KARAMUNTING (*Melastoma malabathricum*) EXTRACTS ON WHITE SHRIMP (*Litopenaeus vannamei*) MATURITY

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ABSTRACT

White shrimp *Litopenaeus vannamei* is one of the prime shrimp commodities cultivated in Indonesia. As such, the discovery of more efficient seed production techniques for this species is deemed necessary. Karamunting (*Melastoma malabathricum*) extract contains the cholesterol precursor called lanosterol, a phytosterol which is used by crustaceans to form the animal steroid hormone that is very crucial in their reproduction. Hence, this research aimed to determine the ovary development of mature *L. vannamei* individuals injected with the Karamunting ethanol extract. The experiment was carried out in several stages. Firstly, injecting the white shrimp at the base of the 5th leg, every 3 days for 15 days with variable control dosage 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), where BW is Body Weight. Secondly, isolating the white shrimp parent ovary. Thirdly, measuring the progesterone level in the ovary using the Radioimmunoassay (RIA) method. Fourthly, observing the histology of white shrimp parent ovary and, finally, analyzing the data. Measurements of the increase in progesterone levels showed that the administration of karamunting ethanol extract significantly affected the progesterone production ($P<0.05$). Histology observations of gonadal development in the control, T5 and T4 showed that the cells developed to previtellogenesis oocytes whereas in treatment T1, T2 and T3 ovary cells developed into endogenous vitellogenesis oocytes and only in T1 did the ovarian cells develop to form exogenous vitellogenesis oocytes. Karamunting extract significantly increased the oocyte sizes ($P<0.05$). At the start of the experiment, the average oocyte sizes were at 15.57 ± 3.15 μm At the end of the experiment, the Control was at 25.29 ± 2.69 μm and the ovarian treatments produced the following oocyte sizes; T1 at 65.65 ± 2.64 μm, T2 at 63.98 ± 3.06 μm, T3 at 39.12 ± 6.01 μm, T4 at 28.08 ± 0.84 μm and T5 at 27.65 ± 0.71 μm. The extract produced oocyte sizes greater than at the beginning of maintenance and control. Apparently, the lanosterol in the karamunting extract had increased the hormone progesterone resulting in an accelerated gonadal maturity and enlargement of oocyte sizes in the parent individuals of the white shrimp.

Keywords: gonadal maturity, histology, *Melastoma malabathricum*, *Litopenaeus vannamei*, progesterone, reproduction

INTRODUCTION

One of the important world-wide aquaculture industries is shrimp farming which accounted for the global 2.9 million tons of shrimp farm production in 2016 (FAO 2017), of which 75% came from Asia-Pacific. Indonesia contributed about 390 tons. One of the shrimp commodities cultivated in Indonesia is the white shrimp (*Litopenaeus vannamei*). For better cultivation, more efficient techniques are needed for white shrimp production. Several ways were tried to increase its seed production, among others; eye ablation, hormone injections, and high protein feeding. However, some of these methods have not been able to 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. Moreover, there is the involvement of other hormones in regulating reproduction in crustacean animals, such as the progesterone, Follicle Stimulating Hormone (FSH), and

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Luteinizing Hormone (LH). The availability of cholesterol strongly influences the development of crustacean ovaries. In the endocrine system, cholesterol is a precursor of steroid hormones that function for the reproduction and maturation of the gonads (Wouters *et al.* 2001). Cholesterol is a sterol compound which is a precursor of steroid hormones and molting hormones (Sheen 2000). This 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 malabathricum*) which is widely known for its medicinal uses particularly its secondary metabolic compounds that consist of saponins, tannins, triterpenoids/steroids, flavonoids. Karamunting extracts obtained by using ethanol solvents contained lanosterol, the cholesterol commonly found in plants (Ridwan *et al.* 2015). Karamunting plants also contained sitosterol α and β amyrin from the hexane fraction (Nuresti *et al.* 2003). Mangrove crabs (*Scylla serrata*) supplemented with serotine cholesterol had experienced an accelerated ripening of the parent ovaries (Pattiasina *et al.* 2010).

Until recently, the use of synthetic cholesterol is still the foundation for accelerating the maturity of shrimp gonads, while the use of natural cholesterol from plants is still very rarely done. Therefore, this research was conducted to prove whether or not the administration of karamunting extract can increase the amount or content/quantity of hormone progesterone and accelerate the maturation of white shrimp gonads.

MATERIALS AND METHODS

Materials

The materials used in this study include karamunting (*Melastoma malabathricum*) ethanol extract, mature individuals of white shrimp (*Litopenaeus vannamei*), feed, formalin, xylol, 100% ethanol, distilled water, paraffin, picric acid, and eosin.

Experimental Design

The shrimp broodstock, with shrimps weighing 32 - 35 g, was placed in a 30 x 30 x 60 cm aquarium. The shrimps were fed with fresh worms and oysters at a dosage of 15% of body weight per day. Feeding was carried out 5 times a day at 04.00 H, 07.00 H, 13.00 H, 18.00 H, and 23.00 H.

This study was conducted using a completely randomized design (CRD) with 6 treatments and 5 repetitions. Using a 1 ml tuberculin syringe, the karamunting extract was injected at the base of the fifth leg of the white shrimp following the method of Tarsim *et al.* (2007) at extract dosages of 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 or without extract (C). The parameters that were used included the increase in the progesterone content by using the Radioimmunoassay (RIA) method and in the ovary development using the histology staining method. At the end of the 15-day observation period, the shrimp gonads were isolated to measure the level of gonad maturity. All data obtained were statistically analyzed by using ANOVA.

RESULTS AND DISCUSSION

Karamunting Ethanol Extract on the White Shrimp Progesterone Hormone

The injection of estradiol-17β into the female white shrimp had stimulated the development of the 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, had stimulated the ovaries to LGM IV in the 11-month-old shrimp weighing 80 - 120 g and LGM II in the 5.5-month-old shrimp weighing 60 - 80 g (Ismail 1991).

The progesterone content at the start of the experiment was at 0.03 ± 0 ng/ml. After the 15-day experiment the contents were measured as follows:, Control (C) was at 0.03 ± 0 ng/ml, T1 at 0.082 ± 0.050 ng/ml), T2 at 0.058 ± 0.023 ng/ml, T3 at 0.034 ± 0.005 ng/ml, T4 at 0.03 ± 0 ng/ml and T5 at 0.03 ± 0 ng/ml). An increase in the hormone progesterone was observed in

the shrimp applied with karamunting extract T1 and T2 (Fig. 1.). This shows that karamunting extract containing lanosterol had increased the content of the cholesterol hormone, resulting in the acceleration of gonadal maturity of the parent white shrimp. Similar results were also observed among the tiger shrimps treated by eye ablation and serotonin hormone injection (Wongprasert *et al.* 2006).

Histology

Gonad development was observed not only morphologically but also histologically. Histological observation was intended to see the development of oocyte cells in the gonads of the parent white shrimp (*L. vannamei*) as influenced by the karamunting ethanol extract. The development of oocyte in shrimp were classified as 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 (Fig. 2).

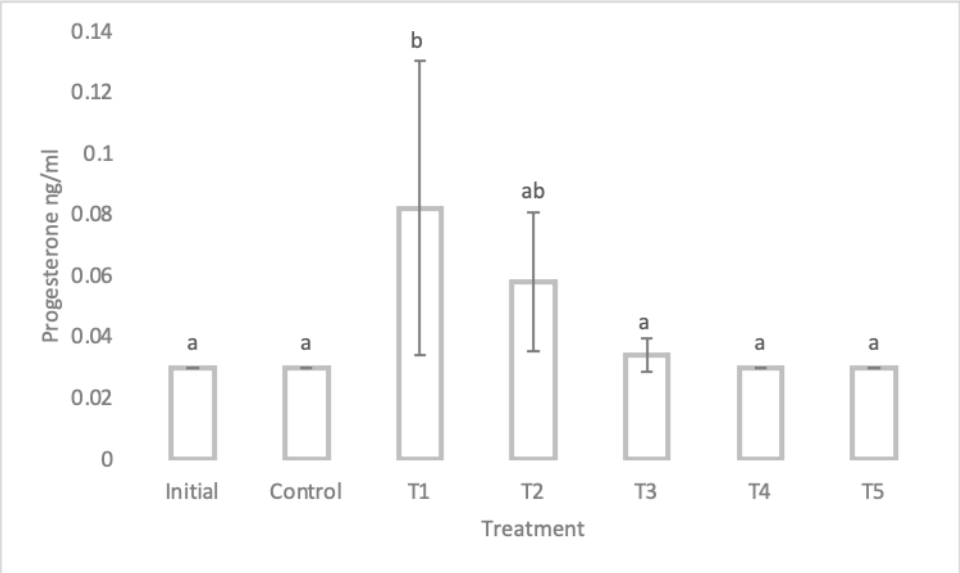

Figure 1 The average progesterone content of white shrimp *L. Vannamei* injected with karamunting *M. malabathricum* ethanol extract

Notes: A = Initial; C = Control; T1 = 10 mg/kg BW; T2 = 7.5 mg/kg BW; T3 = 5 mg/kg BW; T4 = 2 mg/kg BW; T5 = 1 mg/kg BW; a, b = Means with different letters differ significantly from each other at $P<0.05$.

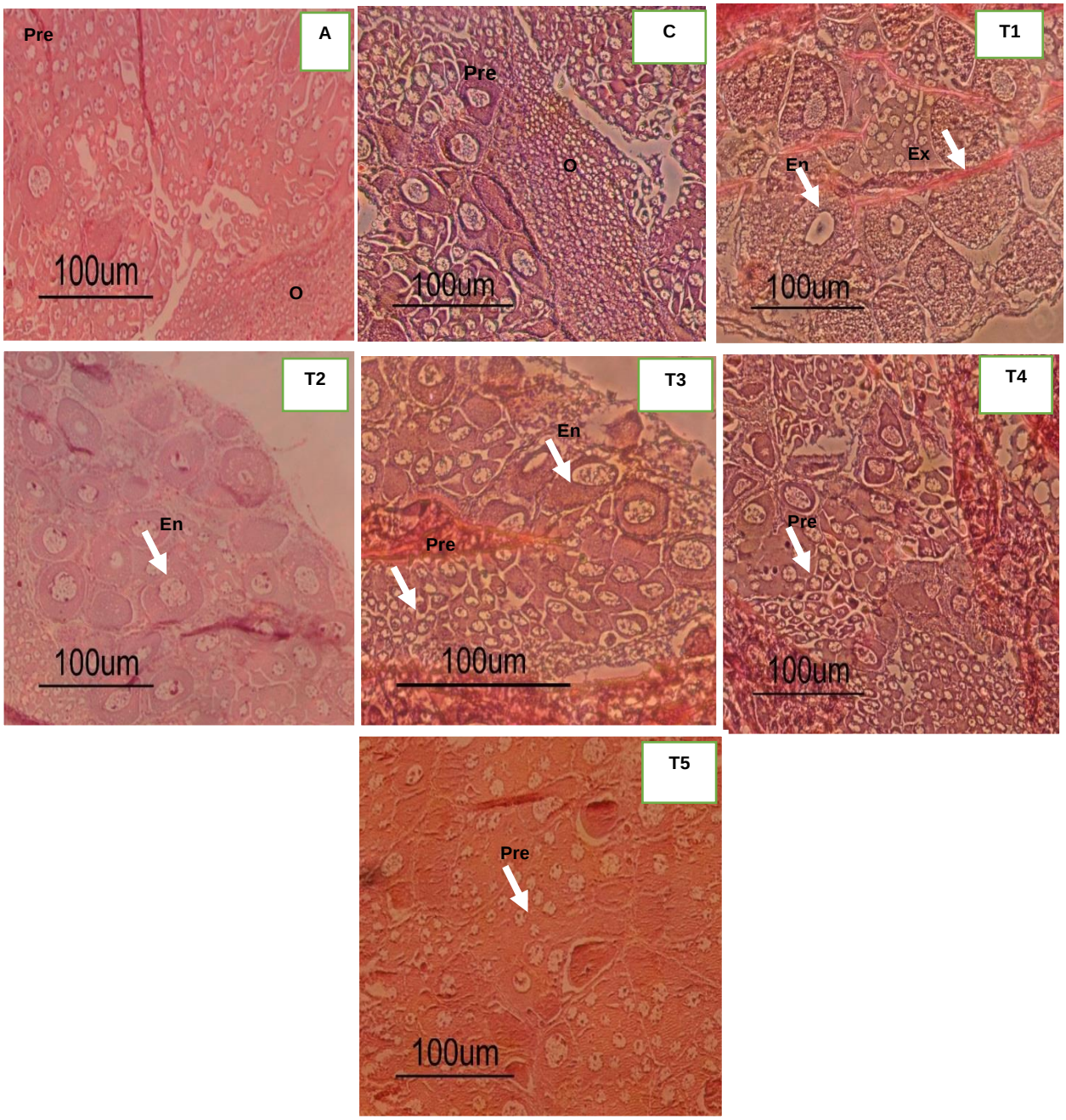

Figure 2 Histology of *Litopenaeus vannamei* gonads
Notes: A = Initial; C = Control; T1 = 10 mg/kg BW; T2 = 7.5 mg/kg BW; T3 = 5 mg/kg BW; T4 = 2 mg/kg BW; T5 = 1 mg/kg BW; a, b = Means of different letters significantly differ from each other at $P<0.05$ using ANOVA; O: Oogonia; Pre: previtellogenic oocytes; En: endogenous vitellogenic oocytes; Ex: exogenous vitellogenic oocytes; 20x enlargement.

Ovarian development among shrimps 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 were generally not developed yet (Fig. 2.A), these gonads were still in the accumulation of oogonia. After the 15 days, the control and all the treatments exhibited gonad cell development. In the Control (Fig. 2.C), the gonads developed into oocytes at the previtellogenic oocytes stage, and part of the ovary was still oogonia. Whereas in T4 (2 mg/kg BW) and T5 (1 mg/kg BW) the development of gonads did not differ from that of the control which was still at the previtellogenic oocytes stage.

In TI (10 mg/kg BW) (Fig. 2.T1), the oocyte development was observed in the endogenous phase of vitellogenic oocytes, and several parts of the gonad have formed exogenous

vitellogenic oocytes and have a larger oocyte size as compared to Control (Fig. 3). In T2 (7.5 mg/kg BW) (Fig. 2.T2), the oocyte development was in the endogenous phase of vitellogenic oocytes as compared with that of the control which was in the previtellogenic oocytes phase. In T3 (5 mg/kg BW) (Fig. 2.T3), the ovary development was in the endogenous phase of vitellogenic oocytes and some still have the previtellogenic oocytes as compared with that of the control which was also in the previtellogenic oocytes phase.

The mean oocyte size at the beginning of the experiment, at 15.57 ± 3.15 μm, was smaller than that of the control at 25.29 ± 2.69 μm (Fig. 3). This indicates that after the 15-day maintenance, the ovary developed in both the control and the treatments. Furthermore, the size of the oocytes in T1 at 65.65 ± 2.64 μm, T2 at 63.98 ± 3.06 μm, T3 at 39.12 ± 6.01 μm, T4 at 28.08 ± 0.84 μm and T5 at 27.65 ± 0.71 μm were also significantly bigger than that of the initial size of the oocyte and control. The control had the smallest oocyte at the end of the observation while T1 had the largest.

The application of karamunting ethanol extracts significantly increased the oocyte diameter sizes among the treatments ($P>0.05$) with the largest diameter at T1 and T2 indicating that karamunting has compounds that can accelerate gonad maturity. In another study, the injection of estradiol 17β has also increased the diameter size of oocytes in *L. vannamei* (Tarsim *et al.* (2007).

Karamunting Ethanol Extract on Gonad Somatic Index (GSI)

The application of Karamunting ethanol extract also increased the gonad somatic index (GSI). The high increase in individual GSI indicated gonadal development manifested by gonads growing bigger (Fig. 4). The use of karamunting ethanol extract has shown that it can meet the average GSI requirements in 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), the GSI increases were not significantly different from the controls.

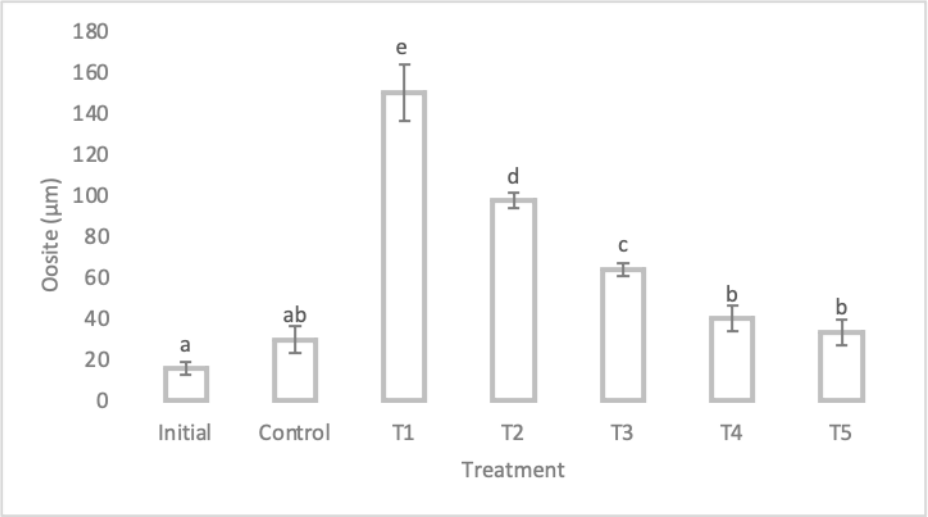

Figure 3 Oocyte average diameter size of the parent *Litopenaeus vannamei* injected with *Melastoma malabathricum* ethanol extract

Notes: A = Initial; C = Control; T1 = 10 mg/kg BW; T2 = 7.5 mg/kg BW; T3 = 5 mg/kg BW; T4 = 2 mg/kg BW; T5 = 1 mg/kg BW; a, b, c, d, e = Means with different letters significantly differ from each other at $P<0.05$.

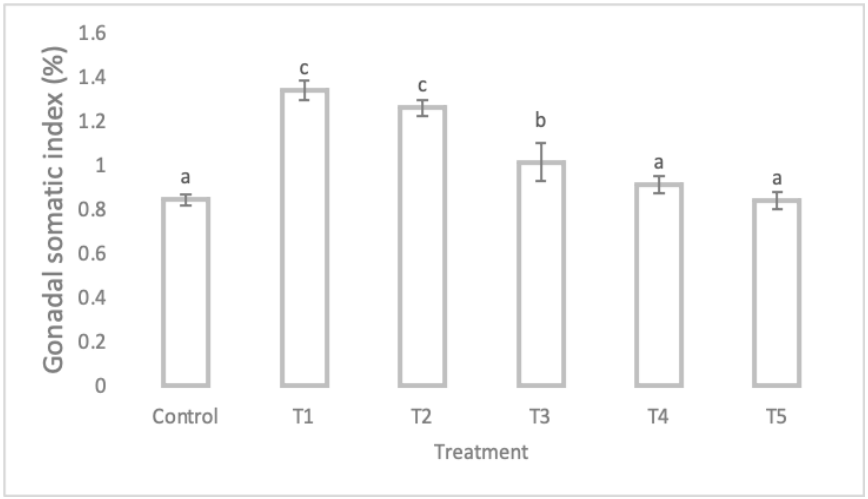

Figure 4 Average Somatic Gonad Index of parent white shrimp *Litopenaeus vannamei* injected with karamunting *Melastoma malabathricum* ethanol extract
Notes: C = Control; T1 = 10 mg/kg BW; T2 = 7.5 mg/kg BW; T3 = 5 mg/kg BW; T4 = 2 mg/kg BW; T5 = 1 mg/kg BW; a, b = Means with different letters significantly differ from each other at P< 0.05.

Based on the Gas Chromatography Mass Spectrometry (GCMS) test, the karamunting extract contained high lanosterol α and β amirin levels. In another study, karamunting plants also contain sitosterol α and β amyrin (Nuresti *et al.* 2003). Moreover, natural feed such as blood worms and squid had also stimulated the ovary maturation in shrimp (Wouter *et al.* 2011). Blood worms and squid are animals that contain high cholesterol (Saidin 2000).

Increases in the GSI values of the treatments were 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 that of the control (value?) at $P <0.05$. The T3 (5 mg/kg BW) increase in GSI was not significantly different from that of the control. A study showed that GSI increase was closely related to protein and lipid levels in the gonads, which also reflected high feed levels (Rodriguez-Gonzalez *et al.* 2009a). The application of cholesterol had also optimized the increase in the ovarian weights of mangrove crabs (Pattiasina *et al.* (2010).

Furthermore, the injection of serotonin on mature female individuals of the freshwater shrimp *Macrobrachium rosenbergii* had stimulated an increase in GSI (Meeratana *et al.* 2006). Increasing the dosage of karamunting plant extract in white shrimp had impacted ovarian development. Irrevocably, crustaceans need cholesterol which functions as precursors of steroid hormones in the process of gonadogenesis, maturation, and (Wouters *et al.* 2001).

GSI of T4 (2 mg/kg BW) and T5 (1 mg/kg BW) did not significantly differ from that of the controls. This is probably due to the small dosage that was not enough to produce the optimum hormonal increase in the parent white shrimp. 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 (Idris *et al.* 2011). The recommended protein levels could produce the highest spawning rate of 30% (Rodriguez-Gonzales *et al.* 2006a).

Water Quality

The optimum qualities for the maintenance medium of the white shrimp is at 28 - 32 °C, salinity 27 - 40 ppt, pH 6.5 - 8.3, and DO 4 - 6 mg/L (Farhan 2006). In this study, the water quality parameters were measured every day to monitor and maintain stable environmental conditions (Table 1). Changes in water quality on broodstock maintenance media will create stress on the shrimp which in turn will disrupt the gonadal maturation process. The growing environment was maintained at optimum conditions for the mature female white shrimp.

To maintain stability of the water quality in the growing media, certain maintenance activities were carried out every day before feeding. Firstly, squeezing the base to remove feces and the rest of the feed; secondly, the water media was replenished every three days at a turnover rate of 30 percent new water.

Table 1 Qualities of the aquatic environment

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

The injection of Karamunting *Melastoma malabathricum* ethanol extract on the mature female individuals of white shrimp *Litopenaeus vannamei* had increased its hormone progesterone and accelerated its gonad maturity indicating that the extract can hasten the reproduction time thereby increasing production volume over a shorter period.

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