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SURVEILLANCE OF *VIBRIO* AND *BLUE-GREEN ALGAE* IN INTENSIVE SYSTEM OF PACIFIC WHITE SHRIMP *(LITOPENAEUS VANNAMEI)* IN SITUBONDO REGENCY, EAST JAVA, INDONESIA

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ABSTRACT

This study aims to investigate changes in the number of *Vibrio* bacteria and *Blue-Green Algae*, water quality, and production performance in farmed Pacific white shrimp with an intensive system in Situbondo, East Java, Indonesia. This study was conducted on seven pacific white shrimp intensive ponds during one cycle of maintenance period (84 days), April 2019-August 2019. Interestingly, the results showed that the highest *Total Vibrio Counts* (TVC) was obtained in the Farms A with a total of 31.2-83.2(2.2±8.9)[b], which followed by Farms C 16.4-71.6 (3.7±18.0)[ab] and Farms B 13.7-47.7 (2.6±13.6)[a] *($p<0.05$)*. Whereas the highest *Total Bacterial Count* (TBC) number was obtained in the Farms C with a total of 0.48-1.7 (95.3±50.7)[a], which followed by Farms A 0.20-4.2 (11.5±61.7)[a] and Farms B 0.13-0.80(28.1±11.4)[a], the highest green colony appeared on Farms C with a total 0-9.0 (1.8±36.0)[a], and followed by Farms B 0-3.8 (9.5±15.8)[a] *($p<0.05$)*. While on yellow colonies, appeared on high amount in Farms A with total 3.1-8.3 (5.7±22.0)[a], followed by Farms C 1.5-7.1 (3.7±18.3)[ab] and Farms B 0.9-4.5 (2.5±13.8)[a] *($p<0.05$)*. Percentage Blue-Green Algae on three Farms undergo fluctuation from May to August. Water quality parameters showed the normal range for all Pacific white shrimp ponds during the observation. The intensive culture system applied to Pacific white shrimp experiences dynamics that can affect aspects of microbiology, water quality, and production performance.

KEY WORD : Pacific White Shrimp, *Vibrio, Blue Green Algae*, Water quality, Production performance

INTRODUCTION

Aquaculture is one of the fastest-growing food production sector, expected to provide 60% of the fish available for human consumption by 2030 (FAO, 2016). Because of that, this sector has emerged as one of the most promising and viable growing industries for keeping the stride in providing nutritional benefits to help curb global food insecurity crises for humans. Around many aquaculture products, shrimp became the most-traded product on the global market, comprising around 15% of the total marine and freshwater production (FAO, 2018).

The Pacific white shrimp *(Litopenaeus vannamei)* became most popular shrimps on the market because of the advantage of growing faster than other shrimp species and able to live in the water column, which allows individuals to be stocked at high densities. Moreover, this species is more resistant to environmental conditions and diseases than other shrimp species, and it is quite popular on the international market because of their taste too (Rakhfid *et al.*, 2018).

Litopenaeus vannamei, like other shrimps, lacks humoral adaptive features and are thus very susceptible to viral, bacterial infections, and phytotoxin (Amoah Kwaku *et al*., 2020). As a result of their high susceptibility and farmers' shifting to intensive culturing in meeting the high growing demand. The application of an intensive system in pacific white shrimp cultivation is one of the success factors for productivity in several Asian countries, including Indonesia (Lukwambe *et al*., 2019). The application of this intensive system can be characterized by a high stocking density, maximum use of additional feed, and little water change activity that make this cultivation simpler to handle (Munjayana, 2019). Situbondo, East Java is one of the regions in Indonesia that implements an intensive system in developing pacific white shrimp cultivation.

However, in recent years, the intensification of shrimp can increase the outbreak of serious bacterial and viral disease problems, because nutrient loads and gradual hypertrophication make the increases of the chronic toxicity of the ammonia and nitrite that can help some viruses and algae grow faster (Thitamadee *et al*., 2015). There two major problems that affect the growth of *Litopenaeus vannamei* in intensive ponds, such as the blooming of Blue-Green Algae (BGA) and the rapid growth of Vibrio bacteria (Jiao *et al*., 2020).

The rapid proliferation of algae, especially cyanobacteria, can damage aquaculture systems, since the release of secondary metabolites and toxins or accumulated biomass affects co-occurring organisms and alters microecological balance on ponds circulation (Lemonnier *et al*., 2016; Sun *et al*., 2018). That will result in water quality deterioration and inflict damage on cultured shrimp during the growing process (Sun *et al*., 2018). The survival, growth, and production of shrimp are very low during and after *harmful algal blooms* (HABs), and it is more often accompanied by the outbreaks of severe diseases, especially can make Vibrio bacteria grows rapidly and become more pathogenic.

Although several studies have reported on disease agents such as bacteria in shrimp (Heri *et al*., 2019; Xiao *et al*., 2017), and algae blooming (Heri *et al*., 2019). Prevention efforts to minimize disease outbreaks in shrimp farming are still not effective. Likewise, the sustainability efforts in the aquaculture sector depend on the management of water circulation which can be useful for preventing the rapid growth of algae and *Vibrio* bacteria. Therefore, an assessment of the main factors such as water quality and fed frequencies must be carried out to be able to control the dynamics of bacteria and algae in the intensive system of Pacific White Shrimp intensive cultivation ponds so that they can obtain maximum production performance. This study aims to investigate changes in the number of Vibrio bacteria and Blue-Green Algae, and water quality in Pacific white shrimp with an intensive system in Situbondo, East Java, Indonesia.

MATERIALS AND METHODS

Time and location of research

The research was conducted in an intensive white shrimp pond in Situbondo, East Java, Indonesia for 84 days or one maintenance period, from May to August 2019. The ponds that were used as research objects were 7 pond units with a size of 2900-4000 m^2 with an average stocking density of 208 fry/m^2. All pond plots are equipped with *high-density polyethylene* (HDPE) on the walls and plastic mulch at the bottom of the pond and are equipped with 26 windmills as an aeration system. The average water level for each pond is 107.14 ± 9.511 cm. The addition of water is carried out about 8-10% of the total volume of water each week in an effort to replace water wasted due to sludge disposal, evaporation, and seepage (Munjaya, 2019).

Research procedure

Ponds preparation follows the procedure from Munjayana (2019). Pond preparation begins with liming the pond bottom using active lime CaO with an average dose of 39.71 ± 7.58 kg and drying for two weeks and installing mulch plastic for the pond bottom. The water used came from Tandon which was disinfected using $CuSO_4$ as much as 1.5 mg/l and left for 24 hours. Furthermore, 1.5 mg/l of dichlorvos was added and aerated using 4 windmills for 4 hours to be distributed throughout the ponds. At a water level of about 80 cm, disinfection was carried out using chlorine with a concentration of 30 mg/l and homogenized with pinwheel for 4 hours and added 1.5 mg/l of $CuSO_4$ and 1.5 mg/l of Pondfos 250 Ec and left for 3 days.

Chlorine was added again at a concentration of 30 mg/l followed by the addition of 40 l saponins the next day. The addition of fermented products is again adjusted to the plankton growth in the pond plots and can be done again when the water transparency increases and the plankton density begins to decline.

The probiotic Herobacillus bacteria (commercial product) was added 24 hours after application of the fementation product to the pond. The seeds are sown after the plankton grows with a water brightness ranging from 50-60 cm. The seeds are propagated by post larva stage (PL). Feed is given as much as 3-5% of the biomass with a frequency of 3-5 times / day which is adjusted to the age of maintenance.

Water sampling

Water sampling was carried out in seven plots of ponds with parameter testing divided into daily, weekly and monthly tests carried out directly (in situ) or through laboratory testing. Water samples were taken at a depth of 20 cm from the top of the pond. Water samples were taken using a sterile 500 mL plastic bottle with an instrument adjusted to the test parameters. Water quality parameters measured include dissolved oxygen (DO), pH, salinity, alkalinity, and ammonia (Wiradana *et al*., 2019).

Biological parameter analysis

Biological parameters were analyzed by measuring total viable *Blue Green Algae*, total bacterial counts (TBC), total *Vibrio* counts (TVC), yellow and green colonies, and *Vibrio* ratio measured every week until harvest time. Bacteria were analyzed using a spread platting method, namely water samples diluted in 1% saline and then spread on *nutrient agar* (NA), *seawater complex* (SWC) agar and *thiosulphate citrate bile salt sucrose* (TCBS) agar which were used to observe the growth of TBC, and TVC. The medium containing the sample was then incubated at a temperature of 30±2 °C for 24-48 hours to calculate the colony forming unit (CFU) (Marwiyah *et al*., 2019).

Data analysis

Data of water quality parameters and biological parameters obtained were collected and processed in Ms. Excel 2016. Furthermore, the data were inputted in SPSS 22.0 with the ANOVA test and to determine the difference in each parameter in each pond followed by the DUNCAN test with a confidence interval of 0.05.

RESULTS AND DISCUSSION

Bacteriology

Based on the data shown in Table 1, the TVC and TBC from the three Farms varied in number, but the highest TVC number was obtained in the Farms A with a total of 31.2-83.2(2.2±8.9)[b], which followed by Farms C 16.4-71.6 (3.7±18.0)[ab] and Farms B 13.7-47.7 (2.6±13.6)[a] ($p<0.05$).Whereas the highest TBC number was obtained in the Farms C with a total of 0.48-1.7 (95.3±50.7)[a], which followed by Farms A 0.20-4.2 (11.5±61.7)[a] and Farms B 0.13-0.80 (28.1±11.4)[a] ($p<0.05$). A study conducted by Alfiansah *et al*. (2018) and Supono *et al*. (2019) reports that the growth of bacteria like Vibrio and others heterotrophic bacteria relative high due to the organic matter deposited at the pond bottom, the leaching process from feed, the release of ammonium from shrimp feces, environmental factors and water exchange.

Nutrients from degradation of organic matter can support bacterial growth, which then causes changes in the physical parameters of the pond environment simultaneously such as pH, DO, suspended particulate matter (SPM), and turbidity (Alfiansah *et al*., 2018). Based on the data obtained, it can be seen that pond A has a high total bacterial abundance, it can be estimated that the feces excreted by the shrimp is quite high or the amount of feed accumulation is also high, resulting in a buildup in the pond sediment, which is then used by bacteria as nutrients to grow. In general, Vibrio is an opportunistic pathogenic bacterium that can be harmful to shrimp health if the shrimp's immune

Table 1. The number of bacteria from samples of pond water for maintenance of Pacific white shrimp with an intensive system in Situbondo District, East Java, Indonesia.

Parameters	Range (Mean±Standard deviation)		
	Farms A	Farms B	Farms C
Total Vibrio Counts (TVC) (10^5 CFU/ml)	31.2-83.2 (2.2±8.9)[b]	13.7-47.7 (2.6±13.6)[a]	16.4-71.6 (3.7±18.0)[ab]
Total Bacterial Counts (TBC) (10^6 CFU/ml)	0.20-4.2 (11.5±61.7)[a]	0.13-0.80 (28.1±11.4)[a]	0.48-1.7 (95.3±50.7)[a]
Green Colony (10^3CFU/ml)	0 (0)[a]	0-3.8 (9.5±15.8)[a]	0-9.0 (1.8±36.0)[a]
Yellow Colony (CFU/ml)	3.1-8.3 (5.7±22.0)[a]	0.9-4.5 (2.5±13.8)[a]	1.5-7.1 (3.7±18.3)[ab]

CFU : Colony Forming Units. No water samples were showing glowing bacterial colonies on SWC agar medium (n = 5). The values sharing common alphabet (a) within the row differed significantly (P <0.05).

system is decreased due to environmental stress (Marwiyah *et al*., 2019).

In this study, green and yellow colonies appeared on TCBS media whose numbers varied in the three observation ponds, with the highest green colonies appearing in pond C with the number 0-9.0 (1.8 ± 36.0)^{a}, and followed by Farm B 0-3.8 (9.5 ± 15.8)^{a}. However, no green colonies were found in Farm A. Whereas in yellow colonies, they appeared in high numbers in Farm A with the number 3.1-8.3 (5.7 ± 22.0)^{a}, followed by Farm C, 5-7.1 (3.7 ± 18.3)^{ab} and Farm B 0.9-4.5 (2.5 ± 13.8)^{a} ($p<0.05$).

The majority of these colors indicate the Vibrio species because they can produce bright colors on the TCBS medium. Studies have shown some vibrios can shift between yellow and green, due to genetic or physiochemical factors (Salomon *et al*., 2015). *Vibrio harveyi* and *Vibrio parahemolyticus* produced green colonies on TCBS agar, whereas *Vibrio alginolyticus* produced yellow colonies (Elle *et al*., 2017). As cited in Cadiz *et al*. (2016), mostly Vibrio spp. pathogens for shrimp will form green colonies when cultured in TCBS agar while yellow colonies are reported to have beneficial effects. The majority of yellow colonies will appear due to the acidification of the fermented sucrose medium by Vibrio which makes the blue bromthymol turn yellow, and this will lower the pH of the medium. The four bacteriological parameters indicate that the three observation ponds are still safe and good for shrimp growth, but must be controlled to ward off or reduce the factors that trigger the growth of pathogenic bacteria.

Blue-Green Algae (BGA)

Shrimp growth and productivity can be disrupted if there are an outbreak and disease (Nimrat *et al*., 2008). Blue-Green Algae fluctuations in intensive ponds can be caused by several factors such as water conditions and feed management. The percentage of BGA observed in the pacific white shrimp rearing pond showed that pond B had the highest percentage, especially at the beginning of the observation in May, which was 21.83% and again drastically decreased in June, namely 11.52% and again experienced a slow increase in July and August. The increase also occurred in Ponds A and C, namely 24% and 21.75%, respectively (Figure 1).

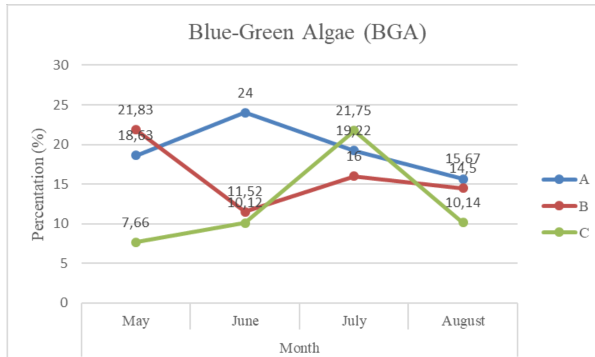

Fig. 1. Percentage (%) of Blue-Green Algae (BGA) fluctuations in the intensive system of Pacific white shrimp in Situbondo District, East Java, Indonesia (n = 7)

The amount of feed given depends on the biomass of the shrimp in the pond and will increase with the length of time for maintenance (Boyd *et al*., 2003). The results of monitoring conducted by Primavera (1994) on intensive ponds stated that 15% of the feed given would dissolve in water, while 85% that was eaten mostly was also returned to the environment in the form of waste (feces).

Blooming of Vibrio and Blue-Green algae can be caused by nitrogen and phosphate concentrations that exceed the quality standards for pond waters, thus triggering microalgae fragmentation and increasing silica concentrations in pond sediments. As much as 87% of silica at the bottom of the pond

Table 2. Physico-chemical characteristics of the ponds water samples of Pacific White Shrimp *(Litopenaeus vannamei)* intensive culture systems of the Situbondo Regency, East Java, Indonesia.

Parameters	Range (Mean±Standard deviation)		
	Farms in area A	Farms in area B	Farms in area C
pH	7.90 (7.90±0.00)^{ab}	7.90-8.00 (7.95±0.05)^{b}	7.80-8.00 (7.86±0.08)^{a}
Salinity (ppt)	45-46 (45.17±0.40)^{b}	43-46 (45.00±1.09)^{ab}	41-46 (43.33±2.06)^{a}
Dissolved Oxygen (DO) (ppm)	3.85-4.39 (4.06±0.17)^{a}	4.00-4.39 (4.17±0.10)^{a}	3.91-4.35 (4.15±0.16)^{a}
Alkalinity (ppm)	222-244 (235.6±8.23)^{a}	200-272 (230.67±27.7)^{a}	192-268 (227.0±31.3)^{a}
Ammonia (ppm)	0.00-0.50 (0.16±0.25)^{a}	0.00-5.0 (1.9±1.85)^{b}	1.00±3.0 (1.66±0.81)^{b}

Note : The values sharing common alphabet (a) within the row differed significantly ($P <0.05$). Each farm area has 6 maintenance ponds (n = 6).

is used by Vibrio and microalgae for their survival (Smith, 1996). In the graph, it is known that Vibrio and Blue-Green Algae have increased and decreased in fluctuating numbers, this can also be caused by the sediment filtering activity which is carried out once every 1-2 days. This aims to reduce the pile of sediment at the bottom of the pond. Putra *et al*., (2014), the more organic matter at the bottom of the feeding pond, the greater the total bacteria. Besides, sediment removal functions to improve water conditions that were initially unfavorable due to piles of sediment.

Water quality

The results of this study showed that the highest levels of DO and pH occurred in Farms B, which is 4.00-4.39 (4.17±0.10)[a] ppm and 7.90-8.00 (7.95±0.05)[b]. Meanwhile, the highest salinity and alkalinity levels were in the Farms A, with 45-46 (45.17±0.40)[b] ppt and 222-244 (235.6±8.23)[a] ppm, respectively. However, the highest ammonia level was in Farms C at 1.00±3.0 (1.66±0.81)[b] ppm. The data can be seen in Table 2.

The highest DO values were found in Farms B which had a large cultivation area, high stocking density, and high frequency of probiotic. This condition does not reduce the quality of oxygen contained so that it can inform that there is sufficient oxygen in the shrimp (Wang *et al*., 2020) and adding probiotic on the water can maintaining water quality so the nutrient cycle for shrimps in balance. Also, a large area of cultivation can reduce the level of competition between shrimp for utilizing oxygen supply on water.

Meanwhile, a high density can increase a high pH value (alkaline conditions). The administration of molasses aims to activate the probiotics that are given into the Farms so that one of the goals is to optimize the growth of shrimp fry. Optimization of this growth causes abundant secretion from shrimp, which affects the higher pH level (Wang *et al*., 2020).

CONCLUSION

The fluctuation and survival of Vibrio and Blue-Green Algae in intensive pacific white shrimp ponds were known to be influenced by water quality and feeding, when water quality and sediment were stable, the growth of Vibrio and Blue-Green Algae did not exceed the limit. Increasing feeding according to shrimp biomass will also increase organic matter in the pond, especially nitrogen and phosphate concentrations, which will ultimately support the growth of Vibrio and Blue-Green Algae. High concentrations of Vibrio and Blue-Green Algae can reduce shrimp productivity and even cause death in shrimp.

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