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Nanobubble Aquaculture System: Its Effect Towards Immune Response and Infection of *Vibrio* sp. in Vannamei Shrimp (*Litopenaeus vannamei*)

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Abstract

The application of nanobubble had a significant effect on the Vannamei shrimp (*Litopenaeus vannamei*) immune response against *Vibrio* sp. with the best THC and DHC results (45.23 cells/ml and 30.23%). The highest average TPC of *Vibrio* sp. in shrimp hepatopancreas was on aerator treatment, which was 3.32 x 10^5 (CFU / g). The highest average TPC of *Vibrio* sp. was on aerator treatment, which was 3.43 x 10^6 (CFU / ml). The results of bacterial identification showed that the types of bacteria were *Vibrio vulnificus* and *Vibrio alginolitycus*.

Key words: *Nanobubble*, Immune Response, Pathogen Infection, Vannamei Shrimp

Water quality is one of the key factors of aquaculture success (Effendi, 2004). Bad water quality gives an opportunity to the *Vibrio* sp. infecting Vannamei shrimp health (Lorenzon *et. al.*, 2001; Supito *et al.*, 2008). Nanobubble is technology can be used to improve water quality by decomposing the organic matter (Ebina *et al.*, 2013; Chiba and Takahashi, 2007; Mahasri, 2016). The purpose of this study was to determine the effect of nanobubble aquaculture systems against immune response and *Vibrio* sp. infection on Vannamei shrimp (*Litopenaeus vannamei*).

Materials and Methods

The materials used in this study were 240 Vannamei shrimp size 7-10 cm with a weight of 4-6 gr, pond water with salinity 18-20 ppt, aerator and nanobubble generator. This study consisted of 2 factors, A (nanobubble and aerator) and B (0 days, 10 days, 20 days, and 30 days). The optimization used in this study is for 1 hour in the morning at 6 am and in the afternoon at 4 pm.

The Vannamei shrimp blood was charged on the hemocytometer and then counted using a hand counter and with its calculation formula for Total Haemocyte Count (THC) (Brock *et al.*, 1991). Making blood-thinning preparations was to take the blood of Vannamei shrimp with a 1 ml syringe that has been given EDTA as an anticoagulant (Mahasri, 2007; Darwantin *et al.*, 2016). The number of Differential Haemocyte Count (DHC) cells was calculated to be up to 100 cells and the percentage was sought (Martin *et al.*, 1985).

The TPC calculation method was carried out aseptically on the laminar flow by means of samples that had been dissolved in sterile physiological NaCl taken 100 µl and diluted multi-level (10-1 to 10-5 dilutions), then 100 µl was taken from 10-3, 10-4 dilutions and 10-5 using the Duplo spread plate method on Nutrient agar media. The next step was incubating the incubator for 18-24 hours at room temperature (28-30°C) and after that, the colony was counted which grows based on SNI 01-2332.3-2006 (Kusdarwati *et al.*, 2016).

Bacterial isolation was carried out aseptically on laminar flow with the streak plate method, by taking one sample which had been dissolved in sterile physiological NaCl and scratched on TCBS media agar and incubated for 18-24 hours at room temperature (28-30°C). The colonies that grew later would be chosen based on differences in colour and size of the colonies that appeared purified. Purification was done by taking a culture selected and grown on NA saline (+ 2% NaCl) with the streak plate method. The purified culture incubated

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Table I. THC and DHC of Vannamei shrimp treated by aerator and *nanobubble*

Technology	Day	THC	DHC
Nanobubble	0	30	18.89
	10	40.68	22.68
	20	41.5	27.65
	30	45.23	30.23
Aerator	0	30	17.98
	10	27.6	16.6
	20	33	17.61
	30	29.63	15.6

Table II. TPC from Vannamei shrimp hepatopancreas and water media treated by aerator and *nanobubble*.

Technology	Day	TPC	
		Hepatopancreas	Water
Nanobubble	0	30.5	3.05
	10	18.75	1.525
	20	12.875	1.0475
	30	3.3	0.33
Aerator	0	33.25	3.425
	10	27	2.55
	20	10.45	0.9575
	30	16	1.35

for 18-24 hours at room temperature (28-30°C) (Letchumanan *et al.*, 2015). The pure colonies are then used for bacterial identification (Lubis *et al.*, 2014). Identification of bacteria was done by morphological observations, Gram staining, and biochemical testing.

Results and Discussion

The results of the THC and DHC could be seen in Table I which showed a significant difference between nanobubble treatment with aerators ($p < 0.05$).

THC and DHC in Vannamei shrimp with nanobubble treatment increased because, in nanobubble systems, oxygen in the waters could last longer so that it is utilized for the biological activity of organisms. Nanobubble systems produced dissolved oxygen for a long time so that it met the needs of organisms and decomposition of organic matter (Chiba and Takahasi, *loc. cit*). An increase in DHC indicates that the shrimp has improved health status.

TPC from shrimp hepatopancreas and in water media showed in Table II.

TPC on the aerator treatment showed a poor value of water quality than nanobubble since the ammonia and nitrite values were above the normal range for Vannamei shrimp. Poor water quality could cause stress on cultivated shrimp so that the growth of shrimp would be disrupted and reduced the body's resistance to infection and cause death (Diansari *et al.*, *loc. cit*).

The results of the identification of *Vibrio* sp. on Vannamei shrimp and water media could be seen in Table III.

The biochemical test results showed that the bacterial samples were *Vibrio vulnificus* and *Vibrio alginolitycus*. Several species of *Vibrio* bacteria were reported to be disease agents that caused a decrease in shrimp production, namely *V. vulnificus*, *V. splendidus* and *V. damsela* infection in *Penaeus monodon* larvae and *Vibrio alginolyticus* infection in *Penaeus orientalis* larvae (Wang *et al.*, 2005).

Nitrite and ammonia levels in the aerator treatment tended to be high, 1.25 ppm and 1.5 - 1.7 ppm. It was considered above the safe limit for Vannamei shrimp cultivation. The quality

Table III. Presence of *Vibrio* sp. in Vannamei shrimp treated by aerator and *nanobubble*

No.	Technology	Sample	Bacteria
1	Aerator	Water	*Vibrio vulnificus* (99,64%)
		Hepatopancreas	*Vibrio alginolitycus* (98,77%)
2	Nanobubble	Water	*Vibrio alginolitycus* (52,04%)
			Vibrio vulnificus (89,58%)
		Hepatopancreas	*Vibrio alginolitycus* (82,41%)

Table IV. Water Quality of Vannamei shrimp culture treated by aerator and *nanobubble*

Parameter	Technology	
	Nanobubble	Aerator
DO (ppm)	3,9 – 10,8	2,81 – 4,65
Temperature (°C)	27 – 29	27 – 29
Salinity (ppt)	19 – 20	19 – 20
pH	8 – 8,6	7,2 – 7,8
Nitrite (ppm)	0,57	1,25
Ammonia (ppm)	0,006	1,5 – 1,7

of nanobubble water was more stable compared to ordinary bubbles (Ebina *et al*., *loc. cit*). If air bubbles were not easily broken, the dissolved oxygen content in the waters would also be more stable.

Summary

Nanobubble could reduce the *Vibrio* sp. infection in Vannamei shrimp culture and maintain the water quality.

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PAGE 1

PAGE 2

PAGE 3