INTERNATIONAL JOURNAL OF LATEST TECHNOLOGY IN ENGINEERING, MANAGEMENT & APPLIED SCIENCE (IJLTEMAS)

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

Management of WSSV Challenge Test with Ig-Y with the Cohabitation Method

Ferdi Fathurohman[1*], Nurul Mukminah [1], Nurizzi Rifqi Ferdian[2]

[1] Agroindustry Study Program, Subang State Polytechnic, Subang, Indonesia
[2] Machine Maintenance Study Program, Subang State Polytechnic, Subang, Indonesia
[*] Corresponding author

Abstract --This study aims to determine the effect and best dose of Ig-Y on WSSV in vannamei shrimp using the cohabitation method in the laboratory and in ponds. The method of this study was passively immunizing shrimp that had been infected with WSSV by immersing Ig-Y doses of 2% and 4% w/v. Statistical analysis using linear regression (T test and Determination Test). The results showed that the positive shrimp group had a mortality rate of 100% on the second day after the shrimp were infected with WSSV. The shrimp group treated with Ig-Y 4% had a mortality rate of 0%. While the shrimp group treated with Ig-Y 2% had a mortality rate of 0%, the morbidity rate reached 20% after contracting WSSV. The results of the T-test statistic test on giving Ig-Y 2% T-count value is greater than T-table (2.079 > 2.037 and Ig-Y 4% T-count value is greater than T-table (2.952 > 2.037) which means Ig -Y 2% and Ig-Y 4% had a positive effect on the healing of WSSV-infected shrimp, the results of the determination analysis of 2% Ig-Y and 4% Ig-Y were able to explain 58.4% of the variation in the shrimp recovery variable from WSSV infection, there was no significant difference between testing in the laboratory and in ponds. The conclusion of the WSSV challenge test with 2% Ig-Y and 4% Ig-Y using the cohabitation method on vannamei shrimp had a significant and positive effect on the recovery of vannamei shrimp.

Keywords: Ig-Y, Cohabitation, Vannamei Shrimp, WSSV

I. Introduction

The Government of the Republic of Indonesia has regulated the Ministry of Maritime Affairs and Fisheries based on Presidential Regulation number 63 in 2015, it stated that marine and fisheries have given a positive impact to increase exports of fishery products which have so far been lagging compared to other agricultural sectors. One of the fishery products with high economic value is the Vannamei shrimp (*Litopenaeus vannamei*), therefore it is expected that the export target for Vannamei shrimp will occupy the main place in the list of exports of fishery products [1]. The demand for this commodity is always high even though there is often a decline in production.

The imports result of fishery products in 2021 reached US$142.7 million or decreased by US$23 million compared to 2020 which reached US$165.7 million. Meanwhile, the import volume in 2021 was 145,227 tons or decreased compared to 2016 which amounted to 184,240 tons. Frozen fresh and processed shrimp experienced a decrease of 5% of the total imports of fishery products in 2021 [2].

The history of the emergence of the Vannamei shrimp farming business in Indonesia began with a decrease in the productivity of tiger shrimp (*Penaeus monodon*) which had triumphed as the flagship of Indonesian fishery exports. The decrease of the productivity is caused by disease, such as White Spot Syndrome Virus (WSSV). It is one of the main causes of the decline in tiger prawn cultivation in Indonesia. The shrimp farming business had slumped for several years, before finally entering Vannamei shrimp which were said to be free of viruses and diseases because they had passed a strict certification process.

The strengths of Vannamei shrimp are not without flaws. Indonesian shrimp farmers are still dependent on other countries because Vannamei shrimp brooders that enter Indonesia must be imported from Hawaii (United States) as the center for the procurement of Vannamei shrimp seeds and brood stock. The mains exported to Indonesia are allegedly not the superior quality ones. As the result, the seeds productions have lower quality than those in their country of origin. Consequently, Vannamei shrimp production in Indonesia continues to decline. In addition, the existence of the White Spot Syndrome Virus (WSSV) also has decreased the productivity of Vannamei shrimp in Indonesia.

Many studies have been conducted to make anti-virus and White Spot Syndrome Virus (WSSV) drugs. There have been research results from [3] and [4] using Ig-Y anti-WSSV by administering it intramuscularly to the shrimp's body. However, this method is less effective for large or massive scale. That is why, from this background, the researchers tried to conduct research to see the efficacy of Ig-Y as passive immunization of Vannamei shrimp against WSSV with the cohabitation method.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

II. Material and Method

A. Time and Settings

This research was carried out from January to November 2020 which took place in the north coast shrimp ponds of Subang Regency, West Java Province and at the Integrated Laboratory in State Polytechnic of Subang.

B. Research Tools and Materials

The tools used during the study were shrimp ponds, aquariums, vortexes, micro pipettes, syringes, micro tips, microtubes (Eppendorf 1.5 ml), refractometers, aerators, and aerator hoses.

The materials used were 30 vannamei shrimp in the WSSV disease-free Juvenile phase (SPF WSSV) which is originally from the maintenance ponds of the Serang Banten Fisheries College. White Spot Syndrome Virus (WSSV) was derived from inoculation results in the laboratory Jakarta Fisheries College with 105 cfu/ml dilution, seawater, chlorine, Sodium, Natrium Tio Sulfat, antibiotics (Penicillin and Streptomycin) and purified Ig-Y.

C. Research Methods in the Laboratory

The research method used was injecting WSSV into shrimp, transmitting WSSV to infected shrimp to healthy shrimp and treatment by immersing the infected shrimp in Ig-Y solution until the shrimp showed no symptoms of WSSV [5] and [6].

1) Preparation before Experiment

Vannamei shrimp were kept in 60x30x35 and 50x50x50 cm aquariums. The used places and equipment are first cleaned by washing with detergent and then rinsing with fresh water. Seawater is collected in a large aquarium and given 100 mg/l of chlorine and then vigorously aerated. Then, it was neutralized with 50 mg/l sodium *Natrium Tio Sulfat* and aeration is given continuously.

2) WSSV Injection in Shrimp

A total of 10 vannamei shrimp were injected with WSSV at a dose of 0.05 ml/head in the 5^{th} segment using a 1 ml syringe. The injected shrimp were put into 60x35x30 cm aquarium. It contained 2 liters of seawater with a salinity of 25 ppt and a temperature of 28^{o}C which had been treated with antibiotics (Penicillin with dose of 0.03 gram and Streptomycin dose of 0.05 gram).

3) Transmission of WSSV Infected Shrimp to Healthy Shrimp

Shrimp infected with WSSV were combined with 20 healthy shrimp in an aquarium measuring 50x50x50 cm containing 6 liters of seawater with a salinity of 25 ppt and a temperature of 28oC and had been given antibiotics (0.03-gram penicillin and 0.05-gram streptomycin). The experiment was carried out until clinical signs of WSSV infection were seen.

4) Treatment by Immersion in Ig-Y Solution

The Treatment was carried out by immersing the shrimp in 2% Ig-Y solution which was mixed into water in the P1 aquarium (treatment 1) and 4% Ig-Y solution in the P2 aquarium (treatment 2). The experiment was carried out until there were no visible clinical signs of WSSV-infected shrimp. As a comparison, K+ (positive control) and K- (negative control) were made. The positive control was WSSV-infected shrimp that were soaked without using Ig-Y and the negative control was healthy shrimp that were not given any treatment.

5) Observed Parameters

The parameters observed during the study were white spots on the carapace, swimming method, body condition (movement), body color, and shrimp appetite.

D. Research Methods in Ponds

The research method was carried out in ponds, namely colonies or groups of shrimps that had been affected by WSSV. The treatment was carried out by immersing the affected shrimp in Ig-Y solution by reducing the volume of water until the shrimp showed no symptoms of WSSV [5] and [6]. The stages of research in ponds include.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

1) Treatment by Immersion in Ig-Y Solution

Treatment was carried out by immersing the shrimp in the pond by first reducing the volume of water in the pond in a 2% Ig-Y solution which was mixed into pond water P1 (treatment 1) and a 4% Ig-Y solution in pond P2 (treatment 2). The experiment was carried out until there were no visible clinical signs of WSSV-infected shrimp. As a comparison, K+ (positive control) and K- (negative control) were made. The positive control was the shrimp infected with WSSV and the negative control was the untreated healthy shrimp.

2) Observed Parameters

The parameters observed during the study were white spots on the carapace, swimming method, body condition (movement), body color, and shrimp appetite.

E. Statistical Test Analysis

1) Linear Regression Analysis

Linear regression analysis was to measure whether there was an effect of 2% Ig-Y and 4% Ig-Y on the recovery of WSSV-infected shrimp by cohabitation or immersion methods. The analysis used is linear regression analysis using the SPSS application.

2) Estimator Parameter Accuracy Test (T Test) Model Accuracy Test (R2 Determination Test)

The T statistical test basically shows how far the influence of one explanatory variable individually explains the variation of the dependent variable. The coefficient of determination R2 test is used to measure how far the model's ability to explain the related variables.

III. Results and Discussions

A. Results in the Laboratory

The infected Shrimp with WSSV by using injection method for 5 days showed clinical symptoms in the form of inactive movements, white spots on the carapace, body redness, lack of appetite and swimming on the surface of the water. The results of injections for 5 days can be seen in Table 1.

TABLE 1.

SHRIMP INFECTION BY INJECTION METHOD

Day	Dead (mortality)	Sick (morbidity)	Healthy	Life	Information
0	-	-	10	10	-
1	-	-	10	10	-
2	1	-	9	9	-
3	3	2	4	6	red body (+)
4	2	4	-	4	red body (+++)
5	-	4	-	4	red body (+++)

Note: -: There is no change; +: Mild infection; ++: Moderate infection; +++: Severe infection

Shrimp that has shown clinical symptoms are shown in Figure 1, Healthy shrimp or shrimp that have not shown clinical symptoms are shown in Figure 2.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

Fig 1. Clinical symptoms of sick shrimp infected with WSSV (White Spot Syndrome Virus) A. Reddened shrimp bodies.

Fig 2. A healthy shrimp

WSSV transmission tested in the laboratory showed the results as shown in Table 2.

TABLE 2. SHRIMP TRANSMISSION

Days	Healthy	Sick	Infected	Dead	Total of Life	Info
0	16	4	-	-	20	
1	16	4	-	-	20	
2	16	4	-	-	20	
3	15	5	1	-	20	red (+)
4	12	8	3	-	20	red (++)
5	10	10	2	-	20	red (++)
6	2	16	6	2	18	red (+++)
7	1	17	1	-	18	red (+++)
8	-	17	-	1	17	red (+++)
9	-	17	-	-	17	red (+++)
10	-	15	-	2	15	red (+++)

Note: -: There is no change; +: Mild infection; ++: Moderate infection; +++: Severe infection

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

On the 2nd day, there was one dead shrimp. Clinical symptoms appeared on the 3rd day after injection. On that day, two tails showed clinical symptoms and three died. On the 4th day there were four tails showing clinical symptoms and two died. On the 4th day all the shrimp were infected.

The shrimp showing clinical symptoms were transmitted to 16 healthy shrimp. On the 1st and 2nd day, the clinical symptoms did not appear. On the 3rd day, there was one tail showing clinical symptoms in the form of redness on the body of the shrimp. On the 4th day, there were three infected shrimp with more severe clinical symptoms than the previous day. On day 5, there were two infected tails with more severe symptoms. On the 6th day, there were six infected rats with the same symptoms as the 5th day, and two of them died. On the 7th day, there was one infected tail with the same symptoms on the 5th day.

On the 7th day, all the shrimp showed symptoms in the form of inactive movements, redness of the body and swimming on the surface of the water. On the 8th day, there was one dead shrimp and on the 10th day there were two dead shrimp. The final number of infected shrimps were 15. Shrimp that experienced healing after being given immunotherapy with Ig-Y can be seen inTable 3.

TABLE 3.

THE NUMBER OF SHRIMPS THAT EXPERIENCED HEALING AFTER BEING TREATED WITH IG-Y

Groups	The number of prawns that experienced healing on day 2								
	0	1	2	3	4	5	6	7	8
P1(Ig-Y 2%)	-	-	-	1	1	2	4	4	4
P2(Ig-Y 4%)	-	-	-	1	2	4	5	5	5
K+	-	-	-	-	-	-	-	-	-
K-	5	5	5	5	5	5	5	5	5

Note: K+: Positif Control, K-: Negative Control, -: no change

The number of sick shrimps that were given immunotherapy with Ig-Y after being infected with WSSV can be seen in TABLE 4.

TABLE 4.

THE NUMBER OF DISEASED SHRIMPS TREATED WITH IG-Y AFTER INFECTION WITH WSSV.

Groups	The number of illnesses (morbidity) on days;								
	0	1	2	3	4	5	6	7	8
P1 (Ig-Y 2%)	5	5	5	4	4	3	1	1	1
P2 (Ig-Y 4%)	5	5	5	4	3	1	-	-	-
K+	5	3	-	-	-	-	-	-	-
K-	-	-	-	-	-	-	-	-	-

Note: K+: Positif Control, K-: Negative Control, - : no change

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

The shrimps began to experience the healing process on the third day after treatment with Ig-Y, namely one shrimp at P1 and one shrimp at P2. There two shrimps died which were occurred in the K+ group on the 2nd day and three shrimps died on the 3rd day, so all the shrimps as K+ had died.

The P2 shrimp group on day 4 began to experience the healing process of one tail. The P1 shrimp group on the 5th day experienced a recovery of one shrimp and two shrimps in P2. The P1 shrimp group on the 6th day experienced two shrimps healed and one shrimp in P2.

Group P2 on the 6th day, all the shrimp showed no clinical symptoms in the form of less active movements, reddening of the body and swimming on the surface of the water. Group P1 on the 7th day, there were no shrimp that experienced the healing process and there was still one shrimp showing clinical symptoms. On the 8th day, the P1 group prawns still showed the same clinical symptoms. The number of shrimps that died after being given immunotherapy with Ig-Y can be seen in Table 5.

TABLE 5.

THE NUMBER OF SHRIMPS THAT DIED AFTER UNDERGOING TREATMENT.

Groups	The number of deaths (mortality) on days;								
	0	1	2	3	4	5	6	7	8
P1 (Ig-Y 2%)	-	-	-	-	-	-	-	-	-
P2 (Ig-Y 4%)	-	-	-	-	-	-	-	-	-
K+	-	2	3	-	-	-	-	-	-
K-	-	-	-	-	-	-	-	-	-

Note: K+: Positif Control, K-: Negative Control, -: no change

Groups P2 and P1 provide 100% protection, but group P1 requires a longer time for the healing process. The K+ group did not provide any protection at all with a 100% mortality rate. This can be seen from the results of morbidity and mortality in the control group and the treated group as shown in Table 6.

TABLE 6.

MORBIDITY AND MORBIDITY IN THE CONTROL GROUP AND THE TREATED GROUP

Groups	Mortality	Morbidity
P1 (Ig-Y 4%)	0%	0%
P2 Ig-Y 2%	20%	0%
K +	100%	100%
K -	0%	0%

Note: K+: Positif Control, K-: Negative Control

B. Linear Regression Analysis Testing in The Laboratory

The data analysis method used is linear regression with the use of the least squares equation (Ordinary Least Square) in the estimation of the model. The results of the regression analysis in the laboratory can be seen in table 7.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

TABLE 7.

LINEAR REGRESSION ANALYSIS RESULTS

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.722	3.146		.547	.588
	Ig-Y 2 %	.179	.086	.357	2.079	.046
	Ig-Y 4%	.221	.075	.383	2.952	.006

a. Shrimp Recovery from WSSV Infection (Y)

Source: Primary data processed, 2022

1) Estimator Parameter Accuracy Test (T Test) Ig-Y 2% in the Laboratory

The t test on linear regression was used to determine whether there was a partial effect between the independent variables (Ig-Y 2%) on the dependent variable (Shrimp Recovery from WSSV Infection), t-count > t-table (2.079 > 2.037), then Ho was rejected, meaning that administration of 2% Ig-Y partially affected the recovery of shrimp from WSSV infection. A positive t-count value means a positive effect, that is, if the shrimp are given Ig-Y 2%, the shrimp recovery rate from WSSV infection will also increase. The results of statistical data analysis proved that there was a positive and significant effect between the administration of 2% Ig-Y on the recovery of shrimp which was indicated by a significance value of 0.046 which means less than 0.05. This study found a positive and significant effect between the administration of 2% Ig-Y on the recovery of shrimp from WSSV infection.

2) Estimator Parameter Accuracy Test (T Test) Ig-Y 4% in the Laboratory

The t test on linear regression was used to determine whether there was a partial effect between the independent variables (Ig-Y 4%) on the dependent variable (Shrimp Recovery from WSSV Infection), tcount > ttable (2.952 > 2.037), then Ho was rejected, meaning that administration of 4% Ig-Y partially affected the recovery of shrimp from WSSV infection. A positive t-count value means a positive effect, that is, if the shrimp are given Ig-Y 4%, the shrimp recovery rate from WSSV infection will also increase. The results of statistical data analysis proved that there was a positive and significant effect between the administration of 4% Ig-Y on the recovery of shrimp which was indicated by a significance value of 0.006 which means less than 0.05. This study found a positive and significant effect between the administration of 4% Ig-Y on the recovery of shrimp from WSSV infection.

3) Analysis of the Coefficient of Determination (Adjusted R2) in the Laboratory

The results of this study obtained an Adjusted R^2 value of 0.584 (58.4%). This shows that the variation of the independent variables used in the model (Ig-Y 2% and Ig-Y 4%) is able to explain 58.4% of the variation in the shrimp recovery variable from WSSV infection, and the rest is explained by other variables not included in the model. this research. The results of the analysis of the coefficient of determination can be seen in Table 8.

TABLE 8.

RESULTS OF THE ANALYSIS OF THE COEFFICIENT OF DETERMINATION

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.787[a]	.620	.584	2.43327

a. Predictors: (Constant), Ig-Y 4% (X2), Ig-Y 2% (X1)

b. Dependent Variable: Shrimp Recovery from WSSV Infection (Y)

Source: Primary data processed, 2022

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

Research on immunization shows unsatisfactory results due to difficulties in daily application and because each method has weaknesses. The injection can be done intra-muscular, intra-peritoneal, and intravenous [7]. This method has several drawbacks, including causing stress on shrimp and being inefficient if immunization is carried out on a large scale because it requires effort, takes a long time and is difficult to apply to small shrimp [8]. This method has the advantage of being faster in the formation of antibodies and does not require large enough doses [9].

The process of immersion and spraying have the disadvantage of causing stress to the shrimp and having low protective power, but these have the advantage of practical method [10]. The oral method has a weakness, namely the dose given must be in large quantities to cause immunity in shrimp, if excess can increase high levels of ammonia in the water and cause stress to the shrimp [11]. This oral method is widely used because it is easy to apply to all ages and sizes of shrimp [12].

The shrimp that will be treated must first be adapted to the temperature and salinity conditions in the aquarium to prevent stress on the shrimp. Stress is very important and can affect the immune reaction of an animal [13]. The success of immunization in causing immunity depends on the temperature of the surrounding environment, because temperature is a stress-causing factor [14]. The formation of antibodies will run slowly at low temperatures and will run quickly at the maximum temperature in accordance with the life temperature of the shrimp [15].

Passive immunization using immersion method by giving specific Ig-Y anti-WSSV doses of 2% and 4% w/v of the amount of water in the aquarium can increase resistance to White Spot Syndrome (WSS). It can be seen from the percentage of mortality and morbidity in the immunized group which is lower when compared to the non-immunized group. Based on statistical tests, the T test and R2 test showed that giving 2% Ig-Y and 4% Ig-Y by immersion method could cure WSSV-infected vannamei shrimp, 4% Ig-Y showed a higher test value compared to Ig-Y 2 %.

The immunization provides 100% protection (0% mortality), while the mortality control shrimp group is up to 100%. The group of shrimps that were not immunized did not have specific antibodies in their bodies against WSSV, when WSSV attacked the shrimp's body, the shrimp's bodies did not give an immune response to that antigen. This causes the shrimp to experience pain and death if they are not given an immune substance from the outside (Ig-Y anti WSSV).

Antigens cause damage when they dissolve in the body. Antibodies play a role in preventing antigens from dissolving in the body. The function of antibodies in neutralizing antigens is as precipitins which precipitate antigens, as agglutinins which agglutinate antigens, as inhibin which obstruct antigen attachment and as opsonin which coat antigens so that they are easier to be phagocytosed by phagocytic cells.

Everyone has different endurance, determined by age, sex, nutritional status and stress [12] and [13]. Pathogens must penetrate the shrimp immune system to cause disease. Natural resistance allows shrimp to be free from pathogen attack because there are no specific tissues or cellular receptors for pathogen colonization or because there is less optimum nutritional adequacy and environmental influences for the growth of these pathogens. Conditions in shrimp will be susceptible to pathogens due to several things including polluted water, the changing seasons, very short temperature changes.

Clinical symptoms that occurred in shrimp during the study were white spots on the carapace, how the shrimp swam on the surface of the water, weak body condition, reddened body color and decreased appetite. White patches are the main feature of WSSV infection [16]. White spots occur due to calcium salts that form in the epidermal layer of the cuticle [17] or caused by lymph fluid contaminated with a virus and mixed with all the blood fluids in the shrimp's body [18].

The decrease in activity is thought to be due to a lack of energy because the response to feed has decreased so that the supply of energy for the activities of the shrimp body is reduced [19]. Changes in body color due to injury to the cuticle chromatophores due to infection from antigens. The cuticle layer is the first defense organ that plays an important role in fighting potential pathogens and physical damage, so a change in shrimp color is a sign of a decrease in body immunity [20]. Changes in the color of the shrimp's body can also be due to the immune system in the shrimp's body working excessively to fight foreign objects in the body [21].

Disease prevention can be done by immunization, namely by activating the immune recognition system and effector systems needed so that the shrimp's body will be more protective. This protection is played by activated phagocytic cells so that they are more efficient in phagocytizing viruses. The results of the study showed that 2% and 4% Ig-Y immersion provided protection against WSSV. This is in accordance with the statement [8] that the immunity that appears varies depending on the number of antibodies ingested by the animal.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

C. Results in the Pond

Shrimp infected with WSSV by injection method in ponds for 5 days showed clinical symptoms in the form of less active movement, white spots on the carapace, body redness, lack of appetite and swimming on the surface of the water, this is like research conducted in the laboratory.

On the 2nd day, there was one dead shrimp. Clinical symptoms appeared on the 3rd day after injection. On that day, two tails showed clinical symptoms and three died. On the 4th day, there were four tails showing clinical symptoms and two died. On the 4th day, all the shrimp were infected. Research in ponds did not show different symptoms like the research conducted in the laboratory.

The shrimp showing clinical symptoms were transmitted to 16 healthy shrimp. On day 1 no clinical symptoms appeared. On the 2nd and 3rd day there was one tail which showed clinical symptoms in the form of redness on the shrimp's body. On the 4th day, there were three infected shrimp with more severe clinical symptoms than the previous day. On day 5, there were two infected tails with more severe symptoms. On the 6th day, there were six infected rats with the same symptoms as the 5th day and two of them died. On the 7th day, there was one infected tail with the same symptoms on the 5th day. Research in ponds shows clinical symptoms faster than in the laboratory. It is possible that there is contamination from the surrounding ponds.

On the 7th day, all the shrimps showed symptoms in the form of inactive movements, redness of the body and swimming on the surface of the water. On the 8th day, there was one dead shrimp and on the 10th day there were two dead shrimp. The final number of infected shrimps was 15. On the 7th and 8th day the research in the ponds showed the same symptoms as the research in the laboratory. Shrimp that experienced healing after being given immunotherapy with Ig-Y can be seen in table 9.

TABLE 9.

THE NUMBER OF SHRIMPS THAT EXPERIENCED HEALING AFTER BEING TREATED WITH IG-Y (IN THE POND)

Groups	The number of prawns that experienced healing on day 2								
	0	1	2	3	4	5	6	7	8
P1 (Ig-Y 2%)	-	-	1	1	1	2	4	4	4
P2 (Ig-Y 4%)	-	-	1	1	2	4	5	5	5
K+	-	-	-	-	-	-	-	-	-
K-	5	5	5	5	5	5	5	5	5

Note: K+: Positif Control, K-: Negative Control, - : no change

The number of sick shrimps that were given immunotherapy with Ig-Y after being infected with WSSV can be seen in Table 10.

TABLE 10.

THE NUMBER OF DISEASED SHRIMPS TREATED WITH IG-Y AFTER INFECTION WITH WSSV (IN THE POND)

Groups	The number of illnesses (morbidity) on days;								
	0	1	2	3	4	5	6	7	8
P1 (Ig-Y 2%)	5	5	5	4	4	3	1	1	1
P2 (Ig-Y 4%)	5	5	5	4	3	1	1	1	-
K+	5	3	-	-	-	-	-	-	-
K-	-	-	-	-	-	-	-	-	-

Note: K+: Positif Control, K-: Negative Control, -: no change

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

The shrimps began to experience the healing process on the third day after treatment with Ig-Y, namely one shrimp at P1 and one shrimp at P2. Two deaths occurred in the K+ group on the 2nd day and three on the 3rd day, so all the shrimps as K+ had died. research in ponds showed the same results with research in the laboratory. The P2 shrimp group on day 4 began to experience the healing process of one tail. The P1 shrimp group on the 5th day experienced a recovery of one shrimp and two shrimps in P2. The P1 shrimp group on the 6th day experienced two shrimps healed and one shrimp in P2. Group P2 on the 6th day, all the shrimp showed no clinical symptoms in the form of less active movements, reddening of the body and swimming on the surface of the water. Group P1 on the 7th day, there were no shrimp that experienced the healing process and there was still one shrimp showing clinical symptoms. On the 8th day, the P1 group prawns still showed the same clinical symptoms. Research in ponds showed the same results with research in the laboratory. The number of shrimps that died after being given immunotherapy with Ig-Y can be seen in Table 11.

TABLE 11.

THE NUMBER OF SHRIMPS THAT DIED AFTER UNDERGOING TREATMENT (IN THE POND)

Groups	The number of deaths (mortality) on days;								
	0	1	2	3	4	5	6	7	8
P1 (Ig-Y 2%)	-	-	-	-	-	-	-	-	-
P2 (Ig-Y 4%)	-	-	-	-	-	-	-	-	-
K+	-	2	3	-	-	-	-	-	-
K-	-	-	-	-	-	-	-	-	-

Note: K+: Positif Control, K-: Negative Control, - : no change

Groups P2 and P1 provide 100% protection, but group P1 requires a longer time for the healing process. The P1 morbidity rate is 20% and the P2 morbidity rate is 0%. The K+ group did not provide any protection at all with a 100% mortality rate. This shows that immersing Ig-Y as much as 2% w/v and 4% w/v has provided protection against WSSV and is in accordance with the statement [8] that the immunity that appears varies depending on the amount of antibody ingested. by animals research on immunization shows unsatisfactory results due to difficulties in daily application and because each method has weaknesses. This can be seen from the results of morbidity and mortality in the control group and the treated group as shown in Table 12.

TABLE 12.

MORBIDITY AND MORBIDITY IN THE CONTROL GROUP AND THE TREATED GROUP (IN THE POND)

Groups	Mortality	Morbidity
P1 (Ig-Y 4%)	0%	0%
P2 (Ig-Y 2%)	20%	0%
K +	100%	100%
K -	0%	0%

Note: K+: Positif Control, K-: Negative Control

1) Multiple Linear Regression Analysis Testing in Ponds

The data analysis method used is linear regression with the use of the Ordinary Least Square equation in the model estimation. The results of the analysis can be seen in Table 13.

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

TABLE 13.

LINEAR REGRESSION ANALYSIS RESULTS

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.832	3.001		.547	.592
	Ig-Y 2 %	.182	.089	.368	2.125	.048
	Ig-Y 4%	.243	.074	.395	2.987	.012

a. Shrimp Recovery from WSSV Infection (Y)

Source: Primary data processed, 2022

2) Estimator Parameter Accuracy Test (T Test) Ig-Y 2% in Ponds

The t test on linear regression was used to determine whether there was a partial effect between the independent variables (Ig-Y 2%) on the dependent variable (Shrimp Recovery from WSSV Infection), t-count > t-table (2.125 > 2.037), then Ho was rejected, meaning that administration of 2% Ig-Y partially affected the recovery of shrimp in ponds from WSSV infection. A positive t-count value means a positive effect, that is, if the shrimp are given Ig-Y 2%, the shrimp recovery rate from WSSV infection will also increase. The results of statistical data analysis proved that there was a positive and significant effect between the administration of 2% Ig-Y on the recovery of shrimp which was indicated by a significance value of 0.048 which means less than 0.05. This study found a positive and significant effect between the administration of 2% Ig-Y on the recovery of shrimp from WSSV infection.

3) Estimator Parameter Accuracy Test (T Test) Ig-Y 4% in Ponds

The t test on linear regression was used to determine whether there was a partial effect between the independent variables (Ig-Y 4%) on the dependent variable (Shrimp Recovery from WSSV Infection), t-count > t-table (2.987 > 2.037), then Ho was rejected, meaning that administration of 4% Ig-Y partially affected the recovery of shrimp from WSSV infection. A positive t-count value means a positive effect, that is, if the shrimp are given Ig-Y 4%, the shrimp recovery rate from WSSV infection will also increase. The results of statistical data analysis proved that there was a positive and significant effect between the administration of 4% Ig-Y on the recovery of shrimp which was indicated by a significance value of 0.012 which means less than 0.05. This study found a positive and significant effect between the administration of 4% Ig-Y on the recovery of shrimp from WSSV infection.

4) Analysis of the Coefficient of Determination (Adjusted R2) in Ponds

The results of this study obtained an Adjusted R^2 value of 0.584 (58.4%). This shows that the variation of the independent variables used in the model (Ig-Y 2% and Ig-Y 4%) is able to explain 52.3% of the variation in the shrimp recovery variable from WSSV infection, and the rest is explained by other variables not included in the model. this research. The results of the analysis of the coefficient of determination testing on ponds can be seen in Table 14.

TABLE 14.

RESULTS OF THE ANALYSIS OF THE COEFFICIENT OF DETERMINATION

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.791[a]	.631	.523	2.63517

a. Predictors: (Constant), Ig-Y 4% (X2), Ig-Y 2% (X1)

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.791[a]	.631	.523	2.63517

a. Predictors: (Constant), Ig-Y 4% (X2), Ig-Y 2% (X1)

b. Dependent Variable: Shrimp Recovery from WSSV Infection (Y)

Source: Primary data processed, 2022

Overall, there is no difference in the results of research conducted in the laboratory with research in ponds. The difference is only in the speed of pain, where research in ponds is faster than in the laboratory. This difference is due to contamination that occurs from outside the study area. But this difference is not significant.

IV. Conclusion and Suggestions

The WSSV challenge test with 2% and 4% Ig-Y using the cohabitation method on vannamei shrimp had a significant and positive effect on the recovery of vannamei shrimp. Ig-Y 4% dose is more effective than Ig-Y 2% w/v. Additionally, the mortality in shrimp that were not immunized reached 100% while the mortality of shrimp that were immunized was 0%. Clinical symptoms are the form of white spots on the carapace, the shrimp had fewer active movements, and the body got angry, decreased appetite, and swam on the water surface. Further research is needed to conduct, especially about the efficacy of Ig-Y with several repetitions and various methods, for example by oral passive immunization and spraying. Importantly, the feed given to the shrimp should contain Ig-Y with a minimum dose of 4% w/v. The understanding of the importance of shrimp health management should be increased so that the farmers will have good knowledge.

References

1. BPS, “Aquaculture Production According to Main Commodities in Indonesia,” *Badan Pusat Statistika*. Badan Pusat Statistik, Jakarta, 2020, [Online]. Available: https://www.bps.go.id.
2. KKP, “Marine Fishery Production Sold at Indonesian Fish Auction Places,” Kementerian Perikanan dan Kelautan Republik Indonesia, Jakarta, 2021. [Online]. Available: https://www.bps.go.id/dynamictable/2018/01/12/1278/produksi-perikanan-laut-yang-dijual-di-tpi-menurut-provinsi-2004-2017.html.
3. B. Sengagau and M. M. Bond, “Anti-WSSV Immunoglobulin Y Activity in Chicken Serum and Eggs,” *Biodidaktika J. Biol. Dan Pembelajarannya*, vol. 15, no. 1, 2020, doi: 10.30870/biodidaktika.v15i1.7821.
4. I. Supriatna, A. Yustiati, and Iskandar, “Sekuen asam amino anti white spot syndrome virus (WSSV) pada udang windu (Penaeus Monodon),” *Bionatura-Jurnal Ilmu-ilmu Hayati dan Fis.*, vol. 16, no. 1, pp. 40–46, 2014.
5. A. S. Sahul Hameed, M. Anilkumar, M. L. S. Raj, and K. Jayaraman, “Studies on the pathogenicity of systemic ectodermal and mesodermal baculovirus and its detection in shrimp by immunological methods,” *Aquaculture*, vol. 160, no. 1–2, pp. 31–45, 1998, doi: 10.1016/S0044-8486(97)00221-4.
6. A. Taslihan, R. Handayani, Suryati, and N. Fahris, “Sensitivity of Litopenaeus stylirostris and Penaeus monodon Against Sembv (Systemic Ectodermal and Mesodermal Baculovirus) Infection,” *J. Perikan. UGM*, vol. 4, no. 1, pp. 1–4, 2020.
7. I. Sudirman, H. Syawal, and I. Lukistyowati, “Erythrocyte Profile of Vanamae (Cyprinus carpio L) Given Feed Containing WSSV Vaccine,” *J. Ilmu Perair. (Aquatic Sci.*, vol. 9, no. 2, pp. 144–151, 2021, doi: 10.31258/jipas.9.2.p.144-151.
8. R. Sari, A. Setyawan, and S. Suparmono, “Peningkatan Imunogenisitas Vaksin Inaktif WSSV dengan Penambahan Adjuvant pada Undang Vanamae,” *e-Jurnal Rekayasa dan Teknol. Budid. Perair.*, vol. 1, no. 2, pp. 87–94, 2018.
9. E. Syafitri, D. Tika Afriani, B. Siregar, D. Yuda Gustiawan, and P. Studi Akuakultur Fakultas Perikanan, “Phytochemical Content and Antibacterial Activity Test of Mangrove (Sonneratia alba) Leaf Extract In Vitro Against Aeromonas Hydrophila,” *J. Ris. Akuakultur*, vol. 15, no. 4, pp. 253–259, 2021.
10. A. M. Amrillah, S. Widyarti, and Y. Kilawati, “Impact of Salinity Stress on White Spot Syndrome Virus (WSSV) Prevalence and Survival Rate of Vannamei Shrimp (Litopenaeus vannamei) Under Controlled Conditions,” *Res. J. Life Sci.*, vol. 2, no. 2, pp. 110–123, 2015, doi: 10.21776/ub.rjls.2015.002.02.5.
11. Muliani, B. R. Tampangallo, and K. Kurniawan, “Several Stressing Methods to Induce the Development of White Spot

ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue II, February 2023

Syndrome Virus (WSSV) in Tiger Shrimp (Penaeus monodon)," *J. Ris. Akuakultur*, vol. 7, no. 3, p. 465, 2012, doi: 10.15578/jra.7.3.2012.465-475.

12. K. K. Khairul, "Stress Test by Reducing Different Salinity To Determine Quality of Tiger Shrimp fry (Penaeus monodon)," *J. Eduscience*, vol. 5, no. 2, pp. 6–10, 2019, doi: 10.36987/jes.v5i2.924.
13. Sarjito, S. B. Prayitno, and A. H. C. Haditomo, *Buku Pengantar Parasit dan Penyakit Ikan*, 1st ed. Semarang: UNDIP Press, 2013.
14. I. K. A. Wirawan, S. A. M. P. Suryani, and I. W. Arya, "Diagnosa, Analisis dan Identifikasi Parasit yang Menyerang Ikan Nila (Oreochromis Niloticus) Pada Kawasan Budidaya Ikan Di Subak 'Baru' Tabanan," *Gema Agro*, vol. 23, no. 1, p. 63, 2018, doi: 10.22225/ga.23.1.661.63-78.
15. W. Widiantoro, "Derajat Kelangsungan Hidup dan kesehatan ikan Sidat (Anguilla bicolor) pada dua wilayah (tempat) pembesaran yang berbeda," *J. Aquafish Saintek*, vol. 1, no. 1, pp. 35–38, 2020, [Online]. Available: https://doi.org/10.1016/j.jnc.2020.125798%0Ahttps://doi.org/10.1016/j.smr.2020.02.002.
16. H. C. Clifford, "Prevention of WSSV : Water management strategies," *Global Aquaculture Advocate*. Global Aquaculture Advocate, pp. 1–11, 2020.
17. D. Wahjuningrum, S. H. Sholeh, and S. Nuryati, "Prevention of White Spot Syndrome Virus Infection on Penaeus monodon by Immersion in CEPM Extract of Avicennia sp. and Sonneratia sp.," *J. Akuakultur Indones.*, vol. 5, no. 1, pp. 65–75, 2017, doi: 10.19027/jai.5.65-75.
18. Muliani, E. Susianingsih, Nurhidayah, and Nurbaya, "Prevention of White Spot Syndrome Virus (WSSV) in tiger shrimp Penaeus monodon using boiled mangrove leaf extract Sonneratia alba in laboratory scale," in *IOP Conference Series: Earth and Environmental Science*, 2021, vol. 860, no. 1, doi: 10.1088/1755-1315/860/1/012049.
19. G. D. Stentiford and D. V. Lightner, "Cases of White Spot Disease (WSD) in European shrimp farms," *Aquaculture*, vol. 319, no. 1–2, pp. 302–306, 2021, doi: 10.1016/j.aquaculture.2011.06.032.
20. H. Ali *et al.*, "A study on health management practices and occupational health hazards in shrimp (Penaeus monodon) and prawn (Macrobrachium rosenbergii)," Bangladesh, 2016. doi: 10.13140/RG.2.1.4919.8961.
21. NACA, "Shrimp Health Management Extension Manual," Bangkok, 2013.