Available online at www.worldnewsnaturalsciences.com

World News of Natural Sciences

An International Scientific Journal

WNOFNS 40 (2022) 91-103 EISSN 2543-5426

The Prevention of *Aeromonas hydrophila* on Tilapia (*Oreochromis niloticus*) Fingerling using Current Generating Circular Tanks

Ibnu Bangkit Bioshina Suryadi, Naufal Muhammad Rizhmi*, **Roffi Grandiosa** and **Emma Rochima**

Fisheries Departement, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Bandung – Sumedang KM. 21, Jatinangor 45363, Indonesia

*E-mail address: naufalmrizhmi@gmail.com

ABSTRACT

This research focused on determining the best survival performance of tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) fingerlings in a current generating circular tanks and challenged by *Aeromonas hydrophila*. This research used a Completely Randomized Design (CRD) with four treatments and four replications. Fish size are ranging from 5 to 7 cm that obtained from Fish Breeding Center of Bandung City, Indonesia. The treatment used are water current and venturi combination with water velocities of 0 m s^{-1} (A), 0.06 m s^{-1} (B), 0.1 m s^{-1} (C), and 0.2 m s^{-1} (D). The research started with fish rearing (40 days) and was followed by a challenged test (14 days). Tilapia fingerlings were challenged by *A. hydrophila* at density 10^8 cfu ml^{-1} using immersion method. The parameters were gross clinical symptoms, survival rate, red blood cells, white blood cells and water quality. The results showed that current and venturi combination of 0.1 m s^{-1} gave the best immune performance seen from the increase in red blood cells and white blood cells with 48% of survival rate, however survival rate was not significantly different ($p \leq 0.05$) among treatments. The increase in red blood cells and white blood cells was from 1.1×10^6 cells mm^{3-1} to 5.1×10^6 cells mm^{3-1} and 4.2×10^5 cells mm^{3-1} to 5.6×10^5 cells mm^{3-1} respectively. Current generating circular tanks also enhanced tilapia immune system against *A. hydrophila* infection since fewer clinical symptoms occurred during challenged period.

***Keywords*:** Aeromonas hydrophila, current circular generating tank, venturi aeration, immunity, tilapia, *Oreochromis niloticus*

(Received 12 November 2021; Accepted 04 December 2021; Date of Publication 05 December 2021)

1. INTRODUCTION

Tilapia (*Oreochromis niloticus* (Linnaeus, 1758)) is a freshwater fish from the family Cichlidae and is an introduced fish from Africa (eastern of the Nile River, Lake Tanganyika, and Kenya) then brought to Europe, America, the Middle East, and Asia. Tilapia fish is one of the commonly cultured fish with high consumer demand due to the thick flesh with few spines and so, the need for tilapia fingerlings from year to year continues to increase.

A current generating circular tank is an aquaculture method categorized as a running water pond with the characteristic of water debit in the media can be completely replaced in minutes. Current generating circular tanks are closer to the aquaculture recirculation systems which is classified as a closed aquaculture system because there is no regular water change during the fish rearing.

Fish health is crucial when practicing aquaculture. Because if the cultured fish is exposed to disease, then the aquaculture activity will fail. One of the diseases that are often found in aquaculture activities is a bacterial disease which is an infectious disease that causes the death of fish in large numbers in a short time. One of the causes is *Aeromonas* bacteria which are widely found in freshwater, causing fish to have a high chance of being infected when the fish's immune system decreases due to stress and unfavorable environmental conditions.

The treatment of bacterial disease so far is by giving antibiotics. However, the use of antibiotics on a large scale is considered to be less efficient because it is uneconomical, can increase the types of bacteria that are resistant to antibiotics, and pollute the environment. Herbs have been widely used in aquaculture too, but they are not as effective as antibiotics. Therefore, the removal of bacteria attached to the fish can be done naturally by using a water current as an alternative to prevent fish disease.

2. MATERIALS AND METHODS

This research is done in Aquaculture Laboratory in Building No. 4 at Faculty of Fisheries and Marine Science, Universitas Padjadjaran. The equipment used was 16 current generating circular tanks which consist of water gallons with a volume of 15L, buckets with a volume of 15 L, PVC pipes with a diameter of ½ inch along with 16 faucets of a similar size, venturi aeration, water pumps with a capacity of 2000 L h^{-1}. The other equipment used was ammonia test kit, water quality checker (DO meter and pH meter), scoop net, and a fiber tank with a volume of 275 L.

The materials used were 160 tilapia fingerlings with size 5-7 cm obtained from Fish Breeding Center of Bandung City, Indonesia; *Aeromonas hydrophila* collected from Microbiology Laboratory at Faculty of Fisheries and Marine Science, Universitas Padjadjaran; and commercial feed with protein content 39%.

The method used was a Completely Randomized Design (CRD) with four treatments and four repetitions with the following details:

1) Treatment A: Tilapia fingerlings without venturi + water current and challenged with *Aeromonas hydrophila* with a density of 10^8 cfu ml^{-1} (control)
2) Treatment B: Tilapia fingerlings with venturi + water current 0.06 m s^{-1} and challenged with *Aeromonas hydrophila* with a density of 10^8 cfu ml^{-1}

3) Treatment C: Tilapia fingerlings with venturi + water current 0.1 m s^{-1} and challenged with *Aeromonas hydrophila* with a density of 10^8 cfu ml^{-1}

4) Treatment D: Tilapia fingerlings with venturi + water current 0.2 m s^{-1} and challenged with *Aeromonas hydrophila* with a density of 10^8 cfu ml^{-1}

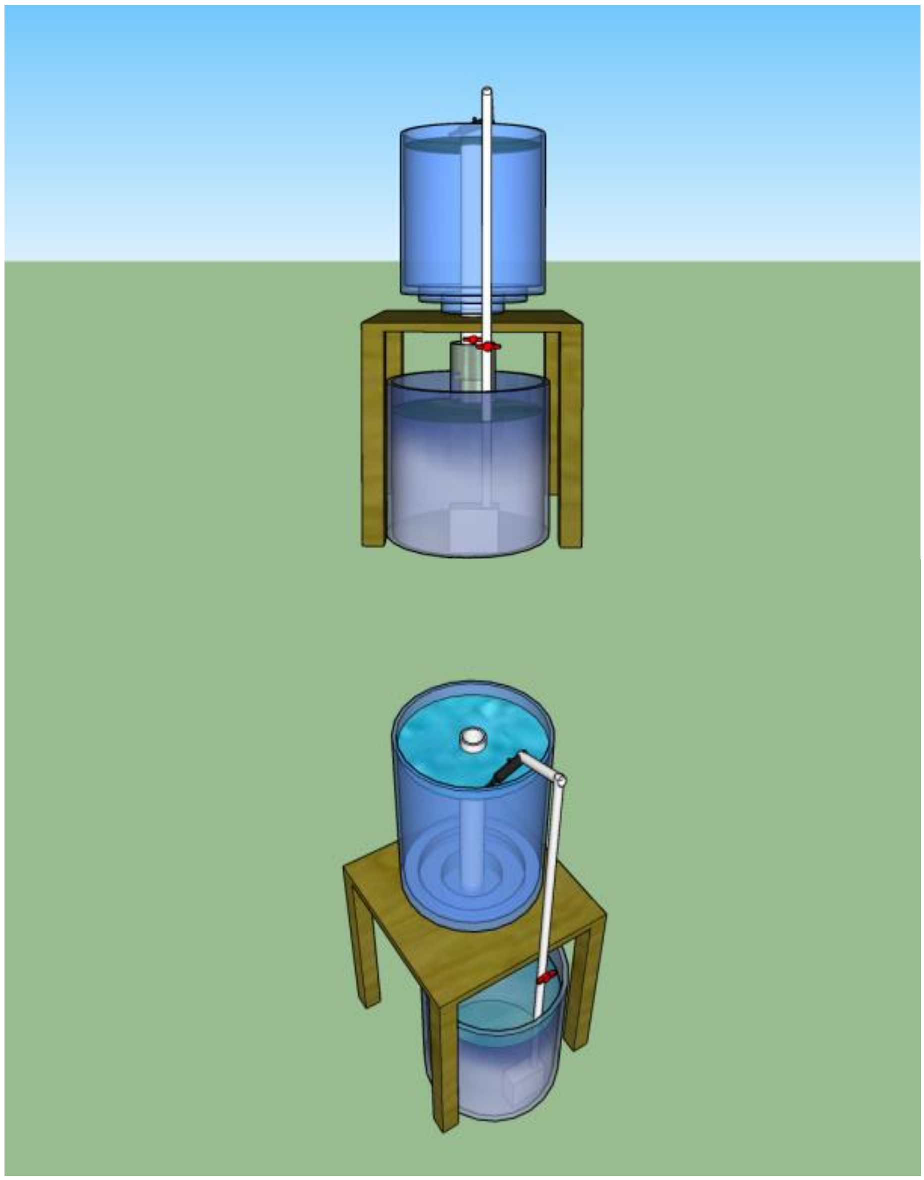


Figure 1. The Current Generating Circular Tank

2. 1. Main Research

This research was carried out for 40 days and was followed by a challenged test with *A. hydrophila* for 14 days using the current generating circular tank. The density of tilapia fingerlings used was 10 fish per unit. The daily feed given to tilapia fingerlings is as much as 4% of its body weight.

2. 2. Observation Parameters

a) Gross Clinical Symptoms

Observation of gross clinical symptoms was carried out by observing the macroscopic symptoms, such as wounds, change of body color, scale loss, fin rot, and dropsy.

b) Survival Rate

The survival rate observations were carried out every day starting from the first day of rearing until the end of challenged test. After 40 days of rearing in current generating circular tanks, the fish density is replenished before the challenged test. The calculation using formula from:

$$SR = \frac{Nt}{N0} x\ 100\%$$

SR = Survival Rate
Nt = Total fish fingerlings at the end of the research
N0 = Total fish fingerlings at the beginning of research

c) Red Blood Cells (RBCs)

Total red blood cells were counted at the beginning and the end of the challenged test and calculated using the formula:

$$\sum RBC \quad = Total\ Mean\ RBC\ \ x\ 50.000\ (Multification\ factor)$$

d) White Blood Cells (WBCs)

Total white blood cells were counted at the beginning and the end of the challenged test and calculated using the formula:

$$\sum WBC \quad = Total\ Mean\ WBC\ \ x\ 3.200\ (Multification\ factor)$$

e) Water Quality

The observation of water quality includes temperature, pH, ammonia (NH_3), and dissolved oxygen (DO). Water quality was observed five times, from the beginning of fish rearing, during treatments (every 10 days), and at the end of the challenged test.

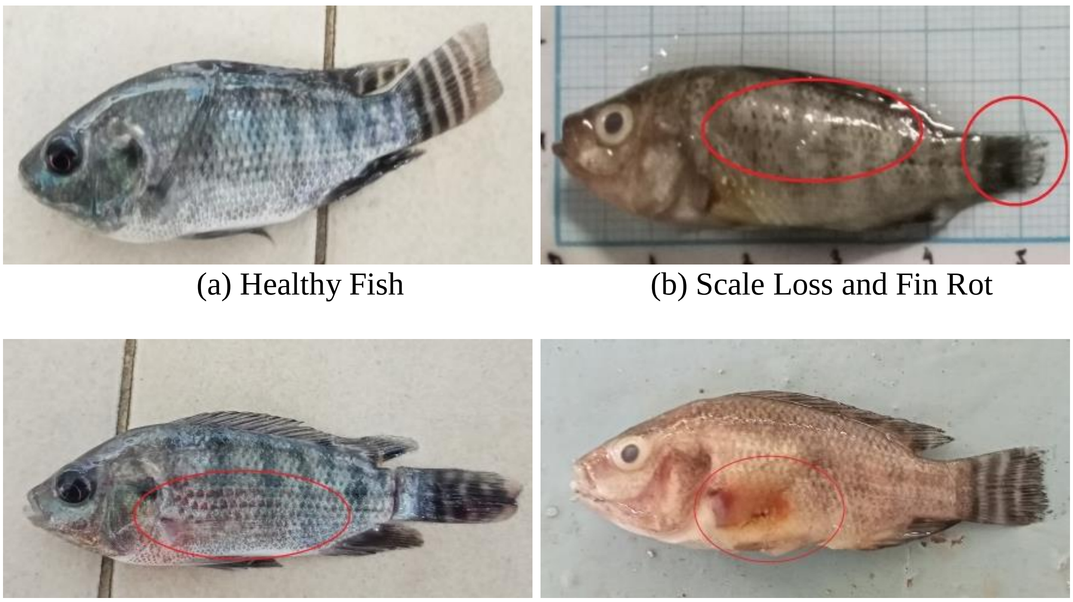
2. 3. Data Analysis

The obtained data were analyzed using ANOVA with p-value ≤ 0.05, and if the results were significantly different, Duncan's test is done. Meanwhile, the gross clinical symptoms and the water quality data were analyzed descriptively.

3. RESULT

3. 1. Gross Clinical Symptoms

The gross clinical symptoms in tilapia fingerlings were observed after being challenged by *A. hydrophila* which lasted for 14 days. The infection method is immersion with a density of 10^8 cfu ml^{-1}.



(a) Healthy Fish (b) Scale Loss and Fin Rot

(c) Red Spots (d) Bleeding (Hemorrhagic)

Figure 2. Gross Clinical Symptoms of Tilapia Fingerlings Challenged with *A. hydrophila*

Table 1. Gross Clinical Symptoms of Tilapia Fingerlings after Challenged by *A. hydrophila*.

Treatments	Replications	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	1	-	-	a	-	-	-	a	ab	b	a			c	
	2	-	-	-	-	-	-	-					c		
	3	-	-	a		b			a	ab		ab			
	4	-	-	-	c			a			a				
B	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	abc			a			bc					
	4	-	-	a	c	c		b			b				

C	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	ac				a			ac				
	4	-	-	ac											
D	1	-	-	-	-	-	-	-	-	-	ac				
	2	-	-	-	-	-	-	-	-	-	ac				
	3	-	-	bc				a							
	4	-	-	-	-	Ab	-	-	-	-	-	C	-	-	-

Note : (a) Peeling Scales and Fin Rot, (b) Red Spots, (c) Bleeding (Hemorrhagic)

The results of gross clinical symptoms during the challenged test are scale loss and fin rot (Figure 2b) the appearance of red spots on the body (Figure 2c) and hemorrhagic (Figure 2d). All of the symptoms are shown on the 3^{rd} day after challenged (Table 1). This shows that the tilapia fingerlings reared in current generating circular tanks in all treatment showed symptoms caused by *A. hydrophila*. However, the symptoms are not evenly distributed in each treatment and replication. In this case, all the water current treatments in this research have not optimally prevented the attachment of bacteria to tilapia fingerlings, but the symptoms they cause are not as severe as control.

This result is presumably caused by the decreased concentration of *A. hydrophila*. *A. hydrophila* with a density of 10^8 cfu ml^{-1} in the current generating tanks installation has not yet reached an effective lethal concentration. As in calm water, *A. hydrophila* will easily attach to fish so the lethal concentration is lower, which is at 10^6 cfu ml^{-1}. In addition, the metabolic ability of fish that live in water current conditions is increased as the stimulation of glucose utilization in the metabolic cycle escalated due to the fish movement against the water current. Thus, this affects the fish ability to resist pathogenic bacteria.

3. 2. Survival Rate

The result of survival rate in tilapia fingerling can be seen in Figure 3. The highest survival before challenged test is in A and B treatments with 58%, followed by C treatment with 55%, and the lowest being D treatment with 48%. Meanwhile, after challenged test, the highest survival is in B treatment with 53%, followed by A treatment with 50%, C treatment with 48%, and the lowest rate is D treatment with 43%.

After analyzing the variance on the survival rate, both before and after challenged by *A. hydrophila*, there are no significance ($p \leq 0.05$). That means all water current treatments show similar results. This could be due to the high adaptability of tilapia fingerlings to water current combined with venturi, although treatment D with velocity of 0.2 ms^{-1} had the lowest survival. This is confirmed by the research which showed that tilapia fingerlings can live at 0.1 ms^{-1} with venturi combined with water current as same as this research. As explained in gross clinical symptoms, tilapia fingerlings that are kept under water current still infected by *A. hydrophila* but showed less symptoms than control.

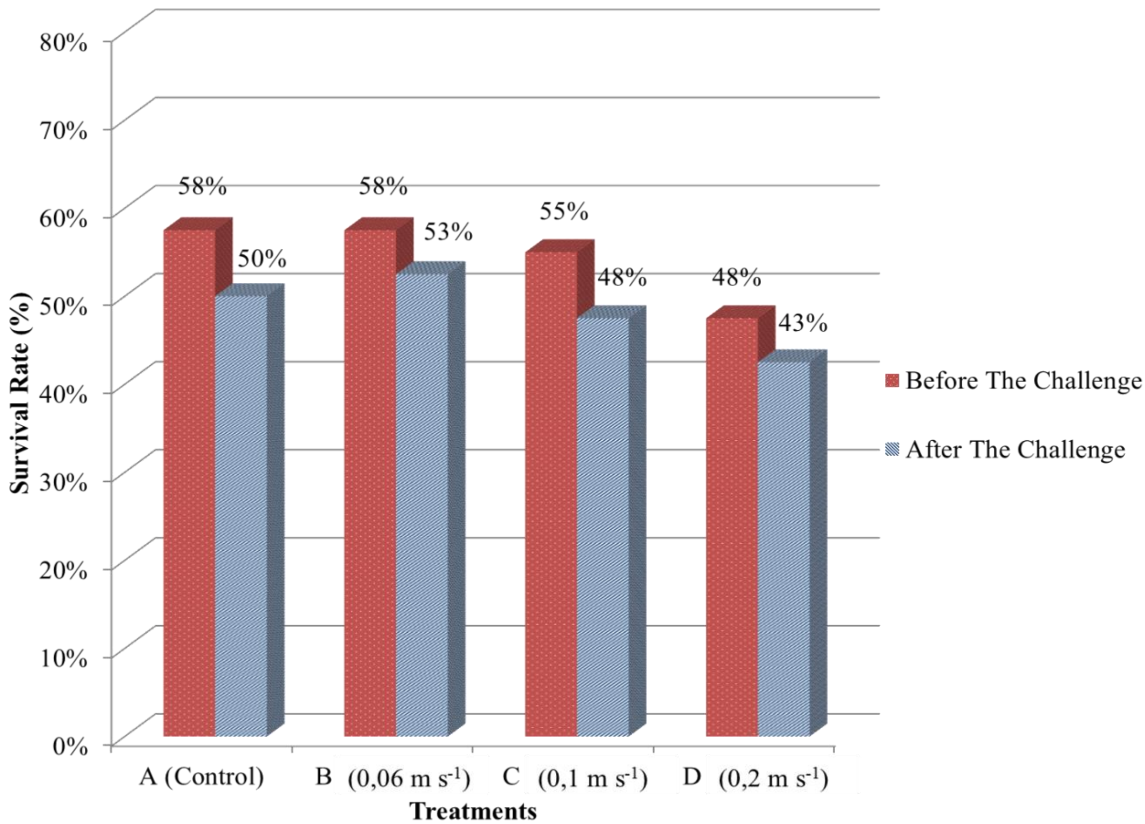


Figure 3. Survival Rate Graph

3. 3. Red Blood Cells (RBCs)

The result of the red blood cells (RBCs) can be seen in Figure 4 and shows that before challenged the total RBCs were in the range of 8.98×10^5 cells $mm^{3\,-1}$ to 1.1×10^6 cells $mm^{3\,-1}$. Then after being challenged for 3 days, the number of RBCs increased to a range of 7.8×10^5 cells $mm^{3\,-1}$ to 1.8×10^6 cells $mm^{3\,-1}$, 2.2×10^6 cells $mm^{3\,-1}$ to 4.2×10^6 cells $mm^{3\,-1}$ on 5th day after challenged, 2.2×10^6 cells $mm^{3\,-1}$ to 5.1×10^6 cells $mm^{3\,-1}$ on 10th day after challenged and decreased in 14th day after challenged to 1.8 x 10^6 cells $mm^{3\,-1}$ to 4.5×10^6 cells $mm^{3\,-1}$.

The ANOVA showed a significant difference ($p \leq 0.05$) between treatments. Furthermore, when the data were re-tested using the Completely Randomized Factorial Design (CRFD) method with the same level of p-value, there was a significant difference between the challenged period. There is an interaction between the number of RBCs at all treatments with the challenged period with the best result obtained by C treatment (0.1 ms^{-1}) after 5 days post-challenged.

Compared to the number of normal tilapia fingerling RBCs ($2 \times 10^5 – 3 \times 10^6$ $mm^{3\,-1}$) RBCs in this study were higher. This increase shows that the tilapia immune status was better than conventional culture. The increasing number of RBCs especially in their nucleic acid has an important role in fish immunity because RBCs modulate specific protein on responding to viruses or pathogens hence the immunity response is better when compared to a lower number of RBCs. In such manner, *A. hydrophila* cannot fully infect tilapia fingerlings, which can be seen from its lesser gross clinical symptoms.

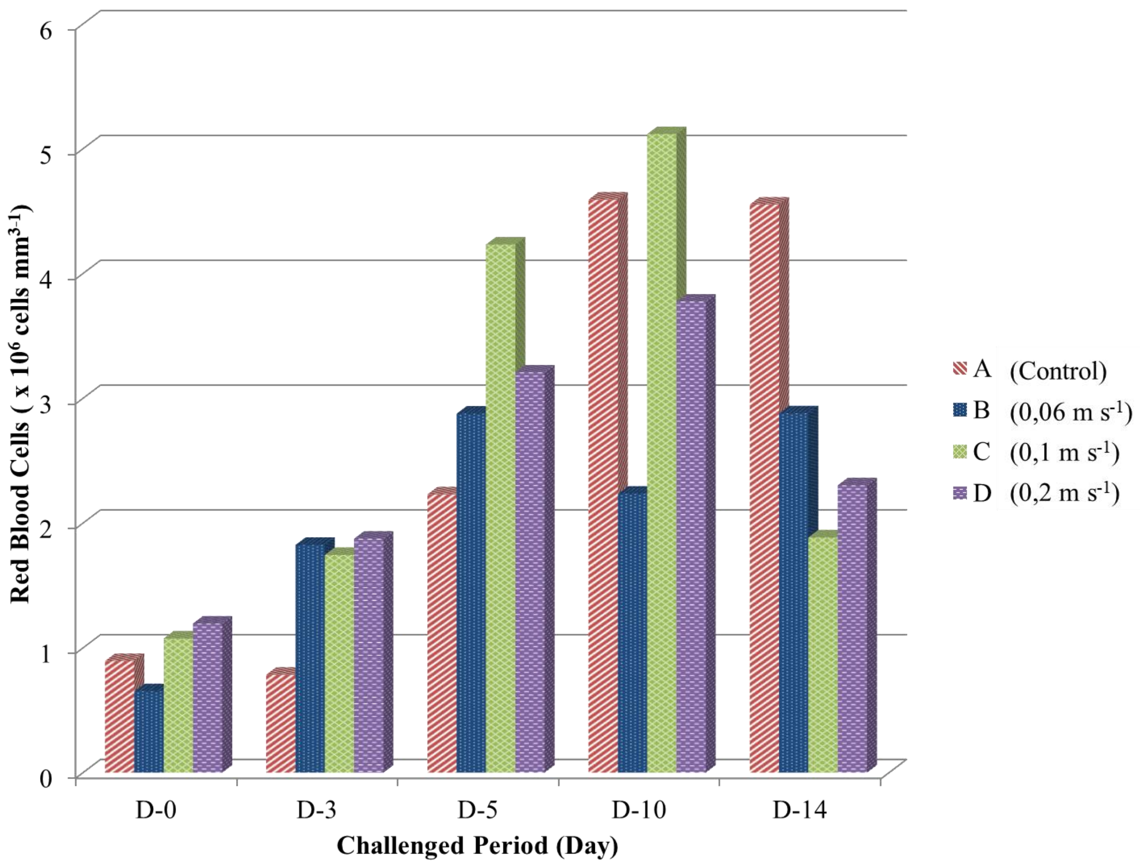


Figure 4. Red Blood Cells Graph

The fluctuation in the number of RBCs occurs because it can be influenced by water flow. This is because when the water velocity is high, the dissolved oxygen in the water and the oxygen content in the RBCs increase so that the metabolic ability of the fish also increases due to the abundant oxygen supply, and the stress level in fish is reduced.

The decreasing RBCs on the 14th day after challenged indicated that the infected tilapia with *A. hydrophila* when reared in a current generating circular tank with velocity of 0.1 m s^{-1} on the 10th day after challenged was sufficient to give the best results on the RBCs. It can be concluded that infected tilapia fingerlings can maintain their metabolism for 10 days in flowing waters, this is due to high energy demand for swimming against the water current.

3. 4. White Blood Cells (WBCs)

The result of white blood cells can be seen in Figure 5 and shows that before the challenged WBCs number were in the range of 1.4×10^5 cells $mm^{3\ -1}$ to 4.2×10^5 cells $mm^{3\ -1}$. On the 3rd day after challenged total WBCs was in the range of 3.3×10^5 cells $mm^{3\ -1}$ to 5.6 ×

10^5 cells $mm^{3\,-1}$, and then decreased on the 5th day after challenged in the range of 2.7×10^5 cells $mm^{3\,-1}$ to 3.5×10^5 cells $mm^{3\,-1}$, on the 10th day after challenged in the range of 1.9×10^5 cells $mm^{3\,-1}$ to 4×10^5 cells $mm^{3\,-1}$ and decreased again in the range of 1.1×10^5 cells $mm^{3\,-1}$ to 2.8×10^5 cells $mm^{3\,-1}$ on 14th day after challenged.

ANOVA shows that there are differences between treatments ($p \leq 0.05$). Similar to the RBCs data, the WBCs were re-analyzed using the CRDF method with the same level of p-value showing a significant difference between challenged periods. There is an interaction between WBCs in all treatments with the challenged period. As a result, C treatment (0.1 ms^{-1}) on 3rd day after infection showed a significant increase in the WBCs compared to the other treatments.

The escalated number of WBCs in C treatment is related to the tilapia's immune response to all kinds of pathogens. WBCs, as explained have the function of protecting the fish from pathogen infections. This indicated that the presence of pathogens responded by the tilapia immune system with producing more WBCs when compared with normal number of WBCs (2×10^4 - 15×10^4 $mm^{3\,-1}$).

Then on the 5th day post-challenged the WBCs numbers decreased which means that the tilapia fingerlings were already withstood *A. hydrophila* infection. However, on the 10th day post-challenged, the number of WBCs increased again but not as high as before and that means the resistance to pathogen occurred once more but on the last day of challenged, it decreased again.

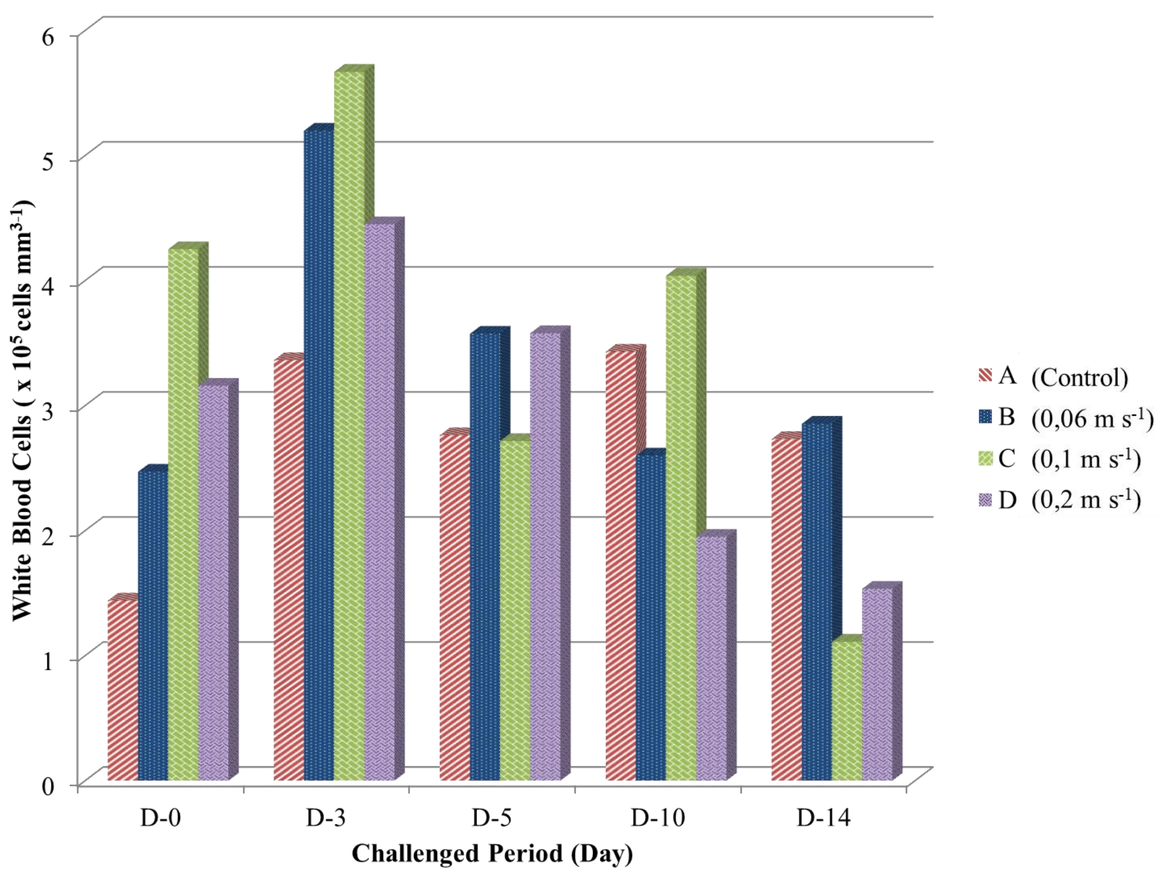


Figure 5. White Blood Cells Graph

This WBCs fluctuation is explained which said that infected fish showed an increase in the number of WBCs as an immune response followed by an increase in antibodies. This means that the tilapia fingerlings immune system reared in the current generating circular tanks after being challenged by *A. hydrophila* has increased. The WBCs fluctuation is also presumably due to the dividing energy of the tilapia fingerlings between swimming against water current and resisting pathogen. In addition, the better response of the WBCs can be seen by the lesser gross clinical symptoms that appear in the tilapia fingerlings. Aside from that, the phagocytic ability on the pathogen in the fish body increases along with the escalation in oxygen utilization since the venturi installed in the circular tank increases the dissolved oxygen so that oxygen utilization by fish becomes more efficient.

3. 5. Water Quality

The results of water quality include parameters such as temperature, pH, ammonia (NH_3), and dissolved oxygen (DO) can be seen in Table 2.

Table 2. Water Quality Range during Research

Treatments	**Parameters**			
	Temperature (°C)	pH	NH_3 (mg L^{-1})	DO (mg L^{-1})
A	24.0 – 27.5	5.72 – 7.62	0.0014	5.4 – 8.6
B	24.4 – 27.5	6.00 – 6.98	0.0014	5.9 – 8.6
C	23.5 – 27.5	6.14 – 7.24	0.0014 – 0.003	6.3 – 8.7
D	24.1 – 27.5	6.50 – 7.38	0.0014	6.2 – 8.7
Optimum	25 – 36	6.5 – 8.5	< 0.2	> 5

Water quality parameters are in an optimum range for maintaining tilapia fingerlings. That means tilapia fingerlings reared in the current generating circular tank have the optimum value for them to live, despite the lowest temperature shows a value slightly below optimal since no heater was used in the installation.

4. CONCLUSIONS

The conclusions from this research are the current generating circular tank with 0.1 m s^{-1} did not affect the survival of tilapia fingerlings when challenged by *A. hydrophila*, hence it resulted in rapid recovery shown by lesser gross clinical symptoms and better RBCs and WBCs numbers in certain period of time (10 days after challenged).

References

[1] Maisyaroh, Latifah Apriliana, Titik Susilowati, Alfabetian Harjuno Condro Haditomo, Fajar Basuki and Tristiana Yuniarti. The Use of Mangosteen Peel Extract (Garcinia mangostana) as Antibacterial to Treat Aeromonas Hydrophila Infection in Tilapia (Oreochromis niloticus). *Indones. J. Sc Trop. Aquac* 2: 2, 36-43, 2018

[2] Wahjuningrum, D., Astrini, R., & Setiawati, M. (2014). Prevention of Aeromonas hydrophila on catfish juvenile using garlic and shatterstone herb. *Jurnal Akuakultur Indonesia*, 12(1), 86-94. https://doi.org/10.19027/jai.12.86-94

[3] S. R. Puente-Marin, Thwaite, L. Mercado, J. Coll, N. Roher, and M. D. M. Ortega-Villaizan. Fish Red Blood Cells Modulate Immune Genes in Response to Bacterial Inclusion Bodies Made of TNFa and a G-VHSV Fragment. *Front. Immunol*. 10 1055, pp. 1-11. 2019.

[4] Afifah Shabirah, Rosidah, Yuniar Mulyani, Walim Lili, Effect of Types Isolated Lactic Acid Bacteria on Hematocrit and Differential Leukocytes Fingerling Common Carp (Cyprinus carpio L.) Infected with Aeromonas hydrophila bacteria. *World News of Natural Sciences* 24 (2019) 22-35

[5] Auryn Ramadhany Geraldine, Rosidah, Heti Herawati, Ibnu Bangkit Bioshina, Isolation and Identification of Potential Pathogenic Bacteria in Living Carp (Cyprinus carpio Linnaeus, 1758) Sold in Supermarkets in Cimahi City, Java. *World News of Natural Sciences* 32 (2020) 21-35

[6] Ibnu Bangkit Bioshina Suryadi, M. Ihsan Fadylah, Iskandar, Ayi Yustiati, The Productivity of Using Current Generating Circular Tanks for Tilapia Fingerling (Oreochromis niloticus (Linnaeus, 1758)) Production. *World News of Natural Sciences* 27 (2019) 85-95

[7] Imtiaz Ahmed, Quseen Mushtaq Reshi, and Francesco Fazio. The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. *Aquaculture international* 28, no. 3 (2020): 869-899. doi: 10.1007/s10499-019-00501-3

[8] Yustiati, A., Nariswari, S., Rostini, I., & Suryadi, I. B. B. (2020). Effect of Stocking Density on Survival Rate and Growth of Tilapia (Oreochromis niloticus Linnaeus, 1758) in Round Container with Water Current Combined with Venturi Aeration System. *Asian Journal of Fisheries and Aquatic Research*, 8(1), 52-61. https://doi.org/10.9734/ajfar/2020/v8i130132

[9] Chauret C, Volk C, Creason R, Jarosh J, Robinson J, Warnes C. Detection of Aeromonas hydrophila in a drinking-water distribution system: a field and pilot study. *Can J Microbiol.* 2001 Aug; 47(8): 782-786

[10] Castro V, Grisdale-Helland B, Helland SJ, Kristensen T, Jørgensen SM, Helgerud J, Claireaux G, Farrell AP, Krasnov A, Takle H. Aerobic training stimulates growth and promotes disease resistance in Atlantic salmon (Salmo salar*). Comp Biochem Physiol A Mol Integr Physiol.* 2011 Oct; 160(2): 278-90. doi: 10.1016/j.cbpa.2011.06.013

[11] Makori, A.J., Abuom, P.O., Kapiyo, R. *et al.* Effects of water physico-chemical parameters on tilapia (Oreochromis niloticus) growth in earthen ponds in Teso North Sub-County, Busia County. *Fish Aquatic Sci* 20, 30 (2017). https://doi.org/10.1186/s41240-017-0075-7

[12] Rieger AM, Barreda DR. Antimicrobial mechanisms of fish leukocytes. *Dev Comp Immunol.* 2011 Dec; 35(12): 1238-45. doi: 10.1016/j.dci.2011.03.009

[13] Palstra AP, Planas JV. Fish under exercise. *Fish Physiol Biochem.* 2011 Jun; 37(2): 259-72. doi: 10.1007/s10695-011-9505-0

[14] M. Dj Ratoe Oedjoe, E. Suprayitno, & E. Y. Herawati. Effect of Flow Water Velocity on Hematology Component in Improving Quality of Tiger Grouper Juvenile (Epinephelus fuscoguttatus). *Journal of Coastal Development*, 15(3), 260–269, 2012

[15] Rida Oktapiani, Kiki Haetami, Evi Liviawaty, Walim Lili, Effect of Using Fermented Coconut Testa by Rhizopus oryzae Went & H.C. Prinsen Geerligs, (1895) on the Growth of Red Tilapia Seeds (Oreochromis niloticus Linnaeus, 1758). *World Scientific News* 162 (2021) 29-42

[16] Shalsabilla Nariswari, Ayi Yustiati, Iis Rostini, Ibnu Bangkit Bioshina Suryadi, Effect of Stocking Density on Water Quality of Tilapia (Oreochromis niloticus Linnaeus, 1758) in Round Container with Current and Venturi Aeration System. *World Scientific News* 145 (2020) 397-408

[17] Dharmaraj Ramesh, Annadurai Vinothkanna, Amit Kumar Rai, and Venkada Subramanian Vignesh, Isolation of potential probiotic Bacillus spp. and assessment of their subcellular components to induce immune responses in Labeo rohita against Aeromonas hydrophila, *Fish & Shellfish Immunology*, 45, 2, (268), (2015).

[18] Sib Sankar Giri, Shib Sankar Sen, Cheng Chi, Hyoun Joong Kim, Saekil Yun, Se Chang Park, and V. Sukumaran, Chlorophytum borivilianumPolysaccharide Fraction Provokes the Immune Function and Disease Resistance ofLabeo rohitaagainstAeromonas hydrophila, *Journal of Immunology Research*, 2015, (1), (2015).

[19] Rosidah, Maria Dewi Yunita, Isni Nurruhwati, Achmad Rizal, Histopathological changes in gold fish (Carassius auratus (Linnaeus, 1758)) infected by Aeromonas hydrophila bacteria with various densities. *World Scientific News* 142 (2020) 150-168

[20] Ayi Yustiati, Sadra Muhammad Haydar Ali Saefulhadjar, Ibnu Bangkit Bioshina Suryadi, Iskandar, Hematological Performance and Survival of Common Carp (Cyprinus carpio Linnaeus, 1758) Strain Majalaya Fingerlings Against Temperature Stress. *World Scientific News* 150 (2020) 92-104

[21] S.S. Giri, V. Sukumaran, S.S. Sen, and P.K. Jena, Effects of dietary supplementation of potential probiotic Bacillus subtilis VSG1 singularly or in combination with Lactobacillus plantarum VSG3 or/and Pseudomonas aeruginosa VSG2 on the growth,

immunity and disease resistance of Labeo rohita, *Aquaculture Nutrition*, 20, 2, (163-171), (2013).

[22] Hien Van Doan, Sompong Doolgindachbaporn, and Amnuaysilpa Suksri, Effects of low molecular weight agar and Lactobacillus plantarum on growth performance, immunity, and disease resistance of basa fish (Pangasius bocourti, Sauvage 1880), *Fish & Shellfish Immunology*, 41, 2, (340), (2014).

[23] Zulfahana Amatulloh, Yuniar Mulyani, Lantun Paradhita Dewanti, Kiki Haetami, Growth Characteristics and Tracing Antagonistic Properties of Bacillus flexus and Bacillus subtilis as Antibacterials to Overcome the Attack of Aeromonas hydrophila Bacteria on Fish. *World Scientific News* 158 (2021) 91-104

[24] Ibnu Bangkit Bioshina Suryadi, Regan Hanifelian Yusa, Grandiosa H. Roffi, Zahidah, The performance of Koi (Cyprinus carpio L.) in a circular current container tested against the Aeromonas hydrophila bacteria. *World Scientific News* 159 (2021) 1-19

[25] Ratchanu Meidong, Kulwadee Khotchanalekha, Sompong Doolgindachbaporn, Takahiro Nagasawa, Miki Nakao, Kenji Sakai, and Saowanit Tongpim, Evaluation of probiotic Bacillus aerius B81e isolated from healthy hybrid catfish on growth, disease resistance and innate immunity of Pla-mong Pangasius bocourti, *Fish & Shellfish Immunology*, 73, (1), (2018).