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Supplementation of astaxanthin and vitamin E in feed on the development of gonads Pacific white shrimp broodstock

Pemberian astaxanthin dan vitamin E dalam pakan terhadap perkembangan gonad calon induk udang vaname

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ABSTRACT

The quality of white shrimp *Litopenaeus vannamei* broodstock can be improved through the addition of astaxanthin and vitamin E in the diet. This study aimed to determine the effect of administration of astaxanthin and vitamin E with different doses in the feed on the maturity gonad of prospective Pacific white shrimp broodstock. Supplementation of 0 mg/kg feed astaxanthin + 0 mg/kg feed vitamin E (control/A), 500 mg/kg feed astaxanthin (B), 350 mg/kg feed vitamin E (C), 500 mg/kg feed astaxanthin and 350 mg/kg feed vitamin E (D), and 250 mg/kg feed astaxanthin and 175 mg/kg feed vitamin E (E) were applied in feed formulation. Shrimp was fed 2% of body weight three times daily at 06.00 am, 13.00 pm, and 20.00 pm. The result showed that the optimum dose for survival, specific growth rate and maturity level of Pacific white shrimp broodstock was obtained in the combination of 175 mg/kg vitamin E and 250 mg/kg astaxanthin. The survival of shrimp by that treatment was 100.00±0.00%, specific growth rate 1.07±0.26%/day, the first level of gonad maturity growth was reached at day 14 (19.45±4.81%), the fourth level of gonad maturity was obtained at day 41, spawning rate 33.33±8.33%, fecundity 87,000±2,000 eggs, and hatching rate reached 49.00±1.53%.

Keywords: astaxanthin, *Litopenaeus vannamei*, vitamin E

ABSTRAK

Peningkatan kualitas induk udang vaname *Litopenaeus vannamei* dapat dilakukan dengan penambahan vitamin E dan astaxanthin pada pakan. Tujuan penelitian ini adalah untuk mengevaluasi pengaruh pemberian astaxanthin dan vitamin E dengan dosis berbeda dalam pakan terhadap tingkat kematangan gonad calon induk udang vaname. Dosis yang digunakan adalah 0 mg/kg pakan astaxanthin + 0 mg/kg pakan vitamin E (kontrol/A), 500 mg/kg pakan astaxanthin (B), 350 mg/kg pakan vitamin E (C), 500 mg/kg pakan astaxanthin and 350 mg/kg pakan vitamin E (D), and 250 mg/kg pakan astaxanthin and 175 mg/kg pakan vitamin E (E). Pemberian pakan dengan penambahan vitamin E dan astaxanthin dilakukan sebanyak tiga kali, yaitu jam 06.00, 13.00, dan 20.00 WIB sebanyak 2% dari bobot udang. Hasil penelitian menunjukkan bahwa dosis optimum untuk sintasan, laju pertumbuhan spesifik, dan tingkat kematangan induk udang vaname diperoleh dengan kombinasi 175 mg/kg vitamin E dan 250 mg/kg astaxanthin. Kelangsungan hidup udang dengan perlakuan tersebut adalah 100,00±0,00%, laju pertumbuhan spesifik 1,07±0,26%/hari, tingkat kematangan gonad pertama dicapai pada hari ke 14 (19,45±4,81%), tingkat kematangan gonad keempat diperoleh pada hari ke 41, tingkat pemijahan 33,33±8,33%, fekunditas 87.000±2.000 telur, dan tingkat penetasan mencapai 49,00±1,53%.

INTRODUCTION

Pacific white shrimp *Litopenaeus vannamei* is one of sought-after fishery commodities in the world due to high market demand. In 2010, the production of exported shrimp amounted to 145,092 tons and it increased to 162,068 tons in 2012(Ministry of Marine Affairs and Fisheries, 2013). The farmed Pacific white shrimp generated 250,300 tons in 2012, meanwhile the farmed tiger prawn *Penaeus monodon* production reached 143,300 tons. Nonetheless, national fishery

production is dominated by farmed white shrimp.

High production of white shrimp depends on the good quality of shrimp larvae. The quality of shrimp larvae decreases over time, such as poor growth performance, the larvae size is not uniform, and prone to environmental changes. Consequently, it leads to low production of cultivated Pacific white shrimp. Several problems of domesticated Pacific white shrimp broodstock are the imperfect maturity of eggs, infertility, low mating frequency (Subaidah *et al*., 2008). Internal factors (feed & health) and external factors (environment) are identified as major causes affecting breeding success. For instances, the diet for broodstock highly affects to egg and sperm maturity. However, feed which has been stored in a period of time may reduce the quality of feed (Subaidah *et al*., 2008).

The addition of vitamin E and astaxanthin in fish feed formulation is recommended (Darvishpour *et al*., 2012; Kurnia *et al*., 2010; Paibulkichakul *et al*., 2008). Additionally, the vitamin E plays a major role as an antioxidant and reproductive performance in fish. Barton-Schuster (2015) reported vitamin E also serves as a protective cell wall from toxic substances such as tin, mercury, benzene, and free radicals that can interfere with endocrine glands and result in a balance of hormone production. The addition of vitamin E to the diet is important because vitamin E cannot be synthesized by the body. Vitamin E is digested in the small intestine and stored in some tissues such as adipose tissue, liver, and other body tissues (Pour *et al*., 2011). Moreover, Aryani *et al*. (2014) stated that the addition of vitamin E in the diet increases the egg diameter size of *Labeobarbus festivus* fish which means improve reproduction quality. In addition α-tocopherol, the most active form of vitamin E is a major form in crustacean tissue (Barim *et al*., 2011).

The addition of astaxanthin accelerates the maturity of the gonad broodstock. For example, the egg and larvae quality of red snapper is improved through the supplementation of astaxanthin in the diet. Similarly, astaxanthin is widely used for gonad maturity of red sea bream fish (Kurnia *et al*., 2010). The carotenoids (astaxanthin) is used as active source of antioxidants and pro-vitamin 'A', enhances immune response, reproductive performance, growth, maturation, and light protection by a variety of aquatic species (Mondal *et al*., 2015). Fish oil (12% total fat) at the level of 8% and 280 mg/kg of astaxanthin can significantly improve maturation of tiger prawn and success in spawning (Paibulkichakul *et al*., 2008).

Supplemented vitamin E in diet formulation plays an important nutrition in reproduction physiology. A dose of 1,000 mg/kg of vitamin E in the feed increases the amount of egg production and reproductive performance of freshwater lobster *Astacus leptodactylus* (Barim, 2009; Barim *et al*., 2011), increased survival of *L. vannamei* larvae (Darvishpour *et al*., 2012), and egg hatching on *L. vannamei* (Darvishpour *et al*., 2012).

Astaxanthin is a natural carotenoid that produces a red color commonly found in yeasts, algae, crustaceans, and predatory fish such as salmon (Tizkar *et al*., 2016). Although the astaxanthin does not completely convert into vitamin A, astaxanthin intake continues to increase the supply of vitamin A for the body. Astaxanthin is absorbed by the body in the form of vitamin A, the remaining part is stored in its original form. In addition to performing physiological functions as vitamin A, astaxanthin is also a superior antioxidant compared to vitamin C, beta carotene, and pycnogenol. Excess of astaxanthin is similar to lipoic acid, which has very strong protection against cytoplasm and cell nucleus, helps vitamin C and E work properly (Lingga, 2012). Another type of antioxidant is vitamin E can be transported from the peripheral tissue to the gonads through the liver with plasma lipoproteins, indicating that vitamin E plays a role in the reproductive process. During the process of vitellogenesis, vitamin E levels in the body decrease to about 10% until maturation. During the course of vitellogenesis, it is suspected that vitamin E is transported from the blood vessels to the liver by high-density lipoproteins.

The influence of astaxanthin on gonad maturity has not been clearly studied, but some studies suggest astaxanthin can increase the size of gonads in tiger shrimp (Paibulkichakul *et al*., 2008). It is suspected astaxanthin can increase essential fatty acids, as the raw material forming prostaglandins. Prostaglandins are one of the hormones that play an important role in the female reproductive process (ovulation, uterine, implantation, and partus) and associated pathogens (Fortier *et al*., 2008). Based on the results of this study, the addition of astaxanthin along with vitamin E in the feed of prospective broodstock of Pacific white shrimp is expected to improve the reproductive performance of Pacific white shrimp namely the spawning success aspects, the number of eggs, egg hatching, and the quality of the larvae.

MATERIALS AND METHODS

Preparation of broodstock tank

Three tanks in different sizes were used in this experiment. A tank with dimension 20×2×1 m^3 was prepared for rearing tank. One tank with a capacity of eight tons was used for mating and 15 tanks of egg hatchings tanks with size 76.5×53.5×45.5 cm^3. All tanks were sterilized with 100 mg/kg chlorine. Disinfection of the tanks was carried out by washing and drying the tanks. Detergent was used to clean the tanks from dirt and germs and rinsed three times with fresh water, sea water, and finally rinsed fresh water. After drying, 50% seawater was filled to tanks with a flow through system (continuous water change). Aerations were installed around the rearing tanks at the distance of 5 cm from the bottom of the tanks.

Broodstock selection and acclimatization

Prospective shrimp broodstock (Vaname Nusantara 1) was obtained from Center of Aquaculture Brackish Water (BBAP) Situbondo. A total of 180 female broodstock were used in the experiment at the initial weight of 28.35±1.11 g and length of 15.90±0.26 cm. The broodstock acclimatization was carried out three days before the study started.

Feed formulation

In the experiment, the feed was in the pellets form (48% protein, 14.5% fat, 2% crude fiber, 14 % ash, 2.2% calcium, 1.7% phosphorus, 6.5% moisture. Astaxanthin (brand ROCHE) with 10% astaxanthin content, vitamin E ROCHE (Roche Ltd.) which contains 78% d-alpha tocopherols were some commercial product used in the feed formulation. A dose of 500 mg/kg of astaxanthin doses and 350 mg/kg of vitamin E doses were supplemented in the feed formulation. The procedure was referred to Paibulkichakul *et al*. (2008) (Table 1).

The astaxanthin was dissolved with distilled water and the vitamin E was dissolved in fish oil. Two chicken eggs were added into 1 kg of feed to glue the astaxanthin and vitamin E. The formulated feed was stirred evenly. The feed was dried at the room temperature for 24 h. The dried feed was given to the broodstock. The feed was stored in the refrigerator.

Feeding

Feeding consisted of artificial feed (in the form of pellets) and live feed (sea worms). Artificial feed was fed by 2% body weight of shrimp which is given three times a day (06.00 am, 01.00 pm, and 09.00 pm). Live feed was fed by 10% body weight of shrimp which is given twice a day at 09.30 am and 04.00 pm.

Broodstock rearing

A total of 12 broodstock were given formulated feed and reared for 28 days. The seawater was filtered and aerated. Water change was made by flow through system. The temperature of the water was maintained between 28–29 °C with salinity between 31–33 g/L.

Eye stalk ablation

Eye ablation was performed after 28 days of feeding treatment. This process was performed on day 35 after treatment.

Experimental design and data analysis

Complete randomised design was applied for experimental design. The experiment was carried out with five treatments each and triplicates. Data was analysed with using SPSS 16.0. Analysis of variance (ANOVA) was applied to survival rate, the gonads maturation rate, growth parameters and significant difference was further tested using Tukey test. After ablation treatment, spawning rate data, fecundity, and egg hatching

Table 1. Dosage of astaxanthin (Asx) and vitamin E (VE) in the feed formulation

Treatment	Dose of tretament
A	0 mg/kg feed Asx + 0 mg/kg feed VE (control)
B	500 mg/kg feed Asx
C	350 mg/kg feed VE
D	500 mg/kg feed Asx and 350 mg/kg feed VE
E	250 mg/kg feed Asx and 175 mg/kg feed VE

Note: Asx: astaxanthin, VE: vitamin E.

were analysed using experimental method. Data was processed using Microsoft excel 2010 and discussed descriptively.

Data collection

The survival rate, gonads maturation rate, growth parameters, spawning rate, fecundity, and egg hatching degree were parameters taken in the experiment.

Broodstock survival

The survival of the shrimp broodstock was determined after day 28 of rearing by using the following formula:

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

Note:
SR = survival (%)
Nt = the number of shrimp at the end of rearing
No = the number of shrimp at the beginning of rearing

Growth parameters

The specific growth rate (SGR) was calculated at the end of the study (day 28 of rearing), which was determined by formula as follows:

$$SGR\ (\%/day) = 100\ (\ln W2 - \ln W1) / t$$

Note:
SGR = specific growth rate (%/day)
W1 = the mean weight of the broodstock at the first observation (g)
W2 = the mean weight of the broodstock in the second observation (g)
T = period of time

Gonad maturity level

The maturity level of the gonads was daily observed in the morning for four weeks (28 days of rearing). Gonads maturity level were recorded during the experiment and accumulated until the end of the experiment. The assessment was only applied to mature gonad of shrimp. The mature gonad of shrimp was indicated by yellowish red on the dorsal part. The assessment of gonad maturity level was performed by observing the development of colour and level of gonad thickness on shrimp's dorsal.

$$MI\ (\%) = \frac{(\sum \text{Broodstock TKG 3 and 4})}{\sum \text{total broodstock}} \times 100$$

Note:
MI = percentage of gonad maturity level (%)
TKG = gonad maturity level

Spawning rate

The female shrimp broodstock was moved to spawning tank which contained male broodstock. The maturity of the gonads in the prospective broodstock of Pacific white shrimp was marked by the development of ovaries located in the dorsal part of the shrimp body with orange colour (Figure 1), the male shrimp gonad maturity was indicated with the white colour in the testes (Figure 2). The spawning rate was checked 5–7 hours after the female and male were situated together. Percentage of spawning rate can be calculated as follows.

$$\text{Spawning rate}\ (\%) = \frac{\sum \text{spawned broodstock}}{\sum \text{total female broodstock}} \times 100$$

Fecundity

To calculate the fecundity, 1 L water from the tank which contained eggs was randomly taken for ten times. Sampling was taken on the same day as the spawning day. The number of

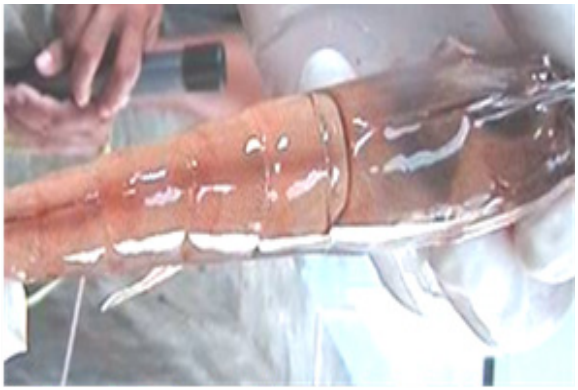

Figure 1. Female broodstock.

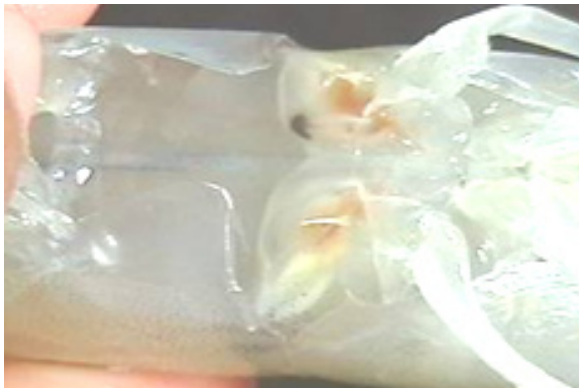

Figure 2. Male broodstock.

egg in the medium was calculated to provide the baseline data for the total of eggs released by the broodstock. The formula can be seen below:

$$Jt = (Bp \times Yt) / (Ps \times Gc)$$

Note:
Jt = number of eggs produced each broodstock (egg/individual)
Bp = water volume of spawning tank
Ps = frequency of egg sampling
Gc = water volume of measuring glass cylinder used in egg sampling
Yt = number of eggs from all samples

Hatching rate

The percentage of eggs hatching is the number of hatching embryos (EM) compared to the number of fertilized eggs (TB). It was calculated after the eggs hatched for 20–24 hours. The formula can be seen below:

$$HR\ (\%) = EM / TB \times 100$$

Note:
HR = egg hatching (%)
EM = percentage of the number of hatching embryos
TB = number of fertilized eggs

RESULTS AND DISCUSSION

Results

Survival and specific growth rate

The result showed that no significant different (P>0.05) related to the survival and specific growth rate (SGR) of prospective broodstock white shrimp for each treatment. The survival rate ranged from 94.44 to 100% and the specific growth rate ranges from 0.74 to 1.07%. Furthermore, the survival rate and specific growth rate of the prospective broodstock white shrimp is presented in Table 2.

Gonad maturity level

The maturity level of gonad (TKG) in the prospective broodstock of Pacific white shrimp for 28 days of experiment can only reach TKG 1 and TKG 2. The cumulative percentage of gonad maturity level of the prospective broodstock of Pacific white shrimp is shown in Table 3.

Based on data analysis, the percentage of broodstock Pacific white shrimp at the first gonad maturity level after day 14 of observation was found in treatment A, B, and D. The results were significantly different with C and E treatment (P<0.05). However, treatment C was not significantly different from treatment D. On day 21, the percentage of second gonad maturity level for each treatment showed significantly different

Table 2. The survival and spesific growth rates of the prospective broodstock Pacific white shrimp

Treatments	Survival (%)	Specific growth rates (%/day)
A	97.22±4.81a	0.74±0.12a
B	100.00±0.00a	0.91±0.12a
C	97.22±4.81a	0.74±0.27a
D	94.44±4.81a	0.74±0.29a
E	100.00±0.00a	1.07±0.26a

Note: mean value ± SD (n = 3). Letters after value on the same line showed no significant difference (P>0.05).

Table 3. Cumulative percentages of prospective broodstock of Pacific white shrimp at first gonad maturity level (TKG1)

Treatments	Cumulative percentages Pacific white shrimp broodstock (TKG 1)				
	day-0	day-7	day-14	day-21	day-28
A	0	0	0.00±0.00a	11.11±4.81a	61.11±4.81a
B	0	0	0.00±0.00a	50.00±8.33b	100.00±0.00b
C	0	0	13.89±4.82bc	66.67±8.34c	97.22±4.82b
D	0	0	5.55±4.81ab	52.78±4.81bc	94.44±4.81b
E	0	0	19.45±4.81c	91.67±0.00d	100.00±0.00b

Note: mean value ± SD (n = 3). Letters after value on the same line showed no significant difference (P>0.05).

results among other treatments (P<0.05). On day 28, treatment A was significantly different from other treatments (P<0.05).

Percentage of shrimp broodstock at second gonad maturity level was achieved on day-21 of treatments A, B, and D. These results were significantly different from treatment C and E (P<0.05). However, treatment C and D showed no significant difference. On day 28 showed the percentage of gonad maturity level for both treatment A was significantly different between treatments (P<0.05).

The cumulative percentages of gonad maturity level in the prospective broodstock of Pacific white shrimp (Table 3). On day 39, 100% of prospective broodstock reached TKG 3 (Treatment C and E), whereas at treatments A, B, and D reached the third gonad maturity level on day-41. The cumulative percentages of gonad maturity level in prospective shrimp broodstock that reached TKG 4 after the ablation of the eye stalk (Table 6). The earliest treatment group which reached final gonad maturity level were treatment C and E at day-41 and then followed by other treatments until reached maximum percentage on day 43.

Measurement of spawning rate in the prospective broodstock of Pacific white shrimp was calculated based on the ratio of the number of fertilized broodstock to the number of prospective broodstock of Pacific white shrimp used in the

Table 4. Cumulative percentages of prospective broodstock of Pacific white shrimp at second gonad maturity level (TKG 2)

Treatments	Cumulative percentages Pacific white shrimp broodstock (TKG2)	
	day-21	day-28
A	0.00±0.00a	11.11±4.81a
B	0.00±0.00a	50.00±8.33b
C	13.89±4.82bc	66.67±8.34c
D	5.55±4.81ab	52.78±4.81bc
E	19.45±4.81c	91.67±0.00d

Note: mean value ± SD (n = 3). Letters after value on the same column showed no significant difference (P>0.05).

Table 5. Percentages of prospective Pacific white shrimp broodstock at third gonad maturity level (TKG 3) after ablation

Treatments	Percentages of prospective Pacific white shrimp broodstock TKG3		
	day-39	day-40	day-41
A	0	0	100±0
B	0	0	100±0
C	100±0	100±0	100±0
D	0	0	100±0
E	100±0	100±0	100±0

Table 6. Percentages of Pacific white shrimp broodstock at fourth gonad maturity level (TKG 4) after ablation

Treatments	Percentages of prospective Pacific white shrimp broodstock TKG3		
	day-39	day-40	day-41
A	0	0	100±0
B	0	0	100±0
C	100±0	100±0	100±0
D	0	0	100±0
E	100±0	100±0	100±0

experiment. The number of eggs and the egg hatching level were calculated on the basis of the sample after spawning the prospective Pacific white shrimp broodstock. Figures 3, 4, and 5 are the results of the spawning rate of the prospective broodstock of Pacific white shrimp, the number of eggs, and the egg hatching level.

Based on Figure 4, the number of Pacific white shrimp eggs ranged from 86,333 to 87,000 eggs, relatively the same between treatments. Based on

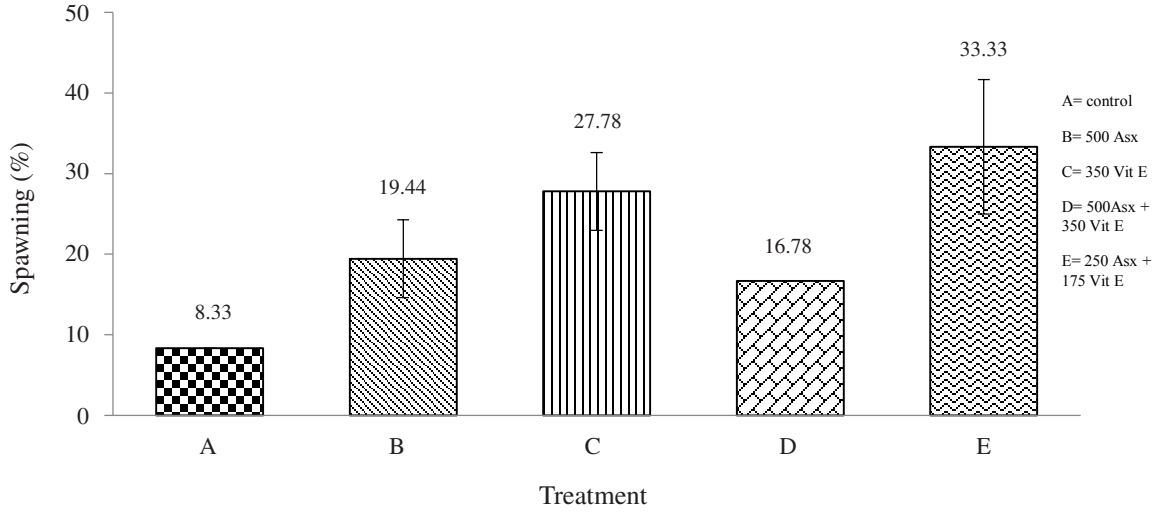

Figure 3. Spawning rate of Pacific white shrimp broodstock after ablation.

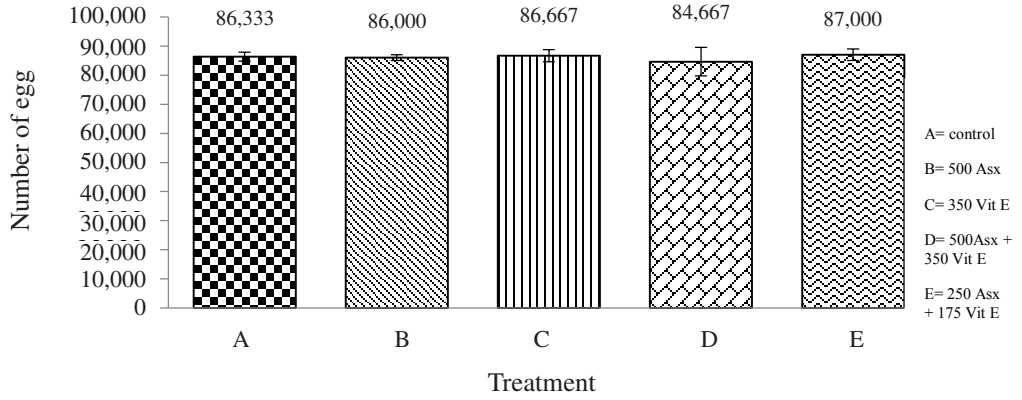

Figure 4. Number of eggs (fecundity) of Pacific white shrimp broodstock after ablation.

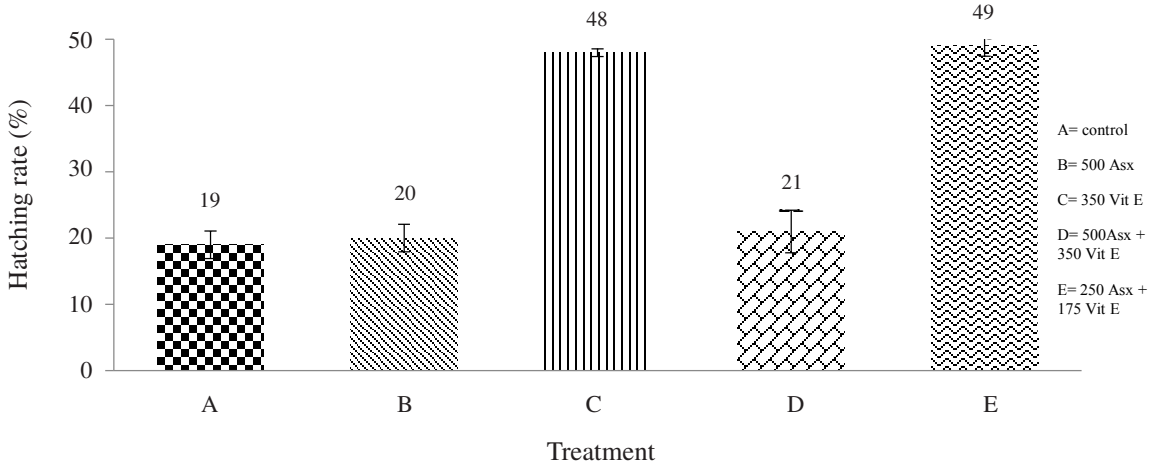

Figure 5. Hatching rate (%) of Pacific white shrimp eggs.

Figure 5, egg hatching ranged from 19 to 49%. The high of egg hatching level was observed in E treatment 49% and C 48%, respectively.

Discussion

A proper selection of Pacific white shrimp broodstock should be in accordance with the criteria and requirements for high-quality broodstock. It is important to ensure the quality of eggs and larvae shrimp. The broodstock which meets the requirement increase probability to success in improving the gonad maturation, mating, and spawning. Furthermore, the quality of diet and rearing media also play a significant role in providing a good quality of eggs and larvae for aquaculture. Similarly, Subaidah *et al*. (2008) noted that reproductive performance also highly affects the egg maturity, spawning rate, frequency, and a number of mating. No physical defect of the body, bright in colour, age 7–8 months, more than 18 cm long, and 40–45 g weight are some criteria for a good prospective female broodstock. While the male broodstock should be more than 17 cm length and 35–40 g weight the reproductive organs are in good condition and free of bacterial or viral diseases (May *et al*., 2008). The weight of shrimp broodstock used in this study was above 25 grams the average broodstock age was eight months.

The survival of prospective Pacific white shrimp broodstock was between 94.44–100%. The result showed no significant difference ($P>0.05$). The survival of shrimp broodstock was good due to a proper adaptation to a new environment. The addition of astaxanthin and vitamin E by the prospective broodstock of Pacific white shrimp did not have a negative impact on the survival rate. Specific growth rate of the broodstock ranged between 0.74% and 1.07% and it did not show significantly different ($P>0.05$). The increase in body weight was greatly affected by the adaptation to new environment and types of feed in the period of rearing. The optimum protein requirement level among the shrimp species varies between 23% and 57%. For instance, the protein requirement of *Metapenaeus macleayi* species is known to be the lowest of 27%, *L. setiperus* (28–32%), and kuruma shrimp *Marsupenaeus japonicus* (40–57%) (Takeuchi & Murakami, 2007; Tantikitti, 2014). In the present study, shrimp broodstock was fed with pellets which contained 48% protein. As a result, it may have effect on the growth of prospective broodstock of Pacific white shrimp. Supplemented egg yolk in the pellet as a binder and produces smell (attractant) stimulate the appetite of shrimp. Live feed, such as nereis marine worms was also fed to the shrimp broodstock during the rearing period. The nereis marine worm contains protein, fat, caretonoid, and high fatty acids (Haryati *et al*., 2010). Subaidah *et al*. (2008) stated that a combination of live feed and artificial feed can be an alternative in accelerating the process of gonad maturity.

The gonad maturity level of Pacific white shrimp broodstock in each treatment was different after rearing period (28 days). The gonad maturity level of broodstock only reached TKG 2 during the rearing period. The percentages of prospective broodstock of Pacific white shrimp with TKG 1 can be seen in Tables 3 and 4. TKG 1 was significantly different on day 14, day 21 and day 28 ($P<0.05$). TKG 2 occurred on day 21 and day 28, it was significantly different ($P<0.05$). Although the broodstock can only reach TKG 2 for 28 days, treatment C and E indicates a positive impact on gonad maturity in prospective broodstock of Pacific white shrimp. Both treatments were faster in achieving TKG 2 compared with other treatments.

The most obvious function of vitamin E is as an antioxidant, especially protecting unsaturated fatty acids on phospholipids in cell membranes. In addition, astaxanthin is also a source of vitamin A in the human body. Although astaxanthin is not perfectly converted to vitamin A, astaxanthin intake adds to the supply of vitamin A to the body. Astaxanthin is absorbed in the body in the form of vitamin A, the remaining part is stored in its original form. Astaxanthin is also a superior antioxidant than vitamin C (beta carotene and pycnogenol). Furthermore, astaxanthin has a similar function to lipoic acid, which has very strong protection against cytoplasm and cell nucleus, helps vitamin C, and E does their function properly (Ni, 2015). The vitamin E and astaxanthin are absorbed by the intestine and metabolized in the liver and is further stored in the skin as well as gonads during maturation and in muscle tissue during growth (Tizkar *et al*., 2016).

Vitamin E and vitellogenesis are closely related in the development of oocytes in fish through prostaglandins, which is enzymatically synthesized using essential fatty acids. The addition of vitamin E maintains the fatty acids synthesis due to it is functional as an antioxidant (Asaikkutti *et al*., 2016). Similarly, supplemented astaxanthin and vitamin E in the feed also

positively impact on the gonad maturity level of prospective Pacific white shrimp broodstock. It occurs through the process of increasing arachidonic acid as essential fatty acids derived from the administration of astaxanthin and vitamin E which has high antioxidants. Thus, it prevents the oxidation of essential fatty acid and it also increases the storage of fatty acids in eggs. Figure 6 illustrates an arachidonic acid metabolism.

The eyestalk ablation stimulates egg development in shrimp. Further development occurs due to the removal of the sinus glands. Katayama *et al*. (2013) noted that sinus glands are not the only organs which play an important role, but also the x-organ as a producer of gonad-inhibiting hormone (GIH). This x-organ works to produce GIH and mandibular organ inhibiting hormone (MOIH). Gonad inhibiting hormones significantly effect on gonad maturation of both males and females due to it naturally inhibits the development of gonads. Similarly, the MOIH hormone serves to inhibit the synthesis process of methyl farnesoate by mandibular organs (Katayama *et al*., 2013). The influence of GIH or MOIH hormone is very dominant in shrimp. Consequently, it can inhibit the gonad maturity development. Therefore, astaxanthin and vitamin E could stimulate the gonad maturity level until TKG IV through eyestalk ablation.

In the present study, spawning rate is directly proportional to the number of eggs and nauplii. The shrimp yielded, which treated with E treatment was the highest spawning performance among all treatments. The percentage of spawning reached 33.33%, the highest number of eggs was 87,000 eggs and the hatching rate reached 49%. The spawning rate on all treatments is classified low between 8.33 and 33.33%. This may be caused by the male shrimp broodstock was the first time to spawn. As said by Parnes *et al*. (2007), spawning is influenced by female gonads maturity levels and the reproductive state of the male broodstock (Parnes *et al*., 2007).

The number of eggs in all treatments group ranged from 86,333 to 87,000 eggs. According to Ibarra and Famula (2008) reported that the number of eggs produced by female shrimp is determined by the size of the body. The large broodstock produces more eggs. The final mean weight of the prospective broodstock was 35.95±2.64 g. The number of eggs in the fourth spawning increased due to the optimum level of triacylglycerides (TG) concentration. Hence, it is used as a survival measurement on shrimp larvae. In addition, high protein, fat, and carotenoid content can produce high-quality eggs. In the present study, the number of eggs is still low between 86,333–87,000 eggs. The shrimp may be affected by the broodstock was spawning the first time and the size of the broodstock used about 35 g. Ibarra and Famula (2008) claimed that fecundity varies depending on the size of the broodstock. The larger broodstock, the more eggs generated.

The hatching rate depends on the quality of the eggs which is produced by the shrimp broodstock. According to Kannan *et al*. (2015) stated that the quality of *P. monodon* tiger shrimp eggs is classified into five types. The first types, the eggs develop normally, positive phototactic responsive larvae, and hatching rate more than 58%. The second type of eggs is poor quality and developed abnormally, the new nauplius come out with a weak condition and the hatching rate 32% only. The third type of eggs is poor quality, unfertilized by irregular cytoplasm, and unable to hatch. The fourth type of poor quality eggs, unfertilized, undeveloped, cytoplasm clumped, and unable to hatch. The fifth type, the bad quality of eggs, unfertilized, the cytoplasm shrinks due to bacterial attack, and unable to hatch. The good quality of eggs is indicated by floating and white colour. However, the poor quality of eggs is precipitating and yellowish. In the present study, C and E treatment of 48±0.58% and 49±1.53% significantly different from the control. The egg hatching rate in this study was not good. Treatment of A, B, and D eggs hatching rate on Pacific white shrimp of 19±2.08%, 20±2.08%, and 21±3.21%, respectively. Based on hatching rate data, the shrimp broodstock is still low compared to shrimp broodstock derived from improvement. this is due to the internal factors (protein, fat, and carbohydrate content in the body and eggs) and external factors (nutrition and environment).The nutrients fed to broodstock are mostly allocated for reproductive purposes and improve the quality of eggs, particularly in the maturation phase of the gonads. Kurnia *et al*. (2010) reported that astaxanthin can be used to improve egg quality and larval production. Supplemented vitamin E can affect the chemical components of egg lipids and egg buoyancy in yellow tail fish. Moreover, the environmental factors contribute to the water treatment system, water temperature (31–32 °C), and water circulation in the rearing tanks.

In this study, astaxanthin and vitamin E were used to stimulate the development of gonads.

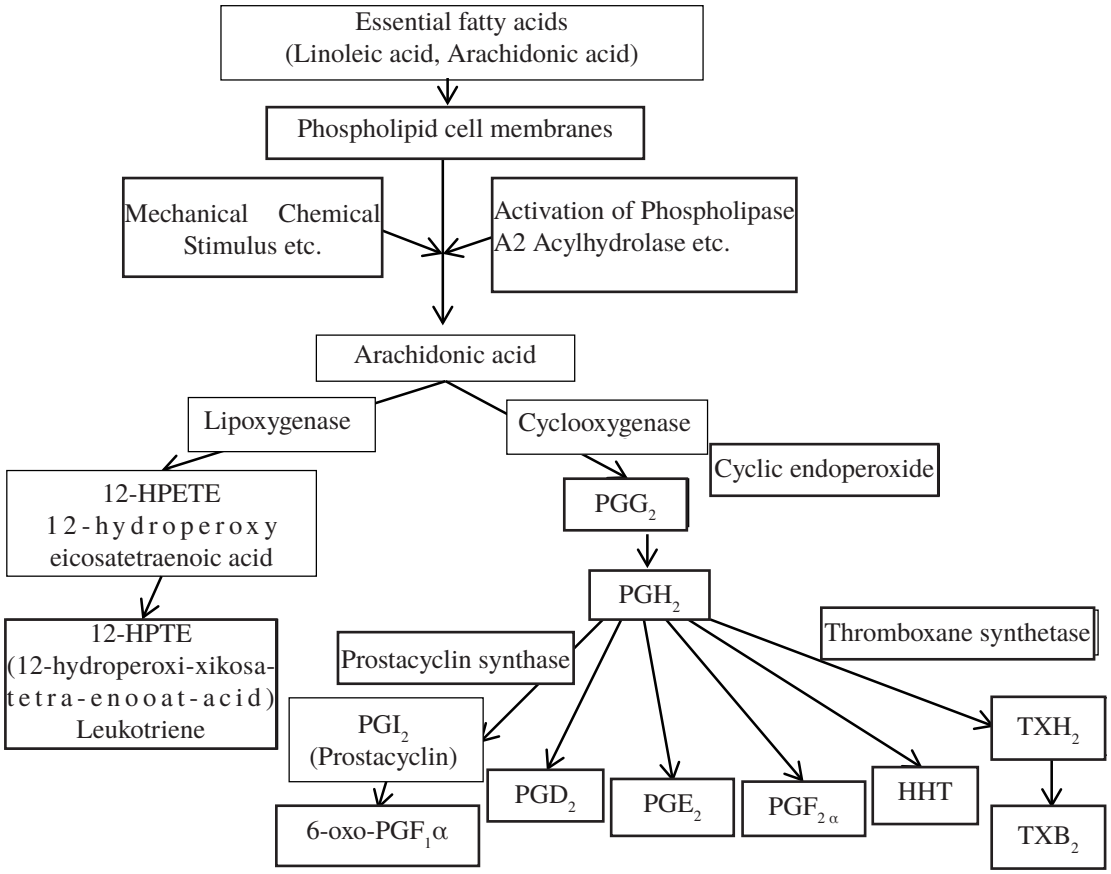

Figure 6. Arachidonic acid metabolism. PGG_2 (prostaglandin G_2), PGH_2 (prostaglandin H_2), TXA_2 (thromboxan A_2), TXB_2 (thromboxan B_2), PGE_2 (prostaglandin E_2), PGD_2 (prostaglandin D_2), $PGF_{2\alpha}$ (prostaglandin) and HHT (10-Heptadecatrienoic acid) (Meirer *et al*., 2012).

Astaxanthin is known as an antioxidant that can protect unsaturated fatty acids on phospholipids in cell membranes and for embryonic development as a constructor of cell membrane structures and prostaglandin precursors, in addition to its main function to provide energy (Mokoginta *et al*., 2002). Vitamin E can be transported from the peripheral tissue to the gonads through the liver with plasma lipoproteins, indicating that vitamin E plays a significant role in the reproductive process. During the process of vitellogenesis, vitamin E levels in the body decrease to about 10% until maturation. Vitamin E is transported from the blood vessels to the liver by high-density lipoprotein. In this study, the best treatment was C and E treatment. This suggests that the supplementation of astaxanthin and vitamin E in feed accelerate the gonad maturity in the shrimp broodstock. It is due to astaxanthin alleviate PGE2 (prostaglandin) hormone when the shrimp reaches the final gonad maturity (Kuedo *et al*., 2016). Therefore, the decrease of PGE2 (prostaglandin) hormones leads gonads to develop properly and the quality of eggs remain good due to lack of egg decay.

CONCLUSION

The supplementation of 250 mg/kg astaxanthin and 175 mg/kg vitamin E in the diet for prospective broodstock of Pacific white shrimp showed the best result with the first gonad maturity level reached on day-14 (19.45%), and the second gonad maturity level reached on day 21 (19.45%).

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