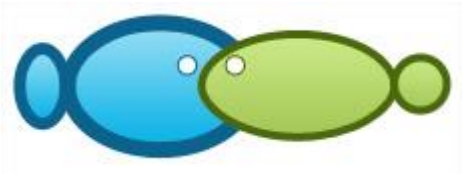

Toxicity of nitrite to vaname shrimp post larvae 15 at rearing salinity of 15 g L^{-1}

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Abstract. Nitrite is a compound that comes from feed waste and metabolic waste which is degraded into ammonia/ammonium with the nitrification process. Nitrite that is > 0.06 mg L^{-1} can be toxic and cause disease in shrimp. This study aims to determine the toxicity of nitrite (LC_{50}) and the physiological response of 15 days post larvae (PL 15) of white vaname shrimp in water with salinity of 15 g L^{-1}. This research has three stages of testing, namely preliminary test, acute test, and sublethal test using a completely randomized design. Preliminary tests resulted in an upper threshold concentration (LC_{100}-24 hours) of 40 mg L^{-1} NO_2-N and a lower threshold concentration (LC_0-48 hours) of 2.5 mg L^{-1} NO_2-N. The acute test in this study resulted in an LC_{50} value of 24, 48, 72, and 96 hours NO_2-N against white shrimp PL 15, respectively 31.24, 21:37, 17.36, and 10.28 mg L^{-1} NO_2-N. The concentrations of nitrite used in the sublethal test were control (0 mg L^{-1}) A (1 mg L^{-1}), B (3 mg L^{-1}), and C (5 mg L^{-1}). The results of the sublethal test showed that nitrite had an effect on decreasing survival rate, absolute weight growth, and absolute length growth as well as increasing the total hemocyte count (THC) value and hemolymph glucose levels of white shrimp.

Key Words: LC_{50}, nitrite, toxicity, white shrimp.

Introduction. Opportunities for Indonesian aquaculturists to achieve increased vannamei shrimp production continue to experience problems with various dynamics of water quality that continues to decline and the increasing frequency of outbreaks of diseases caused by viruses, bacteria or toxins in the environment (Kilawati & Maimunah 2015). Environmental stability in terms of physics, chemistry and biology continues to be pursued to obtain maximum shrimp production. The environment and the parameters in it influence each other which can also have a double effect on the stability of the shrimp. Salinity in Indonesian shrimp rearing water is generally between 15 and 30 g L^{-1} with high stocking density (super intensive system). Bray et al (1994) stated that vannamei shrimp (*Litopenaeus vannamei*) cultivated at a salinity of 15 g L^{-1} had higher survival and specific growth rates than vannamei shrimp cultivated in a salinity of more or less than 15 g L^{-1}. Salinity of living media can determine the level of osmotic work of organisms and affect survival and growth (Hastuti et al 2015).

Along with increasing production with various dynamic rearing media, shrimp aquaculturists are always faced with increasing levels of various inorganic compounds due to feeding activities and biota metabolic waste. Inorganic compounds are sourced from sources of organic nitrogen compounds in the environment from feed residues, metabolic wastes, decomposition products, macro-micro organisms, algae, pond bottom fertilization, and so on (Komarawidjaja 2006). The balance of the shrimp culture environment requires the stability of the activity of bacteria in the water which degrades organic nitrogen compounds into inorganic nitrogen compounds. Inorganic nitrogen compounds that are toxic are ammonia and nitrite, while nitrate in excess will cause a plankton boom and eutrophication. Inorganic nitrogen in excess in the form of ammonia (NH_3-N), nitrite (NO_2-N) and nitrate (NO_3-N) can affect the quality of aquaculture water.

The relationship of toxic excess inorganic nitrogen compounds to various water quality conditions in the environment has not been studied in detail. Nitrite is a nitrogen oxide compound that is toxic to fish. Nitrite can naturally be formed from the activity of nitrifying bacteria that convert ammonia to nitrite (Hastuti 2011) as well as the activity of denitrifying bacteria. Although nitrite in aquaculture waters can be reduced by regular water changes and water dilution. Apart from being inconsistent, this method also has drawbacks, namely the presence of uncontrolled additional costs that lead to the entry of new pathogens into the cultivation container (Sangnoi et al 2017). Conditions in the field show that the level of nitrite toxicity to water quality is not always the same. The concentration of salt content in different environments is considered to be very influential on the toxicity of nitrite. Each phase of the shrimp in its life cycle has its own challenges during its growth. Lin & Chen (2003) stated that the juvenile phase of 3 g of shrimp cultured at salinities of 15, 25, and 35 g L^{-1} had a lethal value of 50% (LC_{50}) to nitrite for 96 consecutive hours with a concentration of 77 mg L^{-1}, 178 mg L^{-1}, and 322 mg L^{-1}. Along with the increasingly dynamic quality of water sources in the environment and for smooth acclimatization of early stage rearing shrimp, it is necessary to evaluate the toxicity of nitrite in the post-larval (PL) vaname shrimp at 15 g L^{-1} salinity. The aim of this study was to evaluate the impact of nitrite toxicity on the physiological response of PL vannamei shrimp 15 at rearing salinity of 15 g L^{-1}.

Material and Method

Time and location of research. This study was conducted at Environmental Laboratory and Fish Health Laboratory Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, during July-August 2019. Analysis of hemolymph cell fragility and body glucose levels were analyzed at the Fish Nutrition Laboratory, Faculty of Fisheries and Marine Sciences, Bogor Agricultural.

Materials. During the study, to test the toxicity of nitrite compounds on vaname shrimp larvae, an aquarium container measuring (20×40×40) cm^3 was used for 10 liters of culture media. The test biota used in this study were 10 vaname shrimp for each treatment (preliminary test and acute test) in the post larval phase (PL) 15 and came from CV *Star Sea* Marunda, Jakarta. The test material used was sodium nitrite ($NaNO_2$) sea salt. The stock solution of nitrite (NO_2-N) was prepared at a concentration of 5000 mg L^{-1}. Then a standard curve was made with the aim of finding the relationship between the nitrite concentration and the measured nitrite absorbance value. The relationship was then entered into a linear equation. The concentration of nitrite used in the preparation of the standard curve was: 0; 0.001; 0.002; 0.004; 0.01; 0.02; 0.025; 0.05; 0.1; 0.2; 0.5 mg L^{-1} NO_2-N. Each nitrite concentration was tested using sulfanilamida-NED method, following APHA (2005).

Preliminary test. Preliminary tests are required to obtain upper and lower threshold concentrations for the toxic substances used. The upper threshold concentration of nitrite is a 100% lethal concentration of nitrite for 24 hours (LC_{100}-24 hours) while the lower threshold concentration of nitrite is a concentration of nitrite that cannot kill 100% of the vaname shrimp population for 48 hours (LC_0-48 hours). The preliminary test was carried out for 48 hours using a completely randomized design (CRD). The concentrations used in the preliminary test stage are control (0), 2.5, 5, 10, 20, and 40 mg L^{-1} NO_2-N each with two replications (Lukmini et al 2016). The water media and test materials used in the preliminary test were replaced every day (Ramírez-Rochín et al 2017). Shrimp mortality was observed at 0, 12, 24, and 48 hours. During the preliminary test, aeration was given and the temperature was maintained at 29-32°C.

Acute test. Acute test aims to obtain LC_{50} (concentration of toxic compounds that can kill the test biota as much as 50% of the population). The acute test was carried out for 96 hours with 15 aquarium containers measuring (20×40×40) cm^3. The water media and test materials used in the preliminary test were replaced every day (renewal method)

(Ramírez-Rochín et al 2017). The test biota used were 10 shrimp with 10 liters of culture media. Observation of vaname shrimp mortality in the acute test was carried out every 24, 48, 72, and 96 hours. The acute test used control, 5, 10, 20, and 40 mg L^{-1} NO_2-N with three replications. The concentration used during the acute test treatment came from the preliminary test which was calculated into the 1985 pesticide commission formula (Anggraini et al 2019).

Sublethal test. The sublethal test uses concentrations below concentrations that can directly cause the death of the test animals. The sublethal test aims to determine the effect of nitrite on survival rate, absolute length growth, absolute weight growth and physiological response of vannamei shrimp (total hemocyte count (THC) and glucose levels). The sublethal test was carried out for 14 days using an aquarium container measuring (20×40×40) cm^3. The water medium used was 10 L and 40 vaname shrimp were used. Media water and toxic materials used in the sublethal test were replaced every day (renewal method) (Ramírez-Rochín et al 2017). The concentrations of sublethal test were derived from percentages of 0% (control), 10% (treatment A), 30% (treatment B) and 50% (treatment C) from LC_{50} at 96 hours.

Water quality parameters. Analysis of water quality parameters on vaname shrimp rearing media was carried out periodically and continuously.

Temperature, dissolved oxygen (DO), pH and salinity. Temperature, DO, pH and salinity were measured periodically three times a day using a digital measuring instrument. While the value of nitrite, nitrate, total ammonia nitrogen (TAN) and alkalinity was carried out continuously every day at the time of water change (APHA 2005).

Nitrite. Nitrite value was measured using the spectrophotometric method with 10 mL of water sample (dilution adjusted to the concentration of the standard curve that has been made) was inserted into a test tube then added 4 drops of sulfanilamide solution and 2 drops of NED solution (APHA 2005).

Nitrate. The nitrate value was measured using the spectrophotometric method with 5 mL of water sample being put into a test tube and then adding 0.5 mL of brucin solution and 5 mL of concentrated H_2SO_2 solution. The cooled solution was measured using a spectrophotometer with a wavelength of 410 nm (APHA 2005).

Total ammonia nitrogen (TAN). TAN measurements were carried out every day during water changes using spectrophotometry and the addition of nitrite stock solution in each treatment. The water sample that has gone through the distillation stage was put into a test tube as much as 10 mL plus 1 drop of $MnSO_4$ solution, 0.5 mL of chlorox and 0.6 mL of phenate. The test tube was closed then the solution was homogenized and allowed to stand for 15 minutes (APHA 2005).

Alkalinity. The alkalinity value was measured every day during water changes and when the nitrite stock solution was added. Alkalinity was measured using the titration method. A total of 25 mL of water sample was put into an erlenmeyer then added 3 drops of BcgMR solution (APHA 2005).

Abundance of nitrifying bacteria. Measurement of the abundance of nitrifying bacteria was carried out at the peak sublethal test (day 14). The abundance of nitrifying bacteria was calculated using the total plate count (TPC) method nitrifying medium (Madigan et al 2018). The single bacterial colonies that grew on the nitrifying medium were then purified and identified the type of bacteria that grew. Identification of bacteria was using morphological observations (shape, margins, elevation, and colony color), biochemical tests (oxidative-fermentative (O/F) test), oxidative test, catalase test, motility test, and oxidase test) and Gram staining and then identified using a table (Barrow & Feltham 1993).

Production parameters

Survival rate. Survival rate (SR) was calculated by comparing the number of vannamei shrimp at the beginning (H0) and end (H14) of the sublethal test stage. The SR can be calculated using the following equation (Zonneveld et al 1991):

$$SR = \frac{Nt}{N0} \times 100$$

where: SR = survival rate (%);
N0 = number of shrimps at the beginning of the rearing period;
Nt = number of shrimps at the end of the rearing period.

Absolute weight growth. The absolute weight growth (AWG) was calculated by subtracting the final weight from the initial weight of vaname shrimp. Data on the initial weight of white vaname shrimp was taken at the sublethal test stage on day 0 (H0) while the final weight data of vaname shrimp was taken on day 14 (H14). The AWG can be calculated using the following equation (Effendie 2002):

$$AWG = Wt - W0$$

where: AWG = absolute weight growth (g);
Wt = shrimp final weight (g);
W0 = shrimp initial weight (g).

Absolute length growth. Absolute length growth (ALG) was calculated by subtracting the final length from the initial length of the white shrimp. Data on the initial length of vaname shrimp were taken at the sub-lethal test stage on day 0 (H0), while data on the final length of vaname shrimp were taken on day 14 (D14). The ALG can be calculated using the following equation (Effendie 1979):

$$ALG = Lt - L0$$

where: ALG = absolute length growth (cm);
Lt = shrimp final length (cm);
L0 = shrimp initial length (cm).

Physiological response test of shrimp biota

Total hemocyte count (THC). The THC value of the test biota was measured by calculating the observed hemolymph of the shrimp as an indicator. Shrimp hemolymph was added with anticoagulant 2 times from the amount of hemolymph then homogenized. Hemolymph was taken with a micropipette and then dropped on the hemacytometer. Total haemocytes were observed under a light microscope with 100 times magnification (Tampangallo et al 2012).

Glucose level. Blood glucose levels in vaname shrimp were measured using a spectrophotometric method by destroying the body of white vaname shrimp. Phenolphthalein was added and then titrated using 30% NaOH until the color changed to pink. Glucose levels can be calculated using this following equation (Wedemeyer & Yasutake 1977):

$$\text{Bloog glucose (mg dL}^{-1}) = \frac{\text{Sample absorbant}}{\text{Blank absorbant}} \times 100$$

Data analysis. Data analysis was carried out quantitatively and descriptively. Probit analysis was carried out with the help of minitab 16 application with 95% confidence interval. The main water quality data (nitrite, nitrate, and TAN), SR, AWG and ALG were statistically analyzed using the SPSS 22.0 IMB.

Results

Preliminary test. Preliminary test stages produced upper and lower threshold concentrations of nitrite by measuring vaname shrimp mortality. Mortality in the preliminary test was observed at 0, 12, 24, and 48 hours. It was found that a concentration of 40 mg L^{-1} NO_2-N produced 100% mortality for 24 hours while the control and a concentration of 2.5 mg L^{-1} NO_2-N produced mortality 0% for 48 hours. The upper threshold value obtained was 40 mg L^{-1} NO_2-N and the lower threshold was 2.5 mg L^{-1} NO_2-N (Table 1).

Table 1

Mortality in the preliminary test

Nitrite concentration (mg L^{-1})	*Number of shrimp (head)*	*Mortality at hour (%)*			
		0	*12*	*24*	*48*
Control	10	0	0	0	0
2.5	10	0	0	0	0
5	10	0	0	5	5
10	10	0	0	10	15
20	10	0	0	20	30
40	10	0	5	100	100

Acute test. The concentrations of nitrite used in the acute test were control, 5, 10, 20, and 40 mg L^{-1} NO_2-N. Mortality in the acute test was observed every 0, 24, 48, 72 and 96 hours. The results of the acute test showed that a concentration of 40 mg L^{-1} NO_2-N resulted in a mortality of 70% at the 24th hour and 100% at the 48, 72 and 96 hours. The lowest mortality was found in controls at 0% at 24, 48, 72, and 96 hours. Mortality in the acute test is presented in Figure 1. LC_{50} nitrite value based on exposure time of 24, 48, 72, and 96 hours were analyzed using probit analysis with the help of minitab 16 software. The longer the exposure time of nitrite, the value of LC_{50} nitrite decreased (Table 2).

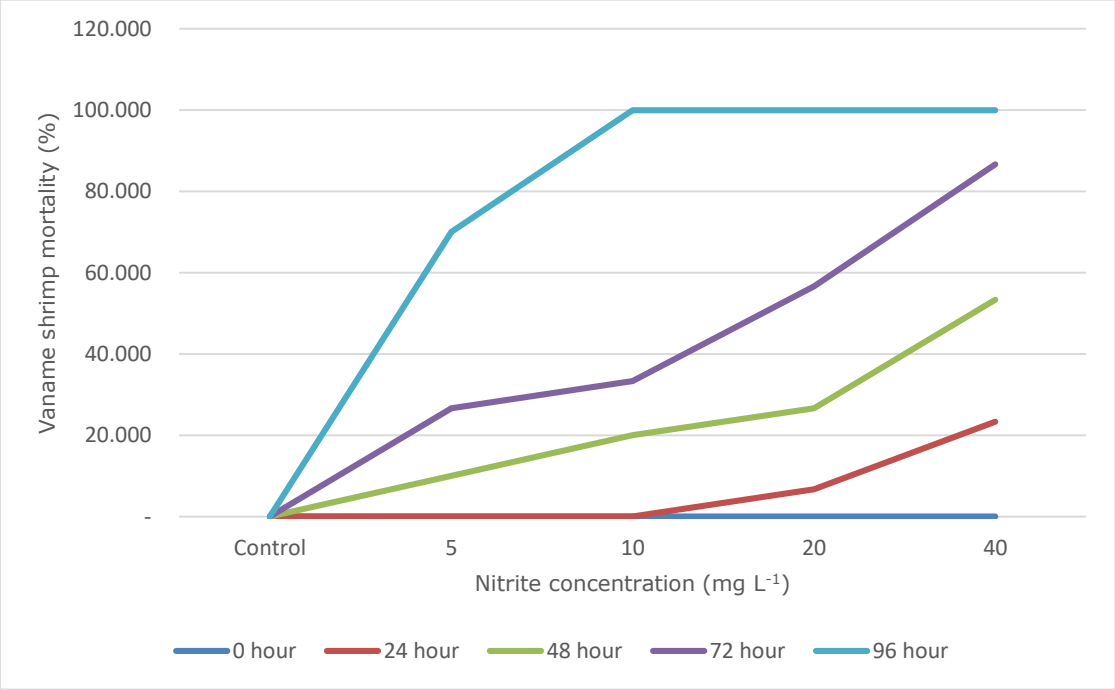

Figure 1. Level of nitrite concentration and mortality of white vaname shrimp at a certain time of exposure.

Table 2

LC_{50} values and nitrite concentration at certain time exposures

LC_{50}	*Nitrite concentration (mg L^{-1})*	*95% confidence interval (mg L^{-1})*
24 hours	31.24	26.89-37.31
48 hours	21.37	18.49-23.36
72 hours	17.36	14.69-20.96
96 hours	10.28	7.57-12.77

Water quality. Temperature during the acute test ranged from 28.5 to 31.5°C, DO ranged from 4.5 to 5.5 mg L^{-1}, pH ranged from 7.1 to 7.3, alkalinity ranged from 52 to 84 mg L^{-1}. Statistical analysis showed that the nitrite concentration was significantly different for each treatment, the nitrate concentration in the 5, 10, 20, and 40 mg L^{-1} nitrite treatments was significantly different from the control treatment with TAN concentrations were not significantly different in each treatment (Table 3).

Table 3

Value of water quality at various concentrations of nitrite

Parameter	*Nitrite concentration (mg L^{-1})*				
	Control	*5*	*10*	*20*	*40*
Temperature (° C)	29-31	29-31	29-31	29-31.5	28.5-30.5
DO (mg L^{-1})	4.5-5.3	4.7-5. 5	4.7-5.4	4.5-5.4	4.6-5.5
pH	7.1-7.2	7.1-7.3	7.1-7.2	7.2-7.1	7.2-7.1
Alkalinity (mg L^{-1})	56-75	60-80	56-84	52-84	60-80
Nitrites (mg L^{-1})	0.009 ± 0.001^{a}	4.996 ± 0.192^{b}	10.04 ± 0.328^{c}	20.2 ± 0.469^{d}	40.129 ± 0.429^{e}
Nitrates (mg L^{-1})	0.063 ± 0.009^{a}	0.17 ± 0.032^{b}	0.171 ± 0.265^{b}	0.155 ± 0.216^{b}	0.174 ± 0.199^{b}
TAN (mg L^{-1})	0.024 ± 0.003^{a}	0.019 ± 0.003^{a}	0.021 ± 0.012^{a}	0.022 ± 0.007^{a}	0.021 ± 0.006^{a}
Salinity (g L^{-1})	15	15	15	15	15

Note: Different superscripts on the same row indicate there are significant differences between treatments.

The water temperature values during the sublethal test ranged from 28.5 to 31°C, DO ranged from 4 to 5.5 mg L^{-1}, pH ranged from 7.1 to 7.3, alkalinity ranged from 48 to 96 mg L^{-1}.

Statistical analysis shows that different nitrite concentration treatments produce different statistical results on nitrite, nitrate and TAN parameters. In the nitrite and nitrate parameters, the nitrite concentration treatment showed that treatment A (1 mg L^{-1}) was significantly different from the control treatment, while treatments B (3 mg L^{-1}) and C (5 mg L^{-1}) were significantly different from treatment A and control. Meanwhile, in terms of TAN parameters, nitrite concentration did not show statistical differences between treatments (Table 4).

Table 4

Quality of sublethal test water

Parameter	*Nitrite treatment*			
	Control	*A (1 mg L^{-1})*	*B (3 mg L^{-1})*	*C (5 mg L^{-1})*
Temperature (°C)	28.5-31	28.5-31	29-31	29-31
DO (mg L^{-1})	4.1-5.5	4-5.4	4.2-5.5	4.3-5.4
pH	7.1-7.2	7.1-7.3	7.2-7.1	7.1-7.3
Alkalinity (mg L^{-1})	48-96	48-84	48-88	52-96
Nitrite (mg L^{-1})	0.002 ± 0.002^{a}	1.001 ± 0.072^{b}	3.06 ± 0.042^{c}	5.013 ± 0.009^{d}
Nitrate (mg L^{-1})	0.033 ± 0.006^{a}	0.145 ± 0.115^{b}	0.168 ± 0.055^{c}	0.174 ± 0.011^{c}
TAN (mg L^{-1})	0.028 ± 0.006^{a}	0.036 ± 0.004^{a}	0.031 ± 0.006^{a}	0.031 ± 0.004^{a}
Salinity (g L^{-1})	15	15	15	15

Note: Different superscripts on the same row indicate there are significant differences between treatments.

Abundance, morphology and biochemical assay of nitrifying bacteria. The abundance of nitrifying bacteria is presented in Table 5. The highest abundance of nitrifying bacteria was found in treatment C of 4.62 x 10^3 CFU mL^{-1}. While the lowest bacterial abundance was found in the control treatment at 1.48×10^3 CFU mL^{-1} (Table 5).

Table 5

The abundance of nitrifying bacteria at different nitrite concentrations

Nitrite treatment	*Abundance (CFU mL^{-1})*
C (control)	1.48 x 10^3
A (1 mg L^{-1})	2.16 x 10^3
B (3 mg L^{-1})	4.40 x 10^3
C (5 mg L^{-1})	4.62 x 10^3

The results of the morphological test were obtained, the colonies that grew on the TPC had a milky white color, entire edges, and flat and raised elevations. Nitrifying bacterial cells are Gram negative and bacilli-shaped (Table 6).

Table 6

Morphology and Gram staining of nitrifying bacteria at different nitrite concentrations

Isolate	*Colony*			*Cell*		
	Color	*Edge*	*Elevation*	*Form*	*Gram*	*Form*
K_1	Milky white	Entire	Flat	Irregular	Negative	Bacilli
K_2	Milky white	Entire	Raised	Irregular	Negative	Bacilli
A_1	Milky white	Entire	Raised	Irregular	Negative	Bacilli
A_2	Milky white	Entire	Raised	Irregular	Negative	Bacilli
B_1	Milky white	Entire	Raised	Irregular	Negative	Bacilli
B_2	Milky white	Entire	Raised	Irregular	Negative	Bacilli
C_1	Milky white	Entire	Flat	Irregular	Negative	Bacilli
C_2	Milky white	Entire	Raised	Irregular	Negative	Bacilli

Biochemical test results showed that all isolates of nitrifying bacteria were oxidizing glucose, oxidase positive, motile and catalase positive (Table 7).

Table 7

Biochemical tests of nitrifying bacteria at different concentrations of NO_2-N

Isolate	*Biochemical test*				*Genus*
	O/F	*Oxidase*	*Motile*	*Catalase*	
K_1	O	+	+	+	*Pseudomonas* sp.
K_2	O	+	+	+	*Pseudomonas* sp.
A_1	O	+	+	+	*Pseudomonas* sp.
A_2	O	+	+	+	*Pseudomonas* sp.
B_1	O	+	+	+	*Pseudomonas* sp.
B_2	O	+	+	+	*Pseudomonas* sp.
C_1	O	+	+	+	*Pseudomonas* sp.
C_2	O	+	+	+	*Pseudomonas* sp.

Survival rate. Survival rate (SR) of vannamei shrimp sublethal test stage is presented in Figure 2. The lowest SR of vannamei shrimp in sublethal test stage was in treatment C which was 83.33±6.291% while the highest SR was in control treatment which was 99.17±1.443%.

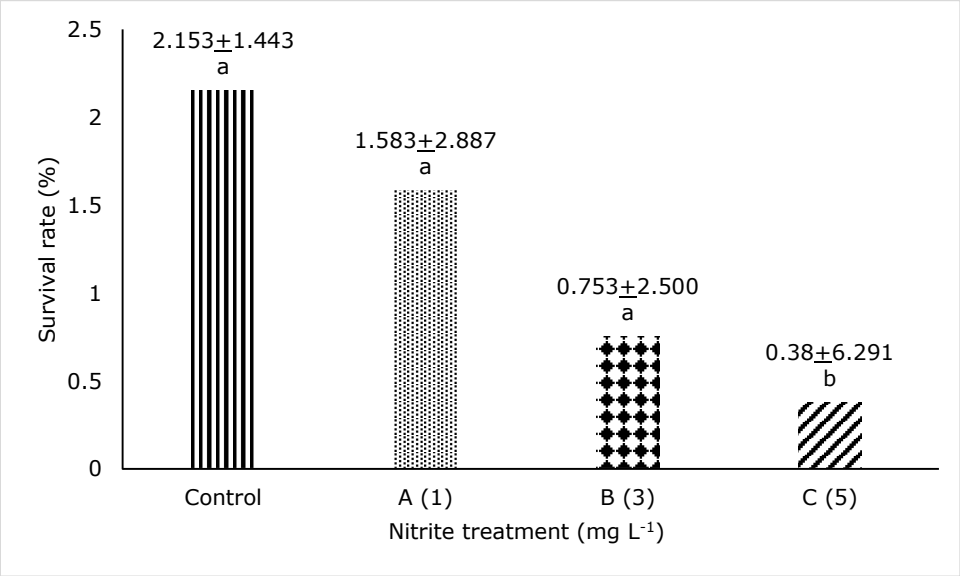

Figure 2. Survival rate of vaname shrimp in the sublethal test during 14 days of maintenance in the control, A (1 mg L^{-1}), B (3 mg L^{-1}), and C (5 mg L^{-1}) treatments.

Absolute weight growth. The absolute weight growth (AWG) of vannamei shrimp in the sublethal test stage is presented in Figure 3. The lowest AWG of vannamei shrimp in the sublethal test stage was found in treatment C, which was 0.018±0.006 g, while the highest result was found in the control treatment, which was 0.076±0.003 g.

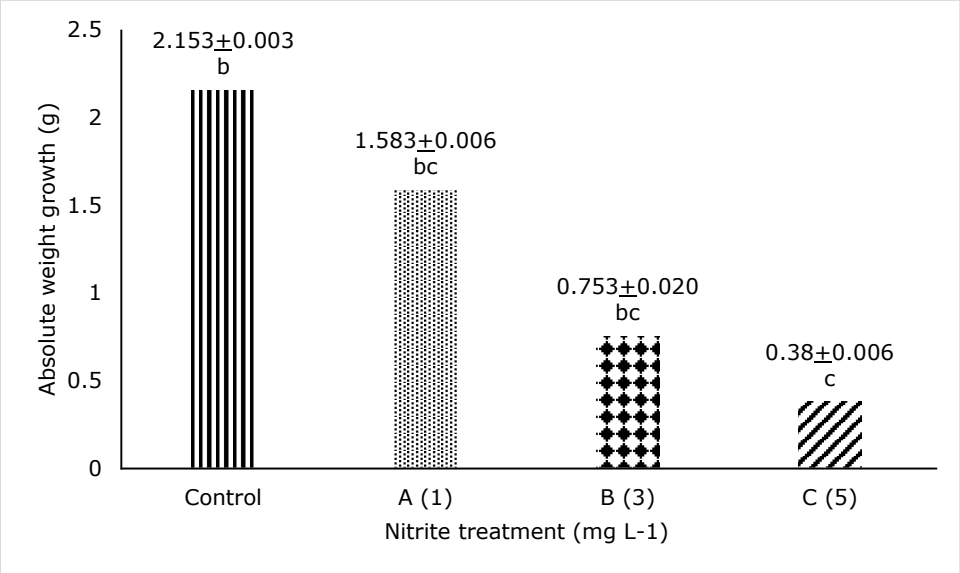

Figure 3. Growth in absolute weight of vannamei shrimp in sublethal test stage for 14 days of rearing in the control, A (1 mg L^{-1}), B (3 mg L^{-1}), and C (5 mg L^{-1}) treatments.

Absolute length growth. The absolute length growth (ALG) of vaname shrimp in the sublethal test stage is presented in Figure 4. The lowest ALG of vaname shrimp in the sublethal test stage was found in treatment C, which was 0.380±0.095 cm, while the highest yield was found in the control treatment, which was 2.153±0.106 cm.

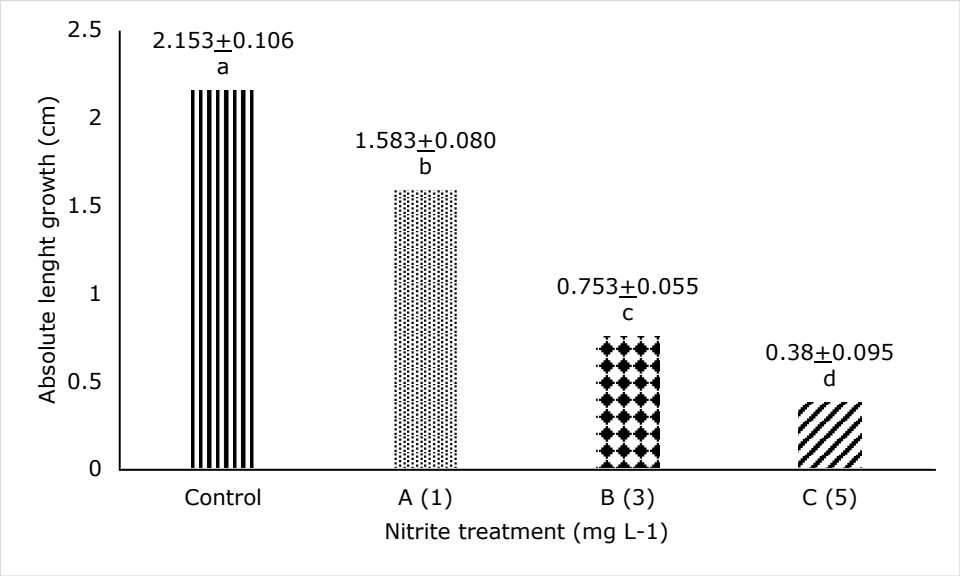

Figure 4. Absolute body length growth of vannamei shrimp sub-lethal test stage for 14 days of rearing in the control, A (1 mg L^{-1}), B (3 mg L^{-1}), and C (5 mg L^{-1}) treatments.

Physiological response test. THC conditions before the sublethal test (H0) ranged from 7-8.3×10^5 cells mL^{-1} and at the end of the sublethal test (H14) there was an increase in THC levels in all treatments. The highest THC level was found in treatment C of 34.7×10^5 cells mL^{-1} and the lowest THC level was found in the control treatment of 8.3×10^5 cells mL^{-1}. H0 glucose conditions ranged from 0.29-0.46 mgdL^{-1} and at H14 there was an increase in glucose levels in treatments A, B, and C (Table 8). In addition, there was an increase in sugar levels before the sublethal test (H0) and at the end of the sublethal test (H14) in each treatment.

Table 8

Physiological response of vannamei shrimp sublethal test stage in control, A (1 mg L^{-1}), B (3 mg L^{-1}), and C (5 mg L^{-1}) treatments

Treatment	*THC (×10 ^{5} cells mL^{-1})*		*Glucose (mg dL^{-1})*	
(mg L^{-1})	*H0*	*H14*	*H0*	*H14*
Control	8	8.3	0.46	0.29
A (1)	7.5	16.5	0.35	0.89
B (3)	7	22.3	0.44	1.81
C (5)	8.3	34.7	0.29	3. 38

Discussion. In the preliminary test, control treatment and a concentration of 2.5 mg L^{-1} NO_2-N produced the lowest vaname shrimp mortality in the preliminary test at 48 hours, namely 0%. Vannamei shrimp in the control did not die, which means that the control treatment did not contain toxicants. The concentration of 2.5 mg L^{-1} NO_2-N was used as the lower threshold concentration. The upper and lower threshold concentrations were then used as the basis for determining the concentration of nitrite (NO_2-N) in the acute test.

In acute test, the highest acute test mortality at 48, 72, and 96 hours was found at a concentration of 40 mg L^{-1} NO_2-N at 100%. Meanwhile, the lowest acute test mortality at 24, 48, 72, and 96 hours was in the control at 0%. LC_{50} value nitrite based on exposure time of 24, 48, 72, and 96 hours were analyzed using probit analysis with the help of minitab 16 software. The longer the exposure time of nitrite, the value of LC_{50} nitrite produced decreased (Table 2). Lin & Chen (2003) conducted a study on nitrite toxicity in vaname prawns with the size of 3.9 g kept in 15 g L^{-1} water salinity and produced LC_{50} nitrite at 24, 48, 72, and 96 hours, respectively, which was 187.9; 142.2;

92.5; and 76.5 mg L^{-1} NO_2-N. The decreasing nitrite LC_{50} results will affect the length of exposure time to nitrite (Lin & Chen 2003). The death of vaname shrimp during the acute test was thought to be due to interference with oxygen binding by the vaname shrimp hemolymph. This is in accordance with the statement of Hastuti et al (2017) that the presence of nitrite in aquaculture waters can form methemoglobin which can no longer bind oxygen. In addition, prolonged exposure to nitrite can increase the concentration of urea in hemolymph (Chen & Cheng 2000).

During the acute and sublethal tests, the environmental conditions of the vaname shrimp were also observed. Water quality testing includes temperature, DO, pH, alkalinity, nitrites, nitrates, and TAN. Temperature 29°C is the best temperature for the growth and survival of vaname shrimp (Panjaitan 2012). According to Rosenberry (1997) vaname shrimp can still grow at a temperature range of 25-35°C. DO in this study ranged from 4.5 to 5.5 mg L^{-1} in the acute test and from 4 to 5.5 mg L^{-1} in the sublethal test. Vannamei shrimp can still live optimally at DO levels > 3 mg L^{-1} and normal pH is 7.1-7.3. The alkalinity in this study ranged from 52 to 84 mg L^{-1} in the acute test and from 48 to 96 mg L^{-1} in the sublethal test. The optimum alkalinity in shrimp culture ranged from 35 to 88 mg L^{-1}. The concentration of TAN in this study was not significantly different in each treatment during the acute and sublethal tests. The concentration of TAN in this study was still at the optimum level that did not cause toxicity to white vaname shrimp, which was < 0.25 mg L^{-1} (Wu et al 2012). Nitrates in each treatment in this study were still included in the optimum standard level of < 80 mg L^{-1} NO_3-N (Talib et al 2017).

In addition to observing environmental conditions, the activity of microorganisms was also observed through counting nitrifying bacteria. The abundance of nitrifying bacteria in the control, A, B, and C treatments, was 1.48×10^3, 2.16×10^3, 4.40×10^3, and $4.62\text{x}10^3$ CFU mL^{-1}, respectively. At the end of the sub-tetal test, the abundance of nitrifying bacteria was calculated for each treatment. There were differences expressed by nitrifying bacteria at each nitrite concentration. Nitrifying bacteria have the nitrogenase enzyme which can convert ammonia into nitrite and nitrite into nitrate. Nitrite can be used by these bacteria for metabolism and to reproduce. Therefore, the higher the nitrite concentration in this study will result in the highest abundance of nitrifying bacteria. In addition to the nitrite factor, the increasing abundance of nitrifying bacteria can be influenced by optimal pH, temperature, light, dissolved oxygen, and salinity (Merani 2017). The presence of nitrifying bacteria can reduce the concentration of nitrite over time. Therefore, to maintain the nitrite concentration in the test container, this study used the new renewal method (water change) every 24 hours as much as 100% (Ramírez-Rochín et al 2017). Based on the results of biochemical tests identified using the table of Barrow & Feltham (1993), nitrifying bacteria are thought to belong to the genus *Pseudomonas* sp. with colony morphology characteristics such entire edge, dominantly raised elevation and milky white color. Bacterial cell morphology is Gram negative and bacilli shaped. Some *Pseudomonas* sp. belong to heterotrophic bacteria (Cheatham 2009). Heterotrophic nitrifying bacteria have a relatively fast growth rate compared to autotrophic nitrifying bacteria (Agustiyani et al 2004). According to Kumar et al (2013), *Pseudomonas* sp. are a bacteria that play a role in degrading nitrogen compounds and are one of the probiotics in the wastewater treatment of the fishing industry.

To determine the effect of 15 g L^{-1} salinity on vaname shrimp, it was necessary to know the SR, absolute weight growth (AWG), and physiological responses which include calculating hemolymph and glucose levels. The SR of vaname shrimp in the sublethal test stage was in the range of 83-99%. The SR in aquaculture production of 80 to 90% can still be said to be good (Kadarini & Prihandani 2011). The results of the statistical test for AWG showed that treatments A and B were not significantly different from the control, while treatment C was significantly different from the control. While the results of the ALG statistical tests for each treatment were significantly different. This was due to an increase in stress on vaname shrimp due to the influence of nitrite. Most of the energy is used for adaptation and body metabolism, while the remaining energy will be used for the growth and development of vaname shrimp. However, if vaname shrimp is stressed, some of their energy will be used to overcome this stress. Therefore, stress conditions

can affect metabolism. The results of the physiological response test can be seen from the hemolymph value through THC calculation. Hemolymph is a body fluid that is very important for crustaceans. Hemocytes are part of hemolymph which has a function as a cellular defense of the body and is able to kill infectious agents (Ridlo & Pramesti 2009). THC values were observed on day 0 (H0) and day 14 (H14) of the sublethal test. Treatment C increased the THC value from 8.3×10^5 cells mL^{-1} at H0 to 34.7×10^5 cells mL^{-1} at H14. While the control treatment THC value increased slightly, namely from 8×10^5 cells mL^{-1} at H0 to 8.3×10^5 cells mL^{-1} at H14. Although the control treatment experienced an increase in the THC value, the THC value in treatment C experienced a much higher increase than the control treatment. The increase in the THC value indicated that the vaname shrimp was stressed to nitrite. This is in accordance with Chakraborty & Ghosh (2013) that hemolymph in the body's defense system in crustaceans can be used as a health assessment parameter. According to Martinez (2007) haemocytes are involved in wound healing whose mechanism is cellular clumping and the initiation of the coagulation process by releasing the factors needed for plasma gelation and bringing and releasing the prophenoloxidase system so that the high number of haemocytes in shrimp hemolymph can cause stress and lowers the immune system (Ekawati et al 2012). An increase in the glucose value indicates that the vaname shrimp is in a state of stress and does not utilize the feed provided, but utilizes the carbohydrates stored in its body to overcome homeostasis (Martínez-Porchas et al 2009). Hepatopancreas will release crustacean hyperglycemic hormone which will then be converted into glucose as a result of the glycogenesis process to increase hemolyzed glucose (Hastuti et al 2004).

Conclusions. Based on the results of the study it can be concluded that nitrite is toxic to vaname shrimp with an LC_{50} value of 96 hours of 10.28 mg L^{-1} NO_2-N. The presence of nitrites can affect the decrease in survival rate, absolute weight growth, and absolute length growth as well as increase THC values and hemolymph glucose levels. The lowest survival rate, absolute weight growth, and absolute length growth were in treatment C while the highest were in the control treatment. The highest THC values and glucose levels were in treatment C and the lowest were in the control.

Conflict of interest. The authors declare that there is no conflict of interest.

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