PROFIL HEMOSIT UDANG VANNAMEI YANG DIINFEKSI WSSV (White Spot Syndrome Virus) DENGAN IMMUNOSTIMULASI ALGA LAUT

HEMOCYTES PROFILE OF VANNAMEI SHRIMP WITH ALGAE IMMUNOSTIMULATION ON WSSV INFECTION

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ABSTRAK

Tujuan dari penelitian ini adalah untuk memperoleh profil hematosit udang Vannamei yang diberikan imunostimulan berupa alga laut melalui pakan terhadap infeksi penyakit WSSV.Penelitian ini dilakukan diLaboratorium Fakultas Perikanan dan Ilmu Kelautan dan Laboratorium Sentral Ilmu Hayati Universitas Brawijaya Malang. Pemberian pakan dengan alga laut sebagai imunostimulan dengan jenis alga yang berbeda sebagai berikut :Kontrol positif (udang diinfeksi WSSV tanpa suplemen pakan alga laut); Kontrol negative (udang tanpa infeksi WSSV dan tanpa suplemen alga laut); A (suplementasi pakan dg *Sargassum* sp 10 g/kg pakan); B (suplementasi pakan dg *Padina* sp 10 g/kg pakan); C (suplementasi pakan dg *Eucheuma cottonii* 10 g/kg pakan); D (suplementasi pakan *Garcillaria* sp 10 g/kg pakan) dengan 3 ulangan. Parameter yang diamati adalah sel Hyalin, granular, semi granular, THC, kelulushidupan dan SOD.Suplementasi pakan dengan ekstrak alga meningkatkan jumlah sel hyaline, granular dan semi granular, serta THC.Kelusluhidupan udang dan SOD mampu ditingkatkan antara 36-108 unit/mL pada suplementasi alga.

Kata kunci :WSSV, Hemosit, vannamei, ekstrak alga, SOD

ABSTRACT

The purpose of this study was to provide an immunostimulatory profile in the form of marine algae through feeding against WSSV disease infection. This research was conducted at the Laboratory of the Faculty of Fisheries and Marine Sciences and Central Life Sciences Laboratory, Brawijaya University, Malang. Feed encapsulated with marine algae as immunostimulants with different types as follows: Positive control (shrimp infected with WSSV without sea algae feed supplements); Negative control (shrimp without WSSV infection and without marine algae supplements); A (feed supplementation with *Sargassum polycystum* 10 g / kg of feed); B (supplementation of feed with *Padina australis* 10 g / kg of feed); C (supplementation of feed with *Eucheuma cottonii* 10 g / kg of feed); D (*Gracillaria verrucosa* feed supplementation 10 g / kg feed) with 3 replications. The parameters that examined were Hyalin, granular, semi-granular, THC, survival and SOD cells. Feed supplementation with algae extract increased the number of hyaline, granular and semi-granular cells, and THC. Shrimp life and SOD are able to improve between 36-108 units / mL in algae supplementation.

Keywords:*WSSV, Hemocytes, vannamei, algae extract, SOD*

INTRODUCTION

verrucosa, can be used as an immunostimulant to improve the immune system both in fish and in shrimp.

Anderson (2004) states that, immunostimulants are a biological compound, which is useful for enhancing the non-specific immune system. Immunostimulants are a synthesis group that has a content to increase non-specific immune responses that are useful in increasing the efficiency of the immune system in fighting pathogens. When compared with non-specific vaccinations, immunostimulants have their own special features, namely immunostimulant materials can stimulate an increase in fish and shrimp body resistance against disease attacks. Commonly used immunostimulants are non-virulent organisms. Materials capable of being used as immunostimulants include synthetic chemicals, polysaccharide bacterial derivatives, vitamins, plant and animal extracts (Ayu, 2013). In Indonesia itself, many use herbal ingredients to improve the immune system because it is believed that the active ingredients contained therein are more able to increase immunostimulants.

White Spot Syndrome Virus (WSSV) in shrimp generally occurs in the rainy season, transition season and winter. The sudden decrease in temperature and salinity can cause stress to the shrimp, so the shrimp are susceptible to diseases such as WSSV (Soetrisno, 2004 in Rahma et al., 2014). Extreme changes in salinity can result in osmotic stress related to the energy needs of shrimp to activate ATPase in ion transport. The presence of pathogenic viruses, namely WSSV, under osmotic stress conditions results in shrimp being unable to activate their response system to diseases, especially phagocytic and inflammatory systems. The mechanism of shrimp defense is suppressed by WSSV through an immunosuppressive mechanism by disrupting cell function to facilitate viral proliferation (Jayasankar et al., 2009; Ramos-Carreno et al., 2014).

In general, prevention of disease in vannamei shrimp is the administration of antibiotics. The number of antibiotics in the market often creates its own problems because in general farmers don't understand how to use them. The composition of the type, time of application, and the right dosage are often not clearly stated, so that the farmers use it inappropriately. This can have an impact on the aquatic environment and cause pathogenic resistance. Various negative consequences of giving antibiotics require other alternatives in

the prevention of vannamei shrimp disease using marine algae. Therefore this study aims to obtain a hematocytic profile of Vannamei shrimp that is given immunostimulant in the form of marine algae through feed on infection with WSSV as an alternative antibiotic for shrimp.

METHODS OF STUDY

Time and Place

Experiment was conducted in Laboratory of Aquaculture and Central Life Science Laboratory in Brawijaya University from February to April 2018.

Materials and Methods

Materials used in the experiment were 216 shrimp size± 10 g, seawater, brown algae (*Padina austrlis* and *Sargassum polycystum*), red algae (G*racillaria verrucosa* and *Eucheuma cottonii*), methanol, shrimp feeds and culture equipments.

The experiment treatments were feeding supplementation using read algae and brown algae from different species as follows;

Algal extraction

Marine algae were airdried for 5-6 days at optimum temperature 40^0C (Sari et al., 2012). Aftrer drying, alge were than milled in powder and filtered. Algae powder was then following maseartion using methanol with 1:3 (algae/solute) for 3x24 hours. This process done using hot plate stirrer to homogenized the solutions. Solutions were then filtered using filter paper to get filtrate. Followed by thickening concentration, the filtrate was evaporated in 45^0C with 70-80 rpm. Algal crude extract were then keep in dark bottle in low temperature (refrigerator).

Freeze dried product of algae crude extract

Ecapsulations of this research using methods by Baranauskiene et al. (2006) as follow, with 30% (w / v) coating formulation on solvents, and 20% extract (w / w) on coatings, then performed a speed of 1800 rpm for 10 minutes, frozen in the freezer. Encapsulation processed with freeze drying for 2 x 24 hours.

Efectivity test of immunostimulan

The administration of immunostimulants through feed is by mixing different active ingredients of marine algae into the feed. The test shrimp is put into a tub with a stocking density of 1 shrimp / 2 liters. The 20-liter container is 24 pieces or containing 9 vaname shrimp. Before immersion, physiological tests on shrimp are first carried out including

feeding response, motion, redness of the body and white spots (Wahjuningrum, et al. 2006).

Vannamei shrimp treated with oral immunostimulants consisted of K + / control (+) groups containing normal shrimp infected with WSSV and without the addition of algae extract on feed. Treatment of K-/ control (-) group of shrimp not infected with WSSV without the addition of algae extract on feed. Whereas in the treatment of immunostimulant feed in group A which was given freeze dry extract *Sargassum polycystum* 10 gr / Kg of feed and infected by WSSV. Group B was given freeze dry *Padina australis* extract 10 gr / Kg of feed and infected by WSSV. Group C was given *Euchema cottonii* extract 10 gr / Kg of feed and infected by WSSV. Group D was given *Gracilaria verrucosa* extract 10 g / Kg of feed and infected by WSSV. Shrimp are maintained during immunostimulant feeding for 4 weeks. Furthermore, the treated shrimp were tested challenged with WSSV. After infection, the hemocyte is taken for analysis of total hemocytes (THC) and Hemocyte Differential (DHC).

Superoxide Dismutase (SOD)

Superoxide dismutase (E.C 1.15.1.1) is a detoxification enzyme that can be found in all aerobic organisms to catalyze dismutation of superoxide radicals into oxygen and hydrogen peroxide molecules. SOD was analyzed based on the spectrophotometer reader method according to Beauchamph and Fridovich, (1971) in Yudiati et al. (2016) as follows: 40 µL Haemolymph shrimp mixed with 10% Na-citrate then mixed with 360 µL buffer phosphat (50mM, pH 7.4), centrifuged 6,000 g, 4^{o}C for 7 minutes. Supernatant (clear liquid) taken 100 µL as much as 3x was taken and heated in an HLC device at 65^{o}C for 5 minutes then added NBT solution 50 µL Hank's Basal Salt Solution (HBSS) solution with a concentration of 0.3% mixed to homogeneous. Then 200 µLsample was inserted into the microplate and then on a spectrophotometer (R-Biopharm Well Reader) with OD 630 nm. SOD value in cell / mL.

Survival rate (SR)

The indicator of shrimp survival is calculated at the end of the observation by calculating the number of live shrimp in each trial container compared to the number of shrimp stocked at the beginning of the study and then expressed in percent (%)(Saraswati, 2014).

Data Analysis

Total Haemocyte Count (THC) is calculated using a haemocytometer using light microscope with 400 x magnification as follows: Observation of the number of hemocytes (hyalin,

semi granular and granular) cells in percentage based on morphological criteria using a 1,000x magnification light microscope THC. Calculation using haemocytometer by procedure (Courdova et al., 2002) as follows:

$$\text{THC} = \frac{number of cells calculated}{volume calculated} x dilution x 10^4$$

$$\text{DHC} = \frac{number of eac \quad emocytes cell}{total \quad emocytes} x\ 100\%$$

Data obtained from the experiment were analyzed using ANOVA to find the best algae treatment using SPSS data processing for statistical testing.

RESULT AND DISCUSSION

Result

THC

Table 1.THC of shrimp with marine algae supplementation before and after WSSV infection

	THC (x107sel/L)	
treatments	before infection	after infection
K-	0.600	0.725
K+	0.575	0.438
A	0.744	1.300
B	0.681	1.013
C	0.769	1.363
D	0.688	1.138

Total hemocyte counts of increased in marine alga treatments compared to controls with the highest THC in C (*E.cottonii*), while K+ has lowered THC than K- (Tabel 1).

DHC

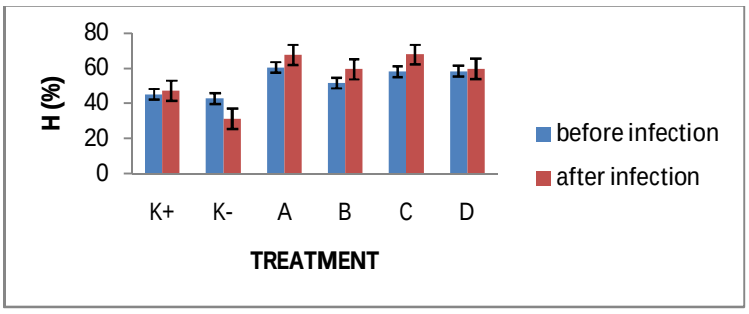

Figure 1. Hyaln cells in L. vannamei with different treatment before and after WSSV infection

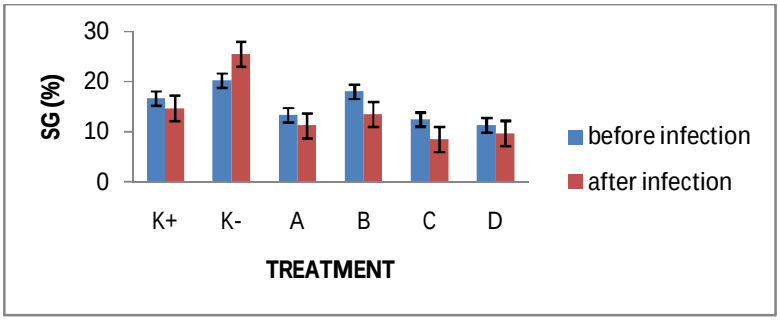

Figure 2. Semigranular cells in L. vannamei with different treatment before and after WSSV infection

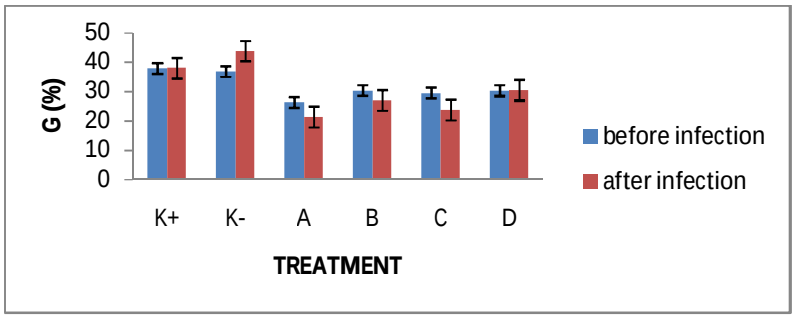

Figure 3. Granular cells in L. vannamei with different treatment before and after WSSV infection

From the figure above shows that the treatments shrimp with using marine algae improve shrimpconditions by increasing hyaline and lowering the semi granular and granular cells. An obvious different trend in the control negative shrimp

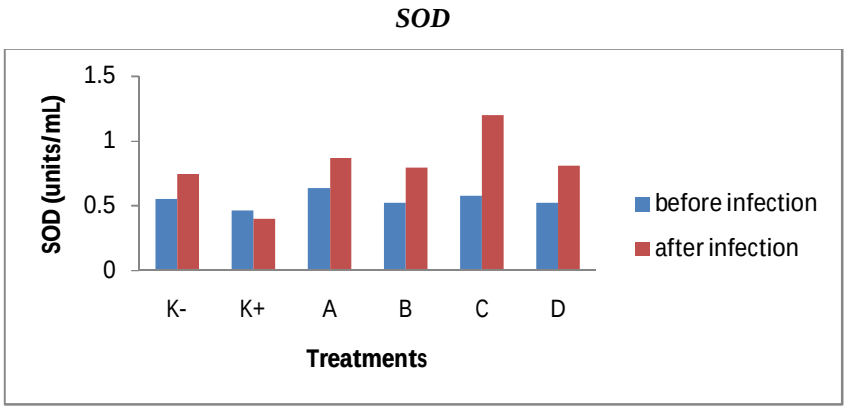

Figure 4.SOD in L. vannamei with different treatment before and after WSSV infection

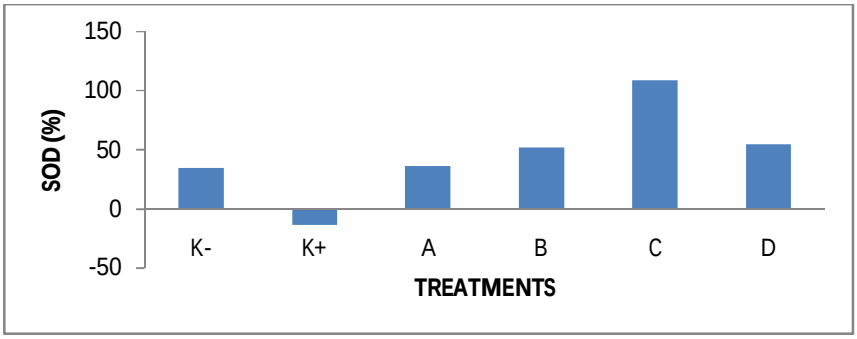

Figure 5.SOD dynamic in L. vannamei with different treatment

Significantly increasing in C (E.cottonii) treatment among others and control treatments. Also, decreasing SOD in positif infected shrimp

Table 21.Average Survival Rate of *L. vannamei* in different treatments

No	Treatments	SR (%)
1	K+	44.4
2	K-	11.11
3	A	22.22
4	B	13.89
5	C	16.67
6	D	27.78

Higher survival rate in the marine algae treatments shrimp compared to positive control.

DISCUSSION

Total hemocytes of vannamei shrimp (Table 1) showed that the treatment of the utilization of E. cottonii for feed encapsulation as shrimp immunostimulant material was higher than other treatments. This finding is in accordance with the research of Yeh et al. (2006) which showed that there was an increase in the number of hemocytes in Litopenaeus vannamei shrimp after administration of *Sargassum duplicatum* extract either through immersion or injection. Hemocytes are synthesized by hematopoietic tissue, a pair of epigastric nodules. The production is carried out to achieve a homeostatic state after the introduction of immunostimulants. The tissue is located right in the dorsal part of the anterior stomach, a site of hemocyanin synthesis. According to Effendy et al. (2004) immunostimulants can increase hemocyanin, which will directly increase hemocytes.

The mechanism of action of hemocytes in fighting viruses is with hemocytes cells will carry out the process of degranulation, cytotoxicity and lysis of the material. Thus the number of hemocytes circulating in the hemolim will be seen to decrease. The result of the degranulation process is the release of peroxinectin which will trigger the appearance of phagocytosis (Effendy et al, 2004). Lectin or agglutinin is a protein in hemolim which has an important role when there are antigens that enter the body that will bind to carbohydrates found in pathogenic cell walls or foreign objects called agglutination. The reaction will be followed by the elimination of the foreign body through the phagocytosis process, melanisasi by the enzyme phenoloksidase and respiration (respiratory burst) (Supamattaya et al. 2000). Hemocytes have an important role in the immune defense system. First, the hemocytes destroy foreign particles in haemacoel through phagocytosis, encapsulation, aggregate nodulation, melanisasi, cytotoxicity and communication between cells (Ekawati et al., 2012). Second, hemocytes contribute to the handling of wounds through cellular reactions and that initiate the coagulation process by carrying and releasing the prophenoloxidase (proPO) system. Third, hemocytes are involved in the formation and alteration of important molecules in hemolim such as α2-macroglobulin (α2M), agglutinin and antimicrobial peptide. Shrimp given crude extract of sea algae were shown to increase the amount of THC shrimp. Along with the increase in total shrimp hemocytes, the shrimp immune

system will also increase so that the rate of virus infection can be reduced.

Semigranular cells play a major role in the encapsulation process and little in the process of phagocytosis, which is able to respond to polysaccharides from bacterial cell walls or β-glucan from mushrooms. These semi-granular cells can carry out the encapsulation process and play a role in the phagocytosis process (Johansson et al. 2000). Encapsulation is a defense reaction against large numbers of particles and is not capable of phagocytes by hemocytic cells (Danwattananusorn, 2009). The function of granular cells is more in the process of producing phenoloxidase enzymes which have an important role in non-specific defense systems. Supamattaya et al. (2000) describe granules in granular cells of hemocytes consisting of propenoloxidase. In activation of prophenoloxidase (proPO) will release an enzyme from granular cells. This system is also driven by the presence of microbial components such as β-glucan.

The process of prophenoloxidase is responsible for the production and secretion of toxic metabolites such as quinon. The final product of this system is the appearance of blackish nodules which are usually around the gills or exoskeleton. When a pathogen attack occurs, granular and semi-granular cells will carry out degranulation, cytotoxicity and lysis of the material so that the number of granular cells circulating in the hemolim will decrease. The result of the degranulation process is the release of peroxinectin which will trigger the appearance of phagocytosis.

SOD enzymes are produced in the body of animals as antioxidants, but the amount will decrease sharply when there is a lot of damage to cell metabolism (Cohen, 1997). When shrimp get infected or get a stressor, hemocytes will produce reactive oxygen species (ROS) and the concentration will be balanced with antioxidant enzymes, one of which is SOD (Anduro et al., 2012). This increase in SOD aims to reduce cellular superoxide explosion during defense against viral infections and to protect shrimp cells from damage. Decreasing the amount of SOD after the shrimp were given synbiotics showed that the shrimp did not experience damage and stress due to synbiotics (Ramadhani et al., 2017).

The survival rate of shrimp is able to be maintained higher than that of shrimp with K +. This is thought to be the role of shrimp self-defense system so that it can suppress shrimp mortality. The low survival rate of shrimp is thought to be due to short cultivation period, which is 21 days, whereas according to Itami, (2002) the virus can survive in the body of shrimp 60-95 days in shrimp immunostimulant and tested challenged with viruses. Therefore, it is necessary to search the duration of feeding using marine algae encapsulation in feed for vannamei shrimp

CONCLUSION

The use of marine algae in feed for shrimp feed encapsulation has a significant effect on increasing the immune response of vannamei shrimp in

treatment (Euchema cottonii) compared to other seaweed treatments. Giving seaweed species of Sargassum polycistum, Padina australis, Euchema cottonii and Gracillaria verrucosa at a dose of 10 grams / kg had a direct effect on vanamei shrimp survival. An increase in SOD as an immune response to shrimp infected with WSSV and has been given antioxidants from seaweed. Shrimp survival rate is higher compared to positive control.

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