

Fortification of chitosan and mangrove flour as windu shrimp feed (*Penaeus monodon*) against infection white spot syndrome virus

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Abstract. Langsa is one of the largest Windu shrimp producing areas in ACEH that holds considerable potential in the cultivation of windu shrimp. But recently the production of tiger shrimp has deteriorated due to by various disease attacks that have caused shrimp deaths including White Spot Syndrome Virus. This disease is a crop failure disease with high levels of morbidity and mortality. Transmission of WSSV was very fast and led to a 100% death within 3-10 days since the clinical symptoms arose. Therefore, research is conducted to prevent and treat WSSV by using shrimp skin and mangrove flour as feed. Chitosan flour from shrimp skin waste has the potential to be used as feed. The aim of this study was to determine the effect of fortification of chitosan and mangrove flour as windu shrimp feed on physiological conditions of windu shrimp. Physiological observations carried out include feeding responses, swimming, body redness (body color changes), white spots on the carapace and counting shrimp that die during maintenance. Physiological observations carried out include feeding responses, swimming, body redness (body color changes), white spots on the carapace and counting shrimp that die during maintenance. Based on the results of the study show that the more mangrove flour and chitosan fortified in shrimp feed, the level of shrimp resistance to white spot syndrome virus infection is increasing which is characterized by a low mortality rate.

1. Introduction

Langsa is one of the largest Windu shrimp producing areas in ACEH that holds considerable potential in the cultivation of windu shrimp. But recently the production of tiger shrimp has deteriorated due to by various disease attacks that have caused shrimp deaths including White Spot Syndrome Virus [1]. White spot Syndrome Virus (WSSV) is a disease that most causes economic loss, this virus infects shrimp at the premolting stage, giving rise a spotting pattern at post molting [2]. Spotting patterns is caused by the destruction of ectodermal cells resulting in the process of calcium deposition to be abnormal on the cuticle resulting in white lesions due to the transfer of exudate from epithelial cells to the cuticle through cuticular pore canal. According to [3] this disease is a crop failure disease with high levels of morbidity and mortality.

The transmission of WSSV was very fast and led to a 100% death within 3-10 days since the clinical symptoms arose. Until now, the treatment of WSSV is unknown; usually prevention is only done by selecting superior seeds, good cultivation management, and vaccines. Therefore, research is conducted to prevent and treat WSSV by using shrimp skin and mangrove flour as feed. Chitosan flour from shrimp skin waste has the potential to be used as feed. This is due to shrimp skin flour containing lysosim enzymes and

aminopolysaccharide groups as antimicrobial substances that can inhibit microbial growth. In addition, shrimp skin also contains chitosan which is quite efficient in inhibiting microbial activity. In this case [4] said that chitosan is able to suppress bacterial growth because chitosan has a positively charged poly cation that can inhibit bacterial and mold growth. Thus the provision of skin flour chitosan is expected to inhibit the activity of White Spot Syndrome Virus.

Mangrove flour derived from mangrove leaves and fruit which are used as shrimp feed. Mangroves contain flavonoids and saponins which can be used as antimicrobial and antiviral agents by inhibiting the virus respiration system [5]. Some flavonoids inhibit phosphodiesterase, aldoreductase, monoamine oxidase, protein kinase, reverse transcriptase, DNA polymerase and lipookigenase in viruses and microbes. Therefore, the addition of chitosan flour and mangrove flour is expected to reduce the level of mortality in tiger shrimp by increasing immunity in tiger shrimp against viral infections, especially White Spot Syndrome Virus.

2. Material and Methods

2.1 Material

The main material were White Spot Syndrome Virus and *Penaeus monodon* from Balai Besar Pengembangan Budidaya Air Payau (BBPABP). All chemicals used for study were analytical grade and obtained from C.V Multikreasi Medan.

2.2 Method

2.2.1 Preparation of feed

a. Manufacture of Mangrove Flour (MF)

The mangrove plants are used in the form of the 3rd leaf to the top and fruit. The method of manufacture mangrove flour using method of [6]

b. Manufacture of Chitosan Flour (CF)

The waste of shrimp skin is washed with flowing water to remove the impurities attached, then dried in the oven with a temperature of 80 °C for 3 hours. After that the dried shrimp skin is ground until smooth with a size of 40 mesh [7].

c. Feed composition

The composition of windu shrimp feed in the form of supplement products from shellfish flour and mangrove flour which will be given when the shrimp is 15-45 days old ((pre molting and post molting) and previously the shrimp was also given feed commonly given by farmers.

2.2.2. Virus Provision

The purification of the spore fungus was carried out by transferring the fungus which has grown by 0.5 cm into the medium PDA. An identification book from Nakagiri [8] is used as a reference to identify morphology and pathogen fungi covering macroscopic and microscopic observations. The same colonies were considered in same isolate, and each representative colony was separated into isolate.

2.2.3 Preparation of laboratory animals and experimental design

a. Maintenance of laboratory animals

This study used PL20 juvenile windu shrimp with a density of 7 tails / liter maintained in an aquarium for 25 days with sufficient aeration for each treatment. Feed given is in the form of supplementation of mangrove and chitosan flour and artificial feed in the form of pellets with

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reddened																
White spots	4	-	-	-	6	-	-	-	6	-	-	-	1	-	-	-
Death post infection WSSV	5	7	8	8	8	-	8	-	9	1	-	-	1	-	-	-
Total death	9	16	16	15	1	-	17	-	21	2	-	-	2	-	-	-

a. Swimming activity

Based on Table 1 shows that on days 1 and 2 there was a change in swimming activity towards a more active one in all treatments except the control on day 8. In this case [8]reported that decreased consumption of food, weak, loose cuticles, and color fading in the hepato pancreas from pink to reddish brown, anorexia, lethargi, redness of the abdomen and white spots which are clinical symptoms in infected shrimp.

b. Decreased appetite

Decrease in appetite occurred on day 26 of the control treatment, day 23 in the treatment of POC1, POC2, and POC3, day 22 in treatment POC3, day 21 in treatment P1C0 and P2C0 and day 18 in treatment P3C0. The decrease in shrimp appetite can be seen from the amount of residual feed in the maintenance medium which reaches half of the feed given. Feeding response is getting better with increasing treatment doses. This is because shrimp is stressed due to WSSV attack [9] reports that characteristics of stressed shrimp can be seen from visual observations such as shrimp loss of appetite seen from lack of response to feed and swimming without direction.

c. Body discoloration (red eyes, clear body and white spots)

The higher the treatment dose, the higher the shrimp health level. It can be seen from the colour of the shrimp body that looks clear, the colour of the eye that is not red and the absence of white spots on the skin of the shrimp. In the control treatment the appearance of white spots and reddish eyes appears faster than other treatments resulting shrimp deaths early (Table 1). White spots are a specific symptom in WSSV-infected shrimp while lethargy such as decreased appetite and swimming activity are common symptoms of animals infected with WSSV ([10]. Spotting patterns is caused by the destruction of ectodermal cells resulting in the process of calcium deposition to be abnormal on the cuticle resulting in white lesions due to the transfer of exudate from epithelial cells to the cuticle through cuticular pore canal.

d. Death post infection WSSV

Based on Table 4.1, it can be seen that the higher the mangrove flour and chitosan fortified in shrimp feed, hence the level of shrimp resistance against white spot syndrome virus infection compared to the control that began to die on the 5th day post infection. It caused mangrove flour and chitosan fortified in feed can increase the resistance of shrimp to infection with whotsptot syndrome virus. Maggrove flour acts as an immune stimulant so that it can increase the body resistance of shrimp.In this case [11] reported that administration of maggrove extract can reduce mortality of tiger shrimp treated with

WSSV. Chitosan flour also plays a role in stimulating the immune system. In this case, [12] reported that the addition of chitosan to the feed can stimulate immune function, improve digestion and absorption of nutrients and increase the protein content of Indian major carp (*Labeo Rohita*) so that it can improve growth performance.

e. Total death

Based on Table 4.1, it can be seen that the more mangrove flour and chitosan are fortified in shrimp feed, the more total time of death of shrimp after white spot syndrome virus infection compared to control died on the 9th day after infection. This is because mangroves fortified in feed contain flavonoids and saponins which can be used as antimicrobial and antiviral agents by inhibiting the virus replication system [13]. Some of flavonoids inhibit phosphodiesterase, aldoreductase, monoamine oxidase, protein kinase, reverse transcriptase, DNA polymerase and lipooxygenase in viruses and microbes. While chitosan as a non-toxic natural polymer can stimulate the development of the immune system, accelerate wound healing and is antibacterial [13]. Chitosan is used for immune stimulants, can improve digestion and absorption of feed nutrients given. In this case [12] reported that the addition of chitosan at a dose of 1% can stimulate the immune function of Indian major carp (*Labeorohita*). The use of immune stimulants as a feed supplement can increase the natural defense of fish so that it is resistant to pathogens during periods of stress [14].

4. Conclusion

Chitosan fortification (300 gr) and mangrove (300 gr) in 1 kg of commercial feed can increase shrimp resistance to white spot syndrome virus infections which are increasingly characterized by low mortality rates.

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