Gonadal Maturity Induction using Karamunting (*Melastoma malabatrhicum*) Ethanol Extract on White Shrimp Female (*Litopenaeus vannamei*)

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Abstract

Common problem that happened in white shrimp culturing is insufficiency of mature female to provide the seed simultaneously. There are several methods to accelerate gonadal maturity infemale white shrimp: eye ablation, environmental manipulation, and providing high cholesterol fresh feed. Till now, high cholesterol feed is not very common method. Karamunting or Malabar Melastome (*Melasthoma malabatrhicum*) is known to have a high cholesterol content, more specifically, lanosterol based on GCMS test. Lanosterol is cholesterol that contained in plants, which assumed as precursor hormone for gonad maturity process in female of white shrimp. The aim of this experiment is to determine whether Karamunting ethanol extract can stimulate and accelerate gonad development in female of white shrimp. This experiment consists of several steps: (1) Karamunting (*Melastoma malabathricum*) extraction, (2) GCMS test for Karamunting, (3) extract injection to female of white shrimp (*Litopenaues vannamel*) for 15 days in 3 days interval with dosage variable 0 (control),), 10 mg/kg BW (P1), 7,5 mg/kg BW (P2), 5 mg/kg BW (P3), 2 mg/kg BW (P4) dan 1 mg/kg BW (P5), (4) The measuring parameters are Karamunting content, morphological gonad development observation and hepatopancreas somatic index (HSI) measurement, (5) Data analysis. Data shows that karamunting ethanol extract with P1 and P2 dosage can result in morphological gonad development response (GML II), meanwhile with P3, P4 and P5 dosage there is no changes observed. All variables didn't affect the level of HSI (P>0.05). Based on the result, it can be concluded that Karamunting ethanol extract indicates an acceleration of gonad maturity process in white shrimp female.

Keywords **:** Gonad maturity; Hepatopancreas Somatic Index; *L. vanname; M. malabatrhicum*

1. Introduction

Quantity of shrimp culturing in Indonesia has been increasing for years but nowadays it doesn't rise significantly [1]. One of shrimp culturing in Indonesia is white shrimp (*Litopenaeus vannamei*) which has declined in production. White shrimp is a common fisherycommodity because it is easy to produce and has high selling price. Most of hatchery catch the female which naturally mature. This can cause over fishing to female white shrimps in their habitat. Many methods have been done to solve availability of female white shrimp to increase seeds quantity so that the culturing process can be done simultaneously. One of the methods is improve the female quality and increase the stock of female white shrimp. Many application has been done to improve female of white shrimp quality that affect gonad maturity acceleration, such as eyestalk ablation, environmental manipulation and feed nutrition improvement. Eyestalk ablation is eyestalk cutting technique to accelerate gonad maturity. Wulandari *et.al.* [2] stated seven unilateral eyestalk ablation can accelerate the spawning of windu shrimp, but it can make permanent damage to the eye and decrease neurohormonal synthesis by sinus gland [3]. Idris *et.al.* [4] stated improving feed nutrition may increase ovarium development in freshwater lobster (*Cherax quadricarinatus*). But this method still has limitation because the protein content can't be controlled if the protein improvement using fresh feed like worm, squid and shrimp.

Gonadal maturity in crustaceans is controlled by two antagonist hormones. First hormone is GIH which is synthesized in X sinus gland (XO-SG) in the eyestalk and Gonad Stimulating Hormone (GSH) which is produced by brain and thoracic ganglion. Gonad maturity phases consist of four phases, I-IV [5]. Based on cytology studies, vitellogenesis or eggyolk synthesis divide to two phases, primary and secondary. Meanwhile oocytes development classification in shrimp consist of previtellogenic, endogenous vitellogenic oocytes and exogenous vitellogenic oocytes [6].

Karamunting plant (*Melastome malabathricum*) is a clump plant which can be easily found in Indonesia. This plant often grows in wide and dry area. Karamunting is wild plant in Borneo which far from attention because it doesn't have economical value. This plant commonly used as medicine because it has metabolic compound which consist of saponin, tannin, triterpenoid/steroid, and flavonoid. Steroid compound is growth and reproduction hormon for crustaceans, which can be used to accelerate gonad maturity. Based preliminary experiment data from Chemical Analyst Academy in Bogor, West Java about compounds that are contained in Karamunting with GCMS method showed ethanol extract from Karamunting has high amount of lanosterol. Nuresti *et.al.* [7] also reported that Karamunting contain cytosterol α and β amyrin from hexane fraction.

Lanosterol is cholesterol from plant. Cholesterol is one of chemical substance that can't be synthesized by crustaceans [8] but very important for ovarium maturity in female shrimp. Wouters *et al.* [9] stated cholesterol has function in endocrine system which is steroid hormones precursor, gonad maturation and reproduction. Therefore, it

is very important to do a research about the role of lanosterol from Karamunting plant (*M. Malabathricum*) for gonadal maturity acceleration in white shrimp (*L. vannamei*).

2. Material and Methods

Experiment was performed from January to August 2015 in Laboratory of Balai Produksi Udang Unggul dan Kekerangan (BPIUUK) Karang Asem, Bali. The specimens used were female of white shrimp (*L. vannamei*) 9 months old, weighed 39 gram from female of nusantara white shrimp, Karang Asem, Bali and ethanol extract of Karamunting (*M. malabatrhicum)*.

2.1. Experiment Design

The experiment was designed to have six variables of dosage and five repetitions for each variables: Control without extract; Variable I 0.01 g/kg BW; Variable II 0.0075 g/kg BW; Variable III 0.005 g/kg BW; Variable IV 0.002 g/kg BW; and Variable V 0.001 g/kg BW. The extract was injected to 5th pereiopods of the white shrimp using 1mL syringe for 15 days in 3 days interval. The females of white shrimp was cultured in a Styrofoam box (70 x 40 x 30 cm), using filtered water with 31 – 32 ppt salinity, temperature 27 – 29^{o}C, DO 6.01, and pH 7.6. The shrimps were given fresh worm and oyster feed and synthetic feed, 15 – 20% of the body weight.

2.2. Parameters Measured

Parameters measured in this experiment are content of ethanol extract in Karamunting using GCMS method and visual observation of gonadal development morphologically and measurement of Hepatopancreas Somatic Index (HSI). Gonad observation is done on daily basis manually by observing specifically on dorsal of white shrimp female. Gonad Somatic Index is ratio of ovary weight versus white shrimp total weight without ovary, using this formula [10]:

$$\text{HSI} = \frac{\text{Hepato Weight}}{\text{Total Weight}}\ x\ 100$$

2.3. Data Analysis

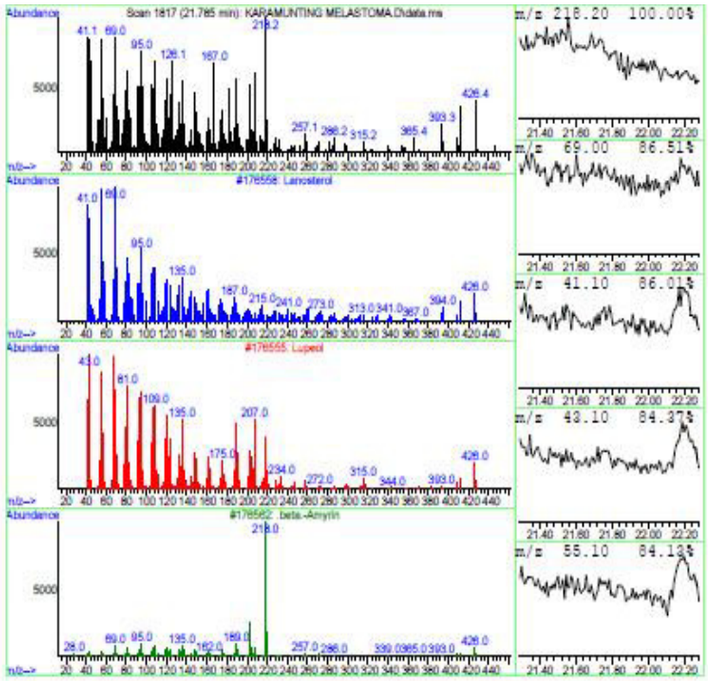
Data obtained are analyzed statistically using One-way ANOVA to determine the significance of mean differences between variables with 95% confidence level. The data analyzed using SPSS 16.0 software.

3. Results and Discussion

3.1. GCMS Test of Karamunting

Chemical compound content of Karamunting was measured with GCMS methods using crude extract of the leaves and ethanol as the solvent. The aim of this measurement is to identify the chemical compound contained in the ethanol extract of Karamunting that can stimulate gonadal maturity of female of white shrimp. According to Figure 1, it can be seen that Karamunting has various chemical compound and it is assumed that one of those compounds is capable of stimulating gonadal maturity. In Figure 2, it shows that Karamunting has high content of lanosterol α and β amyrin. Nuresti, *et.al.* [7] reported that Karamunting also contained sitosterol α and β amyrin from hexane fraction. Lanosterol is a cholesterol compound found in plants and it is the building block of shrimp reproduction, because shrimp cannot produce their own cholesterol.

Figure 1. GCMS test result of Karamunting (*M. malabathricum*)

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Data File: C:\Documents and Settings\gc ms\Desktop\reza\KARAMUNTING MELAST
...        OMA.D
Sample   :

Peak Number:  43    at     21.785 min  Area:    850752  Area % 3.67

 The 3 best hits from each library.          Ref\#    CAS\#       Qual
-------------------------------------------------------------------------
    C:\Database\NIST05a.L
     1 Lanosterol                                176558 000079-63-0   59
     2 Lupeol                                    176555 000545-47-1   58
     3 .beta.-Amyrin                             176562 000559-70-6   52
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Figure 2. Lanosterol measurement result in Karamunting (*M. malabathricum*)

According to [7] in Karamunting (*M. malabatricum*) leaves which extracted using methanol there are three types of pentacyclic triterpenoid isolated :ursolic acid, 2-hydroxyursolic acid, and Asiatic acid, glycerol-1,2-dilinolenyl-3-O-β-D-galactopyranoside and glycerol 1,2-dilinolenyl- 3- O-(4,6-di-O-isopropylidene)-β-D-galactopyranosid. From the GCMS result it can be seen that Karamunting has high content of α and β amyrin. Kanazawa *et.al.* [8] stated that cholesterol is one of lipid that synthesized by crustacean, cholesterol is assumed as diet lipid essential. Cholesterolis a chemical compound that needed by crustaceans but cannot be produced naturally and must be obtained from the environment. Wouters *et al.* [9] mentioned that cholesterol was needed by crustacean as hormone precursor; steroid, gonadogenesis process, ripening and reproduction.

3.2. Effects of Karamunting Ethanol Extract in morphological gonad development

Gonadal development morphology observation is done manually for 15 days to determine the effect of ethanol extract of karamunting (*M. malabathricum*)to gonadal development of white shrimp female (*L. vannamei*). The development of gonad morphology is an indication of gonadala development. Ovary development of shrimp can be classified in four stages. Gonadal Maturity Level I (GML I) – Gonadal Maturity Level IV (GML IV), immature, developing, ripe, and spent stage [11].

The difference between gonadal morphology development and visual observation can be seen in Figures 3. The criteria of gonadal development stages of white shrimp female are referring to a study conducted by [12]. The observation results of gonadal morphology development at the end of experiment can be seen in Table 1. In the data it can be seen that Variable I and Variable II shows visually distinctive gonadal development, meanwhile Variables III, Variable IV, Variable V, and Control didn't show any differences at the end of the experiment. This indicates that Variable I and Variables II has developed very well. In this case, the indication shows that ethanol extract of karamunting can stimulate gonadal development of white shrimp. Pattiasina *et al.* [13] reported that cholesterol can result in changes of ovary tissue of *Scylla seratta* in the fourth period to achieve ripe stage in 18 days.

Gonadal development visual observation in Variable I and II, shows that the gonadal development is in GML II phase, it is indicated from the size of gonad in the specimen.

Figure 3. Gonadal development morphology observation. Note: - n.a; + observed

Visual observation result in Variable III, IV and V shows no difference with control. This happen due to lack of cholesterol that was added, so that the steroid formed is inadequate to stimulate the secretion of *gonad stimulating hormone* (GSH), therefore the ovary maturity takes longer than other variables [13]. According to [14], cholesterol is the source of all steroid hormones which in crustaceans is the the precursor of sexual hormones molting hormones and hypodermal component. According to [15] and [16], steroid hormones have a great role in vittelogenesis regulation, because gonadal development is regulated by a complex hormonal system.

Table 1. Visual Observation of Gonadal Development of Female of White Shrimp (*L. vannamei*). Note: (-) not observed, (+) observed

Variables	Repetition				
	1	**2**	**3**	**4**	**5**
I	+	+	+	+	+
II	+	-	+	+	+
III	+	-	-	-	+
IV	-	-	-	-	-
V	-	-	-	-	-
Control	-	-	-	-	-

3.3. Effects of Karamunting Ethanol Extract in Hepatopancreas Somatic Index

The aim of hepatopancreas index measurement is determine the effect of karamunting extract to hepatopancreas. Vogt [17] stated that hepatopancreas is a detoxification and nutrient storage organ. The measurement result of hepatopancreas somatic indeks (HSI) at the end of experiment are illustrated in Figures 2. Fernandes *et al.* [18] stated that in reproduction period, nutrient is kept in hepatopancreas to produce reproduction products. Figures 2 shows that mean of Variables I (2.06±0.02 g), Variable II (2.06±0.01 g) dan Variables III (2.05±0.01 g) are slightly higher than Control (2.04±0.02 g). Meanwhile HSI of Variables IV (2.04±0.01) and Variables V (2.04±0.01) show no difference with Control.

From the measurement of HIS in females of white shrimp (*L. vannamei*) after injection of ethanol extract of karamunting (*M. malabathricum*) it can be seen that generally there is HIS escalation compared to Control, but according to statistical analysis, theescalation is not significant for all variables ($p>0.05$; ANOVA). Subramoniam [19] explained that the role of hepatopancreas is in vitellin synthesis. This experiment shows ovary development in GML II or oocyt development stage, and not ripening stage meanwhile vittellogenesis refers toproduction of yolkmaterials in process of egg maturation such as distribution of protein, lipid, carbohydrate andother substances [20].

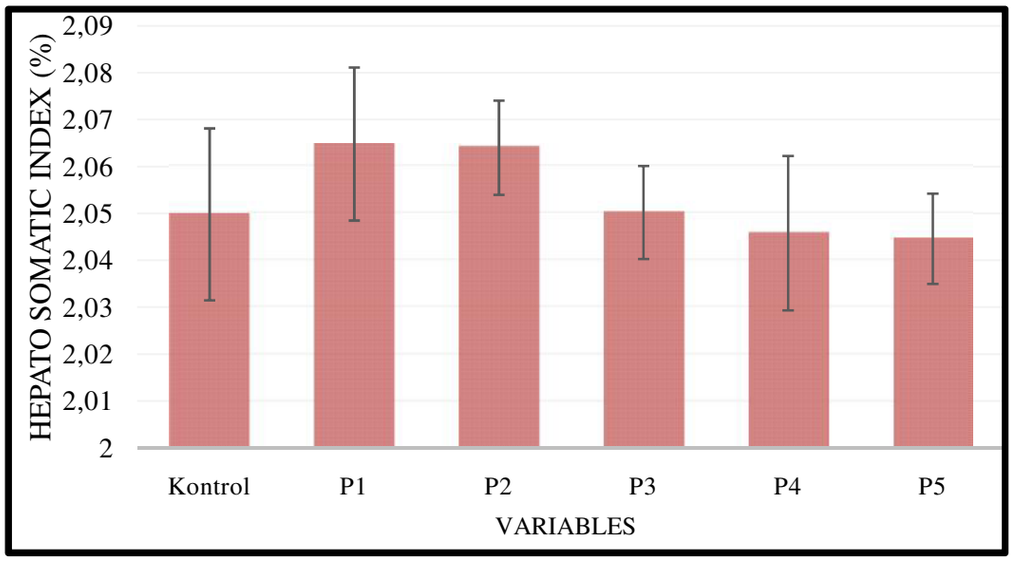

Figure 4. HIS mean of white shrimp female (*L. vannamei*) injected with Karamunting (*M. malabathricum*) ethanol extract at the end of experiment. The control is white shrimp without karamunting ethanol extract injection.

4. Conclusion

GCMS measurement data shows that Kamunting has high content of lanosterol and the observation indicates the stimulation of gonad development in white shrimp female. Meanwhile HIS measurement shows that feeding of Karamunting extract has no significant effect.

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