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THE EFFECTIVENESS OF WHITE TURMERIC EXTRACT (*Curcuma zedoaria*) AGAINST THE IMMUNE SYSTEM OF VANNAMEI SHRIMP (*Litopenaeus vannamei*)

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ABSTRACT

Vannamei shrimp entered Indonesia to replace tiger prawns (*Penaeus monodon*), which then experienced disease attacks and decreased quality. However, with various advantages, the cultivation of vaname shrimp still has problems during the cultivation period. Therefore, this study will determine the effect of adding white turmeric extract (*Curcuma zedoaria*) on the immune system of vannamei shrimp (*Litopenaeus vannamei*). This research was carried out for 60 days using experimental research methods, and the research design used was a completely randomized design (CRD) consisting of 4 treatments with 3 replications were P1: Artificial feed infection, P2: Artificial feed +0, 5% white turmeric extract, P3: Artificial Feed+1% white turmeric extract, P4: Artificial Feed+2% white turmeric extract. The results showed that administration of white turmeric extract (*Curcuma zedoaria*) with a dose of 1% gave the best results and natural effect on the survival rate of 83.3%, Total Haemocyte Count of 1.4x107 cells/mL, and Differential Haemocyte Count (hyaline cells). In addition, by 23%, semi-granulocytes by 18.3%, and granulocytes by 58.7%, which play a role in the immune system of white vaname shrimp. In addition, the value of phagocytic activity was 68.8%.

INTRODUCTION

Vannamei shrimp (*Litopenaeus vannamei*) is one of the most widely cultivated Indonesian fishery export commodities. Vannamei shrimp first entered Indonesia and was then introduced to farmers in 2001. Vannamei shrimp entered Indonesia to replace tiger prawns (Penaeus monodon) which at that time suffered from WSSV (White Spot Syndrome Virus) disease, which caused its quality to decline, so the government allowed shrimp vaname (Litopenaeus vannamei) to enter Indonesia. According to Purnamasari et al. (2017), vaname shrimp has many advantages compared to other types of shrimp. The advantages of vaname shrimp include responsiveness to feed, more resistance to several diseases, high survival rate, faster growth, shorter maintenance time of around 90-100 days per cycle and resistance to environmental quality. In addition, vaname shrimp can be kept with high stocking densities in intensive cultivation systems. This is what causes vaname shrimp to be favoured by shrimp farmers.

The defence mechanism of crustaceans is largely dependent on blood cells and the hemolymph process (Syahailatua, 2009). Hemocytes are a very important factor in the non-specific cellular defence system. Smith et al. (2003) stated that haemocytes store immune

reactions (such as peroxinectin, antibacterial peptide, and clotting components) in the shrimp body so that the increase in the total number of haemocytes (THC) is one indicator of increasing the shrimp's immune system. The ability of hemocytes in phagocytic activity can increase in the event of infection, thus indicating a cellular defence of the body. The presence of the infection will stimulate the non-specific cellular defence system to ward off disease attacks. The increased resistance of the shrimp body can also be seen in the increased phagocytic activity (AF) of haemocyte cells. Phagocytosis is a general, non-specific defence mechanism.

Efforts to increase the shrimp's body defence can use immunostimulants derived from bacterial and fungal cell walls such as -glucans, lipopolysaccharides and peptidoglycans (Shahailatua, 2009). The problem with this type of immunostimulant is that it is not easy to obtain and expensive, so an alternative immunostimulant is needed. White turmeric (Kaempferia rotunda) is one of the herbs with very useful abilities as an immunostimulant and anti-inflammatory (Syukur, 2005). White turmeric is one of the plants that can be used as an immunostimulant (Curcuma zedoaria). The essential oil in white turmeric contains active compounds, including flavonoids and polyphenols Chifdhiyah (2012). The antioxidant content in active polyphenol compounds and immunomodulators in flavonoid compounds can help increase the activity of phagocytic cells in carrying out a phagocytic activity (Afifudin, 2009). According to Chifdhiyah (2012), the addition of immunostimulants in shrimp can be seen from the total number of haemocyte cells, hemocyte differential, and phagocytic activity. Therefore, it is necessary to research the effect of adding white turmeric extract (Curcuma zedoaria) on the immune system of white shrimp (Litopenaeus vannamei).

METHODOLOGY

Research Time and Place

This research was carried out from March to May 2017 at the Fish Production and Reproduction Laboratory, and blood cell testing was carried out at the Fish Health Laboratory, Aquaculture Study Program, Faculty of Agriculture, Mataram University. The study used the Experimental Method and Completely Randomized Design (CRD), which consisted of 5 treatments with 3 replications for each treatment to obtain 15 experimental units. The research conducted was mixing feed with white turmeric extract, the doses used were different, namely: P1 (Control +), P2 (Control -), P3 (0.5% white turmeric extract), P4 (1% white turmeric extract) and P5 (2% white turmeric extract).

Tools and materials

The tools used in this study were Petri dishes, Containers, Haemocytometer, Syringe, analytical balance, micropipette and microscope. In contrast, the materials used included TCBS media, TSA media, liquid SWC media, 0.9% NaCl solution, 10% EDTA, Alcohol 75%, white turmeric extract, methanol and seawater.

Research procedure

Research Preparation

In this study, the vaname shrimp used came from PT. Bibit Unggul, Rempek Village, Kec. Ganga, Kab. North Lombok are shrimp that are in the PL-20 phase. Shrimp acclimatized in advance for 7 days. During the acclimatization process, the shrimp were fed an artificial feed in the form of a crumble with a protein content of 30% with a frequency of feeding 4 times a

day. The maintenance container used in this study was a plastic container with 40 cm x 30 cm x 28 cm, which had been washed clean.

Preparation of White Turmeric Extract (*Curcuma zedoaria*)

The manufacture of white turmeric extract (*Curcuma zedoaria*) in this study used original white turmeric powder without a mixture of other ingredients purchased commercially. 500 grams of powder was extracted using 1.5 litres of 96% ethanol as solvent. First, maceration was carried out for 3 x 24 hours. After that, it was filtered 2 times and then evaporated by evaporation, namely by reducing the pressure using a rotary evaporator until a thick extract was obtained (Ayunda, 2014). The evaporation process using a rotary evaporator was carried out at the Laboratory of Basic Chemistry, Faculty of Mathematics and Natural Sciences, University of Mataram.

Feed Preparation

The feed used in rearing activities during this study was artificial in the form of crumble with a protein content of 30%. Artificial feed was given as much as 5% of the shrimp body biomass, added with white turmeric extract (Curcuma zedoaria) according to a predetermined dose, and then stirred until well mixed. After that, it was aerated before storing. Mixing artificial feed with extract of white turmeric (Curcuma zedoaria) is done once every 10 days.

Research Implementation

Each container was filled with vaname shrimp as test animals, as many as 20 tails. Each container is equipped with aeration and a shelter made of paralon pipe with a length of 10-12 cm. Seawater in containers is replaced periodically every day, as much as 10% of the total volume of containers. Vannamei shrimp were reared for 60 days. Growth sampling was carried out at the beginning of rearing to determine the initial weight of vaname shrimp seeds, and subsequent sampling was carried out once every 10 days. Feed is given 4 times daily at 06.00; 12.00; 17.00, and 22.00 WITA with a predetermined dose.

Shrimp Blood Collection

Shrimp blood samples (hemolymph) were taken 10 days after the challenge test (60th day). Vannamei shrimp hemolymph was taken at the base of the foot of the 5th street with a 1 ml syringe which already contained 0.2 ml of anticoagulant to prevent haemocyte clumping, then homogenized for 5 minutes. The first drop of hemolymph in the syringe is discarded (Chifdhiyah (2012).

Research Parameters

Gas Chromatography-Mass Spectrometry (GC-MS) Test

Gas Chromatography-Mass Spectrometry (GC-MS) combines two analytical methods: gas chromatography and mass spectrometer. The working principle of GC-MS is separating the mixture into components by gas chromatography, and each component can be made into a mass spectrum with higher accuracy. The separation results obtained by gas chromatography produce a chromatogram, while the results of the mass spectrometry examination of each compound are called the spectrum (Sipahelut, 2019). The GC-MS test of white turmeric extract in this study was conducted at the Laboratory of Analytical Chemistry, Faculty of Mathematics and Natural Sciences, University of Mataram.

Total Haemocyte Count (THC)

The hemolymph that has been taken is dropped into the haemocytometer, and the number of cells per ml is counted under a microscope with a magnification of 40 times. Total Haemocyte Count (THC) was calculated using the following formula (Chifdhiyah (2012).

$$\text{Total Hemocytes} = \text{average cell count x } \frac{1}{big\ volume\ box} \text{ x FP x } 1000$$

Information:
FP = Desire Factor

Differential Haemocyte Count (DHC)

Hemolim taken from the test shrimp was then dripped onto a glass slide and prepared for review, then air-dried and fixed with 100% methanol for 5 minutes. After that, it was dried and stained by immersing it in 10% Giemsa solution for 10 minutes and then dried again after drying, rinsed with running water for 30 seconds and then dried. The dried review preparations were then observed using a light microscope with a magnification of 40 times and differentiated by type, namely hyaline, semi-granular and granular cells. The percentage of haemocyte cell types was calculated using the following formula (Jannah et al., 2018).

$$\text{Percentage of haemocyte cell types (\%)} = \frac{Number\ of\ hemosite\ cells}{total\ hemocytes} \text{ x } 100\%$$

Phagocytic Activity

0.1 ml of hemolymph taken from the test shrimp was put into Eppendorf, then 25 l of Staphylococcus bacteria was mixed evenly and incubated for 20 minutes. Next, take 5 l of hemolymph, drop it on a glass object, prepare a smear, and then dry it. The preparations were fixed in 100% methanol for 5 minutes and stained with Giemsa's solution for 15 minutes. Phagocytic Activity was measured based on the percentage of phagocytic cells that carried out phagocytosis (Chifdhiyah (2012). Phagocytic Activity was calculated using the formula:

$$\text{Phagocytic Activity} = \frac{the\ number\ of\ phagocytic\ cells\ that\ carry\ out\ phagocytosis}{number\ of\ phagocytic\ cells} \text{ x } 100\%$$

Data analysis

Analysis of the data from the research results to determine the effect of the treatment in this study using One-Way Anova on SPSS software with a significant level of 5%. If there is a real effect, then further tests are carried out with Duncan.

RESULT

Gas Chromatography-Mass Spectrometry (GC-MS) Test

The results of the GC-MS test of the white turmeric extract used in this study can be seen in Figure 1.

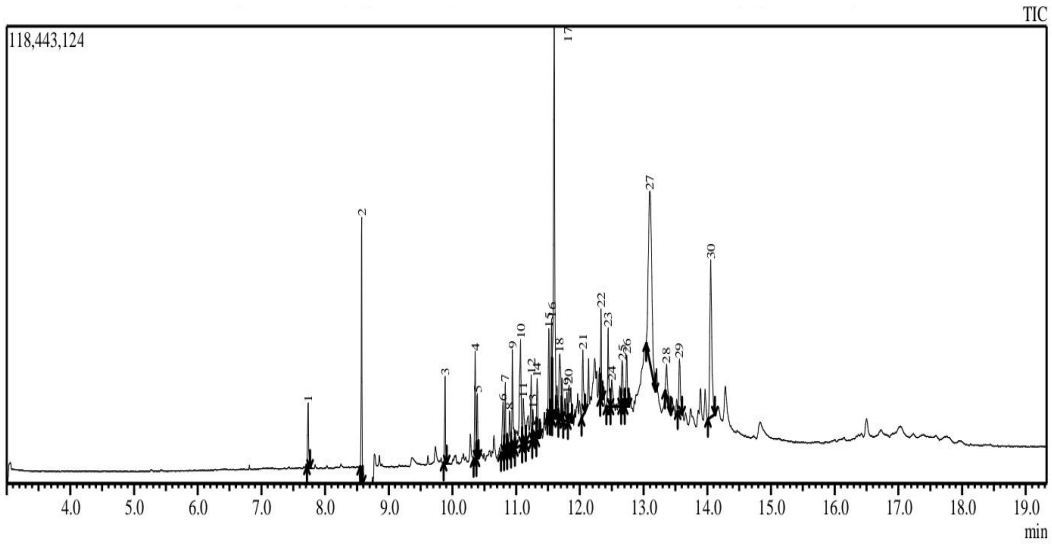

Figure 1. GC-MS Test Results White Turmeric Extract

In Figure 1, it can be seen that there were 30 peaks detected, but only 12 peaks were identified. The compounds that can be identified can be seen in Table 1.

Table 1. Compounds in White Turmeric Extract Based on GC-MS. Test Results

Peak	Compound Name	Compound Group	Other name	Retention Time	Area (%)
1	Eucalyptol (1,8-Cineole)	Monoterpenoid	Eucalyptol	7.733	1.63
2	Camphor	Monoterpen	Formosa	8.573	5.08
3	(-)-.Beta.-Elemene	Seskuiterpen	Vinyl Sikloheksana	9.883	2.08
4	.Beta.-Selinene	Seskuiterpen	Beta-Eudesmol	10.355	2.73
5	(-)-.Alpha.-Selinene	Seskuiterpen	Selina-3,1 1-Diena	10.39	1.49
6	Spathulenol	Seskuiterpen	Spathulenol	10.794	1.85
9	Spathulenol	Seskuiterpen	Spathulenol	10.94	3.03
10	Epiglobulol	Seskuiterpen	Epiglobulol	11.069	5.64
12	Germacrone	Seskuiterpen	Germacrone	11.235	3.53
16	Isopropyl Myristate	Ester	Isopropil Miristat	11.554	2.78
18	Viridiflorol	Terpenoid	Viridiflorol	11.683	3.04
21	Hexadecanoic Acid (CAS) Palmitic Acid	Lipid	Palmitic Acid	12.046	2.12

Survival Rate

The value of the survival rate of vaname shrimp in this study can be seen in Figure 2.

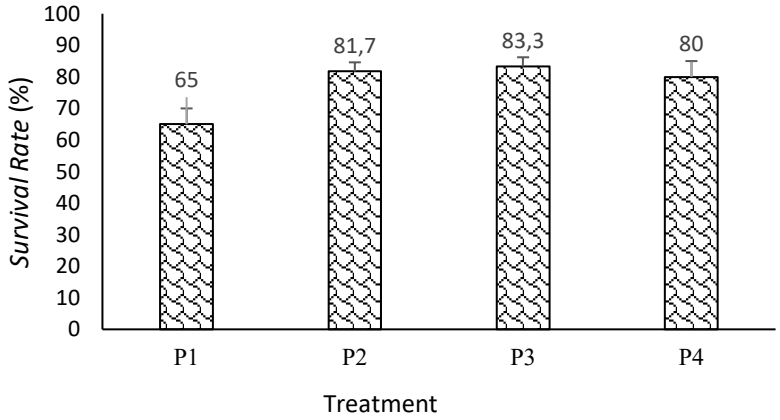

Figure 2. Survival Rate

Total Haemocyte Count (THC)

The value of the Total Haemocyte Count of white shrimp in this study can be seen in Figure 3.

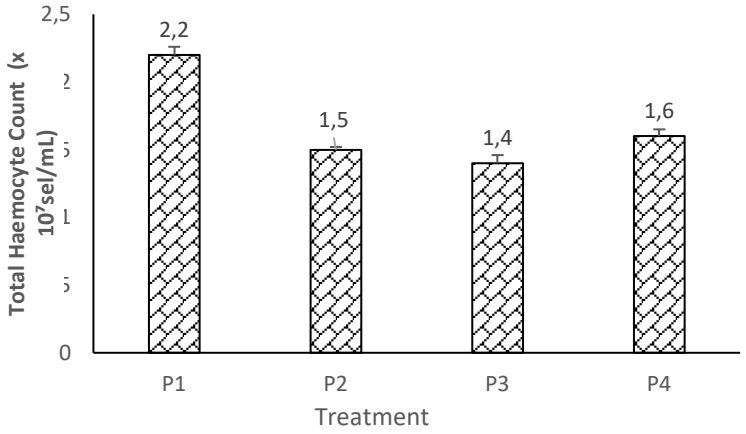

Figure 3. Total Haemocyte Count

Differential Haemocyte Count (DHC)

The value of the Differential Haemocyte Count of white shrimp in this study can be seen in Figure 4.

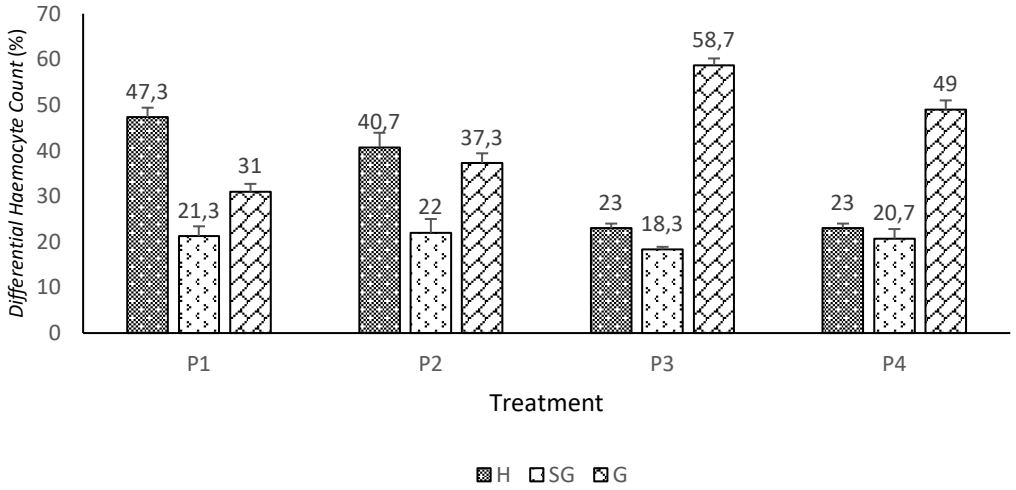

Figure 4*. Differential Haemocyte Count*

Phagocytic Activity

The value of vaname shrimp phagocytosis activity in this study can be seen in Figure 5.

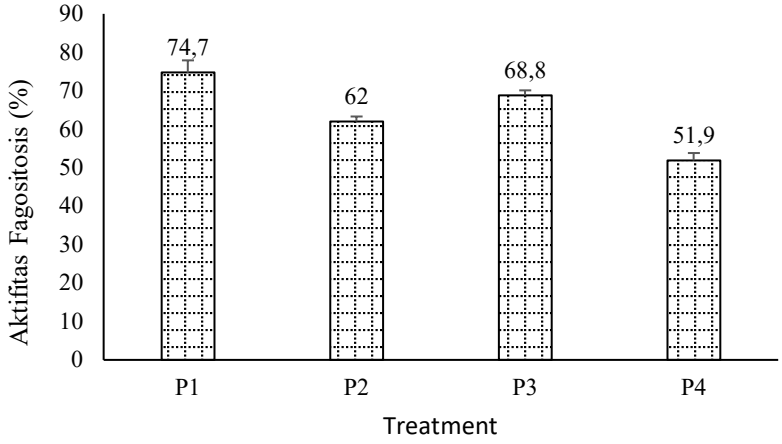

Figure 5. Phagocytic Activity

DISCUSSION

Based on the GC-MS test results that can be identified, it can be seen that white turmeric extract contains the main component in the form of Epiglobulol (5.64%), followed by Camphor (5.08%) and Germacrone (3.53%). Germacrone compounds are known to act as immunostimulants. Arshad et al. (2017) stated that apart from curcuminoids (demethoxycurcumin, bisdemethoxycurcumin, dihydro curcumin and curcumin), other compounds from Curcuma spp. We exhibit significant immune system activity, including xanthorrhizol, turmeronol, curdione, curcuzedoalide, curcumenol, and germacrone.

The level of the vaname shrimp's body's ability to fight pathogens that enter its body can be seen from the survival rate, Total Haemocyte Count (THC), Differential Haemocyte Count (DHC), phagocytic activity, and total bacteria in the intestine. Based on Figure 1, it can be seen that the highest survival rate was found in P3 (negative control), which was 83.3%, and the lowest was in P1 (positive control), which was not given additional extract which was

65%. Therefore, while the survival rate in P2 is 81.7% and P4 is 80% where the percentage is still in the good category for vaname shrimp culture. This is under the statement of Arsad et al. (2017), which states that a good survival rate or Survival Rate for shrimp if the SR value is >70%, for shrimp that get an SR value of 50-60% is in the medium category, while the low category if the SR value <50%.

In the treated vaname shrimp, the highest survival rate was found in P4, namely treatment with a dose of 1% white turmeric extract. This is the best dose to increase the vannamei shrimp immune system because the white turmeric extract can act as an immunostimulant. This is under the statement of Chifdhiyah (2012), which states that white turmeric extract given through feed can act as an immunostimulant for shrimp, which can increase shrimp endurance which can be seen from the increase in the total number of haemocytes. Samuria et al. (2018) also stated that the administration of immunostimulants could increase the survival of white vaname shrimp from 26% to 83%.

Statistically, the results showed a significant effect of giving white turmeric extract on the number of haemocyte cells in vaname shrimp. The highest number of haemocyte cells was obtained at P1 with a value of 2.2x107 cells/mL, and the lowest was at P3 (negative control) of 1.5x107 cells/mL. The number of haemocyte cells obtained in this study is still relatively good and in the normal range. According to Darwantin et al. (2016), the normal amount of THC in shrimp is 1.8 x 107 cells/mL. The higher number of haemocyte cells in treated shrimp compared to shrimp in P1 (control) indicated the ability of white turmeric extract to stimulate the formation of haemocyte cells. According to Suleman et al. (2019), an increase in total haemocytes indicates an increase in the reaction of the body's defences caused by entering foreign particles into the shrimp body.

Hemocytes in shrimp consist of hyaline cells, semi-granulocytes and granulocytes that have their respective functions in fighting pathogens that enter the shrimp body. Based on the results of the One-Way Anova analysis and Duncan's follow-up test, it was shown that giving white turmeric extract significantly affected the number of Differential Haemocyte Count in vaname shrimp. There was a significant increase in DHC (granular hemocyte cells) due to immunostimulants entering the shrimp body that could induce the body's defence mechanism (Darwantin et al., 2016). In Figure 4 can be seen the results of the percentage of the number of Differential Haemocyte Count in each treatment. The number of granulocytes, semi-granulocytes and hyaline cells obtained varied. The average number of hyaline cells obtained ranged from 31%-58.7%, semi-granular cells ranged from 18.33%-23.67%, and granulocytes ranged from 23%-47.3%. The higher percentage of hyaline cells compared to semi-granulocytes and granulocytes is because hyaline cells play an active role in phagocytic activities. This is as stated by Darwantin et al. (2016), where it is said that all types of haemocyte cells can play a role in phagocytic activity. However, hyaline cells generally play a more active role in phagocytic activity. During phagocytosis, hyaline cells will engulf and destroy pathogens that enter the shrimp's body.

Phagocytosis is the main defence for vaname shrimp against pathogens that enter the body. This is as stated by Suleman et al. (2019) that one form of cellular immune response in shrimp when dealing with pathogens is through a phagocytosis mechanism. The results of the One-Way Anova analysis and Duncan's follow-up test showed a significant effect of giving turmeric extract against the phagocytic activity. Although the administration of white turmeric extract increased phagocytic activity, the increase in phagocytic activity was not in line with the increase in the percentage of white turmeric extract. The results showed that the highest percentage of phagocytic activity was found in P3, with a value of 68.8%. Therefore, if given a

dose of more than 1%, although the shrimp body can still tolerate it, its performance as an immunostimulant for white vaname shrimp is less than optimal and tends to decrease. This is like the statement (Manopo, H. & Magdalena, 2015) where it is said that high doses given for a long time can cause the immunostimulant given may not trigger an immune response but can suppress it.

CONCLUSION

Based on this research, it can be concluded that adding white turmeric leaf extract to feed with different doses significantly increases the non-specific immune response of white vaname shrimp and its survival. This is evidenced by the value of immune parameters such as SR, THC, DHC, and AF in the treatment, which was higher than the positive control, which was not given white turmeric leaf extract. The best treatment was P3 (1% white turmeric extract) which had the highest value on the parameters of SR, THC, granular cells (DHC), and phagocytic activity (AF).

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