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Research Article

The Supplementation of *Daphnia magna* Enriched Ascorbic Acid to Improve the Growth and Survival Rate of *Pterophyllum scalare*

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Abstract

Angelfish *(Pterophyllum scalare)* is a tropical ornamental fish in freshwater with considerable potential in the global market and promising cultivation. However, it has a slow growth. The purpose of this research was to find the impact of giving feeding variations, namely *D. magna* that enriched *ascorbic acid* on improving the angelfish growth and its immune response. The research method used was Completely Randomized Design (CRD) using four treatments and three replications. The dosage of ascorbic acid consisted of 0 mg/L, 50 mg/L, 100 mg/L, and 150 mg/L. The fish was fed twice a day and applied with *ad libitum*. The result showed the addition of ascorbic acid had impacted its growth, and it produced SGR 2.393±0.003 %/day and challenge tests. The Survival Rate (SR) after challenge test was 79.99±1.000 %. In conclusion, the highest amount of growth and survival was treatment D (150 mg/L).

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1. Introduction

Freshwater angelfish (*Pterophyllum scalare)* which originates from Amazon River, Guyana, and Orinoco, is one of the most important commercial cichlid species (García-Ulloa and Gomez-Romero, 2005; Ortega-Salas *et al*., 2009). Angelfish is quite popular around the world among aquarium hobbyist (Kasiri *et al*., 2011). Unfortunately, some of the obstacles of angelfish cultivation are its slowgrowth andlowsurvival rate due to low immune system that causes easily infected by pathogens (Perlberg *et al*., 2008; Murphy *et al*., 2009; Putra *et al.*, 2017). Therefore, it is necessary to develop variations in the form of its cultivation. One of them is to give *D. magna* enriched ascorbic acid forthe nutrition to angelfish larvae. *Daphnia* sp. is a natural feed commonly used for the larvae of freshwater fish (Kang *et al*., 2006; Rottmann *et al*., 2011).

The natural feed of *D. magna* is properly consumed as an immature digestive tract of angelfish larvae (Hermawan *et al., 2015*). One of the important nutrient elements in the feed that can affect the growth and survival of fish is ascorbic acid (Adel and Khara, 2016; Ramadhani *et al.*, 2017). This treatment is commonly carried out by most farmers to improve nutrition needed (Putra *et al.*, 2016). According to Dwinanti *et al.* (2019), feeding extra ascorbic acid orally to *Channa* sp. for 14 days shows a good impact on its protection from pathogens and environmental pressure, and it is good for its growth. The numbers obtained of said study showed significant results on absolute weight (1.83 g), absolute length (18.7 mm), resistance to environmental pressure in formalin water of 10% (16.7%), and the percentage of prevalence pathogens (16.7%).

The use of ascorbic acid as a feed supplement can improve the immune response (Nayak *et al.*, 2007; Zhou *et al.*, 2012). Moreover, ascorbic acid in feed can increase the daily growth rate and feed efficiency in anabas fish (Sunarto *et al.*, 2008) and tiger grouper fish (Mudiarti and Kursistiyanto, 2019). So far, there are only few researches about supplementation of *D. magna* enriched with ascorbic acid to Angelfish, therefore, this treatment is necessary to execute.

2. Materials and Methods

2.1 Materials

This research was conducted from February 5th to March 6th, 2020 in Fishery and Biology Laboratories of Universitas Muhammadiyah Malang and Fish Disease Laboratoryof Universitas Brawijaya. The materials used 180 angelfish larvae with the total length of 3±0.56 cm, ascorbic acid (pure), *D. magna*, *Aeromonas hydrophylla*, aquarium size of 40x30x30 cm^3, and water quality measurement tools (thermometer, DO meter, Universal pH, and NH3 *test kit*).

2.2 Methods

2.2.1 Experimental design

The method applied experimental method which purposed to find the influence of certain treatments to another in a control condition. In summary, the doses given at each treatment using Completely Randomized Design (CRD) according to Hanafiah (2012) are as follows:

Treatment A: *D. magna* without ascorbic acid (control)
Treatment B: *D. magna* combined with *ascorbic acid* 50 mg/L
Treatment C: *D. magna* combined with *ascorbic acid* 100 mg/L
Treatment D: *D. magna* combined with *ascorbic acid* 150 mg/L

2.2.2 Addition of D. magna

Each of the twelve aquariums were filled with 1L freshwater containing 100 ind./L *D. magna* combined with ascorbic acid based on the dosage, namely 0 mg/ l\L, 50 mg/L, 100 mg/L, and 150 mg/L. This dosage was done with four treatments and three replications. Then, it was slowly stirred with a spatula and soaked for 3 hours. This determined time was the most effective and efficient time for the treatments. Next, the *D. magna* was given to the angelfish larvae.

2.2.3 Fish rearing

The first step during rearing period was preparing the aquarium and aerating the water to adjust the water as well as the media for the angelfish larvae. Then, the fish was inserted with a density of 1 fish/L (Asma *et al*., 2016) as it is carnivorous and territorial. Fish larvae was reared for 30 days. Feeding was carried out after *D. magna* combined with ascorbic acid based on the specified dose. The feeding was given twice a day at 9:00 a.m. and 3:00 p.m. because their metabolic process took six hours to digest the food

2.2.4 Challenge test

Challenge test is to test the survival rate of angelfish that has been reared for 30 days. This process required 2.5 L of freshwater as the media. The next step was to give *A. hydrophilla* (10^6 CFU/ mL) as already determined through LD 50 (*Lethal Dose* 50%). In

addition, clinical symptoms and mortality rate were observed in each treatment.

2.2.5 Iodimetry titration test

The test of iodimetry titration aims to test the level of ascorbic acid absorbed in the body of *D. magna*. The procedure was preparing 100 ml of ascorbic acid added with 25 ml of sulfuric acid and 0.1 N of titration of iodine solution using starch indicator to form a blue color (Damayanti and Kurniawati, 2017).

2.2.6 Parameter

The main parameters include Specific Growth Rate (SGR), absolute length growth, Survival Rate (SR), and challenge test. The growth of angelfish larvae can be figured out through weight calculation using digital scale and formula as follows (Lugert *et al*, 2014):

$$SGR = \frac{In\ Wt - In\ Wo}{t} \text{ X } 100\%$$

where:
SGR = Spesific Growth Rate (% g/day)
Wt = the final body weight of the fish(g)
W0 = the initial body weight of the fish (g)
T = time (day)

The absolute length growth can be calculated by (Lucas *et al*., 2015):

$$Pm = Lt - Lo$$

where:
Pm = absolute length growth
Lt = the final length (cm)
Lo = the initial length (cm)

The survival rate of the fish can be calculated by (Muchlisin *et al.*, 2016):

$$SR = \left(\frac{Nt}{No}\right) \text{X } 100\%$$

where:
SR = *Survival rate* (%)
No = total number of the initial fish
Nt = total number of the final fish

The other parameters used were pH, temperature, DO, and ammonia which were measured usinguniversal pH, thermometer, DO meter, and ammonia test kit. The measurement of pH, temperature, and DO are conducted every day, while ammonia was only once a week.

2.3 Data Analysis

The data obtained were analyzed using one way ANOVAmethod on SPSS 26.0 and Ms. Excel 2013. The significant impact would be further tested using LSD (Least Significant Difference) to determine the optimum treatment at 95% confidence level.

3. Results and Discussion

3.1 The composition of ascorbic acid and D. magna

The result of analyzing ascorbic acid in the body of *D. magna* through iodometry titration test did not show a significant difference (Table 1). The result of the calculation of all treatments A(0 mg/L), B (50 mg/L), C (100 mg/L), and D (150 mg/L) was 17.6. The iodimetry titration test was executed after 3 hours of immersion process using ascorbic acid combined with *D. magna*. The variance analysis of treatment D-A, D-B, D-C were not significantly different because the ascorbic acid is maximally absorbed through immersion technique. The efficient time of immersion (3 hours) was supported by Jusadi *et al.* (2008) who stated that the length of addition of ascorbic acid 0, 3, 5, and 9 hours will decrease the survival of *D. magna* as the time goes on. The decrease is caused by the incapability of *D. magna* to digest the enzyme released by intestinal microflora in the body of *D. magna* which is lower in volume than ascorbic acid absorbed (Carr and Maggini, 2017).

The analysis result of 17.6 was the minimum tolerance value in the blank of iodimetry titration test. It is proven that there is absorption by *D. magna* as a whole from the total ascorbic acid given in each treatment which shows its increase on the growth of angelfish larvae. The increase in growth is started in treatment A, B, C, and D. Thus, the contents of *D. magna* also increase. According to Setiawati *et al.* (2013), the efficient value towards feed can be interpreted as beneficial absorption by feed from the given total feed. Treatment D (150 mg/L) is a high dose treatment given to *D. magna,* yet it is stillabsorbed. However, when the dose given exceeds the maximum limit on the bodies of *D. magna, it* will cause adverse effects because *D. magna* cannot properly absorb the ascorbic acid. Besides, the body of *D. magna* cannot produce cellulose enzymes, but it digests plan material through microbial intestinal fermentation.

The color can be seen as the most significant change (Figure 1). The control shows yellow color and the color of treatment 50 mg/L (treatment B) becomes darker. Thus, the more ascorbic acid is given, the darker the color will be. This occurrence is caused by instability of ascorbic acid in the form of solution. According to Sediaoetama (2010), dry ascorbic acid is stable yet easily damaged and degraded in the form of solution, particularly exposed to air, metals such as Cu, Fe, and light. Ascorbic acid exposed to light will become brown. The main characteristic is its ability to reduce and being reduced which is catalyzed by metals such as Cu and Ag.

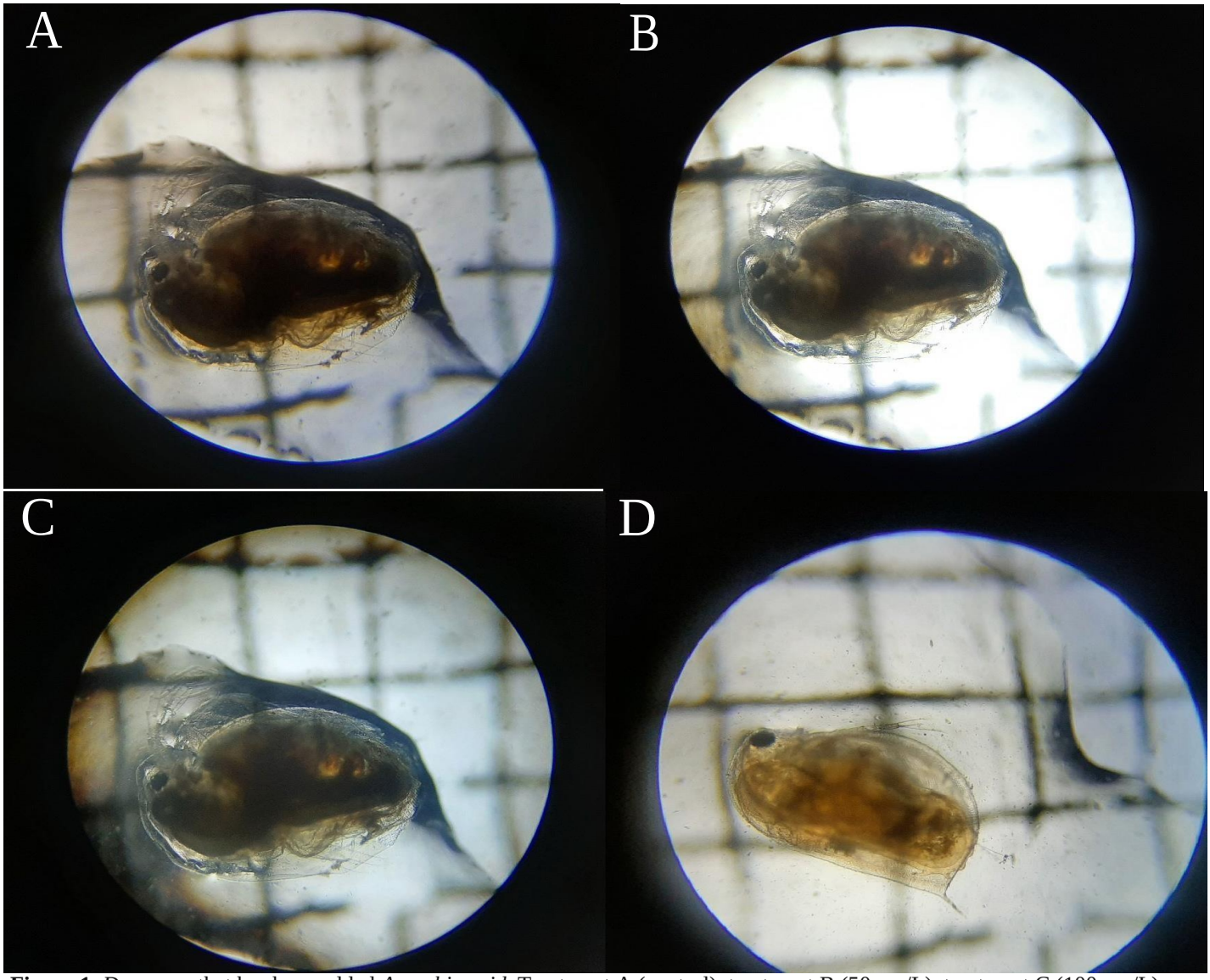

Figure 1. *D. magna* that has been added *Ascorbic acid*. Treatment A (control), treatment B (50 mg/L), treatment C (100 mg/L), and treatment D (150 mg/L)

Table 1. Iodimetry titration test

Treatment	Mean±SD
A (0 mg/L)	17.6^{a}±0
B (50 mg/L)	17.6^{a}±0
C (100 mg/L)	17.6^{a}±0
D (150 mg/L)	17.6^{a}±0

Note: A(control), B(50 mg/L *Ascorbic acid*), C(100 mg/L *Ascorbic acid*), and D(150 mg/L the same average number of *superscript* at the same column is not significantly difference (P>0.05).

Table 2. The range of water quality during maintenance for 30 days

Parameter	Range of water quality	Optimum Range	Source
Dissolved oxygen (ppm)	5.23-5.77	5 mg/l	Muhammad *et al.*, (2017)
pH	7-8	7 – 8 ppm	Affan (2012)
Temperature (°C)	24-30	25 – 30 °C	Muhammad *et al.*, (2017)
Ammonia (ppm)	0.00-0.25	≤ 0.02 mg/l	BSN (2009)

3.2 Absolute length growth and SGR

The analysis result (ANOVA) and advanced $LSD_{0.05}$ test showed that the addition of ascorbic acid was significantly different in term of the Specific Growth Rate (SGR) (Figure 2 & 3). On the other side, it was not significantly different in term of the absolute length growth and survival rate of angelfish larvae.

Based on the analysis data, the result showed that the addition of ascorbic acid in *D. magna* gave a significant impact ($P<0.05$) on the specific growth response of angelfish. The average of SGR of treatment A was 2.373^{a}±0.003%/day, treatment B was 2.384^{ab}±0.004%/day, treatment C was 2.392^{b}±0.007%/day, and treatment D was 2.393^{b}±0.004 %/day. After LSD test was conducted on treatment D-A and treatment D-B, the result showed that there was a significant different from the specific growth response of angelfish. In contrast, the treatment D-C was not significantly different. However, the treatment that mostly showed its significant different is treatment C (100 mg/L) and treatment D (150 mg/L). Therefore, the specific growth response in the treatment C and D could not be linked with the treatment A and B. It could be concluded that the treatment started from the 100 mg/L dose increased the weight of angelfish. The dose of ascorbic acid in *D. magna* can be tolerated at a dose of 150-200 mg/L ascorbic acid (Ramadhani *et al.,* 2017). The addition of ascorbic acid can increase its growth response on angelfish fries. These results aresupported by Setiawati *et al.* (2008) which stated that the addition of vitamin C can increase growth speed 1.23 times compared to fish fed without vitamin C content.

The absolute length growth on LSD test analysis showed the result on treatment A 2.05^{a}±0.06 cm, B 2.05^{a}±0.06 cm, C 2.06^{a}±0.05 cm, and D 2.07^{a}±0.03

cm. Statistically, treatment D-A, D-B, and D-C were not significantly different from the absolute length growth to the angelfish because the addition of ascorbic acid highly increased the appetite of fish. A good growth is supported by good feed quantities as well as provided nutrition (Manush *et al.,* 2013). The growthof angelfish on treatment D (150 mg/L) showed the best result than the other treatments because the number of feed consumption of *D. magna* provided good average, namely 54.06%, while the other treatment showed only 45.8% (treatment A), 47.43% (treatment B), and 50.06% (treatment C). Gunawan *et al.* (2014) argued that the lack of supply of vitamin C in feed can result in decreased appetite, slow growth, increased deaths, and abnormalities in the spine. It is strengthened by Sharma *et al.* (2012) who stated that fish has its own limit to eat and it depends on type and size of the feed. A good nutrition is necessary to provide fish needs including the addition of ascorbic acid (Ambarwati *et al.,* 2014). The addition of ascorbic acid increases the growth of angelfish and provides nutrients for enzyme to easily digest as well as to support the main parameter to increase the fish growth (Mohseni *et al.,* 2012). According to Agus and Tri (2010), feed with good nutrition based on the fish needs will show a good growth.

3.3 Survival rate and challenge test

Thechallenge test aimedtotestimmuneresponse of the fish that had been maintained for 30 days. This test used *A. hydrophilla* with a density of 10^{6} obtained from LD50 test (*Lethal Dose* 50%). The result was analyzed using statistics (Figure 4).

Based on the data obtained and tested using various analysis, the result showed significant difference ($p<0.05$). The advanced LSD test showed that treatment A was significantly different from treatment C and D, treatment B was significantly different from treatment D, and treatment C was significantly different from treatment D. It is proven that the addition of ascorbic acid in the natural feed can increase the survival rate and inhibit the infection process. It is because the ascorbic acid is an immunostimulant which can help to increase the resilience or immune response of angelfish. This statement is in accordance with Zhou *et al.* (2012) who stated that the reason of addition of ascorbic acid will periodically increase growth, non-specific immunity, and be able to protect from disease infection. This is also strengthened by Dwinanti *et al.* (2019) who stated that the addition of ascorbic acid gives an impact to minimize the infection of *A. hydrophila* in cork fish. Ascorbic acid is one of the potential antioxidants to protect cell tissue in fish through activating RB (Parker *et al.,* 2011). The addition of ascorbic acid in the feed for 14 days gives an impact towards the metabolism of cortisol, in which the cortisol regulation is better for stressful period than without ascorbic acid (Peng *et al.,* 2013). According to Leal *et al.* (2017), ascorbic acid functions in physiological process of the fish body including its immune system. Besides, ascorbic acid is also good in boosting osmoregulation, immunity, as well as an antioxidative agent (Dawood and Koshio, 2016). The ascorbic acid is needed in a small amount yet must be provided because of the nutrition impacts on fish immune response and helps to inhibit disease infection (Abdolbaghian *et al.,* 2010). Ascorbic acid is one of the essential components needed by the fish in optimal dose which depends on the type and age of the fish (Ebi *et al.,* 2018). If the fish has not been infected with the disease, immunostimulants will work to protect and increase the immune system of the fish as well as to perform collagen hydroylation to collagen (Gbadamosi *et al.*, 2013).

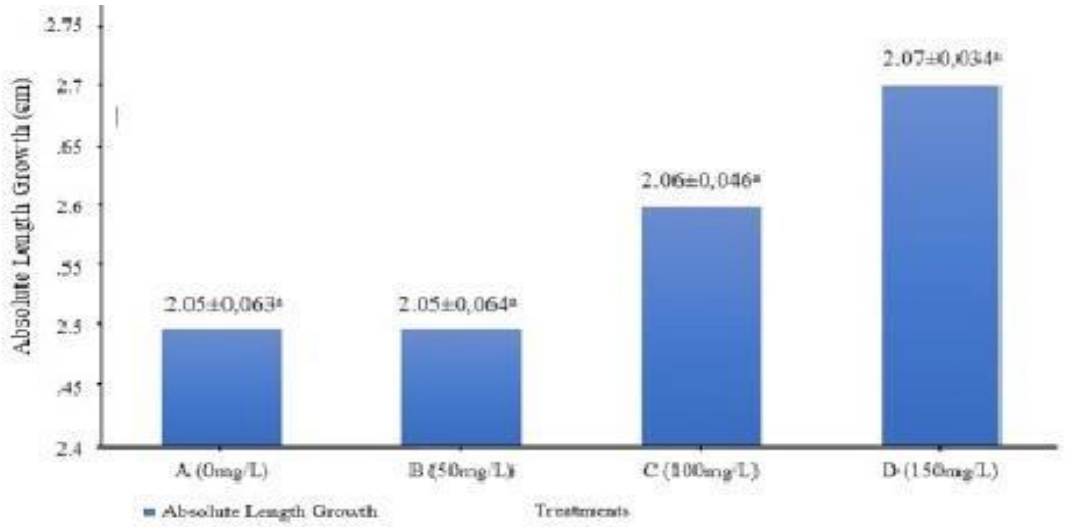

Figure 2. Absolute length growth graphics

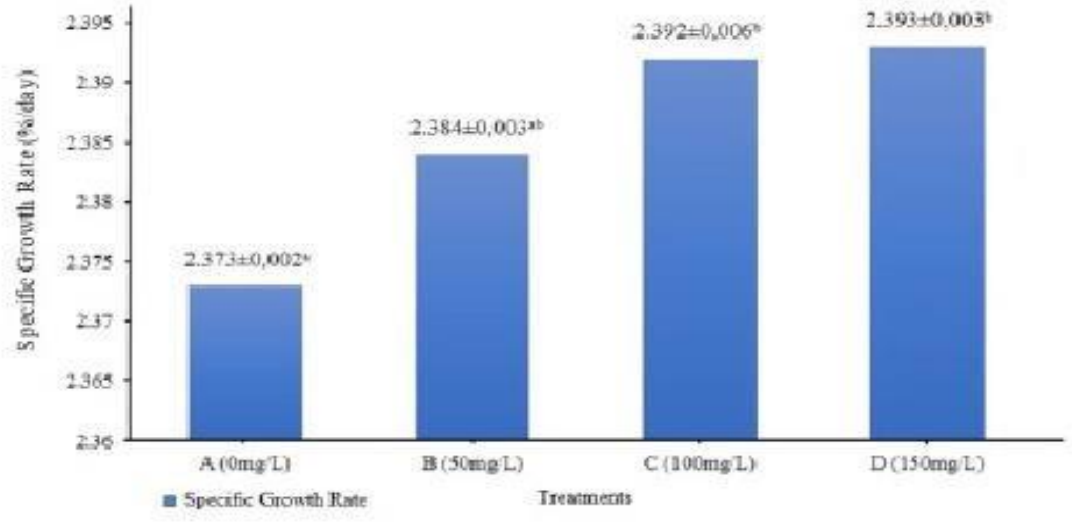

Figure 3. Specific growth rate

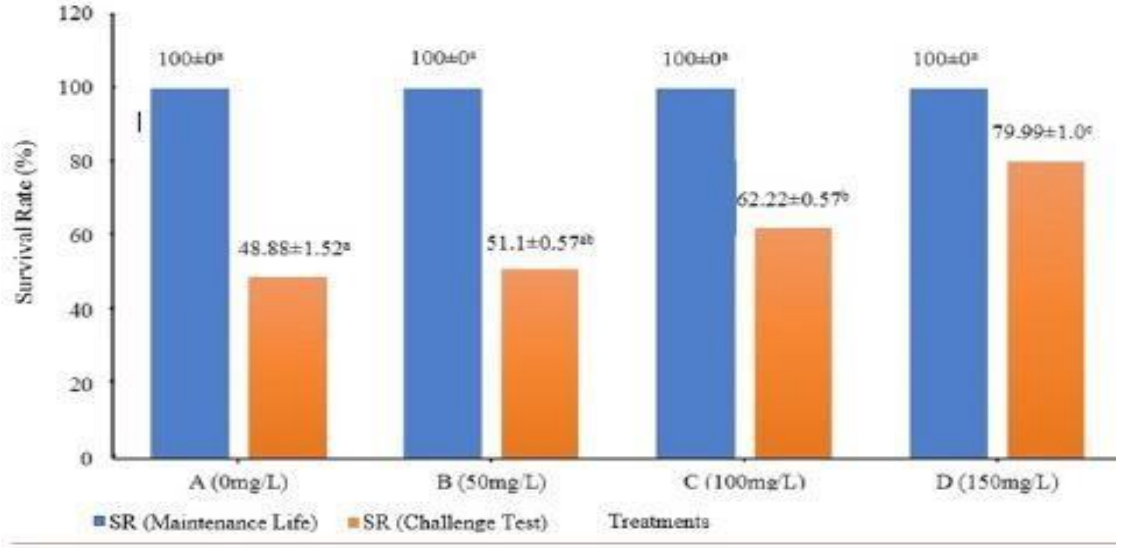

Figure 4. Rearing periode and challenge test graphics

3.4 Water quality

The water quality was checked periodically. The results were pH 7.5 and the temperatures were around 24°-28° C. Fluctuations happened at night but theimpact on *D. magna* was not significant because the crustacea could withstand temperature of 17-24° C (Affan, 2012). The poor water quality will have an impact on the loss of energy in fish, decreased appetite, and easily infected by disease. According to Faziel *et al.* (2017), the optimal cultivation water is about 22.7–23.9° C. A good DO will increase the growth rate and respiratory process, though it must be maintained above 5 ppm. The pH used in this research was based on Tatangindatu *et al.* (2013) who conveyed that a good and optimal pH for freshwater creatures is around 6.8-8.2, too acidic or basic pH fluctuation will cause the fish to move passively.

4. Conclusion

The enrichment of *D. magna* with ascorbic acid as thenatural feedforangelfishgives a significant impact on SGR and survival rate after challenge testing it using *A. hydrophylla*. The best dose of ascorbic acid in *D. magna* is the treatment D 150 mg/L in term of SGR and SR. It gives the survival rate to 100% (before challenge test), and survival rate after challenge test is 79.99^{a}±1.000% and SGR 2.393^{a}±0.003%/day. The addition of ascorbic acid in *D. magna* can become an alternative way to improve the immune response and the growth of angelfish.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follows, Titan Aji Wiratama; designed the methods, collected the data, and wrote the manuscript. Dessy Amitha Kusumaningdyah; collected and processed the data, and wrote the manuscript. All writers discussed and analyzed the data and wrote the final manuscript.

Conflict of Interest

The writer has no conflict of interest.

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