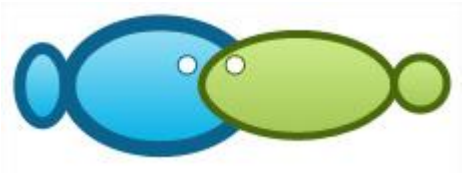

Pathogenicity of bacterial isolate GM 01 in gourami (*Osphronemus goramy*)

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Abstract. Fish farming is inseparable from pests and diseases, one of which is bacteria. Several studies have been carried out to determine the causative agent of bacterial diseases in gourami (*Osphronemus goramy*). However, the studies only reached the Koch postulate test. The purpose of this study was to determine the pathogenicity of GM 01 bacterial isolates in *O. goramy* from Magelang, Central Java, Indonesia. The study was conducted on clinical symptoms, mortality, LD50 value, and blood profile. The present study used an experimental method, which consisted of 4 treatments with three replications. Experimental animals were 270 *O. goramy* with a length of 8-10 cm. The concentration of GM 01 bacterial isolates used in this study was treatment A (10^0 CFU mL^{-1}), B (10^4 CFU mL^{-1}), C (10^5 CFU mL^{-1}), D (10^6 CFU mL^{-1}), E (10^7 CFU mL^{-1}), and F (10^8 CFU mL^{-1}). Clinical symptoms of *O. goramy* infected by GM 01 bacterial isolates swimming abnormally, have low appetite, presents necrosis, reddened mouth, flaking, and bleeding at the fins, blackened body, and exophthalmia. The hihest percentage of fish mortality occurred in treatments E and F, with 100% mortality at the 90th hour post-infection. Treatment D, C, and B were followed at 120, 132, and 144 hours post-infection, also with 100% mortality. LD50 value showed that the concentration of bacteria that could kill *O. goramy* populations by 50% in 96 hours was $4.2209x10^5$ CFU mL^{-1}. Based on these values, GM 01 bacteria are included in the category of pathogens. The percentage of post-infection lymphocyte, neutrophil, and monocyte cells were 72-79%, 12-21%, and 3-8% respectively.

Key Words: blood profile, fish diseases, LD_{50}, mortality, *Yersinia intermedia*.

Introduction. Gourami (*Osphronemus goramy*) is one of the main freshwater commodities that is wildly cultivated in Indonesia (Kusumawaty et al 2017; Rozi et al 2017; Nuryanto et al 2018). *O. goramy* also has a high value in fisheries commodities, as it is evidenced by the price reaching 3.2-3.9 USD kg^{-1}. Consumption of *O. goramy* has increased in recent years. Based on general data of the Ministry of Marine and Fisheries (2014), *O. goramy* production also appears to be increasing every year. In 2010 the production of *O. goramy* only reached 56,889 tons. In 2011 production increased to 64,252 tons, in 2012 increased to 84,681 tons, and in 2013 production reached 86,773 tons. Based on these data, the average increase of *O. goramy* production from 2010 to 2013 was 15.74%.

The emergence of the disease often hampers *O. goramy* culture. Disease arises when the aquaculture environment is not suitable for the needs of the species requirements. Intensive *O. goramy* culture causes environmental changes as a result of high pollution and wrong decision making, one of which is access to the amount of stocking density and feed that results in the emergence of disease problems. The emergence of the disease can cause deflation in production. Incoming disease agents can be contagious or non-communicable. Some infectious disease agents in *O. goramy* are parasites, bacteria, and viruses (Havixbeck et al 2016; Mishra et al 2017; Sen & Mandal 2018). Intensive is the culture, higher is the prevalence of bacteria. Based on previous reports, the bacteria that attack *O. goramy* are *Aeromonas hydrophila*, *Staphylococcus*

saprophyticus, *Aeromonas caviae*, and *Flavobacterium* sp. (Kusumawaty et al 2017; Rozi et al 2017; Rozi et al 2018).

Fish farming activities cannot be separated from fish pests and diseases. The disease can arise through an unbalanced interaction between three factors, namely the host, pathogen, and the environment, which causes stress and lower the immunity and leaves the fish vulnerable to disease (Makrinos & Bowden 2016; Li et al 2017; Yengkhom et al 2019). A bacterial disease that attacks *O. goramy* is a contagious disease (Hidayatullah et al 2018; Sen & Mandal 2018; Kousar et al 2019). Disease agents are one of the important factors that must be investigated to get the certainty of the causes and to find a cure. After that, the Koch challenge test or postulate is tested to ascertain whether the bacterium is a pathogen or not. Koch's postulates are guidelines for testing diseases caused by certain microorganisms or for determining causative agents (Ross & Woodward 2016; Wang & Lai 2018).

Fish stress conditions can facilitate the entry of disease agents. Disease agents in fish can be caused by parasites, fungi, bacteria, and viruses (Idowu et al 2017; Hidayatullah et al 2018). The bacterial disease is one of the obstacles in the culture of *O. goramy* because it can cause death and economic losses. Bacteria that often attack *O. goramy* are *Chryseobacterium aquifrigidense*, *Echerichia ferqusonii*, *Staphylococcus saprophyticus*, and *Bacillus pumilus*. This kind of bacterial invasion can cause death in *O. goramy*; therefore, it can cause economic losses to fish farmers (Xue et al 2017; Luis et al 2019; Kossack et al 2020).

Due to the disease outbreak, which resulted high mortality in *O. goramy* in Magelang District, the isolation of the causative agent of the disease was performed. The preliminary study indicates that isolate GM 01 causes 80% mortality in fish. The purpose of this study was to determine the pathogenicity of GM 01 bacterial isolates in *O. goramy* from Magelang District, Central Java, based on clinical symptoms, mortality, LD_{50} value, and blood profile. Previous experiments only reached the Koch postulate test. Therefore, further research needs to be done regarding the pathogenicity test on bacterial agents that cause disease in *O. goramy*, in order to determine the level of pathogenicity when bacteria infect fish. The results of this study are expected to provide information about the effect of bacterial density with GM 01 codes with different dosage on the blood profile as a representation of the immune response of *O. goramy* originating from Magelang District, Central Java.

Material and Method. The fishes used in this study were 270 healthy *O. goramy* specimens with a length of 8-10 cm. The experimental fishes were stored in 18 aquariums with dimensions of 30 x 40 x 50 cm filled with 15 L freshwater and aerated during the experiment. The fishes were cultivated for six days, and acclimatization was carried out three days before the examination.

This study used an experimental method with a Completely Randomized Design (CRD) consisting of 6 treatments and three repetitions, and then data was analyzed descriptively. The pathogenicity test was done by injecting GM 01 isolates intramuscularly or known by injecting bacteria just below the dorsal fin at an angle of 45°. The bacterial concentrations used were A (10^0 CFU mL^{-1}), B (10^4 mL^{-1}), C (10^5 mL^{-1}), D (10^6 mL^{-1}), E (10^7 mL^{-1}), F (10^8 mL^{-1}). All treatments were performed through intramuscular injection (Sarjito 2010).

The bacterial isolate used in this study was GM 01, isolated from contamined *O. goramy*, from Magelang District, Central Java. The GM 01 bacteria were cultured in Trypticase Soy Broth medium, then incubated at 22°C for 48 hours. Bacteria were harvested and washed using Phosphate-Buffered Saline solution. After harvest, the bacteria density was measured using the McFarland 0.5 method as a standard procedure (Maftuch et al 2016; Mohammed & Omer 2019). The pathogenicity test was carried out to find bacteria that have infected fish which have been previously isolated, while the blood profile preparations process was performed following the steps presented in Figure 1.

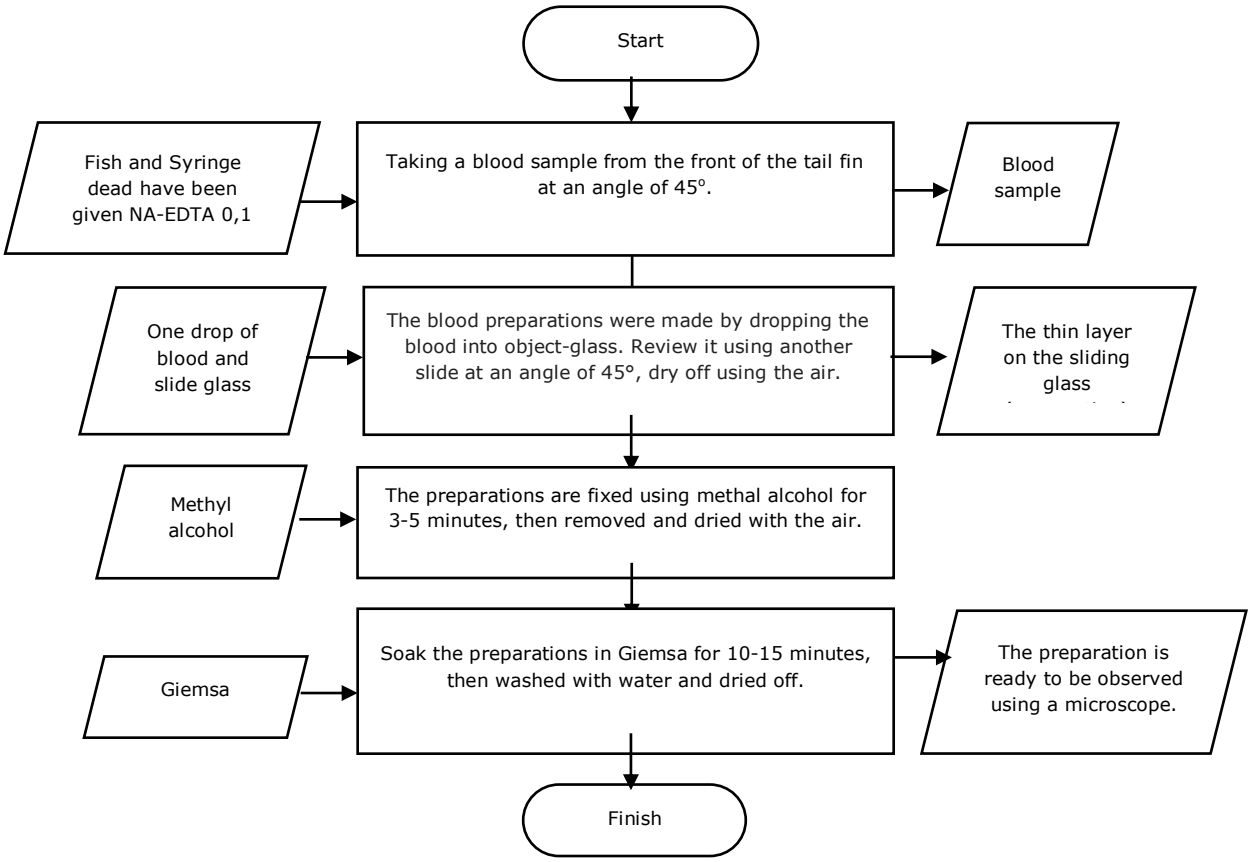

Figure 1. Flowchart of the of the blood sample preparations.

Data analysis

Fish mortality calculation. Total fish mortality was counted using Svobodova & Vykusova (1991) method, with the formula:

$$Z = \frac{N_0 - N_t}{N_0} \times 100\%$$

Where:
Z - mortality rate
No - total fish at the beginning of the experiment
Nt - total fish at the end of the experiment

LD_{50} scores. The LD_{50} scores were determined using the method used in the previous research of Sarjito (2010), which using the formula:

$$m = Xi + d \frac{50 - \%Xi}{\%xi+1 - \%xi}$$

Where:
M - log LD50
Xi - log bacterial dosage below LD50 and above LD50
%xi - accumulation of mortality percentage on bacterial dosage below LD50
Xi+1 - accumulation of mortality percentage on bacterial dosage above LD50

Blood Profile

Lymphocytes. Total lymphocytes were counted using Shabirah et al (2019) method, with the formula:

$$\text{Percentage Limfosit } (\%) = \frac{\text{L}}{100} \text{ x } 100\%$$

Neutrophil. Total neutrophils were counted using a method proposed by Shabirah et al (2019), with the formula:

$$\text{Percentage Neutrofil } (\%) = \frac{\text{N}}{100} \text{ x } 100\%$$

Monocytes. Total monocytes were counted using the method of Shabirah et al (2019), with the formula:

$$\text{Percentage Monosit } (\%) = \frac{\text{M}}{100} \text{ x } 100\%$$

Water quality. Some water parameters in the experimental media were measured concerning temperature, dissolved oxygen (DO), and acidity (pH). Water quality was observed at the beginning and end of the experiment (Puspaningsih et al 2019).

Results

Clinical symptoms. Based on the result observations, the clinical symptoms found in *O. goramy* infected by GM 01 isolate bacteria were exhibited erratic swimming, low appetite, necrosis, reddened mouth, flaking and bleeding at the fins, blackened body, and *exophthalmia* (Table 1 and Figure 2).

Table 1

The clinical symptoms of *Osphronemus goramy* infected with GM 01 bacteria isolate from total sample (n = 45)

No.	*Treatment*	*Percentage of fish*	*Behavior/ Clinical sign*
1	A	100	Swim actively
		100	Swim normally
		100	High appetite
2	B	62.2	Swim normally
		46.67	Low appetite
		31.1	Hemorrhage on the fin base
3	C	51.1	Swim normally
		57.78	Low appetite
		55.56	Reddened mouth
		48.89	Exophthalmia
4	D	68.89	Low appetite
		60	Erratic swimming
		64.4	Reddened mouth
		60	Exophthalmia
5	E	82.2	Low appetite
		75.56	Erratic swimming
		71.1	Reddened mouth
		75.56	Necrosis
		40	Fins flakiness
6	F	91.1	Erratic swimming
		75.56	Reddened mouth
		82.2	Exfoliating scales, necrosis,
		66.67	exophthalmia
		73.3	Color changes, blackened
		48.89	Haemorrhage on the fin

Based on the observations of the clinical symptoms found in *O. goramy* infected with GM 01 bacterial isolates, it appeared that clinical symptoms in all treatments after infection are still normal: fish can still swim actively and has a normal appetite. Clinical symptoms begin to appear at the sixth hour after infection but are only seen on F treatment, where necrosis occurred on the *O. goramy* scale. Clinical symptoms in *O. goramy* infected with GM 01 bacterial isolates, in the form of changes in behavior and morphology of the body, can be clearly seen in Figure 2.

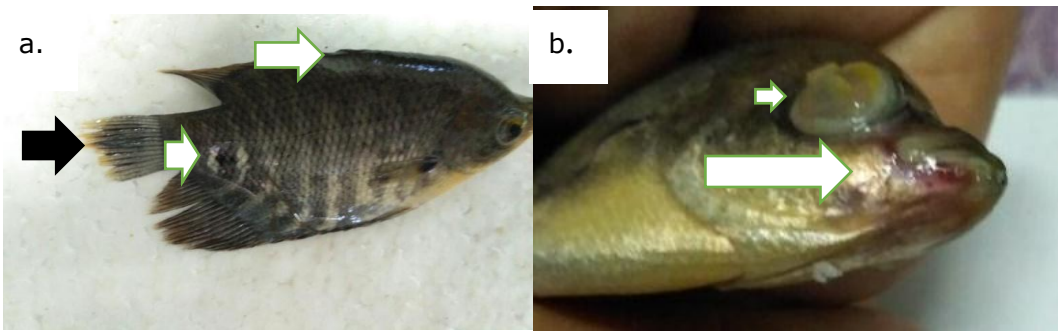

Figure 2. The clinical symptoms arising from fish infected with GM 01 bacteria.

Legend:
a. blackened body
necrosis (peeling scales)
ripped fins
b. *exophthalmia*
reddened mouth

Figure 2 shows that the clinical symptoms found in *O. goramy* infected with GM 01 isolates are characterized by changes in behavior and body morphology. The changes that can be seen from the behavior of fish, among others, are decreased appetite, swim weakly on the bottom of the water and abnormal, such as swim vertically or sloping, while morphological changes consisted of peeling scales, changing body color which becomes more blackened, fins flakiness, reddened mouth, and *exophthalmia* or protruding eyes.

Mortality. The observation of *O. goramy* death time during the pathogenicity test was done every 6 hours for 96 hours and observation 12 hours after that until the end of the study. As for the cumulative mortality pattern of *O. goramy* after being infected with GM 01 bacteria, isolate code is presented in Figure 3.

Based on Figure 3, mortality of infected specimens by GM 01 bacteria isolate, in E and F treatment occurred on the sixth day post-infection, then death of *O. goramy* within the D treatment started at 12 hours after infection, while in treatment B and C *O. goramy* mortality occurred 24 hours after infection. Treatment E and F could kill all individuals within 90 hours after infection, followed by treatment D which has the same results in 120 hours after infection, then treatment C in 132 hours after infection, then all deaths the last fish sample occurred in treatment B, which was within 144 hours after infection. The results of the fastest percentage of fish deaths occurred in treatments E and F, namely as many as 100% of fish died in 90 hours after infection, then followed by treatments D, C, and B at 120 hours, 132 hours, and 144 hours after infection occurred also a 100% mortality.

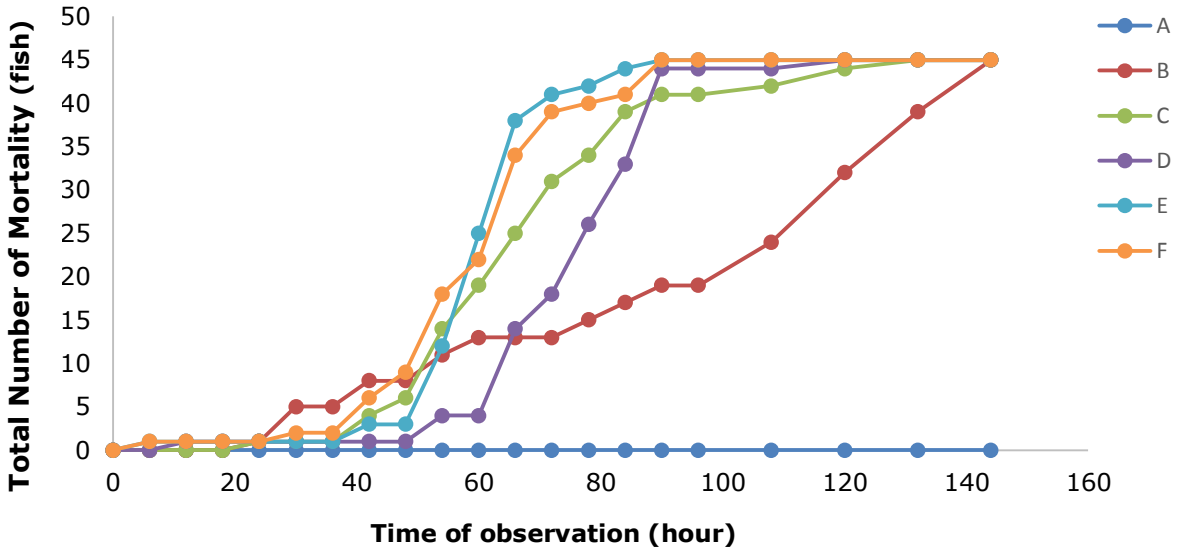

Figure 3. Mortality quantification according time.

Legend: Treatment A (PBS); B (density of bacteria 10^4 CFU mL^{-1}); C (density of bacteria 10^5 CFU mL^{-1}); D (density of bacteria 10^6 CFU mL^{-1}); E (density of bacteria 10^7 CFU mL^{-1}); and F (density of bacteria 10^8 CFU mL^{-1}).

LD_{50} value. LD_{50} test was conducted to determine the level of concentration of bacterial isolates that that cause 50% mortality in *O. goramy* in 96 hours. The results of LD_{50} calculations of GM 01 isolates in *O. goramy* can be seen in Table 2.

Table 2

LD_{50} of GM 01 bacteria isolate in *Osphronemus goramy*

Variables	*Treatment*					
	A	*B*	*C*	*D*	*E*	*F*
Samples (n)	45	45	45	45	45	45
Mortality (n)	0	19	41	44	45	45
Alive (n)	45	26	4	1	0	0
Mortality (%)	0	42.22	91.11	97.78	100	100
LD_{50} (CFU mL^{-1})			4.2209×10^5			

Treatment A (PBS); B (density of bacteria 10^4 CFU mL^{-1}); C (density of bacteria 10^5 CFU mL^{-1}); D (density of bacteria 10^6 CFU mL^{-1}); E (density of bacteria 10^7 CFU mL^{-1}); and F (density of bacteria 10^8 CFU mL^{-1}).

Based on Table 2, the LD_{50} test results with GM 01 isolate which produced 50% mortality of the *O. goramy* population within 96 hours is a bacterium with a density of 4.22×10^5 CFU mL^{-1}, so the bacteria are included in the virulent category.

Percentage of lymphocyte cells. The percentage of lymphocytes was obtained from a comparison between the number of lymphocyte cells with the total number of leukocyte cell samples that are counted as 100 cells, then multiplied with 100. Examination of D-1 blood profile was done before infection, D+1, and D+4 after infection. Examination results of lymphocyte cell percentage in *O. goramy* infected with GM 01 isolates with different bacterial densities can be seen in Table 3.

Based on Table 3, it can be seen that the average percentage of *O. goramy* lymphocyte cells infected by GM 01 isolates has decreased from day to day. The percentage of lymphocyte cells after infection, especially on the 4^{th} day showed that lymphocytes in *O. goramy* blood is abnormal, because the average percentage of lymphocyte counts was less than 80%, which ranged between 72 and 79% while the normal lymphocyte cells for health fish is more than 80%.

Table 3

Average percentage of lymphocyte cell per treatment

Treatment	*D-1*	*D+1*	*D+4*	*Normal*
A	82.00±1.00	82.00±2.00	80.00±1.00	
B	84.00±1.00	81.00±1.73	79.00±2.00	
C	82.00±2.00	79.00±3.00	76.00±1.00	More than 80%
D	80.00±4.00	79.00±2.00	75.00±2.00	(Roberts 2012)
E	80.00±2.00	77.00±2.65	74.00±2.00	
F	79.00±2.00	76.00±3.00	72.00±1.73	

Treatment A (PBS); B (density of bacteria 10^4 CFU mL^{-1}); C (density of bacteria 10^5 CFU mL^{-1}); D (density of bacteria 10^6 CFU mL^{-1}); E (density of bacteria 10^7 CFU mL^{-1}); and F (density of bacteria 10^8 CFU mL^{-1}).

Percentage of neutrophil cells. The percentage of the neutrophils is obtained from the ratio between the number of neutrophil cells and the total number of leukocyte cell samples which were counted as many as 100 cells, then multiplied by 100. Examination of blood profile was done D-1 before infection, D+1, and D+4 after infection. The results of an examination of the average percentage of neutrophil cells in *O. goramy* infected by GM 01 isolates with different bacterial densities can be seen in Table 4.

Table 4

Average percentage of neutrophils cells per treatment

Treatment	*D-1*	*D+1*	*D+4*	*Normal*
A	13.00±2.00	13.00±2.00	12.00±2.65	
B	13.00±2.65	16.00±2.00	17.00±4.58	
C	15.00±1.73	16.00±2.65	18.00±4.36	6–8%
D	15.00±3.00	17.00±4.36	18.00±2.65	(Roberts 2012)
E	16.00±1.73	17.00±3.61	20.00±2.65	
F	18.00±3.00	19.00±1.73	21.00±3.00	

Treatment A (PBS); B (density of bacteria 10^4 CFU mL^{-1}); C (density of bacteria 10^5 CFU mL^{-1}); D (density of bacteria 10^6 CFU mL^{-1}); E (density of bacteria 10^7 CFU mL^{-1}); and F (density of bacteria 10^8 CFU mL^{-1}).

Based on Table 4, it appears that the average percentage of neutrophil cells in *O. goramy* after the infection has increased. The percentage of the neutrophil cells after infection, i.e., on the first day and day 4 showed that the number of neutrophils in *O. goramy* blood is higher than normal, because the average percentage of neutrophil in the treatments was around 12–21%, whereas, according to Roberts (2012) the amount of neutrophil cells for normal fish is approximately 6–8%.

Percentage of monocyte cells. The percentage of monocytes was obtained from the ratio between the number of monocyte cells with the total number of leukocyte cell samples counted, which were 100 cells, then multiplied by 100. Examination of blood profile was done D-1 before infection, D+1, and D+4 after infection. The results of the examination concerning the average percentage of monocyte cells in *O. goramy* infected by GM 01 isolates with various bacterial densities can be seen in Table 5.

Table 5

Average percentage of monocyte cells per treatment

Treatment	*D-1*	*D+1*	*D+4*	*Normal*
A	5.00±2.65	5.00±2.65	8.00±2.00	
B	3.00±2.65	3.00±1.00	4.00±2.00	
C	3.00±1.73	5.00±1.73	6.00±1.73	1–2%
D	4.00±3.00	4.00±1.00	7.00±2.00	(Roberts 2012)
E	4.00±1.00	6.00±2.00	7.00±4.00	
F	3.00±2.00	5.00±2.00	7.00±2.00	

Treatment A (PBS); B (density of bacteria 10^4 CFU mL^{-1}); C (density of bacteria 10^5 CFU mL^{-1}); D (density of bacteria 10^6 CFU mL^{-1}); E (density of bacteria 10^7 CFU mL^{-1}); and F (density of bacteria 10^8 CFU mL^{-1}).

Based on Table 5, it can be seen that the average percentage of monocyte cells in *O. goramy* after the infection begins to increase from day to day at each treatment. The percentage of monocyte cells from before infection to post-infection showed that the number of monocytes in *O. goramy* blood was above normal conditions because the average percentage of monocyte counts in the above treatments was in the range of 3-8%, while according to Roberts (1978) the amount of normal monocyte cells of fish is between 1 and 2%.

Discussion

Clinical symptoms. Based on observations of clinical symptoms found in *O. goramy* infected by GM 01 bacterial isolates, normal parameters could be found in all treatments after infection: fish could swim actively and had normal appetite. Clinical symptoms began to appear at the sixth hour after infection but were only seen in F treatment, where presented necrosis on the fish scale. Clinical symptoms appeared in treatment B, visible at the 12^{th} hour after infection; namely, the appetite of the fish begins to decrease. At the 30^{th} hour, fish begins to move passively, and bleeding at the base of the fin after 48 hours after infection. Then in treatment C, clinical symptoms that appeared were reddish mouth that started appearing at 36 hours after infection and exophthalmia at 42 hours. Not very different from treatment C, the clinical symptoms that occurred in treatment D were passive movements, abnormal swimming, or at the bottom and tilt, reddened mouth, and exophthalmia. It is just that clinical symptoms appeared faster than in previous treatment. The E and F treatments showed almost the same symptoms: in treatment E, the fish were swimming abnormally, presented reddish mouth, dorsal, anal and caudal fins flaked, and necrosis occurred at the 30^{th} hour after infection. While in the treatment F clinical symptoms such as necrosis began to appear after the 6^{th} hour post-infection, followed by redness of the mouth at the 18^{th} hour, besides that the fish were also seen swimming abnormal at the bottom, body-color blacks, and followed by the occurrence of exophthalmia and bleeding at the base of the fins at the 30^{th}-hour post-infection. In contrast to treatment E, in this treatment, were no visible flakes of fish fin.

Based on the results of the clinical symptoms that could be seen *O. goramy* infected by GM 01 in all treatments, the clinical picture indicated a close relationship with species *Yersinia intermedia*. This could be seen from the most prominent clinical symptoms of fish, namely flaky scales, reddened mouth, *exophthalmia*, and blackened body-color (Kumar et al 2015; Yamasaki et al 2017; Acar et al 2018; Kazarnikova et al 2019; Tkachenko et al 2019; Wrobel et al 2019). Morphological changes such as peeling scales cause fish to be more susceptible to skin injury. Clinical symptoms observed in the present study are in accordance with the report of Muziasari (2016), that among the clinical symptoms that arise from infection by *Yersinia* sp. bacteria are behavioral (slow movements) and morphological changes, where the body color becomes darker, and the mouth turns red. *Yersinia* sp. bacteria that have been reported by Hiko et al (2018) have attacked several freshwater fish, such as *Oreochromis niloticus*, *O. goramy*, *Clarias* sp. Rozi et al (2018) also reported *O. goramy* infestation by *Yersinia* sp. where the most prominent clinical symptoms were peeling scales and darker body color.

According to our observation, considering all treatments, fish samples infected by GM 01 bacterial isolates did not showed the same clinical symptoms, but the higher was the concentration of the injected bacteria, faster were the clinical symptoms manifestations. These aspects where more evident in fish samples from treatments E and F, against clinical symptoms of treatment B, C, and D.

Pathogenicity test. Pathogenicity tests included mortality patterns and LD50. During the pathogenicity test, the time of death was observed every 6 hours for 96 hours, and after that observation every 12 hours until the end of the experiment. Death occurred on the first day after infection, in the treatment D, E, and F. According to Table 2, *Y. intermedia* infected *O. goramy* within E and F treatment, were found death starting at 6 hours after infection, followed by D treatment where mortality started at 12 hours after

infection, while specimens from treatment B and C faced mortality at 24 hours after infection. Treatment E and F produced 100% mortality within 90 hours after infection, followed by treatment D, where the 100% mortality occurred within 120 hours after infection. Treatment C produced 100% mortality within 132 hours after infection, and finally, treatment B within 144 hours after infection. The fastest mortality was obtained within treatment E and F, with 100% in 90 hours after infection, followed by treatments D, E, and B, which with 100% mortality within 120 hours, 132 hours, and 144 hours after infection respectively. High levels of bacteria pathogenicity can cause mortality to reach 100% (Rozi et al 2017; Hardi et al 2018; Safinska 2018). It can be concluded that *O. goramy* died due to differences in bacterial concentrations; the higher the concentration of infection in fish, the higher the mortality that occurs.

The alleged cause of occurred mortality was the morphological changes such as flaked scales so that bacterial agents could more easily and quickly infect *O. goramy*. Scales are one of the external protections of fish. Therefore if scales exfoliate, fish will be more susceptible to bacterial infections (Maftuch et al 2016; Putra et al 2016; Yengkhom et al 2019).

LD50 results showed that the concentration of bacteria that could cause 50% mortality of *O. goramy* population in 90 hours was 4.2209x105 CFU mL^{-1}. Hamed et al (2018) reported that bacteria that had LD50 scores of 103-105.5 CFU mL^{-1} are categorized as deadly infectious bacteria, whereas LD50 scores above 10^7 CFU mL^{-1} denotes low-level virulence. Thus *Y. intermedia* used in the present experiment are included in the virulent category.

Blood profile

Lymphocyte cells. The percentage of lymphocyte cells in *O. goramy* after infection with *Y. intermedia* was below the normal range of 80%, namely (72-79%). The percentage of lymphocytes was obtained by comparing the number of lymphocyte cells with the total number of leukocyte cell samples that are counted as 100 cells, then multiplied with 100. Examination of blood profile was done H-1 before infection, H+1, and H+4 after infection.

A decrease in the percentage of lymphocytes from *O. goramy* samples is caused by stress due to poor environment in aquaculture and fishing, whereas larval transportation and aquaculture are stress factors in Teleostei or Elasmobranch fish. Stress and a poor environment condition cause an increase in the hormone cortisol, causing a decrease in lymphocyte cells in the bloodstream. A decrease in lymphocyte cells occurs in immunopathy cases or due to certain stress factors. In the present experiment, *O. goramy* might experience stress, which caused a decrease in lymphocyte cells (Makrinos & Bowden 2016; Fatimah et al 2017; Aceves et al 2019; Lee et al 2019). Under stress conditions, cortisol levels in the blood increase. Cortisol can cause lymphopenia by reducing mitosis or producing lymphocytes. Cortisol also plays a role in reducing lymphocyte cells in the blood circulation due to the redistribution of lymphocytes to the bone marrow and other target locations. Reduced lymphocyte cells can also be caused by *Y. intermedia* infection. Disease attacks can reduce the concentration of antibodies so that fish have low lymphocytes in response to antigens. In other words, lymphocytes functioned to develop cellular responses to immunity (not specific) as triggering response to fish immunity system (Sahan et al 2017; Aliko et al 2018; Shabirah et al 2019; Kossack et al 2020).

Neutrophil cells. Neutrophil cells are the first cells produced by the body to fight antigens. Monocytes and neutrophils have a role of strong phagocytes. One neutrophil cell can phagocyte 20 antigens, while one monocyte can phagocyte up to 100 antigens before the cell becomes inactive. The percentage of monocytes and neutrophils is less than lymphocytes because monocytes and neutrophils are short-lived cells, so the total cells in the blood are less than others (Nahendorf & Swirski 2015; Havixbeck et al 2016; Kordon et al 2018).

The percentage of neuthrophyl cells in *O. goramy* after *Y. intermedia* bacterial infection was above normal, which is between 12-19%, during the normal percentage in

blood. The total percentage of neutrophil is obtained by comparing the total of neutrophil cells with the number of leukocyte cell samples counted, 100 cells, and multiplied with 100. The blood profile was examined D-1 before infection, D+1, and D+4 after infection. In the present experiment, the percentage of neutrophils was less than of lymphocyte cells. The increase in neutrophil cells above normal is caused by stress and bacterial infections. Total neutrophil cells in the blood will increase when bacterial infections occur. Under stress conditions, cortisol can affect the production of neutrophils from bone marrow (Douxfils et al 2017; Maryani et al 2018; Herlina et al 2019). This, results in an increase in neutrophil cells. Physical stress caused by handling and treatment can cause damage to fish skin mucus, which affects the increasing frequency of microorganism infections. Low levels of neutrophils are thought to occur because neutrophils concentrate in the area of bleeding or injury. In addition, the total amount of neutrophil is less than the total blood population.

The increase in neutrophil cells is stimulated by the appearance of foreign matter, which in the present experiment was *Y. intermedia*. An increase in neutrophil cells shows an increase in macrophage collection in the area of infection so that macrophages can easier damage foreign bodies. The function of neutrophils is the destruction of foreign matter through the phagocytosis process is chemical when cells will migrate to particles, cell adhesion, digestive cell particles, and destruction of particles by the enzyme lysozyme in phagolysosomes. Therefore an increase in neutrophil cells occurs due to foreign body reactions, which can be bacterial, viral, or pathogenic neutrophil (Mahasri 2016; Dias et al 2019; Zhang et al 2019).

Monocytes cells. The percentage of monocytes in *O. goramy* after infection with *Y. intermedia* was above normal, which is between 3 and 10%. The normal percentage of monocytes in the blood is between 1 and 2% of the total leukocyte population (Subramani et al 2016; Debouttevillle et al 2019; Lulijiwa et al 2019). The percentage of total monocytes is calculated by comparing the total monocyte cells to the total monocyte cells with the total number of leukocyte cell samples counted, which were 100 cells, then multiplied with 100. The blood profile was examined D-1 before infection, D+1, and D+4 after infection. The increase in monocyte cells occurs due to bacterial infections that cause certain diseases. Monocytes can occur due to chronic diseases, especially if many cell impurities need to be cleaned, for example, fungal infections, granulomatous inflation, and certain other diseases (Chen et al 2016; Ding et al 2019; Niu et al 2019).

Monocytes are large leukocytes, commonly called macrophages. An increase in the number of monocyte cells is related to monocytes themselves as macrophages, which are needed for phagocytes, which are caused by massive infections in the body that stimulate monocyte production so that the number of monocyte cells becomes higher (Fazio 2019; Haghighi et al 2018; Huang et al 2020; Nhu et al 2019). In other words, monocytes can be used as markers of pathogens in T cells so that these pathogens can be identified and killed, or can be used to produce antibodies. Close contact between the lymphocyte and monocyte surfaces is needed for maximum immunological response (Shen et al 2018; Dang et al 2019; Sanchez et al 2019).

Conclusions. Results of mortality and lethal dosage (LD_{50}) in the present research showed that GM 01 bacteria were classified into pathogenic bacteria. The clinical symptoms of *O. goramy* infected with GM 01 isolates showed that the result of a pathogenicity test is similar to the symptoms of a disease caused by *Y. intermedia*.

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