



Pertanika Journal of  
**TROPICAL**  
**AGRICULTURAL SCIENCE**

**JITAS**

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# *Journal of Tropical Agricultural Science*

## About the Journal

### Overview

Pertanika Journal of Tropical Agricultural Science (JTAS) is the official journal of Universiti Putra Malaysia published by UPM Press. It is an open-access online scientific journal which is free of charge. It publishes the scientific outputs. It neither accepts nor commissions third party content.

Recognized internationally as the leading peer-reviewed interdisciplinary journal devoted to the publication of original papers, it serves as a forum for practical approaches to improving quality in issues pertaining to tropical agriculture and its related fields.

JTAS is a **quarterly** (*February, May, August and November*) periodical that considers for publication original articles as per its scope. The journal publishes in **English** and it is open to authors around the world regardless of the nationality.

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The *Introduction* explains the scope and objective of the study in the light of current knowledge on the subject; the *Materials and Methods* describes how the study was conducted; the *Results* section reports what was found in the study; and the *Discussion* section explains meaning and significance of the results and provides suggestions for future directions of research. The manuscript must be prepared according to the Journal's **INSTRUCTIONS TO AUTHORS**.

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Office of the Deputy Vice Chancellor (R&I)  
1<sup>st</sup> Floor, IDEA Tower II  
UPM-MTDC Technology Centre  
Universiti Putra Malaysia  
43400 Serdang, Selangor Malaysia.  
Gen Enq.: +603 8947 1622 | 1616  
E-mail: [executive\\_editor.pertanika@upm.my](mailto:executive_editor.pertanika@upm.my)  
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### PUBLISHER

#### UPM PRESS

Universiti Putra Malaysia  
43400 UPM, Serdang, Selangor, Malaysia.  
Tel: +603 8946 8855, 8946 8854  
Fax: +603 8941 6172  
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## Immunomodulatory Potential of *Eucheuma serra* as Haemocyte Cell Production Enhancer on *Litopenaeus vannamei*

Kartiko Arif Purnomo<sup>1\*</sup>, Merdeka Agus Saputra<sup>2</sup>, Shobrina Silmi Qori Tartila<sup>2</sup>, Fariz Kukuh Harwinda<sup>2</sup>, Sri Umida Setyaningsih<sup>2</sup> and Woro Hastuti Satyantini<sup>2</sup>

<sup>1</sup>Faculty of Pharmacy, Universitas Airlangga, Jl. Dharmawangsa Dalam, 60286 Surabaya, Indonesia

<sup>2</sup>Faculty of Fisheries and Marine, Universitas Airlangga, 60286 Surabaya, Indonesia

### ABSTRACT

*Litopenaeus vannamei* is one of the most well-known fishery products and has high market value. In contrast, production of vannamei shrimp is facing threats from several diseases caused by bacteria, virus, and even parasites that attack the shrimp's immune system. The purpose of this study is to identify the immunomodulatory effect of *Eucheuma serra* extract to enhance haemocyte cells production in *Litopenaeus vannamei* as the non-specific internal immune substance. The research method used was a complete randomised design with 5 concentrations of injected *Eucheuma serra* extract treatments and one placebo as control. *Eucheuma serra* extract administered on shrimp abdomen and the haemocyte cell samples collected from shrimp on day 0 and day 6. Treatment using 8 ppm injected *Eucheuma serra* extract showed the highest haemocyte cell amount increase about 15.90 million cell/ml in 6 days after injection. Statistical calculation using ANOVA test showed a significant difference in the amount of haemocyte cell at before and after treatment.

**Keywords:** *Eucheuma serra*, haemocyte cell, immunomodulator, *Litopenaeus vannamei*

### ARTICLE INFO

#### Article history:

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#### E-mail addresses:

kartiko.purnomo@gmail.com (Kartiko Arif Purnomo)

merdeka.merdekaagussaputra@wur.nl (Merdeka Agus Saputra)

shobrina.silmi@gmail.com (Shobrina Silmi Qori Tartila)

farizkukuh@hotmail.com (Fariz Kukuh Harwinda)

umidas918@gmail.com (Sri Umida Setyaningsih)

worohastuti79@gmail.com (Woro Hastuti Satyantini)

\* Corresponding author

### INTRODUCTION

Crustacean aquaculture represents a major industry in tropical developing countries (Vazquez et al., 2009). *Litopennaeus vannamei* (white leg shrimp) is an important aquaculture species (Sirirustananun et al., 2011). In Indonesia, *L. vannamei* has a major market. Its production volume increased 99.27% from 206.6 tonnes in 2010 to 411.7 tonnes in 2014 (Indonesia Ministry of Fisheries and Marine, 2014).

Shrimp farming has also led to prevalence of disease. Crustaceans are affected by opportunistic pathogens causing huge economic losses (Vazquez et al., 2009). Shrimp farming has suffered from problems linked to deteriorating pond environments, subsequently resulting in stress-induced disease incidences mainly of viral and bacterial aetiologies (Sirirustananun et al., 2011). Commercial shrimp farming has faced disease outbreaks, especially white shrimp *Litopenaeus vannamei*, such as *Vibrio alginolyticus*, *Vibrio damsela*, *Vibrio harveyi*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*, infectious Myonecrosis Virus (IMNV), Monodon Baculovirus (MBV), White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), Yellow Head Virus (YHV) and Taura Syndrome Virus (TSV). In Taiwan, the total farmed production of *L. vannamei* shrimp declined almost 90%, from 82,598 tonnes to 8,878 tonne, in the space of 15 years (Cheng, Liu, Yeh, & Chen, 2004).

There is also evidence of *L. vannamei* infected with WSSV, TSV, IHHNV and IMNV in several regions in Indonesia. Lampung, West Java, East Java and South Sulawesi are the regions which are exposed to various types of infection. The IMNV has led to death of 60% of the shrimps (Sugama, Novita, & Koesharyani, 2006). *V. alginolyticus* bacterium isolated from infected *L. vannamei* is known to cause high mortality rate among shrimp in stressful environments (Wang & Chen, 2005). The WSSV is considered to be an extremely

virulent pathogen and may cause death within a few days post infection (Chou, Huang, Wang, Chiang, & Lo, 1995).

Physical barriers are the biggest challenge to prevent pathogenic microorganisms in most invertebrates (Vazquez et al., 2009). Invertebrates do not possess a specific, adaptive immune system such as found in vertebrates. In the absence of lymphocytes and functional antibody, they have been traditionally thought to rely instead entirely on innate or non-specific immunity for internal defence against parasites and pathogens (Rowley & Powell, 2007; Wei et al., 2012). Haemocyte cell in shrimp has similar function with leukocyte in vertebrate as non-self matter recognition and elimination (Sritunyaluksana & Soderhall, 2000). Circulating haemocytes are generally classified into three types: hyalinocytes, semi-granulocytes and haemocytes with refractile granules. Haemocyte common defence mechanisms include phagocytosis and encapsulation of extrinsic fungal spore, yeast and organisms (Cheng et al., 2004; Vazquez et al., 2009). Other defence mechanisms of haemocytes are activated depending on the pathogen characteristics, such as prophenoloxidase and lectin as sugar-binding properties of crustacean proteins (Vazquez et al., 2009).

Extensive use of human antibiotic drugs in shrimp farming as preventive purpose is potentially damaging and the residues can still enter contaminate the environment and enter the human food chain (Cabello, 2006). Moreover, the European countries have strict regulations



on residues of antibiotics in shrimp product (Food and Agricultural Organization of the United Nations [FAO], 2006). Therefore, proper immune modulators for shrimp are important (Pope et al., 2011).

## MATERIALS AND METHODS

### Materials

Acetone (Merck), Ammonium sulphate (Merck), Ethanol (Merck), Phosphate Buffer Saline pH 7.4 / PBS (Brataco), Sea water (Surabaya, Indonesia), Sodium ethylenediaminetetraacetic / Na-EDTA (Merck).

### Animal and Plant Materials

*Litopenaeus vannamei* shrimp was obtained from a shrimp farmer in Gresik, Indonesia. *Eucheuma serra* was collected in Pandawa Beach, Bali, Indonesia.

### *Eucheuma serra* Extraction

*E. serra* was dried inside a closed and clean room. Dry *E. serra* was later powdered and weighed at 30 gramme. Phosphate buffer saline pH 7.4 solution was dissolved into the dried *E. serra* powder with a ratio 2:1. The solution was stirred using homogeniser for 2 hours at 4°C and then centrifuged at 3200 rpm for 15 minutes. Supernatant was collected then added with acetone 1:1. The solution was centrifuged at 3200 rpm in 15 minutes. The pellet was collected as crude extract of *E. serra*. The yield of extract is 10g.

Lectin concentration in *E. serra* crude extract is determined using spectrophotometry method for protein analysis at wavelength  $\lambda$  540 nm. Protein standard solution is used for quantitative analysis as external standard. The lectin concentration in *E. serra* crude extract is 5.60 % or 2.58 ppm.

### *Litopenaeus vannamei* Treatment

*L. vannamei* shrimp was acclimatised in aquarium with controlled salinity water at 15 ppt and temperature at 29°C for 48 hours. During acclimatisation period and treatment process, the subject was fed using commercial shrimp food three times a day. Probiotic was given only once in three days. The treatment aquarium was cleaned every day to reduce stress impact on shrimp and avoid contamination of ammonia from shrimp waste.

The treatment was conducted using completely randomised design consist of five treatment groups and a control group (Table 1). Each group was replicated four times. Each replication contained four *L. vannamei*.

### Haemolymph Collection and Total Haemocyte Count

Haemolymph was collected (0.50 ml) at fourth segment of the shrimp abdomen using disposable syringe and later stored inside an Eppendorf tube which already contained Na-EDTA in cold temperature storage. Haemocytometer was used for Total

Table 1  
Prerequisite of dosage injection on treatment subject

Group	Treatment
Control (G0)	0.50 ml injection of PBS
Treatment 1 (G1)	0.50 ml injection of 2 µg/ml extract solution
Treatment 2 (G2)	0.50 ml injection of 4 µg/ml extract solution
Treatment 3 (G3)	0.50 ml injection of 6 µg/ml extract solution
Treatment 4 (G4)	0.50 ml injection of 8 µg/ml extract solution
Treatment 5 (G5)	0.50 ml injection of 10 µg/ml extract solution

Haemocyte Count (THC) of haemolymph of each group. Blank haemocyte (H0) was collected from haemolymph before lectin was injected. Final haemocyte result for THC was collected from haemolymph after six days (H6).

### Survival Rate Count

Survival rate was counted as percentage of shrimps still available until the end of treatment compared with the amount before the treatment. Survival rate count was conducted in every treatment and control group.

### Statistical Analysis

Analysis of Variance (ANOVA) statistical test was conducted to test the differences between the results.

## RESULTS AND DISCUSSION

Total haemocyte count (THC) measures innate immunity and non-specific immune activity of invertebrates (Cheng et al., 2004). THC before treatment was calculated as average from all group. While THC after treatment was calculated as average

haemocyte on a shrimp in each treatment group. According to THC result (Figure 1), shrimp in Group 4 (G4) via treatment with 8 µg/ml extract injection, has the highest haemocyte cell increase compared with another treatment group, including control group (G0). Haemocyte cell amount in G4 increased 15.29 million cell/ml from  $3.16 \times 10^6$  cells/ml at H0 to  $18.45 \times 10^6$  cells/ml at H6. Other treatment groups also have various increase in haemocyte cell. The lowest increase is group 3 (G3), which saw an increase of 9.88 million cell/ml from H0.

Control group also shows a slight increase in haemocyte after six days, even though the subject did not get any lectin treatment injection. The haemocyte number increase in control group is caused by the influence of substances and nutrition from feed and probiotic which is given to all of subject (Olmos, Ochoa, Michael, & Contreras, 2011). ANOVA test give the result of F calculation ( $F_{calc}$ ) as 19.04 and F table 0,01 ( $F_{tab 0.01}$ ) as 4.25. The higher value of  $F_{calc}$  compared with  $F_{tab 0.01}$  shows the significant difference of *L. vannamei* haemocyte amount at before and after treatment using lectin extract solution via

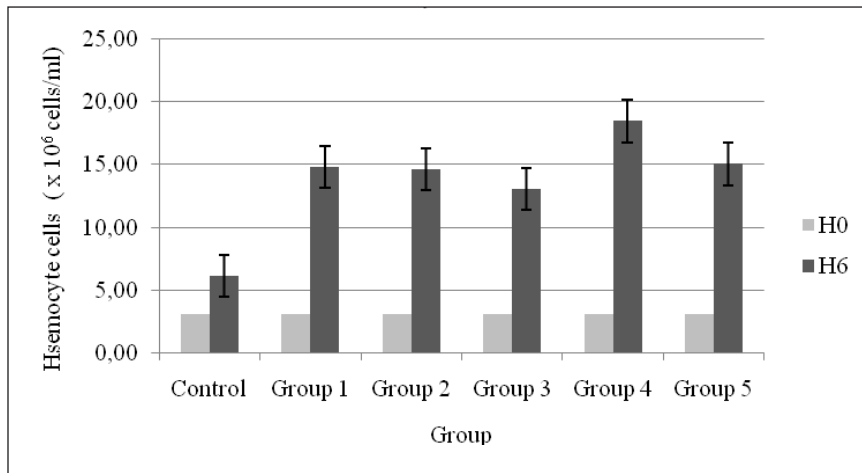


Figure 1. THC comparison of each treatment at H0 and H6

injection method. The group with the highest survival rate is G0 (87.50%) in control group and the lowest is G5 with 56.25%.

Based on the THC result of treatment groups, *Eucheuma serra* extract could be considered as the novel treatment as immunomodulator agent for *L. vannamei*. The increase in haemocyte cell means the innate immunity system has become stronger to encounter infectious pathogens, such as virus and bacteria. *Eucheuma serra* extract is more acceptable than using antibiotic to deter microorganism growth in pond.

*Eucheuma serra* gives high yields of isolectins - ESA-1 and ESA-2. The yield could be up to 1% from powdered algae with 0.1% as ESA-1 and 0.9% as ESA-2. *E. serra* could be a valuable source of lectin molecules that work even at relatively high temperatures and over a wide pH range that have preferential affinity for glycoproteins bearing high mannose-type N-glycans (Hori et al., 2007; Kawakubo, Makino,

Ohnishi, Hirohara, & Hori, 1997). Lectin is defined as carbohydrate-binding proteins of non-immune origin that agglutinate cells or as carbohydrate-binding proteins other than antibodies or enzymes. It is a group of non-immunogenic proteins possessing at least one noncatalytic domain that binds reversibly to specific carbohydrates (Janeway & Medzhitov, 2002; Teixeira et al., 2012). Lectin has been regarded as primary candidates for pattern recognition receptors in animal innate immunity due to its ability to bind to specific carbohydrates on the surfaces of microorganisms (Zhao et al., 2009). Lectin can specifically recognise the carbohydrates from the membrane or surface of cell. Moreover, it is able to induce agglutination of these cell or can lead to diverse cellular events, such as phagocytosis (Marques & Barracco, 2000).

The carbohydrate-binding profile of ESA-2 is examined as 45 different complexes. N-acetyl glucosamine (GlcNAc) is related in 35 different complexes and

N-acetyl galactosamine (GalNAc) take part in 4 different complexes (Hori et al., 2007). Lectin with structural characteristics and specificity has been identified in *L. vannamei*, known as C-type lectin (Ma, Tin, He, & Chan, 2007; Vazquez et al., 2009). C-type lectins has ligand binding specificities for carbohydrates, such as GlcNAc, GalNAc, sialic acid and lipopolysaccharide, indicate antimicrobial activity against several bacteria and fungi, also in response to WSSV (Vazquez et al., 2009; Zhao et al., 2009). The similarity of ligand binding carbohydrates of ESA-2 and C-type lectin is necessary for increase the immunity on *L. vannamei*.

Furthermore, immune memory in invertebrates that is referred as immune priming or specific immune priming, appears to be passed from brood stock to offspring (Pope et al., 2011). *L. vannamei* subjected to treatment and has an increase of haemocyte cell can be maintained as particular brood stock called Specific Pathogen Free (SPF). *L. vannamei* SPF could produce progeny that have can resist pathogens (Sugama et al., 2006).

## CONCLUSION

*L. vannamei* is vulnerable to infection by pathogen microorganisms, such as virus and bacteria. *E. serra* extract contains lectin which serves as immunomodulator for *L. vannamei* (to provide an innate immunity). *Eucheuma serra* extract as injection treatment for *L. vannamei* leads to a significant increase in haemocyte. Treatment of 8 µg/ml extract injection

enhances haemocyte production as much as 15.29 million cell/ml in six days. Lectin from *E. serra* is a novel solution to enhance immunity of *L. vannamei* against pathogens.

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## CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

## REFERENCES

- Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8, 1137-1144.
- Cheng, W., Liu, C. H., Yeh, S. T., & Chen, J. C. (2004). The immune stimulatory effect of sodium alginate on the white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. *Fish & Shellfish Immunology*, 17, 41-51.
- Chou, H. Y., Huang, C. Y., Wang, C. H., Chiang, H. C., & Lo, C. F. (1995). Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. *Diseases of Aquatic Organisms*, 23, 165-173.
- Food and Agricultural Organization of the United Nations. (2006). *Cultured aquatic species information and programme: *Panaeus vannamei**

- (Boone, 1931). Rome, Italy: FAO Fisheries and Aquaculture Department.
- Hori, K., Sato, Y., Ito, K., Fujiwara, Y., Iwamoto, Y., Makino, H., & Kawakubo, A. (2007). Strict specificity for high-mannose type N-glycans and primary structure of a red alga *Eucheuma serra* lectin. *Glycobiology*, 17(5), 479-491.
- Indonesia Ministry of Fisheries and Marine. (2014). *Udang vaname dan udang windu masih andalan ekspor Indonesia* [Vaname shrimp and tiger shrimp are still the mainstay of Indonesian exports]. Retrieved from [http://www.djpb.kkp.go.id/arsip/c/246/Udang-Vaname-dan-Udang-Windu-Masih-Andalan-Ekspor-Indonesia/?category\\_id=13](http://www.djpb.kkp.go.id/arsip/c/246/Udang-Vaname-dan-Udang-Windu-Masih-Andalan-Ekspor-Indonesia/?category_id=13)
- Janeway, C. A., & Medzhitov, R. (2002). Innate immune recognition. *Annual review of immunology*, 20, 197-216.
- Kawakubo, A., Makino, H., Ohnishi, J., Hirohara, H., & Hori, K. (1997). The marine red alga *Eucheuma serra* J. Agardh, a high yielding source of two isolectins. *Journal of Applied Phycology*, 9, 331-338.
- Ma, T. H. T., Tin, S. H. K., He, J. G., & Chan, S. M. (2007). Molecular cloning of C-type lectin (LvCTL) from the shrimp *Litopenaeus vannamei*: early gene down-regulation after WSSV infection. *Fish & Shellfish Immunology*, 23, 430-437.
- Marques, M. R. F., & Barracco, M. A. (2000). Lectins as non-self recognition factors in crustaceans. *Aquaculture*, 191, 23-44.
- Olmos, J., Ochoa, L., Michael, P. J., & Contreras, R. (2011). Functional feed assessment on *Litopenaeus vannamei* using 100% fish meal replacement by soybean meal, high levels of complex carbohydrates and *Bacillus* probiotic strains. *Article of Marine Drugs*, 9, 1119-1132.
- Pope, E. C., Powell, A., Roberts, E. C., Shields, R. J., Wardle, R., & Rowley, A. F. (2011). Enhanced cellular immunity in shrimp (*Litopenaeus vannamei*) after 'vaccination'. *PLoS ONE*, 6(6), 1-7.
- Rowley, A. F., & Powell, A. (2007). Invertebrate immune system-specific, quasi-specific or nonspecific? *The Journal of Immunology*, 79, 7209-7214.
- Sirirustananun, N., Chen, J. C., Lin, Y. C., Yeh, S. T., Liou, C. H., Chen, L. L., ... Chiew, S. L. (2011). Dietary administration of a *Gracililaria tenuistipata* extract enhances the immune response and resistance against *Vibrio alginolyticus* and white spot syndrome virus in the white shrimp *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, 31, 848-855.
- Sritunyalucksana, K., & Soderhall, K. (2000). The proPO and clotting system in crustaceans. *Aquaculture*, 191, 53-69.
- Sugama, K., Novita, H., & Koesharyani, I. (2006). Production performance, disease, SPF-Breeding and risk issues concerning white shrimp, *Penaeus vannamei*, introduction into Indonesia. *Indonesia Aquaculture Journal*, 1(1), 71-78.
- Teixeira, E. H., Arruda, F. V. S., do Nascimento, K. S., Carneiro, V. A., Nagano, C. S., da Silva, B. R., ... Cavada, B. S. (2012). *Biological applications of plants and algae lectins: An overview*. In *Carbohydrates – Comprehensive Studies on Glycobiology and Glycotechnology*. Rijeka, Croatia: InTech.
- Wang, L. U., & Chen, J. C. (2005). The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* at different salinity levels. *Fish & Shellfish Immunology*, 18, 269-278.
- Wei, X., Liu, X., Yang, J., Fang, J., Qiao, H., Zhang, Y., & Yang, J. (2012). Two c-type lectins from shrimp *Litopenaeus vannamei* that might be involved in immune response against bacteria and virus. *Fish & Shellfish Immunology*, 32, 132-140.

Kartiko Arif Purnomo, Merdeka Agus Saputra, Shobrina Silmi Qori Tartila, Fariz Kukuh Harwinda,  
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- Vazquez, L., Alpuche, J., Maldonado, G., Agundis, C., Pereyra-Morales, A., & Zenteno, E. (2009). Immunity mechanisms in crustaceans. *Innate Immunity*, 15(3), 179-188.
- Zhao, Z. Y., Yin, Z. X., Xu, X. P., Weng, S. P., Rao, X. Y., Dai, Z. X., ... He, J. G. (2009). A novel C-type lectin from the shrimp *Litopenaeus vannamei* possesses anti-white spot syndrome virus activity. *Journal of Virology*, 83(1), 347-356.