Potential of milkfish waste protein extract as an immune supplement for Covid-19 prevention

[1]Shafira Rahmania, [1]Yusuf Novaldo, [1]Anisa Lutfiaturrohmah, [2]Titan Ababil, [1]Riza R. Hakim, [1]Hariyadi,[1,3]Soni Andriawan

[1] Department of Aquaculture, Faculty of Agribusiness and Animal Science, Muhammadiyah Malang University; [2] Department of Pharmacy, Faculty of Health Sciences, Muhammadiyah Malang University; [3] Biotechnology Development Center, Muhammadiyah Malang University, Indonesia. Corresponding author: S. Andriawan, soniandriawan@umm.ac.id

Abstract. Indonesia is currently experiencing a Covid-19 pandemic, which reached 4.06 million cases on September 9, 2021. It infects humans when their immune systems is at a low level. Some references suggest consuming fish to boost immunity. Milkfish (*Chanos chanos*) contains 19.15% of the protein that is believed to elevate lymphocytes. In the current investigation, milkfish production reaches 170,852,179 tons in Java, Indonesia. As a result, the increase in those fish's production and consumption led to huge waste (byproduct). Therefore, the study aimed to investigate milkfish waste, including heads, tails, spines, and offal, as a protein supplement for Covid-19 prevention. They were collected from Langdungsari Market, Malang, East Java, Indonesia, and selected based on their quality and freshness. The waste was then cleaned and extracted employing 0.1 M of HCL. Afterwards, the extract was mixed with Na-CMC (sodium carboxymethyl cellulose). Furthermore, the test was carried out by inducing milkfish waste protein (MWP) extract to mice (*Mus musculus*) with three dosages (6.522 mg (T1), 9.730 mg (T2), and 15.332 mg (T3)) and one control. A complete hematology test, such as red blood cells (RBS), white blood cells (WBC), hemoglobin (Hb), hematocrit (PCV), differential leucocytes, absolute lymphocyte count (ALC), and blood cell indices was assessed to evaluate the immune responses of mice. Data was analyzed employing SPSS to determine the differences among treatments. The results showed that the administration of MPW extracts significantly affected the number of ALC, RBC, WBC, Hb, PCV, and differential leucocytes, particularly T1. Unfortunately, only eosinophils did not give response to the treatments. Regarding those results, it was found that the administration of milkfish MPW extract has the potential to be used as a supplement for boosting immunity.
Key Words: blood parameters, byproduct, *Chanos chanos*, defense.

Introduction. Currently, the world is facing an outbreak caused by a new coronavirus called the Novel Severe Acute Respiratory Syndrome Corona Virus, abbreviated as SARS-CoV2. Covid-19 cases in Indonesia reached 4.06 million cases, with a total of 131,000 deaths on August 28, 2021 (Nafrin & Hudaidah 2021). Humans with weakened immune systems are victims of the Covid-19 sickness. It is possible to boost immunity by eating foods high in protein, such as fish. Fish is a source of animal protein that increases immunity (Li et al 2021). The proteins' benefits are replacing damaged body tissues and those that need to be overhauled and maintaining existing tissues (Stern & Cui 2019). The protein content in fish can increase lymphocytes and play a role in forming immunoglobulins (Ig). According to Sumarmi (2020), protein can boost the body's immunity. Fish contains animal protein in the range of 20-24%, and its quality is indicated by the completeness of its essential amino acids (Djunaidah et al 2017). One of the fish species that have a high protein content is milkfish, *Chanos chanos*.

It was reported that fish consumption increased in May 2020, reaching 56.39 kg per capita. *C. chanos* is a consumption fish that contains 19.15% high protein (Prasetyo et al 2015). According to the Department of Marine Affairs and Fisheries of East Java, Indonesia, *C. chanos* production reached 170,852,179 tons in 2020, increasing waste production. Koli et al (2015) showed that fish waste is approximately 30 to 40% of the

total weight. As a result, fish waste becomes a resource that could negatively impact the environment (Wibowo et al 2017).

The protein content of fishery waste is quite diverse. For instance, offal is known to have a protein content of 32.12% on a dry basis (Aditya et al 2018). According to Rijal (2016), fish waste such as offal and fish heads contains protein, omega 6 and omega 9, while the bone contains a lot of calcium. According to the study of Nurhayati & Desniar (2013), hydrolysis of sturgeon fish innards produces fish hydrolyzate, which has a high protein content of 65.82%. Therefore, this study aimed to determine the level of *C. chanos* waste protein (MWP) and its effect on mice (*Mus musculus*), Absolute Lymphocyte Count (ALC), and blood parameters. An increase in the immune system could prevent the infection of COVID-19 in the future using the administration of MWP.

Material and Method

Description of the study sites. The research was conducted in May-August 2021 at the Fisheries Laboratory, Molecular Microbiology Laboratory, and Pharmacy Laboratory, University of Muhammadiyah Malang. Implementation is carried out in a blinded manner. Research activities are conducted offline, while consultation activities are coordinated with accompanying lecturers and teams. The sampled fish used in this study was *C. chanos* waste (heads, spines, tails, and offal), obtained from a fish distributor in Landungsari Market, Malang, East Java, Indonesia. Meanwhile, *M. musculus* was provided by the Pharmacy Laboratory, University of Muhammadiyah Malang, based on the following criteria: healthy-eyed mice, 15-20 g of body weight, regular agile movements, and no disease. Furthermore, the protein extract was mixed with Na-CMC and then homogenized. The mixture was tested on mice orally using a needle probe with various dosages, 6.522 mg (T1), 9.730 mg (T2), and 15.332 mg (T3).

Protein extraction. The extraction was based on Ghaly et al (2013) method, with slight modification. *C. chanos* waste was weighed and cleaned before blended with 0.1 M HCL of 1:1 (w/v). Afterward, It was centrifuged and then heated to 60ºC. The samples were then separated and filtered using a layered filter cloth. Then the sample was added with isopropanol to remove the residual fat, with a ratio of sample and solvent of 1:1. Following the next step, the sample was stirred at low speed for 5 min to divide the filtrate and oil. The filtrate was dried in an oven at 55ºC for 12 hours and stored in a dry place.

Proximate analysis. According to the protocol, the proximate analysis was performed at the Fish Nutrition Laboratory of the University of Muhammadiyah in Malang, Indonesia. All proximate studies were conducted following the AOAC (2012).

Parameter measurements. All blood measurements were facilitated by the Medical School of the University of Muhammadiyah Malang, Indonesia. The Red Blood Cells (RBS) count was modified by Pal (2009) by mixing blood with Hayem's solution.

$$N = n \times 10^4$$

Where:
N - erythrocyte total in 1 mL of blood;
n - erythrocyte total in the 80 areas of a hemocytometer.

Moreover, White Blood Cells (WBC) calculated by fish blood were diluted with Turk solution up to 20 times before counting. The leukocyte count was measured using a 10 × 40 microscope.

$$\text{Total numbers of WBCs present in 1 mm}^3\text{ of blood} = \frac{\text{The number of cells counted}}{\text{The number of 1 mm}^2\text{ counted}} \times \text{dilution}$$

The Sahli method assessed the mice's hemoglobin by dissolving 20 mm^3 of blood in 3.8% sodium citrate in a Sahli pipette (Barduagni et al 2003; Faatih 2017). Meanwhile, hematocrit level calculation using a microcapillary tube was filled with mice blood and paraffin. The tube was centrifuged for 5 minutes at 5000 rpm, and then the HCT value was determined with a ruler (Hudson et al 2008). Moreover, the derived hematological, including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), were calculated based on Mostakim et al (2015):

$$MCV = (HCT \div RBC \text{ in millions}) \times 10\ \mu m^3$$

$$MCH = (Hb \text{ in g} \div RBC \text{ in millions}) \times 10\ pg$$

$$MCHC = (Hb \div HCT) \times 100\ g / 100\ mL$$

The blood samples for the ALC test were taken from the heart of mice by injection of 1 mL. Then the samples were collected in an EDTA tube. Blood samples were collected in the tube and tested through a hematocrit test. The absolute lymphocytes count can be calculated by multiplying the total number of WBC against the percentage of white blood cells that were lymphocytes. The ALC calculation followed Nasrani (2022):

$$ALC\ (cells\ \mu L^{-1}) = \text{number of lymphocytes (\%) x number of leukocytes } (\mu L) \times 10^{-3}$$

Interpretation values: alert ALC<1.500, suspicious ALC<1.100 and danger ALC<500.

Statistical analysis. Observational data were processed by one-way analysis of variance (ANOVA); if there was an effect on the treatment, testing continued with Duncan's test to determine the difference between treatments. Data analysis used SPSS and Excel.

Results. After the extraction process, the present study found that MWP had high protein content, reaching 76.70% (Figure 1). The number could be determined as MWP having the possibility to be a food supplement.

No.	Sample Name	Water content			ASH	DM (Dry Meter LAB)	PROTEIN		Coarse FAT		Rough FIBER		TDN	Gross Energy
		I (60°C)	II (150 C)	Total			LAB analysis	Conversion Results*	LAB analysis	Conversion Results*	LAB analysis	Conversion Results*		
1.	Milkfish Waste Protein Extract	-	9,61	-	7,88	90,39	**76,70**	84,19	6,72	7,44	0,28	0,31	-	-
Unit		%	%	%	%	%	%	%	%	%	%	%	%	Cal/g
Test Method		SNI-2001-1992 Item 5.1					AOAC 2016, Chapter 4 item 4.1.10 Method 942.05		IK PM 5.4.1.3.e		SNI-2001-1992-Item 8.1			KA C2000

Figure 1. The proximate analysis of MWP after treatment.

Absolute Lymphocyte Count (ALC) value. Based on the data in Figure 2, it is known that during two weeks of observation, T2 was the best treatment, while T3 was not recommended for increasing ALC in mice. In the first week, the ALC level of all treatments was $2.58x10^{-3}$, and all samples were homogeneous. Based on the analysis data, the ALC values began to increase in the first week, showing a significant difference ($p<0.05$). Regarding the observation, T1 (6.522 mg) was the best, with an ALC of $3.72x10^{-3}$±0.12, followed by T2 (9.370 mg), with an ALC of $2.49x10^{-3}$±0.05. In contrast, T3 (15.332 mg) became the worst treatment, and its number was below the control group level (T0).

In the second week, the ALC value leveled off in all treatments. The T1 was still the best treatment compared to others, reaching 3.67×10^{-3}±0.02. In contrast, T3 was the worse treatment, $1.90x10^{-3}$±0.09. To sum up, administering milkfish waste to mice could significantly affect the ALC value ($p<0.05$) for two-week trials.

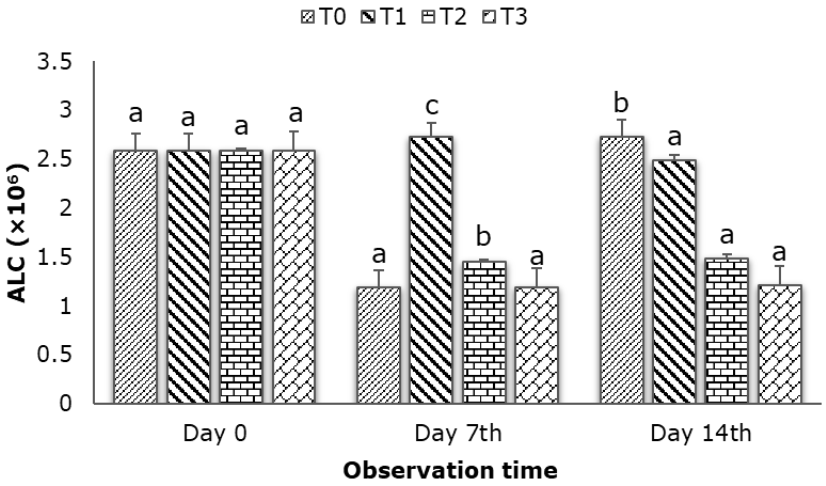

Figure 2. ALC values of *Mus musculus* after two weeks of treatments (different letters represent significant differences between treatments).

Red blood cells. The results of data analysis showed that the treatment of protein extract at various doses had a significantly different effect ($p<0.05$) on the number of red blood cells (erythrocytes) in mice. The average number of cells is presented in Figure 3.

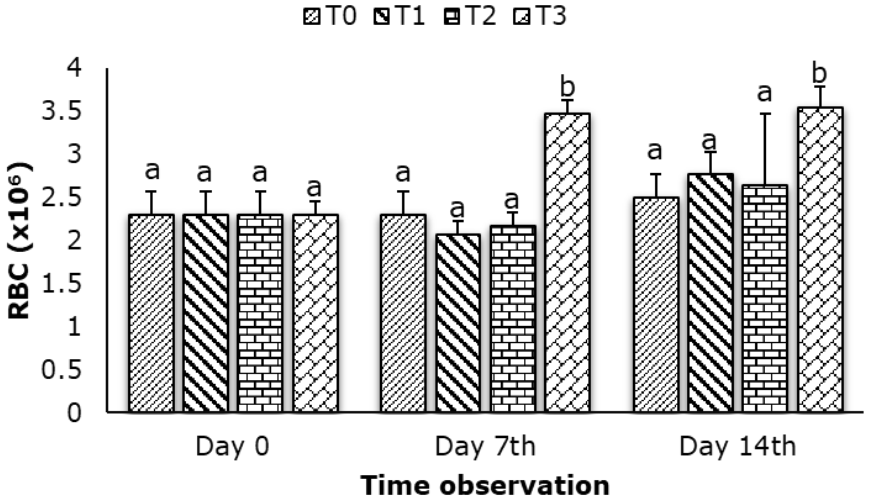

Figure 3. The red blood cells of *Mus musculus* after two weeks of treatments (different letters represent significant differences between treatments).

In general, the number of red blood cells in mice that received treatment with T1 in the first and second weeks was higher than in the control mice (control), reaching 3.90±0.10. The increase in the number of red blood cells occurred due to the administration of protein extract.

White blood cell (WBC). The results of data analysis showed that the treatment of protein extract at various doses was significantly different ($p<0.05$) in the white blood cell (leukocyte) count of mice. The average number of cells is presented in Figure 4.

It was noticed that the number of WBC of T1 in both the first and second weeks was higher than for the control (T0), consisting of mice who did not receive treatment. Duncan's test showed that control significantly differed from treatment T1, T2, and T3. The best treatment was at T1 in the first and second weeks, reaching 6.1±1 and 6.0±1, respectively.

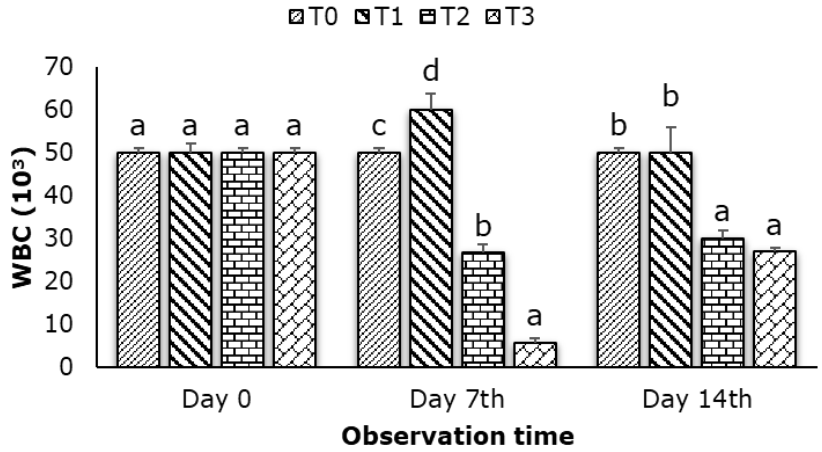

Figure 4. The white blood cells of *Mus musculus* after two weeks of treatments (various letters represent significant differences between treatments).

Average hematocrit/PVC (%). The data analysis showed that the treatment of protein extract at various doses had significantly different effects ($p<0.05$) on the hematocrit count of mice. The data is presented in Figure 5.

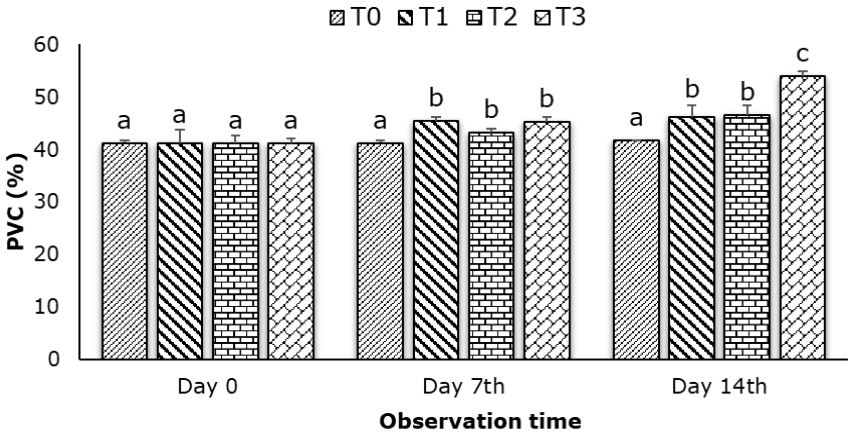

Figure 5. Hematocrit/PVC (%) of *Mus musculus* after two weeks of treatments (different letters represent significant differences between treatments).

It illustrates the number of PVC in mice in each treatment for two weeks. The T3 was the best method to accelerate the PCV level, while the T0 was the worst treatment. In the initial observation, all treatments showed insignificant ($p>0.05$), accounting for 40% before most treatments rose slightly. On day 7th, the present study discovered that T1, T2, and T3 inclined gradually to 45.43%, 43.19%, and 45.26%, respectively. In contrast, T0 remained stable at 40%, even in the last trial. Finally, there was a steady increase in T2 and T3 (43.19±2.34 versus 53.9±1.24), while the number of PCV in T1 leveled off.

Hemoglobin (Hb). The bar chart presents the level of Hb mice treated with the protein extract from *C. chanos* waste in various dosages. According to the data analysis, the administration of protein extract had a positive effect ($p<0.05$) on the average Hb of mice for two weeks presented in Figure 6. The observation was conducted three times to evaluate the number of Hb.

In the initial observation, the number of Hb was eventually used as the control point. It started to rise by T1 and T2 to 1.58±0.09 and 1.56±0.61 g dL^{-1}, respectively, on day 7th, while the others remained stable at the same level. In the following recording, T3 reached the peak of 2.13±0.25 g dL^{-1}, while there was a steady increase in T1 and T2 to 1.63±0.11 and 1.56±0.61 g dL^{-1}, respectively. On the other hand, T0 decreased

gradually on day 14th by 0.08 g dL^{-1}. Regarding those discoveries, the treatment worked well in promoting mice's Hb level for two weeks.

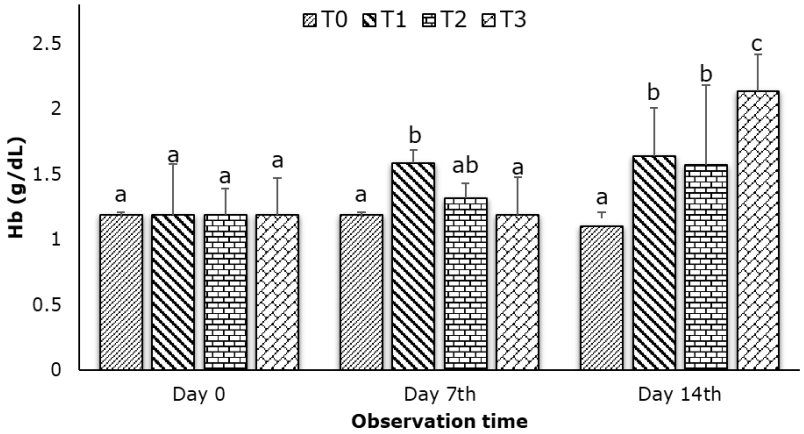

Figure 6. Hemoglobin (Hb) level of *Mus musculus* after two weeks of treatments (various letters represent significant differences between treatments).

Average erythrocyte index (MCH, MCHC, MCV). The MCH and MCHC data analysis showed that the treatment of protein extract at various doses was not significantly different (P<0.05) from the average MCH and MCHC of mice. However, the MCV data showed that the treatment of protein extract at various doses was significantly different (P<0.05) from the average MCV of mice. The average values are presented in Figure 7.

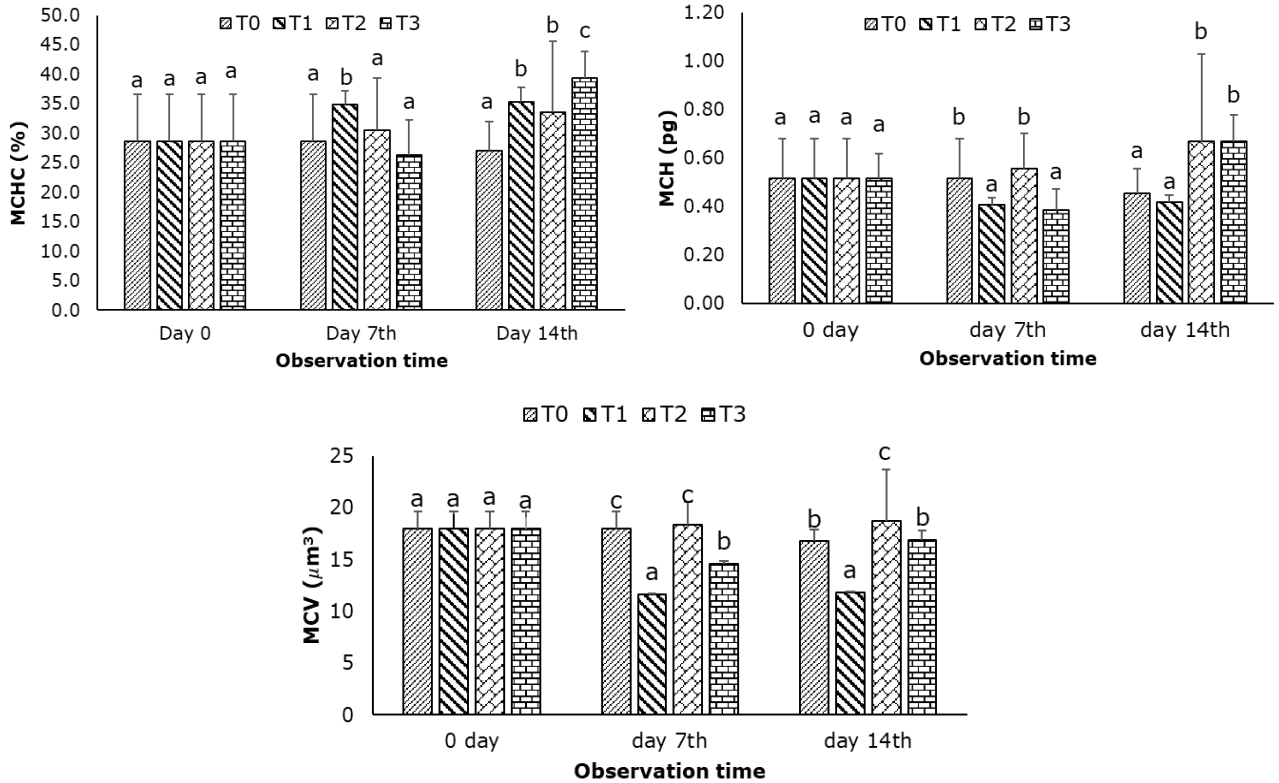

Figure 7. MCHC, MCH, and MCV levels of *Mus musculus* after two weeks of treatments (various letters represent significant differences between treatments).

The data of MCHC did not have a difference in the initial observation (Day 0) statistically (p>0.05) before T1 increased gradually to 34.84% on day 7th. Although there was a moderate rise in the MCHC level of T2, reaching 30.4%, it could not be determined as significantly different from T0 and T3. Moreover, the MCH level in T3 peaked at 39.36%,

while the T2 inclined steadily to 33.55% on day 14th. On the one hand, the T1 was not as big as in the previous observation, while the T0 fell gradually to 27.06%.

Secondly, level of MCH in all treatments had similar number on Day 0 of 0.52 pg ($p>0.05$). The following observation showed a noticeable decrease in two treatments (T1 and T3) to 0.41 pg and 0.38 pg, respectively. Meanwhile, the other two remained stable as last time and still showed insignificant different ($p>0.05$). On day 14th, T3 rocketed to 0.67 pg, as big as T3, which rose slightly to 0.12 pg from the previous level. In contrast, the T0 fell markedly to 0.07 pg, while T1 leveled off with 0.41 pg.

Finally, there was a similar condition of MCV level as MCHC and MCH at the beginning of the study. However, the T1 and T3 dipped significantly on day 7th, accounting for 11.65 μm^3 and 14.59 μm^3, respectively, while the T0 and T2 maintained the same level. Furthermore, at the end of the period, there was a slight increase in MCV levels of T1, T2, and T3 to 11.82 μm^3, 18.73 μm^3, and 16.87 μm^3, respectively. Meanwhile, there was a minimally decline in T0 by 1.24 μm^3. To sum up, MWP's administration to mice has promoted erythrocyte derivation considerably for two weeks trials. As a result, MWP has the potential to be a supplement for stimulating immunity.

Differential leucocytes. The various differential leucocytes data of mice after treatments are shown in Tables 1 and 2. Overall, there was insignificant data ($p>0.05$), particularly on monocytes, basophils, and eosin during the study. Moreover, the present study discovered that the T1 was the best treatment compared to others. All data was measured in percentage.

Table 1

The average value of differential leukocytes in the blood of mice given protein extract with various doses in the first week

Parameters	*Data sampling*			
	Day 7th			
	T0	*T1*	*T2*	*T3*
Lymphocytes (%)	50.4 ± 2.5^a	61 ± 1^b	59.4 ± 0.7^b	52.4 ± 1.3^a
Monocytes (%)	1.7 ± 0.6^a	1.9 ± 0.5^a	1.6 ± 0.3^a	1.6 ± 0.2^a
Neutrophils (%)	44.7 ± 0.8^a	58.4 ± 0.9^b	45.4 ± 2.6^a	45.1 ± 1^a
Basophils (%)	0^a	0^a	0^a	0^a
Eosinophils (%)	1.9 ± 3.23^a	0^a	0^a	0.9 ± 1.5^a

The different letters in the same row represent significant differences between treatments.

Table 2

The average value of differential leukocytes in the blood of mice given protein extract with various doses in the second week

Parameters	*Data sampling*			
	Day 14th			
	T0	*T1*	*T2*	*T3*
Lymphocytes (%)	54.6 ± 0.7^a	61.2 ± 0.8^b	60.4 ± 0.7^a	54.3 ± 1.1^a
Monocytes (%)	1.4 ± 0.1^a	1.6 ± 0.3^{ab}	2 ± 0.4^b	1.5 ± 0.2^a
Neutrophils (%)	44 ± 0.7^a	57.7 ± 0.6^c	48.4 ± 2.5^b	44.8 ± 2.2^a
Basophils (%)	0^a	0.5 ± 0.9^a	0.7 ± 1.1^a	2.9 ± 0.6^b
Eosinophils (%)	1.7 ± 3^a	0^a	0^a	0^a

The different letters in the same row represent significant differences between treatments.

On day 7th, it could be seen that only lymphocytes and neutrophils presented a substantial difference among other parameters ($p<0.05$). The T1 and T2 managed to have the same level of lymphocytes statistically by 61±1% and 59.4±0.7%, respectively. Meanwhile, in the case of neutrophils, only T1 shared the highest level (58.4±0.9%) among treatments.

On day 14th, the lymphocyte percentage of mice increased moderately in all treatments, around 0.8% to 4%. There was a huge increase in lymphocyte level of T0 of 54.6%. However, its number had been considered no different from T2 and T3 ($p>0.05$). The T1 shared the highest number of all medicines, accounting for 61.2%. Moreover, the monocyte and neutrophils number dipped gradually to approximately 20% for all treatments except T2. It rose steadily, reaching 2 and 48.4% of the monocytes and neutrophils level, respectively. Meanwhile, the number of basophils was led by T3 at 2.9%, while others did not have a positive influence compared to the control group. Unfortunately, the eosin level remained stable and showed an insignificant difference until day 14th. Regarding those discoveries, several differential leucocytes could be stimulated by administrating of the MWP.

Discussions. The immune system consists of the innate and adaptive immune systems, including lymphocytes and macrophages (Smith et al 2019). According to Sumarmi (2020), proteins play a role in forming immunoglobulins and lymphocytes. The T cells, a type of white blood cell (lymphocyte), are responsible for locating and destroying infections (Blumenreich 1990). They serve to produce antibodies when an antigen enters the body (Schwartz 1990; Willard-Mack 2006). Protein is an essential substance in the body, which functions as a regulator of the body's defense against various microbes and foreign substances (Mariani et al 2019). The result showed that the protein content of MWP was relatively high (76.70%). According to Rosalina and Istiqomah (2017), protein has a function in forming the body's defense system to produce cellular components. A declining protein will damage the immune system needed to fight foreign substances.

Data on the prognostic factors of Covid-19 are still scarce. It is known that lymphocytopenia, defined as absolute lymphocyte count (ALC)<1,000 cells μL^{-1}, occurs in COVID-19 survivors and correlates with increasing disease severity (Fu et al 2020). Lymphocytes are mononuclear cells consisting of T and B lymphocytes that serve as a specific immune response (Janossy & Greaves 1971). Therefore, the ALC value could be used as an inflammation marker in interpreting the Covid-19 disease (Nasrani 2022). Figure 2 shows that the MPW extract could significantly affect the ALC value ($p<0.05$) compared to the control group. Lymphocytes play a role in fighting infections caused by viruses or bacteria. Consequently, the ALC value is usually used to interpret the data that a person has been infected with Covid-19 (Illg et al 2021). Some studies revealed that SARS-CoV-2 could significantly reduce the number of lymphocyte subsets, such as $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and B cells, according to the results of the flow cytometry (Huang et al 2020; Qin et al 2020; Xu et al 2020). According to Tan et al (2020), lymphocytes are crucial for immunological homeostasis and inflammatory response. Understanding how blood lymphocyte counts drop could help cure COVID-19. Although the cause of considerable lymphocyte decrease in severe COVID-19 is unknown, other hypotheses include lymphocyte infiltration and sequestration in the lungs, GI tracts, and lymphoid organs (Huang & Pranata 2020). It carries the ACE2 receptor, which might be an infection's primary target (Xu et al 2020). A rise in pro-inflammatory cytokines, particularly IL-6, in COVID-19 may trigger additional lymphocyte depletion (Lin et al 2020). Regarding those references, lymphocytes treatment might have an opportunity to solve the COVID-19 problem. For instance, the insertion of MPW extract could boost mice lymphocyte that was treated for two weeks (Table 1).

Moreover, white blood cells are generally classified into granulocytes and agranulocytes. Granulocytes consist of eosinophils, neutrophils, and basophils, while agranulocytes comprise lymphocytes and monocytes (Hidayanti et al 2014). These blood cell components function as the body's defense against foreign objects. In observing the lymphocyte values, it was found that the treatments promoted the number of lymphocytes better than the control group. Neutrophils and lymphocytes are the two types of leukocytes that are found the most frequently and together make up about 90% of the total population of leukocytes (Ramoji et al 2012) and have a role in immune responses such as natural killer (NK) and lymphoid cells. Both cells have a role in tissues that experience infection and inflammation (Gasteiger & Rudensky 2014). This indicates

that the administration of protein extract could potentially increase the lymphocyte value, indirectly increasing the body's immunity.

Monocytes are a part of leukocyte blood cells produced in the bone marrow and circulated in the bloodstream (Samour & Hart 2020). They can phagocytose or eat foreign objects, such as bacteria (Arai et al 2015), and play a role in the inflammatory response. The present study found that the treatments mainly decreased the number of monocytes gradually for two weeks. The absence believes in reducing monocyte numbers of phagocytic activity against foreign objects or slow phagocytic ability (Hidayanti et al 2014). The neutrophil value showed a tendency to increase the number of neutrophil cells compared to the control. According to Chervenick et al (1968), mice's standard number of neutrophils is 12-30%. There was a moderate increase in mice's neutrophils level in T1 and T2 compared to the control group (without treatment). An increase in mice's neutrophil cells is due to chemotactic factors in the tissue caused by inflammation or injury (Ley et al 2018). Our results seem related to Rusu et al (2010) study that bovine whey proteins activated the synthesis and storage of specific cytokines and chemokines, directly controlling human blood neutrophils. Therefore, the present study assumed that MPW extract did the same pathway as bovine whey proteins to stimulate mice's neutrophils. In addition, mice's basophils number increased gradually by T3 on day 14th. A similar study proved that peanut protein promoted the number of basophils through a Western blotting experiment.

Furthermore, there was no eosinophils increase until the period's end. Eosinophils are unquestionably involved in the pathophysiology of allergy disorders and play effector roles (Shi 2004). It is also supported by Humbles Alison et al (2004) and Moussa et al (2019), they are primarily responsible for allergic reactions and immunological responses to parasite infections. Regarding those statements, the present study believed that the MPW extract did not generate allergies in the mice. It has been demonstrated that red blood cells, often known as RBCs, can affect the immune system's function and cause inflammatory responses following transfusion (Karsten et al 2018). Meanwhile, hemoglobin is a protein in red blood cells that transports oxygen to the tissues (Marengo-Rowe 2006; Wisanuvej et al 2021). Moreover, there is a strong relationship between hemoglobin and hematocrit (Hematyar & Ekhtiari 2008; Wisanuvej et al 2021). Therefore, our study revealed that the MPW extract could significantly stimulate RBC, HB, and PCV compared to the control group. Furthermore, our results also confirmed a close relation between RBC deformability and blood indices (MCV, MCH, and MCHC). An increase in RBC correlated to the incline of MCH and MCHC, but the MCV had a different story. According to Alwan et al (2009), despite a rise in RBC count, a fall in MCV may indicate the amount of cell size reduction. In line with our findings, Bosch et al (1994) found that elevated MCHC and decreased MCV accompanied increased RBCs.

Conclusions. There was a positive impact on the application of MPW extract toward the immunity of *M. musculus*. The MPW could effectively stimulate the immune responses of mice on day 14^{th,} almost in all parameters. In the current case, only Eosinophils showed insignificant differences compared to other parameters. The present study suggests that it needs to conduct further research on whether the MPW extract could directly prevent the invasion of COVID-19.

Acknowledgements. The authors would like to thank the Director-General of Higher Education, Indonesia, who funded this research, and the laboratory of fisheries provided facilities for supporting the research.

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

References

Aditya D., Deanti H., Ma'arif J., Dewi E., 2018 [Production of protein hydrolyzate of tilapia innards (*Oreochromis niloticus*) using the bromeilin enzyme of pineapple (Ananascomosus)]. Prosiding Seminar Nasional Kelautan XIII "Implementasi Hasil

Riset Sumber Daya Laut dan Pesisir dalam Rangka Mencapai Kemandirian Ekonomi Nasional", Fakultas Teknik dan Ilmu Kelautan Universitas Hang Tuah, Surabaya, pp. 56-70. [In Indonesian].
Alwan S., Hadi A., Shokr A., 2009 Alterations in hematological parameters of fresh water fish, Tilapia zillii, exposed to aluminum. Journal of Science and its Applications 3(1):12-19.
Arai Y., Mizugishi K., Nonomura K., Naitoh K., Takaori-Kondo A., Yamashita K., 2015 Phagocytosis by human monocytes is required for the secretion of presepsin. Journal of Infection and Chemotherapy 21(8):564-569.
Barduagni P., Ahmed A. S., Curtale F., Raafat M., Soliman L., 2003 Performance of Sahli and colour scale methods in diagnosing anaemia among school children in low prevalence areas. Tropical Medicine & International Health 8(7):615-618.
Blumenreich M. S., 1990 The white blood cell and differential count. Clinical methods: The history, physical, and laboratory examinations. 3rd Edition. Butterworths, Boston, 276 p.
Bosch F., Werre J., Schipper L., Roerdinkholder-Stoelwinder B., Huls T., Willekens F., Wichers G., Halie M., 1994 Determinants of red blood cell deformability in relation to cell age. European Journal of Haematology 52(1):35-41.
Chervenick P. A., Boggs D. R., Marsh J. C., Cartwright G. E., Wintrobe M. M., 1968 Quantitative studies of blood and bone marrow neutrophils in normal mice. American Journal of Physiology-Legacy Content 215(2):353-360.
Djunaidah I., Ramadhona M., Hakim D., Nurdianto A., Notosudjono D., Soebagia H., Uddin N., Rashid M., Mostafa M., Salam S., 2017 Level of fish consumption in Indonesia: irony in the nautical country. Jurnal Penyuluhan Perikanan Dan Kelautan 11(1):12-24.
Faatih M., 2017 [Use of hemoglobin measuring devices in Puskesmas, Polindes and Pustu]. Jurnal Penelitian dan Pengembangan Pelayanan Kesehatan 1(1):32-39. [In Indonesian].
Fu Z., Tang N., Chen Y., Ma L., Wei Y., Lu Y., Ye K., Liu H., Tang F., Huang G., 2020 CT features of COVID-19 patients with two consecutive negative RT-PCR tests after treatment. Scientific Reports 10(1):1-6.
Gasteiger G., Rudensky A. Y., 2014 Interactions between innate and adaptive lymphocytes. Nature Reviews Immunology 14(9):631-639.
Ghaly A., Ramakrishnan V., Brooks M., Budge S., Dave D., 2013 Fish processing wastes as a potential source of proteins. Amino acids and oils: A critical review. Journal of Microbial and Biochemical Technology 5(4):107-129.
Hematyar M., Ekhtiari A., 2008 Correlation between neonatal cord blood hemoglobin and hematocrit with mode of delivery. Journal of Inflammatory Diseases 12(3):21-25.
Hidayanti M. D., Astuti S., Kustyawati M. E., 2014 [The effect of giving "Kombucha" rosella tea on the blood profile of mice (*Mus musculus* L)]. Agritech 34(4):382-389. [In Indonesian].
Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., Zhang L., Fan G., Xu J., Gu X., 2020 Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet 395(10223):497-506.
Huang I., Pranata R., 2020 Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. Journal of Intensive Care 8(1):36-46.
Hudson H., Brauer P., Scofield M., Petzel D., 2008 Effects of warm acclimation on serum osmolality, cortisol and hematocrit levels in the Antarctic fish, Trematomus bernacchii. Polar Biology 31(8):991-997.
Humbles A. A., Lloyd C. M., McMillan S. J., Friend D. S., Xanthou G., McKenna E. E., Ghiran S., Gerard N. P., Yu C., Orkin S. H., Gerard C., 2004 A critical role for eosinophils in allergic airways remodeling. Science 305(5691):1776-1779.
Illg Z., Muller G., Mueller M., Nippert J., Allen B., 2021 Analysis of absolute lymphocyte count in patients with COVID-19. The American Journal of Emergency Medicine 46:16-19.

Janossy G., Greaves M., 1971 Lymphocyte activation: I. Response of T and B lymphocytes to phytomitogens. Clinical and Experimental Immunology 9(4):483-498.
Karsten E., Breen E., Herbert B. R., 2018 Red blood cells are dynamic reservoirs of cytokines. Scientific Reports 8(1):3101-3113.
Ley K., Hoffman H. M., Kubes P., Cassatella M. A., Zychlinsky A., Hedrick C. C., Catz S. D., 2018 Neutrophils: new insights and open questions. Science Immunology 3(30):4579-4593.
Li X., Zheng S., Wu G., 2021 Nutrition and functions of amino acids in fish. Amino acids in nutrition and health. Springer, USA, pp. 133-168.
Lin L., Lu L., Cao W., Li T., 2020 Hypothesis for potential pathogenesis of SARS-CoV-2 infection-a review of immune changes in patients with viral pneumonia. Emerging Microbes & Infections 9(1):727-732.
Marengo-Rowe A. J., 2006 Structure-function relations of human hemoglobins. Proceedings, Baylor University, Medical Center 19(3):239-245.
Mariani E., Lisignoli G., Borzì R. M., Pulsatelli L., 2019 Biomaterials: foreign bodies or tuners for the immune response? International Journal of Molecular Sciences 20(3):636-678.
Mostakim G. M., Zahangir M. M., Monir M. M., Rahman M. K., Islam M. S., 2015 Alteration of blood parameters and histoarchitecture of liver and kidney of silver barb after chronic exposure to quinalphos. Journal of Toxicology 415984-415992.
Moussa K., Gurung P., Adams-Huet B., Devaraj S., Jialal I., 2019 Increased eosinophils in adipose tissue of metabolic syndrome. Journal of Diabetes and its Complications 33(8):535-538.
Nafrin I. A., Hudaidah, 2021 [Development of Indonesian education during the Covid-19 pandemic]. Edukatif: Jurnal Ilmu Pendidikan 3(2):456-462. [In Indonesian].
Nasrani L., 2022 [The relationship between neutrophil-lymphocyte ratio, absolute lymphocyte count, and platelet lymphocyte ratio to the severity of COVID-19]. Intisari Sains Medis 13(1):127-130. [In Indonesian].
Nurhayati T., Desniar S. M., 2013 [Enzymatic production of peptone using raw material of tuna fish innards]. Jurnal Pengolahan Hasil Perikanan Indonesia 16(1):1-11. [In Indonesian].
Pal G. K., 2009 Textbook of practical physiology. Orient Blackswan, India, 456 p.
Prasetyo D. Y. B., Darmanto Y. S., Swastawati F., 2015 [Effect of temperature difference and smoking time on the quality of milkfish (*Chanos chanos* Forsk) remove smoked spines]. Jurnal Aplikasi Teknologi Pangan 4(3):94-98. [In Indonesian].
Qin C., Zhou L., Hu Z., Zhang S., Yang S., Tao Y., Xie C., Ma K., Shang K., Wang W., 2020 Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. Clinical Infectious Diseases 71(15):762-768.
Ramoji A., Neugebauer U., Bocklitz T., Foerster M., Kiehntopf M., Bauer M., Popp J., 2012 Toward a spectroscopic hemogram: raman spectroscopic differentiation of the two most abundant leukocytes from peripheral blood. Analytical Chemistry 84(12):5335-5342.
Rijal M., 2016 [Processing and increasing the protein content of fish nuggets made from fish waste by giving pineapple peel extract]. BIOSEL (Biology Science and Education): Jurnal Penelitian Science dan Pendidikan 5(1):84-92. [In Indonesian].
Rosalina S., Istiqomah S. B. T., 2017 [The relationship between calorie and protein intake of postpartum mothers with the duration of perineal wound healing at the Balowerti Public Health Center, Kediri, Indonesia]. Jurnal EDUMidwifery 1(2):87-95. [In Indonesian].
Rusu D., Drouin R. J., Pouliot Y., Gauthier S., Poubelle P. E., 2010 A bovine whey protein extract stimulates human neutrophils to generate bioactive IL-1Ra through a NF-κB- and MAPK-dependent mechanism. The Journal of Nutrition 140(2):382-391.
Samour J., Hart M., 2020. Normal and abnormal white blood cells. Hawkey's atlas of wild and exotic animal haematology. CRC Press, Boca Raton, pp. 31-34.
Schwartz R. H., 1990 A cell culture model for T lymphocyte clonal anergy. Science 248(4961):1349-1356.

Shi H.-Z., 2004 Eosinophils function as antigen-presenting cells. Journal of Leukocyte Biology 76(3):520-527.
Smith N. C., Rise M. L., Christian S. L., 2019 A comparison of the innate and adaptive immune systems in cartilaginous fish, ray-finned fish, and lobe-finned fish. Frontiers in Immunology 10:2292-2315.
Stern D., Cui H., 2019 Crafting polymeric and peptidic hydrogels for improved wound healing. Advanced Healthcare Materials 8(9):1900104-1900121.
Sumarmi S., 2020 [Harmony work of nutrients in increasing the body's immunity against Covid-19: mini review]. Amerta Nutrition 4(3):250-256. [In Indonesian].
Tan L., Wang Q., Zhang D., Ding J., Huang Q., Tang Y.-Q., Wang Q., Miao H., 2020 Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduction and Targeted Therapy 5(1):33-35.
Wibowo T., Purwanto P., Yulianto B., 2017 Environmental management efforts at fillet fish processing industry in Coastal Fishing Port Tegalsari Tegal—Indonesia. Advanced Science Letters 23:2542-2544.
Willard-Mack C. L., 2006 Normal structure, function, and histology of lymph nodes. Toxicologic Pathology 34(5):409-424.
Wisanuvej K., Boonyawat K., Savetamornkul C., Virapongsiri S., Krongvorakul J., Sungkanuparph S., Phuphuakrat A., 2021 Comparison between blood hemoglobin concentration determined by point-of-care device and complete blood count in adult patients with dengue. PLOS Neglected Tropical Diseases 15(8):0009692-0009703.
Xu H., Zhong L., Deng J., Peng J., Dan H., Zeng X., Li T., Chen Q., 2020 High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. International Journal of Oral Science 12(1):1-5.
Xu Z., Shi L., Wang Y., Zhang J., Huang L., Zhang C., Liu S., Zhao P., Liu H., Zhu L., Tai Y., Bai C., Gao T., Song J., Xia P., Dong J., Zhao J., Wang F.-S., 2020 Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet Respiratory Medicine 8(4):420-422.
Zendrato W., 2020 [Movement to prevent rather than treat the covid-19 pandemic]. Jurnal Education and development 8(2):242-242. [In Indonesian].
*** AOAC, 2012 Official method of analysis. Association of Official Analytical Chemists, Washington DC, pp. 121-130.

Received: 31 January 2022. Accepted: 03 August 2022. Published online: 20 August 2022.
Authors:
Shafira Rahmania, Muhammadiyah Malang University, Faculty of Agribusiness and Animal Husbandry, Program Study of Aquaculture, e-mail: shafirarahmania@webmail.umm.ac.id
Yusuf Novaldo, Muhammadiyah Malang University, Faculty of Agribusiness and Animal Husbandry, Program Study of Aquaculture, e-mail: yusufnovaldo@webmail.umm.ac.id
Anisa Lutfiaturrohmah, Program Study of Aquaculture, Faculty of Agribusiness and Animal Husbandry, Muhammadiyah Malang University, e-mail: anisarohmah10@webmail.umm.ac.id
Titan Ababil, Muhammadiyah Malang University, Faculty of Health Sciences, Program Study of Pharmacy, e-mail: titanfarm000@webmail.umm.ac.id
Riza Rahman Hakim, Muhammadiyah Malang University, Faculty of Agribusiness and Animal Husbandry, Program Study of Aquaculture, e-mail: rizarahman@umm.ac.id
Hariyadi, Muhammadiyah Malang University, Faculty of Agribusiness and Animal Husbandry, Program Study of Aquaculture, e-mail: hariyadi@umm.ac.id
Soni Andriawan, Program Study of Aquaculture, Faculty of Agribusiness and Animal Husbandry, Muhammadiyah Malang University, e-mail: soniandriawan@umm.ac.id
How to cite this article:
Rahmania S., Novaldo Y., Lutfiaturrohmah A., Ababil T., Hakim R. R., Hariyadi, Andriawan S., 2022 Potential of milkfish waste protein extract as an immune supplement for Covid-19 outbreak prevention. AACL Bioflux 15(4):2090-2101.