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MEASUREMENT HORMONE PROGESTERONE AND GONADAL MATURITY ON WHITE SHRIMP (*Litopenaeus vannamei*) AFTER THE ADMINISTRATION OF THE KARAMUNTING (*Melastoma malabhtricum*) ETHANOL EXTRACTS

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ABSTRACT

Lanosterol is a phytosterol that is used by crustacea to form animal steroid hormone that plays a role in the reproduction of the shrimp. This research aims to determine the response of ovary development in white shrimp parent after given Karamunting ethanol extract. This research was carried out in several stages: 1. Injecting the white shrimp every 3 days, at the base of the 5th leg, for 15 days with variable control dose 0 (C), 10 mg / kg BW (T1), 7.5 mg / kg BW (T2), 5 mg / kg BW (T3), 2 mg / kg BW (T4) and 1 mg / kg BW (T5), (2). Isolation of white shrimp parent ovary, (3). Measurement of progesterone level in the ovary using the Radioimmunoassay (RIA) method, (4) Histology of white shrimp parent ovary, and (5). Data analysis. The statistical results of measuring the increase in progesterone showed that administration of ethanol extract of karamunting at the end of maintenance had a significant difference ($P<0.05$). Histology observations of gonadal development in control, T5 and T4 showed cells to develop to previtellogenesis oocytes whereas in treatment T1, T2 and T3 ovary cells developed into endogenous vitellogenesis oocytes and only in T1 ovarian cells developed to form exogenous vitellogenesis oocytes. Statistical results of oocyte size after administration of the karamunting extract at the end of maintenance were significantly different ($P<0.05$). The average oocyte size at the beginning of maintenance (15.57 ± 3.15 µm), C (25.29 ± 2.69 µm) indicating that at the end of the ovarian treatment development occurred both in control and treatment. T1 (65.65 ± 2.64 µm), T2 (63.98 ± 3.06 µm), T3 (39.12 ± 6.01 µm), T4 (28.08 ± 0.84 µm) and T5 (27.65 ± 0.71 µm) also appear to be greater than the oocyte size at the beginning of maintenance and control. Based on the results of this study it can be concluded that the content of lanosterol in karamunting plants can increase the hormone progesterone which indicates an acceleration of gonadal maturity and enlargement of oocyte size in the parent shrimp of White shrimp.

Keywords: *Melastoma malabhtricum*, *Litopenaeus vannamei*, Hormone progesterone, Histology Gonadal Maturity, Reproduction

INTRODUCTION

Shrimp farming is one of the important aquaculture industries in the whole world. Based on FAO data (2017) shrimp farm production in the world in 2016 globally produces 2.9 million tons, and about 75% of production comes from Asia-Pacific. Shrimp production in Indonesia is 390 tons. One of the commodities of shrimp cultivated in Indonesia is White shrimp (*Litopenaeus vannamei*). In further cultivation development, more efficient white shrimp seed production techniques are needed. There are several ways to increase seed production, among others, eye ablation, hormone

injections, and high protein feeding. Some of these methods have not been able to help significantly improve shrimp reproduction.

Gonad maturity in white shrimp is influenced by two antagonistic hormones, namely Gonad Inhibitng Hormone (GIH), which is synthesized in the X sinus organ glands (XO-SG) in the eye and Gonad Stimulating Hormone (GSH) produced by the brain and chest ganglion. Besides that, there is the involvement of other hormones in regulating reproduction in crustacean animals such as progesterone, Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH). The availability of cholesterol strongly influences the development of ovary crustaceans. Wouters *et al*. (2001) stated that in the endocrine system, cholesterol is a precursor of steroid hormones that function for the reproduction and maturation of the gonads. Cholesterol is a sterol compound which is a precursor of steroid hormones and molting hormones (Sheen 2000). Cholesterol is one of the chemical compounds that cannot be synthesized by crustaceans (Kanazawa *et al*. 1988) but is very much needed by the parent shrimp. Therefore, to meet the cholesterol requirements, crustaceans obtain it from outside the body through feed intake.

Cholesterol sources can be obtained from plants that form secondary metabolites. One plant that contains cholesterol is Karamunting (*Melastoma malabatrhicum*). Karamunting plants (*M. malabathricum*) are widely used as medicines because they have secondary metabolic compounds consisting of saponins, tannins, triterpenoids/steroids, flavonoids. Ridwan *et al*. (2015) proved that karamunting plants extracted using ethanol solvents contained lanosterol. This lanosterol is cholesterol commonly found in plants. Nuresti *et al*. (2003) also reported that karamunting plants contained sitosterol α and β amyrin from the hexane fraction. Pattiasina *et al*. (2010) state that mangrove crabs (*Scylla serrata*) supplemented with serotine cholesterol can accelerate the ripening process of mangrove crab parent ovaries.

Until now the use of synthetic cholesterol is still the foundation in accelerating the maturity of shrimp gonads, while the use of natural cholesterol from plants is still very rarely done. Therefore, research is needed to prove whether the administration of karamunting extract can increase the hormone progesterone and accelerate the maturation of white shrimp gonads.

MATERIALS AND METHODS

Materials

The materials used in this study include karamunting ethanol extract, parent of White shrimp (*L. vannamei*), feed, formalin, xylol, 100% ethanol, distilled water, paraffin, picric acid, and eosin.

Experimental Design

Shrimp broodstock is placed in an aquarium measuring 30x30x60 cm. The weight of shrimp used ranges from 32-35 grams. Shrimp were given fresh food such as worms and oystersh at a dose of 15% of body weight per day. Feeding is carried out 5 times a day at 04.00; 07.00 AM; 13:00; 18:00; and 23:00.

This study used a completely randomized design (CRD) with 6 treatments and 5 repetitions. Karamunting extract injection was given through the base of the fifth leg of tiger shrimp (Tarsim *et al*. 2007) using a 1 ml tuberculin syringe.The dose of the extract given was 10 mg / kg BW (T1); 7.5 mg / kg BW (T2); 5 mg / kg BW (T3); 2.5 mg / kg BW (T4); 1 mg / kg BW (T5); and control without extract (C). The parameters measured in this study included an increase in the content of progesterone by using the Radioimmunoassay (RIA) method and ovary development using the histology staining method. Observation were carried out for 15 days. At the end of the observation the shrimp gonads were isolated to measure the level of gonad maturity. Furthermore, all data obtained were analyzed statistically.

RESULTS AND DISCUSSION

The Effect of Karamunting Ethanol Extract on Increased Progesterone Hormone in White Shrimp

In female white shrimp by injection of estradiol-17β, it can stimulate the development of gonads to the Level of Gonad Maturity (LGM) I (Tarsim *et al*. 2007). Whereas white shrimp with Human Chorionic Gonadotropin, progesterone, or a combination of both in the form of feed and injection, can stimulate the ovaries to LGM IV in 11-month-old shrimp weighing 80-120 g and LGM II in 5.5-month-old shrimp with weight 60-80 g (Ismail 1991).

The progesterone content from the results of this study was as follows: content at the start of the experiment (0.03 ± 0 ng / ml), C (0.03 ± 0 ng / ml), T3 (0.034 ± 0.005 ng / ml), T4 (0 , 03 ± 0 ng / ml) and T5 (0.03 ± 0 ng / ml) while in T1 (0.082 ± 0.050 ng / ml), T2 (0.058 ± 0.023 ng / ml). It was seen that there was an increase in the hormone progesterone in the main shrimp treated with karamunting extract with treatments T1 and T2 (Figure 1.). This shows that karamunting extract containing lanosterol can increase the content of the cholesterol hormone, which shows the acceleration of gonadal maturity from the parent White shrimp. This results similar on the tiger shrimp condition which was treated by eye ablation and serotonin hormone injection (Wongprasert *et al*. 2006).

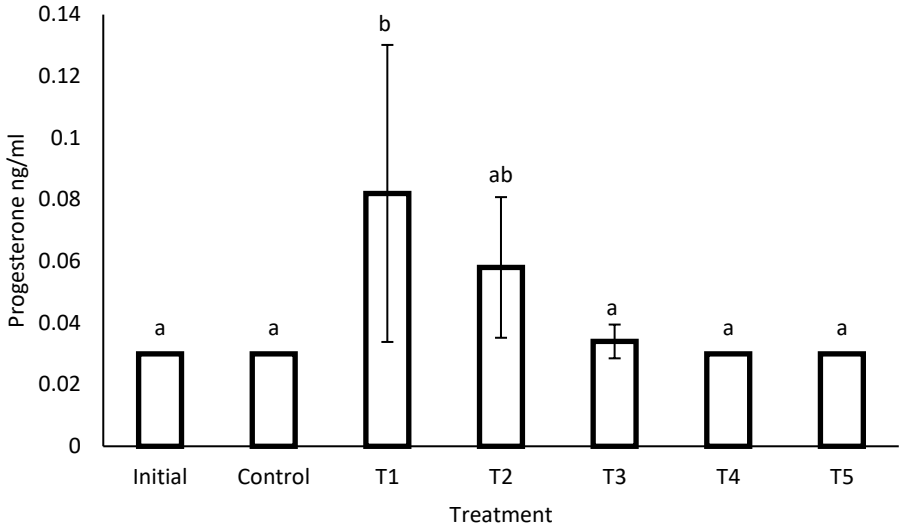

Figure 1 The average content of progesterone in white shrimp (*L. vannamei*) in the treatment of injection of ethanol extract of karamunting (*M. malabathricum*). A: Initial; C: Control; T1 (treatment1): 10 mg / kg BW; T2 (treatment2): 7.5 mg / kg BW; T3 (treatment3): 5 mg / kg BW; T4 (treatment4): 2 mg / kg BW; T5 (treatment5): 1 mg / kg BW; a and ab: Significant differences ($p < 0.05$) between means were determined using ANOVA.

Histology

Gonad development was observed besides morphologically also histologically. Histology observation is intended to see the development of oocyte cells in the gonads of the parent White shrimp (*L. vannamei*) due to the influence of the ethanol extract of karamunting. The classification of oocyte development in shrimp consists of previtellogenic, endogenous vitellogenic oocytes, and exogenous vitellogenic oocytes (Wilder *et al*. 2010). Based on observations of oocyte development after exposure to the karamunting ethanol extract, oocyte development results were obtained at the beginning of the observation and the end of the observation (Figure 2) .

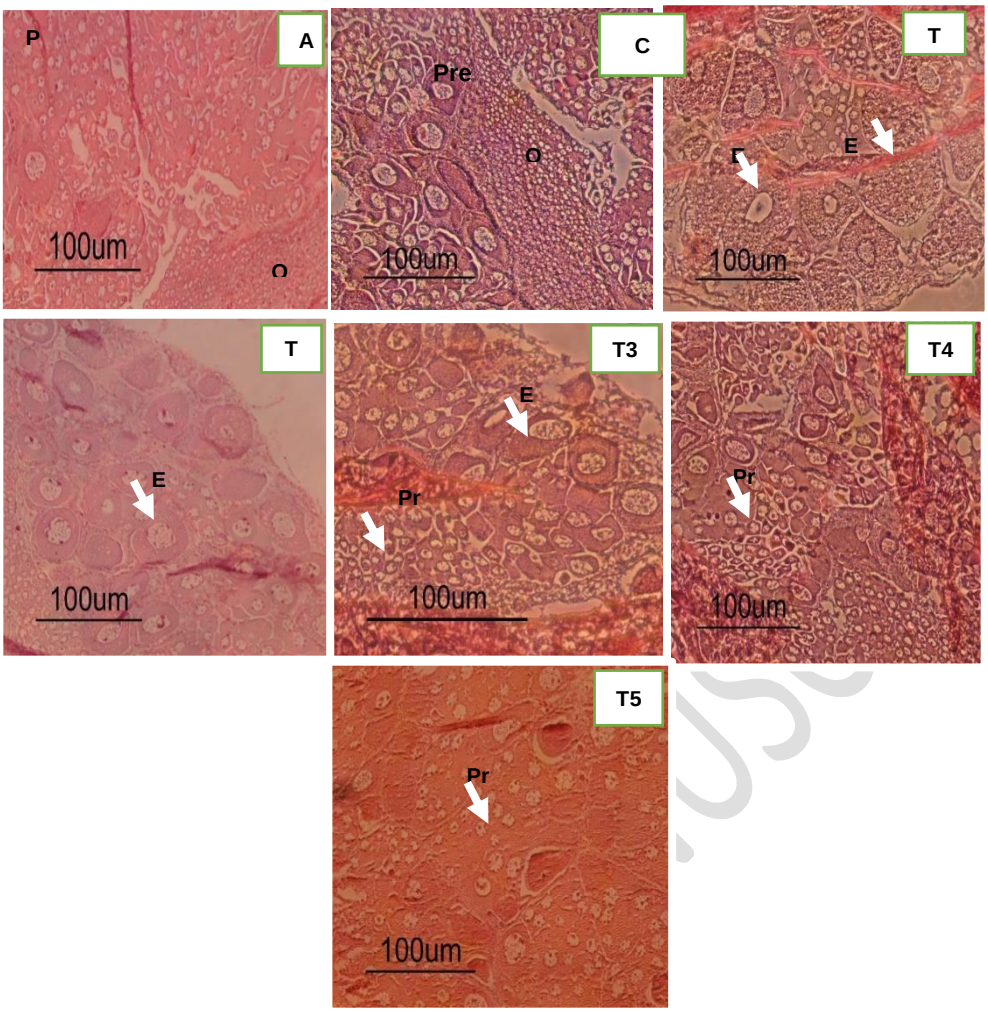

Figure 2 Histology of gonads of white shrimp (*L. Vannamei*) Description: A A: Initial; C: Control; T1 (treatment1): 10 mg / kg BW; T2 (treatment2): 7.5 mg / kg BW; T3 (treatment3): 5 mg / kg BW; T4 (treatment4): 2 mg / kg BW; T5 (treatment5): 1 mg / kg BW; a and ab: Significant differences ($p < 0.05$) between means were determined using ANOVA.; O: Oogonia; Pre: previtellogenic oocytes; En: endogenous vitellogenic oocytes; Ex: exogenous vitellogenic oocytes; 20x enlargement.

In shrimp, ovarian development is characterized by the formation of cortical rods in the oocytes after the accumulation of yolk (Clark *et al*. 1980). At the beginning of the treatment, the gonads are generally not developed (Figure 2.A), the gonads are still in the accumulation of oogonia. At the end of maintenance for 15 days, both control and all treatments experienced cell development. In control (Figure 2.C), the gonads develop into oocytes at the previtellogenic oocytes stage, and part of the ovary is still oogonia. Whereas in T4 (2 mg/kg BW) and T5 (1 mg/kg BW) the development of gonads is not different from the control still at the previtellogenic oocytes stage.

Histological observations on TI (10 mg/kg BW) (Figure 2.T1) oocyte development shows in the endogenous phase of vitellogenic oocytes, and several parts of the gonad have formed exogenous vitellogenic oocytes and have a larger oocyte size when compared to controls (Figure 3). In T2 (7.5 mg/kg BW) (Figure 2.T2 development of oocytes in the endogenous phase of vitellogenic oocytes

when compared with the control of the development of oocytes in the previtellogenic oocytes phase. In T3 (5 mg/kg BW) (Figure 2.T3) ovary development in the endogenous phase of vitellogenic oocytes and some still have the previtellogenic oocytes when compared with the control of oocyte development in the previtellogenic oocytes phase.

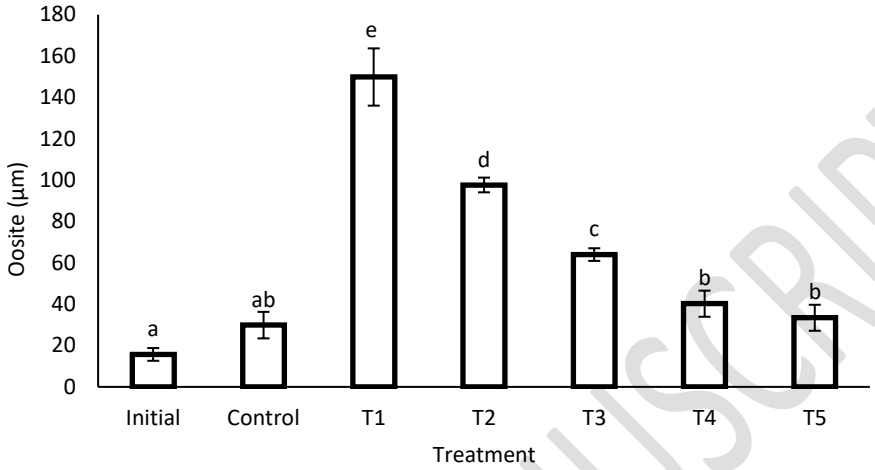

Figure 3 Average Oocyte Diameter Size in the parent white shrimp (*L. vannamei*) in the treatment of injecting ethanol extract of karamunting (*M. malabathricum* A: Initial; C: Control; T1 (treatment1): 10 mg / kg BW; T2 (treatment2): 7.5 mg / kg BW; T3 (treatment3): 5 mg / kg BW; T4 (treatment4): 2 mg / kg BW; T5 (treatment5): 1 mg / kg BW; a and ab: Significant differences ($p < 0.05$) between means were determined using ANOVA.. a, ab, b, c, d, and e: Significant differences ($p < 0.05$) between means were determined using ANOVA.

The results of measurements of ovarian oocyte diameter before and after exposure to the karamunting ethanol extract (Figure 3). Based on these images, it can be seen that the mean oocyte size at the beginning of maintenance (15.57 ± 3.15 µm) looks smaller than the control (25.29 ± 2.69 µm). This indicates that at the end of the maintenance, there is ovarian development both control and treatment. Furthermore the size of the oocytes in the treatment of T1 (65.65 ± 2.64 µm), T2 (63.98 ± 3.06 µm), T3 (39.12 ± 6.01 µm), T4 (28.08 ± 0.84 µm) and T5 (27.65 ± 0.71 µm) also looks bigger when compared to the size of the oocyte at the beginning of observation and control. Based on Figure 3, it is also known that the smallest oocyte size at the end of the observation was found in the control while the largest oocyte size in treatment T1.

The results of statistical analysis of the size of the oocyte diameter at the end of the observation showed that the administration of ethanol extract of karamunting produced significant differences between treatments ($P > 0.05$) with the largest diameter at T1 and T2. Tarsim *et al*. (2007) stated that injection of estradiol 17β was able to increase the size of oocytes in White shrimp (*L. vannamei*). In line with this, it can be assumed that there are compounds in the karamunting extract that can accelerate gonad maturity as indicated by an increase in oocyte diameter.

Effect of Karamunting Ethanol Extract on Increased Gonad Somatic Index (GSI)

The increase in gonad somatic index (GSI) was obtained in this study to determine the increase in the gonad of White shrimp (*L. vannamei*) on gonadal development responses by administering ethanol extract of Karamunting (*M. malabathricum*). A high increase in GSI in individuals is an indication of gonadal development caused by gonads that develop into large ones. Based on the calculation of the increase in GSI at the end of maintenance with the administration of karamunting ethanol extract shown in Figure 4, it can meet the average GSI requirements in setting T1 (1.39 ± 0.04), T2 (1.26 ± 0.03) and T3 (1.01 ± 0.08) higher than the control (0.84 ± 0.02). At T4 (0.91 ± 0.03) and T5 (0.83 ± 0.04) higher mean values were compared with controls.

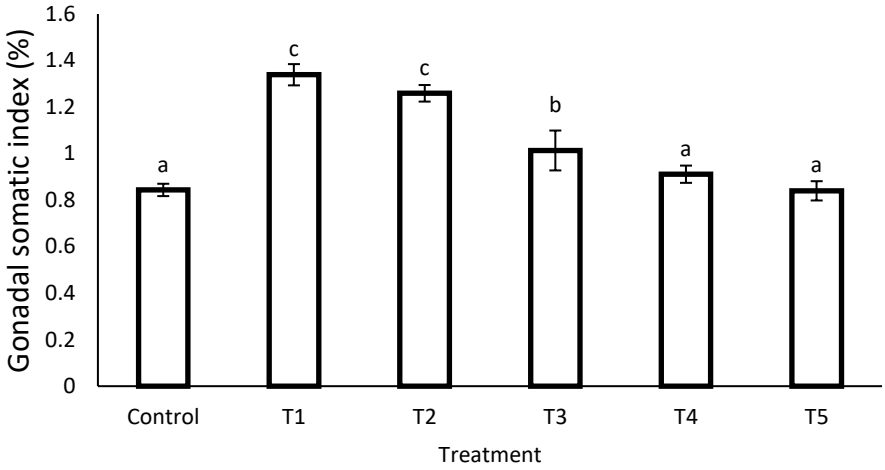

Figure 4 Average Somatic Gonad Index on parent white shrimp (*L. vannamei*) in the treatment of injecting ethanol extract of karamunting (*M. malabathricum*) at the end of maintenance. C: Control; T1 (treatment1): 10 mg / kg BW; T2 (treatment2): 7.5 mg / kg BW; T3 (treatment3): 5 mg / kg BW; T4 (treatment4): 2 mg / kg BW; T5 (treatment5): 1 mg / kg BW; a and ab: Significant differences ($p < 0.05$) between means were determined using ANOVA.. a, b and c: Significant differences ($p < 0.05$) between means were determined using ANOVA.

The karamunting extract with GCMS testing showed that the extract contained high lanosterol α and β amirin. Nuresti *et al*. (2003) reported that karamunting plants contain sitosterol α and β amyrin. Wouter *et al*. (2011) reported that by providing natural feed such as blood worms and squid, it could stimulate ovary maturation in shrimp. Blood worms and squid are animals that contain high cholesterol (Saidin 2000).

Based on the results of statistical testing on the treatment with administration of ethanol extract karamunting showed that the data increase in the value of GSI was significantly different from the control ($P<0.05$). In T1 (10 mg/kg BW) and T2 (7.5 mg/kg BW) the increase in GSI was higher

than the control (P <0.05), T3 (5 mg/kg BW) was able to increase GSI more high compared to controls, but did not exceed the increase compared to T1 and T2 (P<0.05). Rodriguez-Gonzalez *et al*. (2009a) reported that it was closely related to protein and lipid levels in the gonads, which also reflected feed levels. This is reinforced by research conducted by Pattiasina *et al*. (2010) with cholesterol administration can optimize the increase in ovarian weight in mangrove crabs.

In the research conducted by Meeratana *et al*. (2006) by injecting serotonin on freshwater shrimp mother *Macrobrachium rosenbergii* can stimulate an increase in somatic gonad index. Increasing the dose of karamunting plant extract in white shrimp exposure has an impact on ovarian development. This is following the statement of Wouters *et al*. (2001) that cholesterol is needed by crustaceans, which function as precursors of steroid hormones in the process of gonadogenesis, maturation, and reproduction.

The results of GSI measurements on T4 (2 mg/kg BW) and T5 (1 mg/kg BW) statistically did not show significant differences with controls (P >0.05). This is probably due to the small amount of dosing so that it is not enough to meet the increase in hormone in the parent white shrimp. As explained by Idris *et al*. (2011) The parent shrimp has an optimum level of protein requirements, where increasing the level of protein above the optimum level will not affect the nutritional condition. Protein levels recommended by Rodriguez-Gonzales *et al*. (2006a) which produces the highest spawning rate of 30%.

Water Quality

The optimal maintenance media for White shrimp is 28-32 ^{o}C, salinity 27-40 ppt, pH 6.5-8.3, and DO 4-6 mg / L (Farhan 2006). The results of measuring media during maintenance can be seen in Table 1. Maintenance water quality measurements are carried out every day. Measurement of water quality parameters is carried out every day to monitor environmental conditions to remain stable. Changes in water quality on broodstock maintenance media will cause stress to the shrimp, which will disrupt the gonadal maturation process. Maintenance media is relatively stable due to controlled maintenance. Maintenance media is suitable for the maintenance of mother white shrimp. To maintain the stability of water quality in the maintenance media every day before feeding, first, we need to squeeze the base to remove feces and the rest of the feed, while the maintenance of water media is done every three days with a turnover of 30 percent new water.

Table 1 Water quality

No	Parameter	Avarage range		
		Morning	Afternoon	Evening
1	Salinity	33	34	34
2	Temperature	28	29	28
3	pH	7,6	7,8	7,8
4	DO	6,56	6,01	6,57

CONCLUSION

Based on the results of this study it can be concluded that the administration of caramunting ethanol extract to the parent of white shrimp can increase the hormone progesterone and accelerate gonad maturity.

REFERENCES

Clark, W. H., Lynn, J. W., Yudin, A. I., and Persyn, H. O. (1980). Morphology of the Cortical Reaction In The Eggs Ofpenaeus aztecus. *The Biological Bulletin, 158(2), 175–186.* doi:10.2307/1540929

Farchan M. 2006. Teknik Budidaya Udang Vannamei. Serang. In: BAPPL-STP.

Food and Agriculture Organization. 2017. Globefish Highlights. A Quaterly Update On World Seafood markets: (FAO) of the United Nations

Huberman. 2000. Shrimp endocrinology: a review. *Aquaculture*, 191, 191-208.

Idris M. 2011. Energy pathway on two repeated spawning of Australian red claw crayfish (Cherax quadricarinatus) [Dissertation] Retrieved from Institut Teknologi Bandung

Ismail A. 1991. *Pengaruh Rangsangan Hormon terhadap Perkembangan Gonad Individu Betina dan Kualitas Telur Udang windu* (Penaeus monodon Farb), Disertasi, Fakultas Pascasarjana, Institut Pertanian Bogor, Bogor.

Kanazawa A, Chim L, Laubier. 1988. Tissue uptake of radioactive cholesterol in the prawn Penaeus japonicas Bate during ovarion maturation. Aquat. Living Resour Ed ke-1. 85-91.

Meeratanaa P, Withyachumnarnkulb B, Damrongpholc P, Wongprasertb K, Suseangthamb A, Sobhonb P. 2006. Serotonin induces ovarian maturation in giant freshwater prawn broodstock, *Macrobrachium rosenbergii* de Man, Aquaculture 260:315-325.

Nuresti, S., Baek, S.H and Asari, A. 2003. Chemical Components of *Melastoma Malabathricum*. ACGC Chemical Research Communications. 16:28–33.

Pattiasina, B, J., Zairin, M., Mokoginta, I., Affandi, R., Manalu, W. 2010. Perkembangan ovari induk kepiting bakau *Scylla serrata* yang disuplementasi kolesterol dan disuntik serotonin. Jurnal Akuakultur Indonesia 9 (1), 67-76.

Ridwan A, Awaludin, Anggraeni T. 2015. Gonadal Maturity Induction using Karamunting (*Melastoma malabatrhicum*) Ethanol Extract on White Shrimp Female (*Litopenaeus vannamei*). Proceeding of 6th ICGRC. p.69-74

Rodrigiez-Gonzales H, Garcia-Ulloa M, Hernandez-Llamas A, dan Villarrel H. 2006a. Effect of Dietary Protein Level on Spawning and Egg Quality of Redclaw Crayfish *Cherax quadricarinatus*, Aquaculture **257**:412-419.

Rodrigiez-Gonzales H, Villarrel H, Garcia-Ulloa M, dan Hernandez-Llamas A. 2009a. Evaluation of Practical Diets Containing Different Protein Levels on Gonad Development of Female Redclaw Crayfish *Cherax quadricarinatus*, Aquacult. Nutrition **15**:347-355.

Saidin M. 1999. Cholesterol Content of Foods Originating From Animal Tissue. Bulletin of Health Research, p-ISSN: 0125-9695

Sheen S S. 2000. Dietary cholesterolrequirement of juvenile mud crab (*Scylla serrata*). Aquaculture 189:277-285.

Tarsim, Zairin M, Riani E. 2007. Rangsangan Perkembangan Ovari Udang Putih (Litopenaeus vannamei) dengan Penyuntikan Estradiol-17β. Jurnal ilmu kelautan ISSN 0853-7291.

Wilder MN, Okumura T, Tsutsui N. 2010. Reproductive Mechanisms in Crustacea Focusing on Selected Prawn Species: Vitellogenin Structure, Processing and Synthetic Control, Aqua-BioScience Monographs 3, No. 3:73–110

Wongprasert K, Asuvapongpatana S, Poltana P, Tiensuwan M, Withyachumnarnkul B. 2006. Serotonin stimulates Ovarian Maturation and Spawning in the Black Tiger Shrimp *Penaeus monodon*, Aquaculture 261:1447-1454.

Wouters R, Piguave X, Bastidas L, Calderon J, Sorgeloos P. 2001. Ovarian maturation and hemolymphatic vitellogenin concentration of pacific white shrimp *Litopenaeus vannamei* (Boone) fed increasing levels of total dietary lipids and HUFA. Aquaculture Research 32:573-582.

Wulandari A, Pancoro A, Suhandono S. 2010. Construct and Production Gonad-Inhibiting Hormone dsRNA of Black Tiger Prawn (Penaeus monodon) by in vitro and in vivo Technique in L44440 Expression Vector. [Thesis] Retrieved from Institut Teknologi Bandung.