The analysis of challenges test of catfish (Clarias sp.) with fatty acid compounds from starfish (Acanaster planci)

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Abstract. Catfish (Clarias sp.) is one of the fresh water fish commodities widely cultivated and very popular in community for being simple in breeding and fast in growth. The demand of people for catfish has led to the increase of production in meeting the demand. This study aimed to analyze the values of SR, RPS, MT and MTD of catfish using the challenge test with bacterium A. hydrophila and given the fatty acid compounds of A. planci. The results of this study showed that the highest average value of SR was found in the treatment with 0.7 mg/kg BBI (26 - 76%), the highest average of RPS was found in the treatment with 0.7 mg/kg BBI (65%), the lowest average values of MR was found in the treatment with 0.7 mg/kg BBI (23,33%), and the average values of MTD were in the range of 94,24 to 142,67%. Meanwhile, the water quality parameter in the range of the optimum value of DO on day 1 was in the range of 5 to 6,8, on day 7 it was in the range of 5,2 to 6,8, and on day 15, it was in the range of 5,5 to 6. The level of oxygen was at 4 ppm and the water temperature was in the range of 26 to 28°C. The results of the identification towards the fatty acid compounds with GS-MS showed two fatty acid compounds; those are Hexadecanoic acid and 13-Octadecenoic acid. From this study, it can be concluded that there was an effect of giving the injection doses of A. planci compound on the analyses of SR, RPS, MR and MTD in the challenge test with the bacterium Aeromonas hydrophila in comparison to the fish not given with fatty acid compounds of A. planci.

1. Introduction

Clarias sp. is one of species of fresh water fish and it is included in phylum chordate. It is one of fresh water fish commodities that have high economic values as it is popular among societies [1]. The demand of society to catfish then leads to the increase of fish production to fulfil the demand. The increase of the intensification of cultivation also brings an impact on the attack of disease caused by the bacteria, virus, fungi or parasites. The emergence of disease commonly is not caused by a single factor, but it is the result of the complex interaction between the cultured fish (quality), environment of cultivation (intern and extern), and organism of disease factors [2].

The dominating disease in the cultivation of Clarias sp. is motile aeromonas septicemia (MAS) caused by Aeromonas hydrophila. MAS disease can cause the death of MAS 80-100% within 1 to 2 weeks towards catfish [3]. One of solutions to cope with MAS is through the use of immunostimulant, seen to be effective and efficient to control MAS. Immunostimulant works by increasing the functions
of body and immune system by stimulating the macrophage in the formation of white blood cells that are able to remove any foreign materials [4], [5], [6].

The benefits of using immunostimulant include environmentally friendly, availability, affordability, and being relatively safer to be consumed as it does not leave any residues in fish comparing to the use of chemical drugs that bring some impacts on water environment, pathogens becoming resistant and even health of customers due to the antibiotic residues [7], [8], [9],[10]. In providing immunostimulant, it is deemed essential to measure the condition of water quality as the protection level caused by immunostimulant highly relies on the condition of fish and its living environment (water quality) [11], [12], [13].

Natural compounds from the sea in the form of secondary and primary metabolic that have widely been reported in terms of their use in immunostimulant include polymer polysaccharide, alginate, fucoidan from seaweed and glycoside from sea cucumber [14], [9], [15], [16], [17], [18], [19], [20]. The report about the results of the research on the challenge test for the immune of fatty acid compound of A. planci so far has never been given. This study aimed to analyze the values of SR, RPS, MT and MTD of catfish using the challenge test of bacterium A. hydrophila and given with the fatty acid compound of A. planci.

2. Research methodology
2.1 Challenge test
Challenge test was conducted to observe the impact of fatty acid compound of A. planci on protecting Clarias sp. tested with A. hydrophilla, conducted by injecting the fatty acid compound of A. planci with the dose of 0.4 mg/kg to Clarias sp. Three days after the injection of active fraction, Clarias sp. was injected with bacterium A. hydrophilla.

2.2 Revirulence of Bacterium Aeromonas hydrophila
The virulence of Aeromonas hydrophila used to infect the fish was increased by infecting the healthy fish with the suspension of bacteria. The sample from the fish showing the symptoms to be attacked by the bacteria was taken. Here, the sample was taken from the kidney, isolated in the medium of rimlershotts (medium R-S) and incubated within 24 - 48 hours. The bacteria growing in the medium were used to again infect the healthy fish. The reinfection was conducted three times to obtain Aeromonas sp.

2.3 The calculation of bacteria density
The calculation of the bacteria density was conducted using the total plate count method. The pure culture of bacteria was grown in the TSB media, after being incubated for 24 hours. Then the dilution with PBS until the dilution of $10^{-4}$, $10^{-5}$, and $10^{-6}$ was conducted in the medium of TSB contained with bacteria and 100 µl were taken and grown in the jelly media (TSA). Once incubated within 12 hours, the density of 0 (control), 2.18x10$^5$; 2.18x10$^6$; 2.18x10$^7$; 2.18x10$^8$; 2.18x10$^9$; dan 2.18x10$^{10}$ CFU/ml of bacteria was calculated.

\[
\text{Calculation: } \mu \times P \times 10^{10} = \ldots (\text{cfu})
\]

Remark: $\mu = \Sigma$ bacteria, and $P = \text{dilution}$

2.4 Determination of LD$_{50}$
The test examination of LD$_{50}$ was conducted towards the calculation of number of fish (n) that were dead when being infected with certain concentration bacteria (x). Then, the data obtained were inserted in the table and calculated using the following formula:

\[
\text{Mortality (\%)} = \frac{\Sigma \text{dead}}{\text{Total}} \times 100\%
\]
\[ m = X_1 + d \left( \frac{50 - \% X_1}{\% X_1 + 1 - \% X_1} \right) \Rightarrow \text{LD}_{50} = \text{ANTILOG} m \]

2.5 Data analysis
Data of SR, MTD, RPS and SGR were analyzed using the analysis of variance (ANOVA) to observe the effect of each treatment. If the data that have been analyzed with ANOVA had a significant difference (P < 0.05), it was then continued with the test of duncan multiple range test (DMRT) to know the best doses of food. Survival rate (SR) was calculated in the end of symbiotic provision treatment in the post-challenge test referring to the following formula:

\[ SR = \frac{N_t}{N_0} \times 100 \]

Where, \( N_t \) = Number of individuals in the end of treatment of day 1 and \( N_0 \) = Number of individuals in the beginning of treatment-0

Relative percent survival (RPS)
RPS (relative percent survival) post-challenge test was calculated based upon the formula:

\[ \text{RPS} = \frac{\text{Mortality of Catfish infected by Pathogen}}{\text{Mortality}} \times 100 \]

Mean time to death (MTD)
Mean time of death (MTD) was calculated based on the reference of OIE

\[ \text{MTD} = \frac{\sum_{i=1}^{n} a_i b_i}{\sum_{i=1}^{n} b_i} \]

Where, MTD = mean time to death, \( a_i \): time to death at hour \( i \) (hour), \( b_i \): number of the tested animals that were dead at hour \( i \)

2.5.1 The observation of water quality
Parameters of water quality observed during the research included temperature, pH, dissolved oxygen (DO), and ammonia (NH₃). The parameter of temperature was observed every day, meanwhile DO, pH and ammonia was conducted three times (beginning, mid and the end of research).

2.5.2 The identification of compounds with gas chromatography-mass spectrometry (GS-MS)
Purification was conducted using the Activity Guided Purification method using the fractionation of Column Chromatography (CC), Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GS-MS).

3. Results and discussion
3.1 Results
3.1.1 Re-virulence
The re-virulence phase was conducted three times by injecting \textit{A. hydrophila} to \textit{Clarias} sp. and isolating by means of GSP medium. The results obtained showed that time to death of the infected \textit{Clarias} sp. got faster. The death of \textit{Clarias} sp. in the first re-virulence occurred on day 3 (72 hours after injection). The symptoms coming out from the dead \textit{Clarias} sp. included the bulging intestine accompanied by red spots on the stomach and bleeding mouth. The confirmation of \textit{A. hydrophila} was conducted using the test of KOH and the test of Gram. Furthermore, re-isolation and bacteria were re-injected to \textit{Clarias} sp. The death of \textit{Clarias} sp. in the second re-virulence was faster than the first one i.e. on day 2 after infection. The death in the third revirulence occurred one day after infection.
Lethal Dose$_{50}$ (LD$_{50}$) A. hydrophila towards Clarias sp. Pathogenicity of A. hydrophila to Clarias sp. was shown with the density of bacteria causing the death of fish by 50%, called as Lethal Dose$_{50}$ (LD$_{50}$). LD$_{50}$ was then used as the dose of infection of bacterium A. hydrophila in the challenge test. LD$_{50}$ lied between $2,18 \times 10^7$ cfu/ml and $2,18 \times 10^6$ cfu/ml. Based on the method of Reed-Muench (Anderson, 1974) LD$_{50}$ obtained was $5,47 \times 10^6$ cfu/ml. The data of mortality of Clarias sp. infected with A. hydrophila for the test of determination of LD$_{50}$ can be seen in Table 1.

**Table 1.** Mortality of Clarias sp. infected with A. hydrophila for the test of determination of LD$_{50}$

| No | Number of bacteria (CFU) | Level of Mortality (%) |
|----|--------------------------|------------------------|
| 1  | $2,18 \times 10^{10}$    | 100,0                  |
| 2  | $2,18 \times 10^9$       | 100,0                  |
| 3  | $2,18 \times 10^8$       | 100,0                  |
| 4  | $2,18 \times 10^7$       | 67,5                   |
| 5  | $2,18 \times 10^6$       | 35,0                   |
| 6  | $2,18 \times 10^5$       | 22,5                   |
| 7  | Control                  | 0                      |

The value of LD$_{50}$ showed that isolate A. hydrophila strain used in the research was quite malignant as it was in the range of $10^4$-$10^6$ cell/ml isolate A. hydrophila with LD$_{50}$ of $10^4$-$10^5$ CFU/ml declared as virulent. In contrast, the isolate A. hydrophila with LD$_{50}$ was $10^7$ CFU/ml or declared as nonvirulent.

3.1.2 Survival rate (SR)

The survival rate of Clarias sp. infected with A. hydrophila was in the range of 26 to 76%. The highest SR was found in the treatment of 0.7 mg/kg BB, and the lowest one was in the control. The results of the analysis of variance (ANOVA) of Clarias sp. infected with A. hydrophila that has been tested showed a significant difference. The results of DMRT test showed the survival rate of control (K) was significantly different (P<0.05) with Clarias sp. given the fraction of A. planci. This indicated that the provision of fraction in each treatment was able to increase the immune and Clarias sp. against the infection of A. hydrophila. Table 2 presents the values of survival rate Clarias sp. infected with A. hydrophila.

**Table 2.** The Level of Survival Rate Clarias sp. given the fatty acid compound of A. planci after the challenge test with A. hydrophila

| Treatment (Doses mg/kg BB) | Repetition | Mean       |
|---------------------------|------------|------------|
|                           | 1          | 2          | 3          |              |
| 0                         | 20,00      | 40,00      | 20,00      | 26,00±16,66 $^b$ |
| 0,1                       | 50,00      | 60,00      | 40,00      | 50,00±10,00 $^b$ |
| 0,3                       | 30,00      | 60,00      | 60,00      | 50,00±17,32 $^{bc}$ |
| 0,5                       | 60,00      | 70,00      | 70,00      | 70,00±66,67 $^b$ |
| 0,7                       | 80,00      | 70,00      | 80,00      | 76,00±5,77 $^c$ |
Remark: the significant difference at P<0.05 indicated with the provision of different notation

Remark: Control (-) = PBS (1 ml)

3.1.3 Relative percent survival (RPS) Level
The RPS level of *Clarias* sp. in this research was in the range of 31 - 65%. The highest RPS was found in the treatment of 0.7 mg/kg BBI (65%), while the lowest RPS was found in the treatment 0.1 mg/kg BBI that is 31, 93%. The results of RPS *Clarias* sp. during the challenge test are presented in Table 2. The results of ANOVA of RPS *Clarias* sp. during the challenge test from the data whose homogeneity has been tested showed a significant difference (Appendix). The results of DMRT test showed a significant difference (P<0.05). The treatments of 0.5 mg/kg and 0.7 mg/kg were significantly different with 0.1 mg/kg and 0.3 mg/kg, but 0.1 mg/kg and 0.3 mg/kg were not significantly different. The results showed that the RPS level of treatments 0.5 mg/kg and 0.7 mg/kg was better than 0,1 mg/kg and 0.3 mg/kg. Table 3 presents the values of RPS level.

Table 3. Relative Percent Survival Values of *Clarias* sp. given with the fatty acid compound of *A. planci* after the challenge test with *A. hydrophila*

| Treatment (dose of mg/kg BB) | Repetition | Mean       |
|-----------------------------|------------|------------|
| 0.1                         | 37,50      | 33,30      | 25,00      | 31,93±6,36 a |
| 0.3                         | 12,50      | 33,33      | 50,00      | 31,94±18,78 b |
| 0.5                         | 50,00      | 50,00      | 62,50      | 54,17±7,21 b |
| 0.7                         | 70,00      | 50,00      | 75,00      | 65,00±13,23 a |

Remark: Significant Difference in P<0.05 indicated with the provision of different notation.

3.1.4 Mortality rate
The percentage of mortality rate of *Clarias* sp. in this research was in the range of 23 to 73%. The lowest mean of MR was found in the treatment of 0.7 mg/kg BBI (23.33%), while the highest one was found in the control (73.33%). The results of the ANOVA test showed a significant difference (P<0.05). The treatments of 0.5mg/kg and 0.7 mg/kg were significantly different from the treatments of 0.1 mg/kg and 0.3 mg/kg and control. The results showed that the best treatment was found in 0.5 mg/kg and 0.7 mg/kg. Table 4 shows the values of Mortality.

Table 4. The level of mortality rate (%) of *Clarias* sp. given with the fatty acid compound of *A. planci* after the challenge test with *A. hydrophila*

| Treatment (doses of mg/kg BB) | Repetition | Means       |
|-----------------------------|------------|-------------|
| 0                           | 80,00      | 60,00       | 80,00      | 73,33±11,55 a |
| 0.1                         | 50,00      | 40,00       | 60,00      | 50,00±10,00 b |
| 0.3                         | 70,00      | 40,00       | 40,00      | 50,00±17,32 b |
| 0.5                         | 40,00      | 30,00       | 30,00      | 33,33±5,77 c |
| 0.7                         | 20,00      | 30,00       | 20,00      | 23,33±5,77 c |

Remark: Significant difference in P<0.05 indicated with the provision of different notation.

Remark: Control (-) = PBS (1 ml)

3.1.5 Mean time to death (MTD)
Mean Time to Death (hour) in *Clarias* sp. infected with *A. hydrophila* was in the range of 94.24 to 142.67%. The highest MTD was found in the treatment of 0.5 mg/kg BBI, while the lowest MTD was found in the treatment of 0.3 mg/kg BBI. The results of the analysis showed that there was no any
significant difference between treatment and control (p<0.05). Table 5 and Figure 1 present the Mean Time to Death and the values of comparison of SR, MTD, RPS and SGR, respectively.

**Table 5.** The level of mean time to death (%) of *Clarias* sp. given with the fatty acid compound of *A. planci* after the challenge test with *A. hydrophila*

| Treatment (doses of mg/kg BB) | Repetition | Mean |
|-------------------------------|------------|------|
|                               | 1          | 2    | 3    |       |
| 0                             | 93,00      | 136,00 | 75,00 | 101,30±31,34<sup>a</sup> |
| 0,1                           | 129,00     | 168,00 | 100,00 | 132,30±34,11<sup>a</sup> |
| 0,3                           | 102,85     | 78,00  | 102,00 | 94,28±14,10<sup>a</sup> |
| 0,5                           | 132,00     | 152,00 | 144,00 | 142,67±10,06<sup>b</sup> |
| 0,7                           | 156,00     | 128,00 | 108,00 | 130,67±24,11<sup>a</sup> |

Remark: the significant difference in P<0,05 was indicated with the provision of different notation

Remark: Control (-) = PBS (1 ml)

![Figure 1. Percentage (%) of SR, RPS, MR and MTD from fatty acid compound of *A. planci* on *Clarias* sp.](image)

### 3.1.6 Clinical symptoms

The results of the observation towards the clinical symptoms on *Clarias* sp. infected showed a number of external clinical symptoms such as fish that tended to be secluded, decreased appetite, bleeding on anus, dorsal fin, pectoral and mouth fins. Meanwhile, the internal clinical symptoms include abdomen bulging and filled with yellow fluid and kidney that was pale kidneys. The clinical symptoms observed in each treatment showed the similar symptoms. This indicated that the infection of *A. hydrophila* have caused the death of fish. Figure 2 shows the internal clinical symptoms of bulging kidney stomach containing the fluid (A) and the pale kidney.
The clinical symptoms of sample *Clarias* sp. induced with *A. hydrophila*:

Internal symptoms:
- Bulging stomach
- Filled with yellow fluid
- Pale kidney

External symptoms:
- Fish tended to be secluded,
- Decreased appetite,
- Bleeding on anus, dorsal fin, pectoral and mouth fins

**Figure 2.** Internal clinical symptoms of *Clarias* sp. sample

3.1.7 Water quality

The results of observation on the water quality during the challenge test showed the temperature at the range of 23.9 to 24.7°C, pH 7.42 to 7.78, the level of dissolved oxygen (DO) 1.52-3.93 mg/l, and ammonia 0.0005 - 0.2316 mg/l. Table 5 shows the results of the observation on the water quality in the challenge test.

| Treatment | Temperature (°C) | pH (mg/l) | DO (mg/l) | CO₂ (mg/l) | NH₃ (mg/l) |
|-----------|------------------|-----------|-----------|------------|------------|
| 0         | 23.8-23.9        | 7.42-7.03 | 1.52-3.90 | 4.4-13.2   | 0.0005-0.216 |
| 0,1       | 23.9-24.7        | 7.42-7.75 | 1.53-3.93 | 4.4-13.2   | 0.0005-0.232 |
| 0,3       | 24.5-23.8        | 7.42-7.68 | 1.53-3.90 | 4.4-13.2   | 0.0005-0.222 |
| 0,5       | 24.4-24.6        | 7.42-7.78 | 1.54-3.92 | 4.4-13.2   | 0.0005-0.231 |
| 0,7       | 24.3-24.5        | 7.42-7.64 | 1.80-3.91 | 4.4-13.2   | 0.0005-0.232 |

3.1.8 Identification of immunomodulatory compounds with gas chromatography-mass spectrometry (GC-MS)

The analysis of chromatogram of GS-MS showed 45 peaks (Figure 3), while the analysis on the compound molecule of peak no 27 with the retention time of 23.25 minutes (Figure 4) as the fatty acid
compound *Hexadecanoic acid* (Figure 5) and the compound molecule of peak no. 38 with the retention time of 25.07 minutes was *13-Octadecenoic acid* (Figure 5).

Chromatogram

![Chromatogram](image)

**Figure 3.** Chromatogram *A. planci*

![MS peak no:27](image)

**Figure 4.** MS peak no:27 with the retention time of 23.25 minutes

![Hexadecanoic acid](image)

**Figure 5.** Prediction of the structure of compound molecule of peak number 27.

![MS peak no:38](image)

**Figure 6.** MS peak no:38 retention time of 25.07 minutes

![13-Octadecenoic acid](image)

**Figure 7.** Prediction of structure of compound molecule no:38

### 3.2 Discussion

#### 3.2.1 Challenge test

Challenge Test is important to observe to what extent the effectiveness of immunostimulant given in giving the protection against the infection of pathogen. The disease of *motile aeromonas septicemia* (MAS) caused by the infection of bacterium *A. hydrophila* is one of diseases causing the quite high rate of mortality in the cultured fish. Revirulence before the challenge test was conducted to return the
The virulence of bacteria. The time to death and the symptoms of MAS disease that got faster after infection showed the more increasing virulence of bacteria. The survival rate of *Clarias* sp. injected with fatty acid was higher than that of control. The results of SR analysis showed that the provision of fatty acids as the immunostimulant can prevent any infection of *A. hydrophila* to *Clarias* sp. the injection of fatty acids can increase the survival rate of *Clarias* sp. by 76% compared to the survival rate of *Clarias* sp. control by 26% as *A. hydrophila* is included into the opportunistic pathogen that is almost always in water and ready to carry a disease if the fish are not in a good condition. Therefore, preventive measure is highly deemed very important to manage this disease [21], [14]. *Clarias* sp. is one of fresh water fish that are vulnerable with MAS disease. This is related to the absence of the scales on fish causing *Clarias* sp. more vulnerable than other species.

The effectiveness of fatty acids as the immunostimulant can be seen from relative percent survival (RPS). The highest RPS was found in the doses of 0.7 mg/kg BB (65%). The lowest mortality rate was at 23.33 hours with the doses of 0.7 mg/kg BBI, and the highest was found in *Clarias* sp. control with 73.33 hours. The relative protection level of cumulative mortality during the observation showed that *Clarias* sp. with the concentration of low doses had the lower mortality rate that of *Clarias* sp. of control. The mortality of *Clarias* sp. of control occurred since the day 2 after infection with the number of dead fish reaching more than 18%. While, *Clarias* sp. with the fatty acid only experienced the mortality of 3% on day 2 after infection.

The increase of mortality rate in *Clarias* sp. with the injection of fatty acid occurred gradually during the observation. The proportion of fatty acid to *Clarias* sp. functioned as immunostimulant that was able to increase the non-specific immune system. The values of MTD showed that the injection with fatty acid caused an increase in the doses of 0.5 - 0.7 mg/kg BBI, while the lowest MTD was found in 0.3 mg/kg BBI. The high value of MTD and the low value of SR in this research showed that the provision of fatty acids was able to give a protection for the fish against the disease of *A. hydrophila*. This was because the activities of compounds when the immunostimulant entered the body that would be recognized by pattern recognition receptors (PRR) or pattern recognition protein (PRP) [22], [23]. According to [22] and [12] PRR is dissolved in the protein of C3 complement, lectine and components of other humoral non-specific immune systems. PRP/Rs in leukocyte can stimulate the phagocytosis activities in neutrophil and macrophage. Both cells have the phagocytosis. The activities of phagocytosis was characterized with the production of oxygen reaction during the respiratory burst process. Neutrophils have myeloperoxidase in cytoplasmic granules, which can secrete halides and hydrogen peroxide enabling them to heterogenize bacterial cell walls [2], [24].

Pathogens must be able to penetrate the immune system of seeds to cause disease. The natural resistance of seeds allows each individual to be free from the pathogen attack. Each individual has a different endurance determined by age, sex, nutritional status, and stress [3].

The introduction by PRP/Rs will also induce the cytokines that have a function in non-specific and specific immunity [21]. In non-specific immune system, cytokines are in the form of interleukins (IL) -1β, tumor necrosis factor-α and IL-6 and anti-viral cytokines like interferon (IFNs). Cytokines in the specific immune system are in type I and type II IFN, IFN-γ, IL-18 and IL-2, all types Th1, IL-4, and Th2 [25]. The biological effect of immunostimulant administration relies on the target cell receptor that can recognize immunostimulant as a foreign material and can trigger the body’s defense system [21].

Clinical symptoms that emerge such as hemorrhage on the dorsal, pectoral, oral and anal fins are caused by hemolysis toxin with the target to break down the red blood cells enabling them to come out of blood vessels, and causing the reddish color to the skin [7].

Each different strain of aeromonas bacteria produces a number of different enzymes. *A. hydrophila*, in addition to enzymes, also produces toxins such as hemolysis, cytotoxins and enterotoxins. Virulence *A. hydrophila* involves many very complex factors of virulence. Hemolysis and enzymes work together in opening the surface tissue of the skin and fish scales [11].
3.2.2 Water quality

Water quality is one of factors triggering the emergence of disease to fish. It is because the disease can come out from the interaction among the host, pathogen and environment [14], [26], [27]. The water quality was measured in the beginning, middle and the end of the research purposely to control the condition of water quality during the fish farming. The condition of water quality during fish farming in each treatment was still based on the quality standards set by the Indonesian National Standard (2015). Thus, it can be assumed that the values of SR, RPS, MR and MTD, clinical symptoms and tissue damage found in each treatment was not caused by the water quality in the media of fish farming but caused by the treatment given. Water quality beyond the optimum range of life needs of fish can lead the fish to be stressful and then more vulnerable to disease [21], [26]. The condition of water quality during treatment, therefore, must be a concern to make it remains in the normal range. The values of DO on day 1 was in the range of 5 to 6.8, on day 7 it was in the range of 5.2 to 6.8, and on day 15 it was in the range of 5.5 to 6. These conditions indicated that DO was still relatively good [21].

The level of oxygen needed by Clarias sp. to live normally is 4 ppm [28], [17], [29]. The water temperature based on the observation was in the range of 26 -28°C. It meant that the condition of this temperature was still optimal for catfish that can grow well at the temperature of 25 - 30°C [21]. The value of water pH from the results of observation was in the range of 6.5 - 7.8. The optimal condition of pH to support the life of Clarias sp. is in the range of 6 - 8.5 [21], [27].

The dominant peaks in different retention time analyzed and identified from the compound of A. planci were Hexadecanoic acid and 13-Octadecenoic acid. The fatty acids were used as the proliferation of T cell inhibition, the inhibition of Interleukin 5 and 13 and the inhibition of macrophagephagocytosis [30], [31].

As stated by [25], fatty acid of omega-3 (n-3) Hexadecanoic acid and 13-Octadecenoic acid, together with (3)-linolenat and antioxidant modulate the response of systemic inflammation and increase the oxygenation and the results with the patients with acute lung injury. [29] reported that A. planci has a low lipid content, and good fatty acid composition profile manifested by the results that unsaturated fatty acids could reach 59.84 to 68.36% of the total of fatty acids; here, the polyunsaturated fatty acids contribute half of the unsaturated fatty acids. Also, the polyunsaturated fatty acids contain timnodonate acids (EPA) and Docosahexaenoic acid, Hexadecanoic acid and 13-Octadecenoic acid that have as an important physiological function for both human and animals. The fatty acid compounds in A. planci originated from algae and corals are the main food of this organism [32].

4. Conclusion

Based on the results of analysis, it can be concluded that there was an impact of providing the doses of fatty acid compound from A. planci, on the analysis of SR, RPS, and MTD in the challenge test with bacterium A.hydrophila compared to the fish not given the compound of A. planci.

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