diet. However, high doses of vitamin E had an inverse effect and inhibited the egg incubation period. This phenomenon was observed in the treatments with the 600-900 IU kg^{-1} vitamin E in the formulated diet, with the egg incubation period of berried female of the *P. pelagicus* being longer compared to those females receiving the treatment of 300 IU/kg vitamin E in the formulated diet. The difference in the achievement time of the incubation period is thought to be due to differences in the quality of the diet given, which affects the quality of the eggs produced. Vitamin E plays an essential role in gonad development, namely for the process of fertilization and influences fecundity (Izquierdo et al 2001). Vitamin E can be added to feed to accelerate the phase of follicular formation (Verakunpiriya et al 1997). Vitamin E is transported from the peripheral tissues during vitellogenesis even though the plasma content of the vitellogenin is not affected, and it is suspected that lipoproteins might be involved in the transport of vitamin E during the vitellogenesis (Izquierdo et al 2001).

Compared to the results of the studies related to the time of incubation of crustaceans under the influence of other environmental factors such as temperature, the control of the environmental factors in the diet, as in the current study, is more capable of accelerating a decrease in the *P. pelagicus* egg incubation period. Such control was shown by the results reported by Hamasaki et al (2002) who found that controlling the temperature in the range from 20.3-30°C is only able to reduce the incubation period for 10-30 days in *S. serrata*. For the same species, *P. pelagicus*, the incubation period was found to range from 8.33-6.67 days at 28-34°C (Efrizal et al 2006).

The differences in the egg diameters observed in this study and analyzed quantitatively are presumably attributable to the biological response to the quality of feed given to the target cells. In this case, the feed stimulated the process of biosynthesis of vitellogenesis and stimulated the germinal epithelium in the secretion of maturation-inducing steroids (MIS) that stimulate the hydration of eggs. For example, a low protein, high-calorie diet caused a reduction in red seabream (*Pagellus bogaraveo*) reproductive performance (Watanabe et al 1984). In another sparid, the gilthead seabream (*Sparus aurata*), a well-balanced broodstock diet in essential amino acids, improved the vitellogenin synthesis (Tandler et al 1995). Moreover, a reduction in the dietary protein levels from 51 to 34%, together with an increase in dietary carbohydrate levels from 10 to 32%, reportedly reduced egg viability in seabass (Cerda et al 1994). These diets have been shown to cause alterations in GnRH release in seabass broodstock during spawning (Kah et al 1994) and in the hormonal plasma levels of the gonadotropin GtH II. The latter is known to play an essential role in oocyte maturation and ovulation (Navas et al 1996).

As shown in Table 3, a significant difference ($P<0.05$) can be seen between Fdiet 1 and Fdiets 2, 3, and 4. The difference in the percentage value of the degree of fertilization of the eggs between treatments was related to the incubation period, and to the difference in egg diameter obtained, as described above. This finding is in line with the results of the study by Efrizal et al (2012), who reported that the degree of egg fertilization of the crab female parent decreased with the length of the egg incubation time. Furthermore, one of the nutrients known to be necessary for the fertilization is vitamin E (Izquierdo & Fernandez-Palacios 1997; Izquierdo et al 2001). Vitamin E has been shown to play an essential role in reproduction (Watanabe et al 1991). Its role as an inter- and intracellular antioxidant, maintaining homeostasis of labile metabolites in the cell and tissue plasma, is well known (Izquierdo et al 2001). The antioxidant function of vitamin E can provide an essential protective role for the sperm cells during spermatogenesis and until fertilization by reducing the risk of lipid peroxidation, which is detrimental for sperm motility (Izquierdo et al 2001).

The hatching rate with the Fdiet 1 treatment when compared with the provision of Fdiets 2, 3, and 4, was related to the low percentage of fertilization, as presented in Table 8 and Figure 4. Efrizal et al (2012) reported that the hatching rate of the crab eggs tended to be in line with the percentage of egg fertilization. Besides, the hatching rate was also related to the incubation period, and the diameter of the egg produced. Davis (1981) stated that the diameters of decapod crustacean eggs increased during the incubation period, which was due to a slow enlargement of the perfect osmotic inner egg

membrane or to an enlargement of the embryo itself. Furthermore, an increase in size resulted in the absorption of water by increasing the solution concentration inside the egg until hatching time.

Besides, the mean hatching rates cited in this study may be relatively high (78.29-88.46%), these rates are probably derived from the vitamin E in the formulated diet. Vitamin E is a potent scavenger of active oxygen species and has been shown to have a protective role against the action of free radicals. Free radicals can deteriorate egg membranes and membrane integrity. Although the harmful effects of vitamin E deficiency on the reproductive performance of higher vertebrates has been demonstrated since the early 1920s, dietary vitamin E has only been shown to be an essential nutrient for fish reproduction in 1990. Its deficiency results in immature gonads in carp and *Plecoglossus altivelis*, and in reduced hatching and fry survival rates in *P. altivelis* (Watanabe 1990).

The difference in the fecundity of female crabs in quantity is thought to be the result of giving a diet supplemented with vitamin E at different doses. The difference in the dose of vitamin E in artificial feed is thought to cause changes in the vitellogenesis process in the gonad, especially in producing a balance of essential fatty acids. Furuita et al (2000) states that one of the factors that influence fecundity is the quality of feed given to test animals. Millamena & Quinitio (2000) also stated that the ratio between the levels and types of fatty acids omega-3 and omega-6 affected the fecundity of the mud crab *S. serrata*.

A difference in the OSI values was observed between dietary treatments given to the female crab parent that are allegedly related to the dose of vitamin E, which causes a balance of nutrient content in the vitellogenesis process in the gonads of the test animals. With Fdiet 2, the essential fatty acids omega-3 and omega-6 were in a more balanced composition compared to the Fdiet 3, Fdiet 4 and Fdiet 1 during the vitellogenesis process. Rusdi & Ahmad (1993) reported that the administration of a combination of fresh feed, namely, lemuru and clam meat, was the best way to increase the maturation of the gonads (100%) and to spawn (91.6%) in mangrove crabs *S. serrata*. Furthermore, lemuru and clam meat, are foods naturally rich in essential fatty acids omega-3 and omega-6, which are necessary to the process of gonadal maturation. Mokoginta (1992) explained that the levels and types of fatty acids omega-3 and omega-6 contained in the ration affected the development of the parent and the hatchability of catfish eggs. Watanabe et al (1991) reported that the lack of essential fatty acids affected the fish spawning activities.

Conclusions. The following conclusions can be drawn from the results of this experiment: (1) the manipulation of a formulated diet supplemented with different doses of vitamin E can reduce the incubation period of the female crab parent and increase the egg quality (egg diameter, fertilization rate, hatching rate, and fecundity) and ovi somatic index of the female crab parent; (2) the feeding supplemented with different vitamin E doses had a significant effect ($P<0.05$) on the egg fertilization rate, the egg hatching rate, and the ovi somatic index (OSI), with highest values of 96.42%, 88.46%, and 22.94, respectively, with Fdiet 2; (3) the feeding supplemented with different doses of vitamin E had no significant effect ($P>0.05$) on the incubation period (6.80-7.60 days), egg diameter (0.334-0.357 mm) and egg fecundity (693,087.62-754,742.57 egg crab^{-1}); (4) a quadratic relationship existed between the dose of vitamin E (IU kg^{-1}) in the formulated diet and the egg quality and ovi somatic index of female *P. pelagicus* broodstock.

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