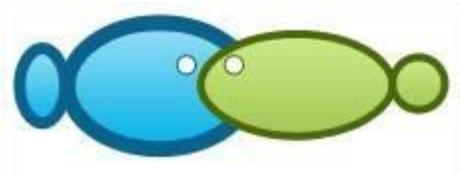

Health performance of Asian redtail catfish (*Hemibagrus nemurus*) treated with vitamin C and thyroxine hormone

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Abstract. Fluctuations in the quality of cultivation water cause physiological changes such as the adaptation system and decreased fish body strength. This research aimed to determine the effect and dose of the best combination of vitamin C and the hormone thyroxine (T4) to improve *Hemibagrus nemurus* health. The results obtained showed the health performance of *H. nemurus* subjected to the treatments. The research was carried out between January-July 2021 with maintenance and blood analysis performed at the Fish Parasite and Disease Laboratory. The juveniles used were 8-10 cm in size. They were treated with a mixture of vitamin C and thyroxine hormone by combining each of the following doses of vitamin C of 0, 500 and 1,000 mg kg^{-1}, with thyroxine hormone doses of 0, 4, 6 and 8 mg kg^{-1}. A completely randomized design was used, with 2 factors. The best fish health conditions were obtained for a combination of 1,000 mg kg^{-1} of vitamin C with 8 mg kg^{-1} of thyroxine hormone, indicated by oxygen consumption levels ranging from 0.58 to 0.99 mg L^{-1}, total erythrocyte levels oxygen consumption 0.99 mg L^{-1}, total erythrocytes 2.76 x 106 cells mm^{-3}, hematocrit 37.67%, hemoglobin 10.33 g dL^{-1}, and total leukocytes 12.47 x 104 cells mm^{-3}.
Key Words: blood, combination, immune response, oxygen consumption, physiological.

Introduction. Asian redtail fish (*Hemibagrus nemurus*) is a common aquatic fish species that lives in lakes and rivers in Sumatera and Kalimantan (Tang 2003). Asian redtail fish has a high economic value, with prices ranging from 3.15-3.75 USD kg^{-1} (Saputra et al 2017). *H. nemurus* production is unable to meet market needs that increase every year (Wardani 2018). This is due to fluctuations in the quality of aquaculture water which causes physiological changes such as adaptation systems and decreased fish body power (Braga et al 2017). The solution for improving the immune system of fish, investigated in this study, consists of giving vitamin C and thyroxine hormone into the feed. Vitamin C serves as a catalyst in metabolic and physiological processes in fish, so that it can increase the immune response of fish (Wirdona et al 2019). Vitamin C supplementation into feed will also accelerate the absorption of Fe (in the form of Fe^{2+}) so that hemoglobin levels will increase (Siregar & Adelina 2009). Several studies have shown that giving vitamin C trough feed improves the fish health performance at doses of 200 mg kg^{-1} feed (Yani et al 2019), 600 mg kg^{-1} feed (Rahayu et al 2019), 1,000 mg kg^{-1} feed (Hidayat et al 2017) and 2,000 mg kg^{-1} feed (Wirdona et al 2019). In addition, the addition of thyroxine hormone (T4) to the feed helps accelerate the metabolic process and growth rate of fish (Yandra et al 2020).

This study uses a recirculation system that aims to reduce ammonia levels and organic waste produced by fish so that water quality can support fish life (Verawati 2017). Poor water quality can trigger fish stress which results in reduced fish appetite and disruption of the metabolic system (Mulyadi et al 2014). Hematological or fish blood profiles can be used for various purposes in monitoring the health of fish and their farming environment. To monitor the changes of the state of health of fish, both due to pathogenic infections and to the use of immunoregulatory substances (immunostimulants and vaccines), blood images can be used because they have high sensitivity and specificity. In addition, the use of blood images to monitor the condition of waters has also begun to be

used because this parameter has a correlation with the condition of waters, in the long term (Siagian et al 2021).

This study aimed to determine the effect and dose of the best combination of vitamin C and thyroxine hormone (T4), in order to improve fish health. The results obtained show the health performance of Asian redtail fish treated with vitamin C and thyroxine hormone.

Material and Method

Time and location. This research was conducted in the period January-July 2021. Maintenance and distillation were conducted by weight and oxygen consumption rate measuring in *H. nemurus*, at the Marine Science Biotechnology Laboratory. A blood analysis was carried out at the Fish Parasites and Disease Laboratory. An analysis of protein and fat levels of *H. nemurus* was carried out in the Basic Chemistry Laboratory; the sampling and drying of muscles and liver of Asian redtail fish were carried out at the Integrated Service Laboratory of the Faculty of Fisheries and Marine, University of Riau. The analysis of muscle and liver glycogen levels of Asian redtail fish was carried out at the Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University.

Materials. The test fish used was *H. nemurus* with a size of 8-10 cm. The pellets used were Hi-Provite 781-2, with 31% protein, administered following a satiating feeding method, 3 times a day. Vitamin C was used in a powder form (L-Ascorbic-Acid), with the trademark CSPC (Weisheng Pharmaceutical, LTD-China). The thyroxine hormone was used in the form of tablets with the trademark Euthyrox® and each tablet contained 100 µg of levothyroxine. The tablet was crushed until smooth, by mixing vitamin C and levothyroxine into the test feed, according to the treatment dose, then homogenized and put into a sprayer device; when sprayed, vitamin C and levothyroxine were mixed with the pellet, without residue. The container used for the enlargement of Asian redtail fish was round, made of PE plastic, with a diameter of 56 cm and a height of 48 cm, and it was stocked with 36 specimens.

Experimental design. The dose of hormone (T4) given in this study refers to Yandra et al (2020) in *H. nemurus*, where the best treatment is a thyroxine dose of 6 mg kg^{-1}. In the current study, the thyroxine hormone (T4) was administered in 4 dose treatments: 0 mg kg^{-1} (H0), 4 mg kg^{-1} (H1), 6 mg kg^{-1} (H2), 8 mg kg^{-1} (H3). All combinations with vitamin C can be seen in Table 1.

Table 1

Combined Vitamin C and thyroxine (T4) doses treatments

No.	*Treatments*	*Concentration*
1.	V_0H_0	Without Vitamin C + Without T_4
2.	V_0H_1	Without Vitamin C + T4 4 mg kg^{-1}
3.	V_0H_2	Without Vitamin C + T4 6 mg kg^{-1}
4.	V_0H_3	Without Vitamin C + T4 8 mg kg^{-1}
5.	V_1H_0	Vitamin C 500 mg kg^{-1} + Without T4
6.	V_1H_1	Vitamin C 500 mg kg^{-1} + T4 4 mg kg^{-1}
7.	V_1H_2	Vitamin C 500 mg kg^{-1} + T4 6 mg kg^{-1}
8.	V_1H_3	Vitamin C 500 mg kg^{-1} + T4 8 mg kg^{-1}
9.	V_2H_0	Vitamin C 1,000 mg kg^{-1} + Without T4
10.	V_2H_1	Vitamin C 1,000 mg kg^{-1} + T4 4 mg kg^{-1}
11.	V_2H_2	Vitamin C 1,000 mg kg^{-1} + T4 6 mg kg^{-1}
12.	V_2H_3	Without C 1,000 mg kg^{-1} + T4 8 mg kg^{-1}

Data analysis. The data obtained were quantitative, consisting of the level of oxygen consumption, total erythrocytes, hematocrit values, hemoglobin levels and total

leukocytes. Quantitative data were statistically processed with analysis of variance (ANOVA). If it showed a real effect, then it was continued with Duncan's follow-up test to see the difference between treatments so that the best treatment can be identified. To analyze the effect of giving a combination of Vitamin C and thyroxine hormone on the health condition of fish, testing was carried out using the help of SPSS 21, at a significance level of 5%, to determine the real difference.

Results and discussion

Oxygen consumption rate. The level of oxygen consumption of *H. nemurus* during the study can be seen in Figure 1.

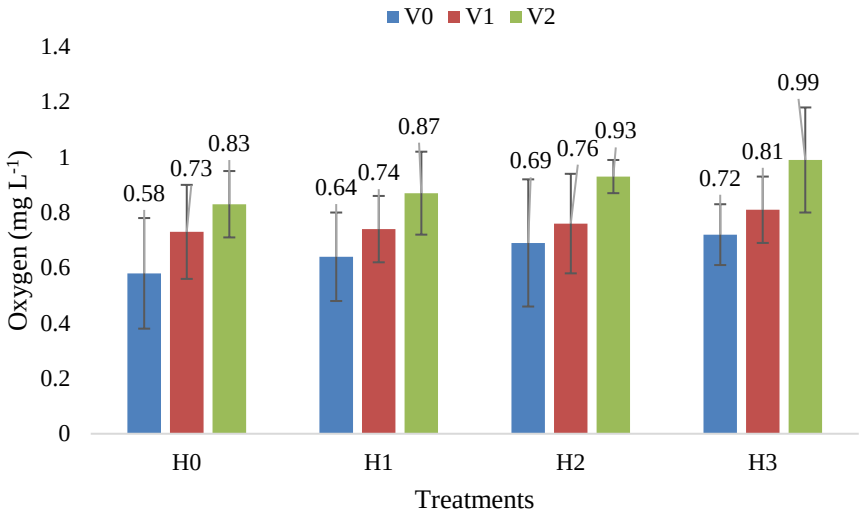

Figure 1. Oxygen consumption rate of *Hemibagrus nemurus* during the study.

The results of the calculation of the amount of oxygen consumption level of *H. nemurus* at the end of the study ranged from 0.58-0.99 mg L^{-1}. The results of variance analysis (ANOVA) showed that the combination of vitamin C and T4 hormone added to the feed had no effect on the level of fish oxygen consumption ($P>0.05$). However, the value of the amount of oxygen level tends to increase with increasing doses of vitamin C and the hormone thyroxine (Figure 1). The highest level of oxygen consumption, 0.99 mg L^{-1}, was observed in the treatment with a combination of vitamin C 1,000 mg kg^{-1} feed + T4 hormone 8 mg kg^{-1} feed, and the lowest, 0.55 mg L^{-1}, in the treatment without vitamin C and thyroxine hormone. The addition of vitamin C to feed has an influence on the level of oxygen consumption of Asian redtail fish ($P<0.05$). The addition of as much as 1,000 mg kg^{-1} vitamin C in feed resulted in the highest level of oxygen consumption, which is 0.99 mg L^{-1}. The addition of thyroxine hormone to feed had an influence, between combinations, on the level of oxygen consumption of Asian redtail fish ($P<0.05$). The addition of thyroxine hormone as much as 8 mg kg^{-1} of feed resulted in the highest oxygen consumption rate of 0.83 mg L^{-1}. Manurung et al (2017) stated that the thyroxine hormone has an important role in regulating metabolism, growth and development in fish. The thyroxine hormone can also stimulate the rate of oxidation of foodstuffs, increasing the rate of oxygen consumption and growth (Khalil et al 2011).

Total erythrocytes. Erythrocytes (red blood cells) are the most numerous cells of the blood. Total erythrocyte reduction was carried out to see changes of the total erythrocyte value in the baung fish fed at different doses and with various combinations. The average total erythrocytes of the *H. nemurus* after 50 days of maintenance ranged from 1.73 to 2.76 x 10^6 cells mm^{-3}. The results of the observation of total erythrocytes are presented in Figure 2.

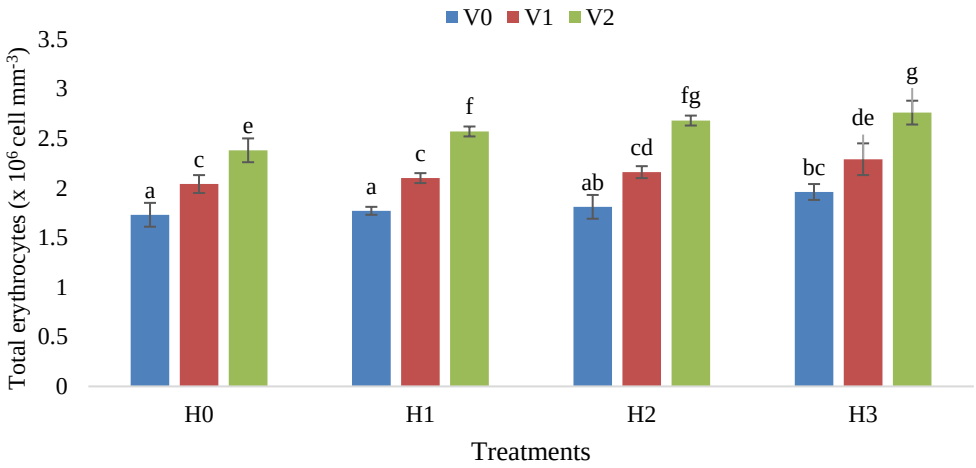

Figure 2. Total erythrocytes of *Hemibagrus nemurus* during the study (Different superscript letters following mean values (± standard error, n=3) in the same row indicate significant differences (P <0.05).

Giving different doses of vitamin C through feed has an effect on the total erythrocytes of *H. nemurus* ranging from 1.82 to 2.60 x 10^6 cells mm^{-3} (P<0.05). The highest total erythrocytes concentration was 2.60 x 10^6 cells mm^3 with the addition of vitamin C dose of 1,000 mg kg^{-1} feed, while the lowest, 1.82 x 10^6 cells mm^{-3}, was recorded in the treatments without vitamin C. This shows that the addition of vitamin C can increase the production of erythrocytes in fish, allegedly due to the ability of vitamin C to act as an immunostimulant that can support fish health and fish growth.

One of the vitamins that has a very important role in the physiological process of fish is vitamin C. It is a nutrient that is present in micro amounts in feed, but it must also be available. Although needed in small amounts, vitamin C must be obtained from feed, because the body cannot make it on its own (Jusadi et al 2006). Vitamin C is an antioxidant that functions to prevent the breaking of fatty acid chains into various compounds that are toxic to cells, such as aldehydes, ethane and pentane, which can cause severe damage to cell membranes, including erythrocyte membranes (Syawal et al 2021).

Thyroxine hormone supplementation at different doses in feed has an influence between treatments on the total erythrocytes of *H. nemurus* (P<0.05), which ranges from 2.05-2.34 x 10^6 cells mm^{-3}. Administration of thyroxine hormone at a dose of 8 mg kg^{-1} feed gave the highest total erythrocytes number, of 2.34 x 10^6 cells mm^{-3}, while the lowest, 2.05 x 10^6 cells mm^{-3}, was recorded after the treatment without an addition of thyroxine hormone (H0). This shows that the addition of the hormone thyroxine is able to increase the production of erythrocyte cells. According to Manurung et al (2017), the thyroxine hormone is able to increase oxygen consumption in blood plasma, which stimulates an increase in the rate of oxidation of cells and fish growth.

The results of the variance analysis (ANOVA) of vitamin C and thyroxine hormone added to the feed had an influence on the total erythrocytes of Asian redtail fish (P<0.05). The highest total erythrocyte value of *H. nemurus* during maintenance was found in the combination treatment of vitamin C + thyroxine hormone with a dose of vitamin C 1000 mg and T4 hormone 8 mg kg^{-1} feed, namely 2.76 x 10^6 cells mm^{-3}, while the lowest value was found in the treatment without the addition of vitamin C and thyroxine hormone, with a total erythrocyte value of 1.73 x 10^6 cells mm^{-3}. The total erythrocytes of *H. nemurus* during the study were still in the normal range. According to Shilman et al (2020), the total erythrocytes of Asian redtail fish ranged from 2.73 to 2.79 x 10^6 cells mm^{-3}. The increase in erythrocyte cells is caused by several factors including species, sex, age, feed nutrition, size, physical activity, and age (Wientarsih et al 2013). An increase in the size of the fish will affect the need for oxygen. Oxygen is needed by fish for respiration, blood circulation and metabolism, so larger fish have more erythrocytes than small ones (Syawal et al 2021).

Total hematocrit. Hematocrit is a percentage of the volume of erythrocytes in the blood of fish. *H. nemurus* fed artificial feed with the addition of vitamin C and thyroxine hormone had an inter-treatment influence on hematocrit values after 50 days of maintenance (P<0.05), ranging from 26.00-37.67%. The result of hematocrit levels can be seen in Table 2.

Table 2

Results of measuring hematocrit levels (%) during the study

Hormon (H) (mg kg^{-1})	*Vitamin (V) (mg kg^{-1})*			*Average*
	V0 (0)	*V1 (500)*	*V2 (1,000)*	
H0 (0)	26.00±1.00^{a}	30.33±0.58^{c}	34.67±0.58^{e}	30.33±3.81^{a}
H1 (4)	26.33±0.58^{a}	31.33±0.58^{c}	35.33±0.58^{ef}	31.00±3.94^{a}
H2 (6)	27.00±1.00^{a}	32.67±1.53^{d}	36.33±0.58^{f}	32.00±4.18^{b}
H3 (8)	28.67±0.58^{b}	33.33±0.58^{d}	37.67±0.58^{g}	33.22±3.93^{c}
Average	28.00±1.28^{a}	31.92±1.44^{b}	36.00±1.28^{c}	

Superscript in the same column and row shows a noticeable difference (P<0.05).

Giving a combination treatment of vitamin C and thyroxine hormone with a dose of vitamin C 1,000 mg kg^{-1} feed + thyroxine hormone 8 mg kg^{-1} feed gave the highest hematocrit value of 37.67%, while the lowest value was found in the treatment without vitamin C and thyroxine hormone, which was 26.00%. The hematocrit value of *H. nemurus* maintained for 50 days was increased, but still within the normal range. The addition of vitamin C at different doses had a different level of influence between treatments on the hematocrit value of baung fish maintained with a recirculation system (P<0.05). A dose of 1,000 mg kg^{-1} of feed produced the highest hematocrit value, which was 36.00%. This shows that the administration of vitamin C provides an increase in hematocrit levels of the redtail fish. Asian redtail fish given commercial pellets have a hematocrit content of 28.00%.

H. nemurus, given the addition of thyroxine hormone at different doses, recorded a different level of influence between treatments on their hematocrit values (P<0.05). A dose of 8 mg/kg of feed resulted in the highest hematocrit value of Asian redtail fish, which was 36.00%. The addition of thyroxine hormone is able to provide an increase in hematocrit levels of *H. nemurus*, allegedly influenced by the growth of fish weight.

The hematocrit level of baung fish during maintenance is in the normal range. Fitria et al (2019) stated that normal *H. nemurus* hematocrit levels can reach 38.8%. Increased hematocrit levels are associated with increased total erythrocytes. According to Kuswardani (2006), the percentage of hematocrit levels is related to the number of red blood cells. Furthermore, according to Fadil et al (2011), an increase in hematocrit values in fish blood shows a relationship with the large number of red blood cells formed by hematopoiesis tissue, where the number of red blood cells is directly proportional to the hematocrit value. Nursatia et al (2017) stated that hematocrit values can change depending on the season, temperature and feeding, and the impact of immunostimulant administration. The hematocrit value varies depending on nutritional factors, the age of the fish, sex, body size and spawning period.

Hemoglobin levels. In *H. nemurus* fed with vitamin C and thyroxine hormone at different doses, during the 50 days of maintenance, a different effect between treatments (P<0.05) was observed on the hemoglobin, whose value ranged from 7.07-10.33 g dL^{-1}. The results of measuring hemoglobin levels of *H. nemurus* during the study can be seen in Figure 3. The highest hemoglobin level during 50 days of maintenance was observed in the combination treatment of vitamin C 1,000 mg kg^{-1} feed + thyroxine hormone 8 mg kg^{-1} feed, which is 10.33 g dL^{-1}, while the lowest level was observed in treatment without vitamin C and thyroxine hormone, which is 7.07 g dL^{-1}. The results of variance analysis (ANOVA) showed that the combination of the addition of thyroxine and vitamin C hormones had a different level of influence between treatments on the hemoglobin levels of the *H. nemurus* maintained in the recirculation system (P<0.05). Vitamin C at different doses, added to feed, in the rearing of *H. nemurus*, had a different effect on the hemoglobin levels

(P<0.05). A dose of 1,000 mg kg^{-1} in feed produced the highest hemoglobin level, which is 10.00 g dL^{-1}, while the lowest level, 8.17 g dL^{-1}, was recorded in feed without vitamin C. This shows that the addition of vitamin C can increase the hemoglobin levels of *H. nemurus*.

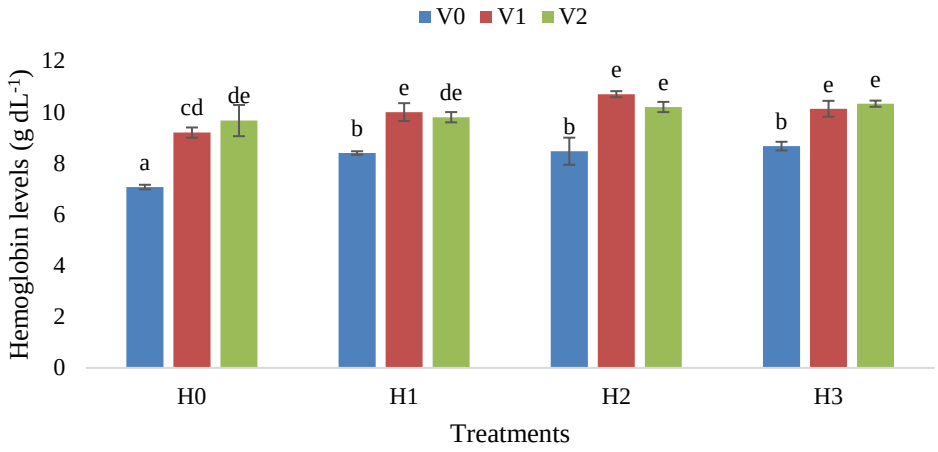

Figure 3. Hemoglobin levels of *Hemibagrus nemurus* during the study (Different superscript letters following mean values (± standard error, n=3) in the same row indicate significant differences (P <0.05)).

According to Said (2003), the vitamin C content functions as an activator in various fermentations that break down proteins and lipids, in the process of oxidation and dehydration in cells, which is important in the formation of platelets, increasing the body's immunity, and as an antioxidant. Siregar & Adelina (2009) stated that vitamin C helps the absorption of iron from food so that it can be processed into red blood cells again. By increasing hemoglobin in the blood, the intake of food and oxygen in the blood can be circulated to all body tissues which can eventually support the survival and growth of fish. High levels of hemoglobin support the oxygen storage and carry out blood-buffering functions in fish (Lavabetha et al 2015).

The addition of thyroxine hormone to feed had different levels of influence between treatments on the hemoglobin levels of *H. nemurus* (P<0.05). The addition of 8 mg kg^{-1} of thyroxine hormone to feed gave the highest increase in the hemoglobin levels, which was 9.73 g dL^{-1}, higher than in feed without thyroxine hormone (8.64 g dL^{-1}). The hemoglobin level of *H. nemurus* during the maintenance is in the normal range. According to Fitria et al (2019), hemoglobin levels of *H. nemurus* range from 5.4 to 8.5 g dL^{-1}. The hemoglobin level is affected by the number of erythrocytes and hematocrit. The correlation between hemoglobin and hematocrit is that erythrocytes contain Hb which functions to bind oxygen used for the catabolism process so that energy is produced. Increased Hb is closely related to an increase in the number of erythrocytes; this condition is caused by increased iron content and serum iron concentration in the blood (Purwanti et al 2014). Hemoglobin determines the degree of resistance of the body of fish, due to its binding to oxygen. The level of hemoglobin in the blood is strongly correlated with hematocrit values. The lower the number of red blood cells, the lower the hemoglobin level in the blood (Lagler et al 1977).

Total leukocytes. The total leukocytes of *H. nemurus* given a combination of vitamin C and thyroxine hormone during the study can be seen in Figure 4. The total leukocytes of *H. nemurus* fed with vitamin C and thyroxine hormone in the feed, during the 50 days of maintenance, had a different effect among treatments (P<0.05), which ranged from 5.63 to 12.47 (10^4 cells mm^{-3}). The results of the variance analysis (ANOVA) of vitamin C and thyroxine hormone added to feed showed different influence levels between treatments on the total leukocytes of *H. nemurus* (P<0.05). The highest total leukocytes, 12.47 x 10^4 cells mm^{-3}, was observed in the vitamin C and thyroxine hormone combination treatment (H3), while the lowest total leukocytes were found in the treatment without vitamin C and

thyroxine hormone, which was 5.63 x 10^4 cells mm^{-3}. This shows that the combination of vitamin C and thyroxine hormone is able to increase the total leukocytes of *H. nemurus*, but still within the normal range. According to Apriyandi (2008), the normal range of leukocyte count in healthy *H. nemurus* with a size of 20-25 cm ranges from 190,000-265,000 cells mm^{-3}.

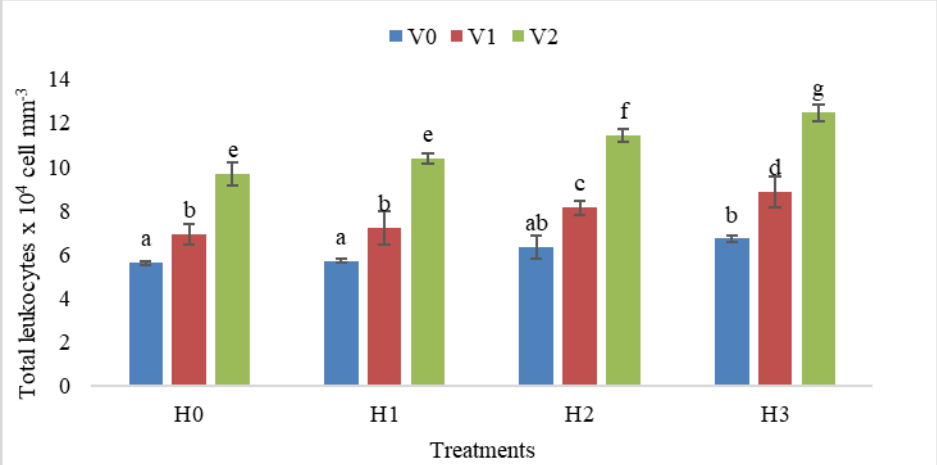

Figure 4. Total leukocytes of *Hemibagrus nemurus* during the study (Different superscript letters following mean values (± standard error, n=3) in the same row indicate significant differences (P<0.05)).

The addition of vitamin C to the feed at different doses, had a different effect on the total leukocytes of *H. nemurus* during the maintenance (P<0.05). A dose of 1,000 mg kg^{-1} of vitamin C in feed produced the highest total concentration of leukocytes, which is 10.99 x 10^4 cells mm^{-3}. This shows that there has been an increase in the immune system of fish. This is thought to be caused by the function of vitamin C which can increase the resistance of the fish body. Affandi & Tang (2017) stated that vitamin C can increase the resistance of the fish body. Vitamin C has a role in the synthesis of proteins needed in the formation of immune responses and in the collagen biosynthesis, to accelerate the wound healing process. Leukocytes store large concentrations of vitamin C in addition to the thymus gland, spleen and immune cells. In fish that experience stress, the number of lymphocyte cells in the blood and lymphoid organs (bone marrow, spleen glands and spleen) will decrease (Ismail 2010). Hazzuli et al (2015) stated that vitamin C stimulates the activity and reactivity of cellular and humoral defense cells, besides that it can provide increased phagocytosis activity (non-specific immune response) in fish.

Giving thyroxine hormone through feed in *H. nemurus* rearing with different dose treatments resulted in an average total leukocyte of 7.41-9.35 x 10^4 cells mm^{-3}. The results of variance analysis (ANOVA) showed that the addition of thyroxine hormone to the feed had different effects on total leukocytes in *H. nemurus* (P<0.05), among the treatments. The dose of 8 mg kg^{-1} of thyroxine hormone feed produces the highest hemoglobin level, 9.35 x 10^4 cells mm^{-3}. This is thought to be influenced by an increase in the growth rate of Asian redtail fish. Fluctuations in total leukocytes in each treatment are influenced by certain conditions such as; stress, age, weight and physiological activity. Adult fish certainly have a stronger body defense system than larvae or juveniles, in accordance with Rosidah et al (2019), stating that the age and weight of fish affect their blood system, by increasing white blood cells used as body defense.

Conclusions. The best *H. nemurus* health condition during the study was found in the treatment of adding a combination of 1,000 mg kg^{-1} of vitamin C and 8 mg kg^{-1} of thyroxine hormone, with a total oxygen consumption rate by erythrocytes of 0.99 mg L^{-1}, total erythrocytes of 2.76 x 10^6 cells mm^{-3}, a hematocrit of 37.67%, a hemoglobin of 10.33 g dL^{-1}, and total leukocytes of 12.47 x 10^4 cells mm^{-3}.

Conflict of interest. The authors declare no conflict of interest.

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