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Original Paper

# EFFECT OF FLOW WATER VELOCITY ON HEMATOLOGY COMPONENT IN IMPROVING QUALITY OF TIGER GROUPER JUVENILE (*Epinephelus fuscoguttatus*)

Marcelien Dj.Ratoe Oedjoe[1], E. Suprayitno[2], Aulanni'am[3], E. Y. Herawati[2]
[1]Faculty of Agriculture, University of Nusa Cendana, Indonesia
[2]Faculty of Fishery and Marine Science, University of Brawijaya, Malang, Indonesia
[3]Faculty of Mathematic and Natural Science, University of Brawijaya, Malang, Indonesia

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## ABSTRACT

*The flow water velocity is closely correlated to oxygen demand. Oxygen plays an important role as the indicator of fish blood. The objective of research is to acknowledge red blood cell, hematocrit, hemoglobin, and white blood cell of grouper larvae given with flow water velocity treatments. Research begans from June 2011 to September 2011 at Lampung Marine Aquaculture Center. Juvenile with 7-9 cm length and 15-17 g/fish weight were tested using complete random design with three treatments of flow water velocity, i.e. 1.25 m/sec (A), 1.00 m/sec (B), and 0.75 m/sec and control treatment. All treatments had 3 replications. Result of research indicates that flow water velocity had obvious effect on red blood cell, hematocrit, hemoglobin, and white blood cell. Red blood cell total was the highest in treatment of 1.25 m/second, counted for 2,922,666 cells/ml. It was followed by treatment B with 2,816,000 cell/ml, treatment C with 2,763,000 cell/ml and control with 2,573,333 cell/ml. The highest hemoglobin was found in treatment A, followed by B, C, and control treatments. The highest hematocrit rate was seen in treatment A, followed by B, C and control treatments. The highest white blood cell total was discovered in control, followed by C, B and A treatments.*

**Keywords:** flow water velocity; hematology component; tiger grouper juvenile
**Correspondence :** Phone: +62-380-811560 ; E-mail : lien_tallo@yahoo.com.au

# INTRODUCTION

Grouper fish is distributed throughout tropic and subtropic area (Yushimitsu *at al*., 1986). In general, grouper fish is subsumed into protogenous hermaphrodite class. Tiger grouper (*Epinephels fuscoguttatus*) is protogeny fish which is widely cultivated in Indonesia (Zainuddin, *et.al.,* 2008). Tiger grouper represents one economically valuable fish in the range between Rp 50,000,- and Rp 70,000,- per kilogram at cultivator/fisher level. Such fact urges the fishing enterprise to develop. However, the preservation of environment is disturbed, while the reef environment is damaged due to less environmental friendly fishing methods, such as the use of potassium or cyanide. The cultivation to produce the qualified tiger grouper larvae may be important to consider.

Germinating is an activity to supply germ for cultivation work or natural restocking. Indonesian germinating technology has been developed and advanced. However, this technology cannot be applied in any conditions. Different geographic sites may result in different weather and natural condition (waters).The limited structure and infrastructure requires the technology to be subjected for adjustment and modification based on the existing condition. It is important to ensure the compatibility between technology and community that people easily adopt and apply the technology to obtain better germ production. According to Nana *et al.,* (2007) a simple technology to consider is artificial current stimulation. Tajerin *et.al.*, (2000). This method will control the stability of water quality to increase the quality of the fish because fish quality is given greater consideration by consumer, especially for the attributes such as slimmer body and more compact meat texture. (Sorta and Yuwono, 2005). A dynamic condition in the streamed

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water may stimulate fish to move because dynamic activity will be correlated to oxygen demand. (Edwan, 1998) mentioned that oxygen plays important role as the indicator of fish blood. The good dissolved oxygen rate is more than 4 mg/l. The oxygen rate of waters can influence fish hematology. According to Stoskopf (1993) hematology test must be useful to diagnose health status of fish. Hematology is closely related to fish health to ensure whether the fish is sick or healthy. Further Fange, (1994) red blood cell functions as transporting the respiratory gas, and its concentration is related to the dissolved oxygen rate and habitat. Smith, (2007) red blood cell count in the highl activate fish is higher than the slow activate. Improving the quality of tiger grouper larvae, therefore, the research of the effect of flow water velocity on hematology is required.

The recent research is aimed at understanding the performance of red blood cell, hemoglobin, hematocrit, and white blood cell of tiger grouper juvenile.

## MATERIALS AND METHODS

The experiment was started from June to September 2011. It was located at Lampung Marine Cultivation Center. Research design was Completely Randomised Design. Recirculation system used comprising to use three treatments of flow water velocity, i.e 1.25 m/sec (A), 1.00 m/sec (B), and 0.75 m/sec, with three replications. The fish observed was tiger grouper germ with 7-8 cm length and 10-12 grams weight. Spreading density was 5 fishes/ batch. Research batch was plastic container with the dimension of 35 cm top diameter, 29 down diameter, 35 cm height, 24 l volume, and 25 cm water content (20 l). Fish was cultured for 90 days (3 months) for hematology test using Wirawan and Silman (2000). The hematological features included red blood cell, white blood cell, hemoglobin and hematocrit. During the keeping, fish was fed (artificial food) three times a day, at 6 am, 12 pm, and 5 pm toward saturated *(ad libitum*). Food used in the research was supervigro 5 mm pellet type which was composed of 45 % protein, 13 % fat, 4 % fiber, 5 % BETN, 3.6 call/g, and 20 % water content. Water quality was retained by siphoning food remnant and fish feces once of two days.

After 90 days rearing, blood sample was taken for test. Blood parameters observed were red blood cell count, white blood cell count, hemoglobin rate and hematocrit rate. All were subjected to F-test to observe the effect of treatment. If the difference was found between treatments, it is followed by Tukey Test (Fowler *at al.*, 2000).

## RESULTS AND DISCUSSION

Result of the observation onto tiger grouper hematology during 90 days provides the following results.

*Mechanisme of effect flow water velocity on red blood cell count (Erythrocyte)*

Erythrocyte total of tiger grouper from flow water velocity treatments is shown in **Fig. 1.**

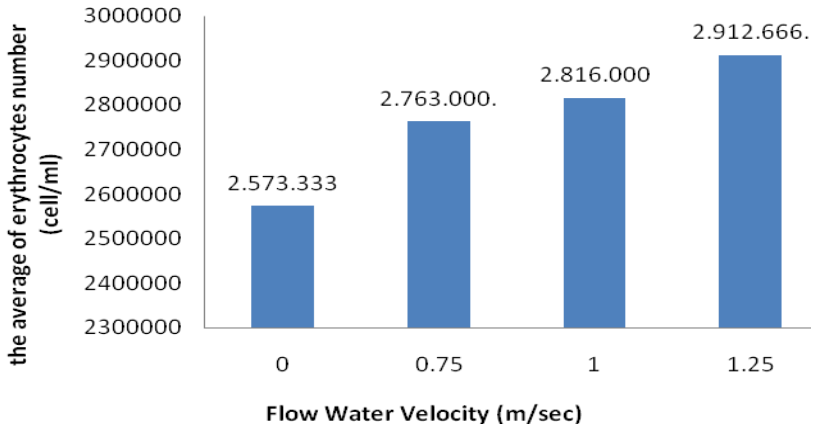


**Fig. 1.** Histogram of average erythrocyte concentration (cell/ml) of tiger grouper in different flow water velocity

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**Fig. 1,** shows that the highest erythrocyte average was achieved at water velocity of 1.25 m/sec which was counted for 2,922,666 cells/ml. It was followed by treatment of 1 m/sec with 2,816,000 cell/ml, treatment of 1.00 m/second with 2,763,000 cell/ml and control with 2,573,333 cell/ml.One of the hematological parameters were observed to determine the effect of flow water velocity treatment on fish health was the total erythrocytes. Erythrocytes mature fish shaped were oval, the cell nucleus and its size varied from one species to another. There are oval shaped and has a core is shown in **Fig. 2.**

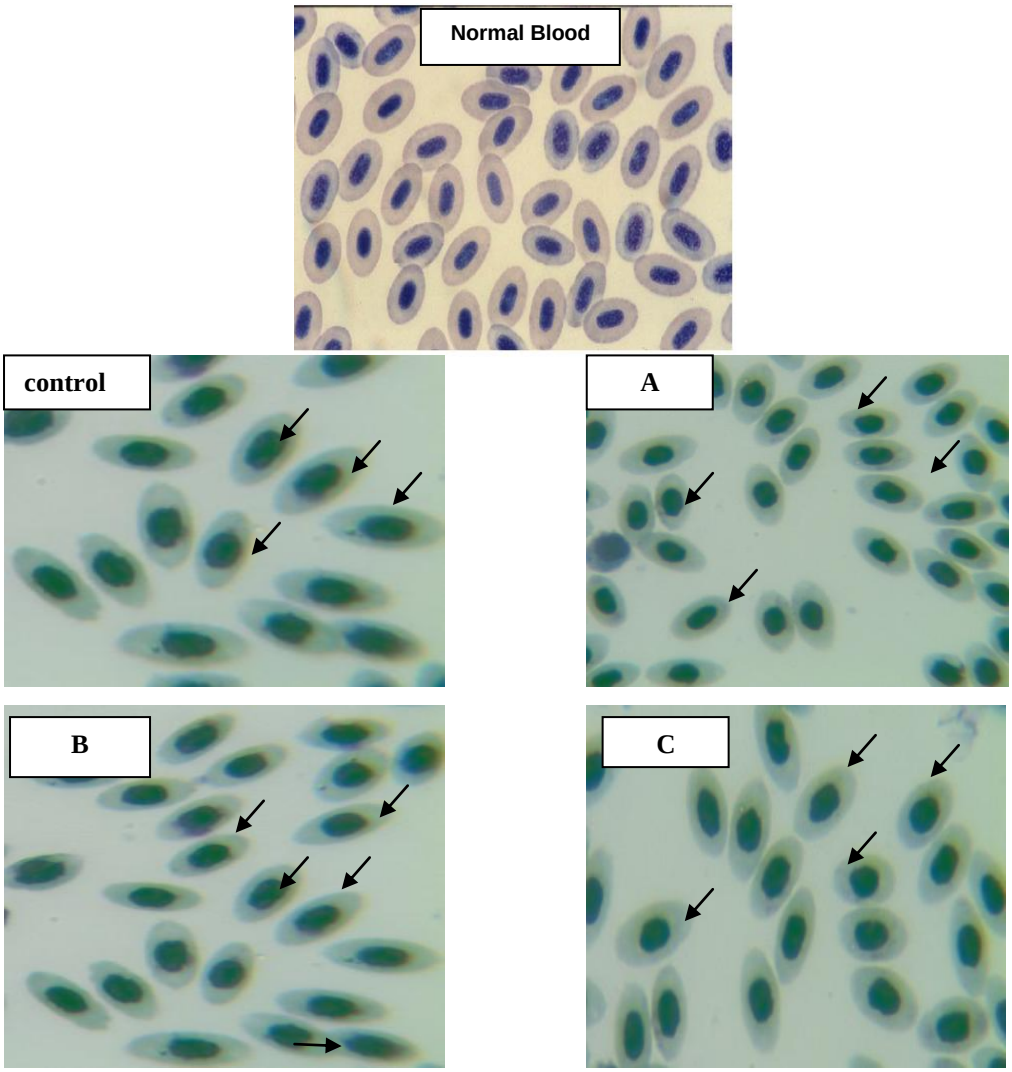


**Fig. 2.** Erythrocytes of grouper fish *(Epinephelus fuscoguttatus*). (Control), A (flow water velocity 1,25 cm/sec), B (flow water velocity 1,00 cm/sec),C (flow water velocity 0,75 cm/sec) , Erythrocytes, Enlargement 400X

Result of F-test against the erythrocyte average count of tiger grouper after 90 days rearing with flow water velocity treatment indicates the obvious effect of $F_{count}$ (5.565) > $F_{table}$ (4.757). It was then confirmed that flow water velocity has different effect on erythrocyte count of tiger grouper. Tukey Test, it was found that that erythrocyte count average at control was different from those in treatment of 1.00 m/second and 1.25 m/second, which was the flow water velocity with 0 m / sec ( the control of ) and 0.75 m / sec (c) treatment not influenced significantly to the red blood cells . Treatment 0.75 m / sec (C) and treatment of 1,00 m /sec were not influence significantly to the red blood cells. Likewise to the treatment of 1,00 m / second (B) and treatment 1.25 m / sec (A) treatment were not influence significantly

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to the red blood cells. However, treatment to 1.25 m / sec treatment ( A ) and 0,00 m / sec ( the control ) gave influence significantly to red blood cells . The best treatment to improve fish erythrocyte was the treatment of 1.25 m/second. It means that flow water velocity treatment can increase erythrocyte count average of tiger carp because flow water velocity may improve oxygen rate in the cells of various tissues, thus keeping those cells worked appropriately. High erythrocyte count in tiger grouper may be developed by environmental condition (water velocity increases oxygen rate) which is compatible for fish life, thus reducing stress rate. The increased erythrocyte count will increase oxygen rate because the main function of erythrocyte is transporting the oxygen. This oxygen is bonded by hemoglobin in the erythrocyte. Dynamic condition of the streaming water will stimulate fish to move. According Brett (1988) and Tajerin *et al.*, (2000) the moving activity is highly correlated with great demand of oxygen because fish must need high energy source for swimming. While Ye *et al.*, (2007) reported that the number of red blood cells of healthy grouper 2.3700.000 cells / ml and Qi *et al.*, (2007) reported a red blood cell grouper healthy 2.53.000 cells / ml. Further Salosso (2011) adds that erythrocyte count of healthy tiger grouper was 2,825,000 cells/ml. The results of the total erythrocyte tiger grouper, was still in the range of normal fish erythrocytes as pointed out by Nabib and Pasaribu (1989) and Stoskopf, (1993) that the number of red blood cells at each different species depending on the species, environmental conditions and activity of the fish, generally ranging from 1.05 - 3.0 x $10^6$ cells / ml.

Regression equation was made to confirm the relationship between flow water velocity treatment and erythrocyte rate of tiger grouper. It is Y = 26,323 x + 06 with $R^2$= 0.991. The equation shows that flow water velocity and erythrocyte rate of tiger grouper juvenile is positively associated. Based on $R^2$ value, the association must be very strong ($R^2$ > 0.5) like shown in **Fig. 3.**

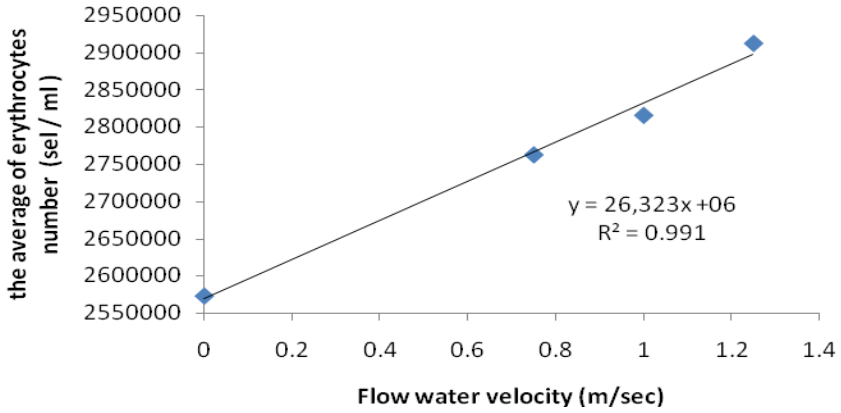


**Fig. 3**. Regression equation between flow water velocity and the average of erythrocytes number Tiger grouper

**Fig. 3,** shows that the increase in red blood cells had a close relationship with the flow water flow, this suggests that the provision of flow water velocity of red blood cell count increase compared with control. The main function of red blood cells are oxygen transport. Bijanti, (2005), the oxygen bound to hemoglobin (Hb) found in erythrocytes, and a hemoglobin molecule contains four atoms of iron (Fe) and can carry four oxygen molecules. Ye *et al.*, (2007) explains that the physical solubility of oxygen in the plasma is strongly influenced by the oxygen partial pressure, because if the pressure increases, the oxygen binds to hemoglobin. Further, Smith (2007) explain that the factors that influence the levels of hemoglobin is the oxygen in the blood supply, network activity and the age and species. The results of the analysis of amino acids: isoleucine: 4.61% (control); 4.64% (C) 4.66% (B) and 4.79% (A), histidine: 2.44 %(control), 2.45 % (C) 2, 53% (B) and 2.67%

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(A). Isoleucine and Histidine are amino acid essential amino acids. The results of analysis of amino acids isoleucine and histidine were relatively higher than thair needs. According to (Koolman and Roehm, 2005; Mokoginta *et al*., 2000; Buentello *et al*., 2011), the function of the amino acids isoleucine and histidine are to form and stabilize the red blood cell and hemoglobin formation.

*Hematocrit*

Hematocrit of the fish varies depending on physiology, health and activity of the fish. The highest hematocrit average at 1.25 m/second treatment is 31.89 %, followed by 1.00 m/second treatment with 31.33 %, 0.75 m/second treatment with 29.33 and control with 28.33 %. The hematocrit average rate of tiger grouper from flow water velocity treatments is shown in **Fig. 3.**

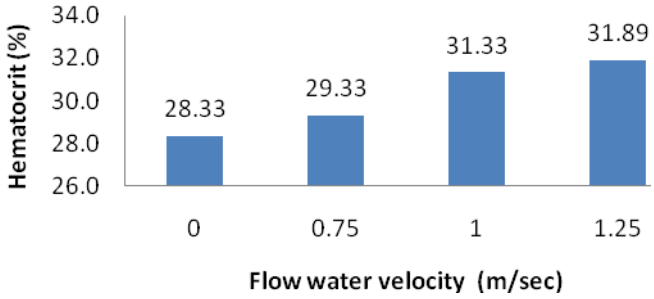


**Fig. 3.** Histogram of hematocrit of tiger grouper in different flow water velocity (%)

F-test result determines that hematocrit average rate of tiger grouper after 90 days rearing with flow water velocity treatments has obvious effect, as illustrated by $F_{count}$ (13.14) > $F_{table}$ (4.757).

From Tukey Test, which is the average hematocrit value of control and 0,75 m/sec (C) not influenced significantly. Hematocrit average of 0.75 m/sec ( C ) and 1,00 m/sec (B) were not influenced significantly. So also to 1.00 m/sec (B) and 0.75 m/sec ( C ) there was no significant influence. However, for 1.00 m/sec with 0,00 m/sec (control) give influence significantly. It was also applied 0.75 m/sec and 1.25 m/sec ( A) gave influence significant. The best treatment with the increased fish hematocrit was 1.25 m/sec. It was then confirmed that flow water velocity could give various results. Bond, (1979) mentioned that marine fish hematocrit rate may be in range of 30-42 %. According Anderson and Siwicki, (1993) fish health condition can be observed through measuring hematocrit rate. Bijanti, (2006) reported that highly activated fish has hematocrit rate higher than 30 %. Cheng *et al*., (2005) explains that the hematocrit value can be used as a guide to find out more or less the number of red blood cells.

While Velesek *et al.,* (2009) states that the hematocrit value is used to determine the average value of the erythrocytes and the amount of hemoglobin. Further Ye *et al.,* (2007) stated that fish have a higher mean hematocrit values have a lot of red blood cells, or fish that have a high metabolic rate.

Regression equation was prepared to ascertain the relationship between water velocity treatment and hematocrit rate of tiger grouper. That was Y = 2.761 x + 28.09 with $R^2$= 0.870. The equation indicates that the relationship between water velocity and hematocrit rate of tiger grouper larvae was positive like shown in **Fig. 4.**

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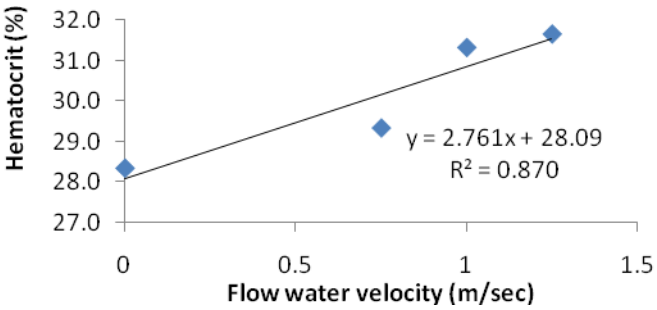


**Fig. 4.** Regression equation between flow water velocity and the average of hematocrit percentage tiger grouper.

*Hemoglobin*

Hemoglobin rate in the blood of tiger grouper with flow water velocity treatments is shown in **Fig. 5.**

From **Fig. 5**, it was found that the highest hemoglobin average was 7.94 g/100 ml at 1.25 m/second treatment, while the lowest hemoglobin average was observed at control, was 6.23 g/100 ml.

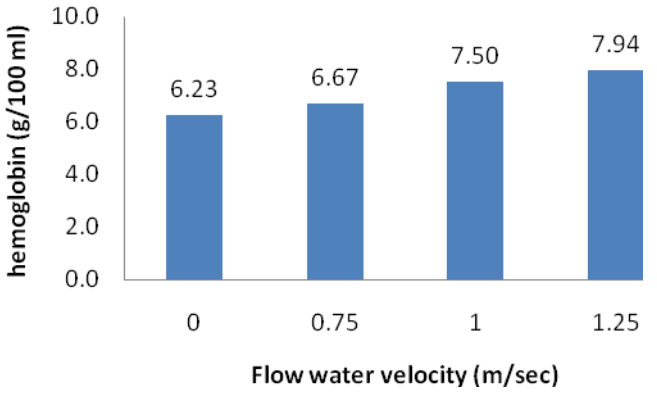


**Fig. 5.** Histogram of hemoglobin of tiger grouper in different on flow water velocity (g/ 100 ml)

Hemoglobin value in the fish is highly associated with red blood cell count because hemoglobin is contained within red blood cell. Erythrocyte must have hemoglobin within because hemoglobin transports oxygen from gill to body tissues (Moyle and Cech, 2004). Therefore, hemoglobin in the blood has close relationship with erythrocyte.

Based on $F_{count}$ (29.490) > $F_{table}$ (4.757), it is confirmed that flow water velocity treatments gave different effect on hemoglobin. Tukey Test indicates that the different in treatment, which is 0.00 m/sec (control) and 0.75 m/sec ( C ) no influence significant. 1.00 m/second ( B) and 1.25 m/sec (A) no influence significantly. But 0.75 m/sec ( C ) and 1.00 m/sec ( B) provide influence significantly to hemoglobin. Therefore, flow water velocity treatments successfully improving fish hemoglobin was 1.25 m/sec (A)

Regression equation to understand the relationship between flow water velocity treatment and hemoglobin rate of tiger grouper was Y = 1.134 x + 6.08 with $R^2$= 0.869. The equation shows that flow water velocity and hemoglobin value of tiger grouper juvenile was positively associated like shown in **Fig. 6**.

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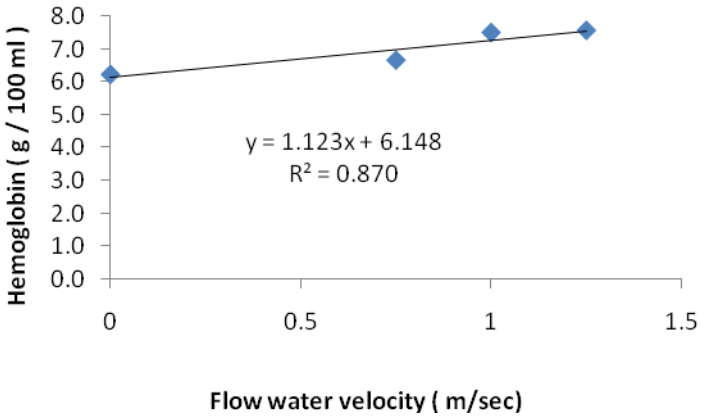


**Fig.6.** Regression equation between flow water velocity with the average of hemoglobin of tiger grouper

Erythrocyte, hematocrit and hemoglobin rates were related to each other. Flow water velocity treatments could improve erythrocyte, hematocrit and hemoglobin rates compared to control. It means that water stream speed increases oxygen rate. Arif (2003), reported that the highest dissolved oxygen was 5.71 ± 0.10 mg/l, while the lowest at control was 4.01 ± 0.06 mg/l. Agus (2005) explains that the dissolved oxygen was greater than 5 mg/liter represents optimum oxygen for fish health. If it was only reduced to lower than 5 mg/liter, it was enough for fish to get stressed.

*White Blood Cell (leucocyte)*

A parameter which was also observed was leukocyte total. The examination on leucocyte total was aimed at understanding the fish health status. Flow water velocity treatment had close relationship with the change of leukocyte total average. Highest leucocyte total was found at control, counted to 28,967 cells/ml, while the lowest was observed at 1.25 m/sec treatment with 24,140 cells/ml. It means that fish leucocyte at control 6-8 higher than those at water velocity treatments (**Fig. 7**).

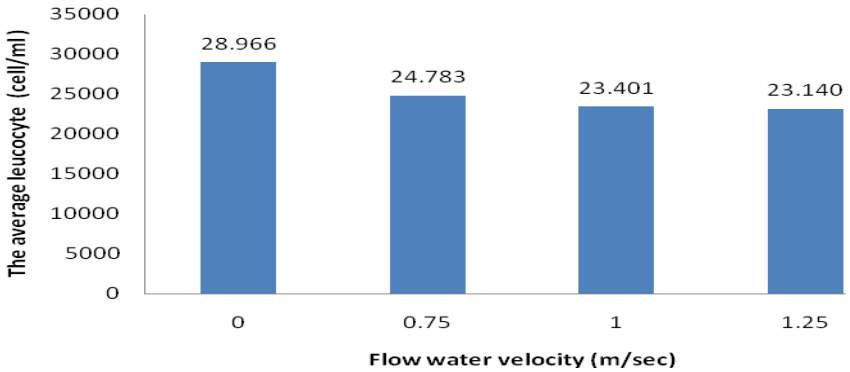


**Fig.7.** Histogram of average leucocyte concentration (cell/ml) of tiger grouper in different flow water velocity

Salosso (2011) asserts that the change pattern of leukocyte was different from erythrocyte because leukocyte increases either at sick fish or at healthy fish.

Based on F-test, $F_{count}$ (9,319) > $F_{table}$ (4,757) such that it was estimated that water velocity treatments have different effect on leucocyte. Tukey Test finds that leucocyte average at control was different from those at 1.25 m/sec, 1.00 m/sec and 0.75 m/sec treatments. The differences of treatment between 1.00 m/sec (B) and 1.25 m/sec (A) had

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no influence significantly. 0.75 m/sec ( C ) and 1.00 m/sec (B) no influence significantly. Similarly 0.00 m/sec (control) and 0.75 m/sec (C) had no influence significant. However 1.25 m/sec (A) and 0.00 m/sec ( control) provides influence of significant. Thus, also can be concluded that in the treatment of the flow water velocity 1.25 m/sec produce white blood cells concentration was as least as compared to the control.

Regression equation to confirm the relationship between flow water velocity and leucocyte rate of tiger grouper was Y = -4919 x + 28763 with $R^2$ = 0.972. This equation means that water velocity and leucocyte rate of tiger grouper was negatively associated. Based on its $R^2$, this association was very strong ($R^2$ > 0.5). Leucocyte total in this research remains in the range of 24,140 cells/ml – 28,967 cells/ml, but it still stands for normal rate, like shown in **Fig.8.**

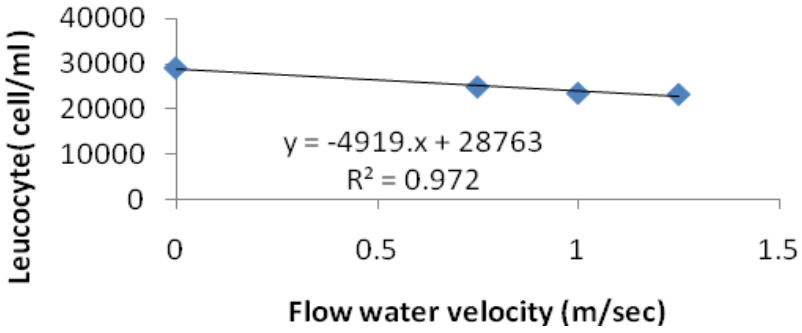


**Fig.8.** Regression equation between flow water velocity with the average of leucocyte concentration tiger grouper

## CONCLUSION

It is concluded that flow water velocity was able to raise red blood cell, hematocrit and hemoglobin rates of tiger grouper juvenile, but reduced white blood cell total, in comparison to control. Flow water velocity generally does not distort fish health, although it increases erythrocyte, hematocrit, and hemoglobin rates, and reduces leucocyte rate, the range was still normal.

It may be suggested that further research should observe muscle, gill, and liver of tiger grouper juvenile at some flow water velocity treatments.

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