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Effectiveness of Herbal Diet *Nigella sativa* and *Gracilaria verrucosa* Against Non-specific Immunity of Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The addition of *Nigella sativa* and *Gracilaria verrucosa* to feed is used as a supplement to increase non-specific immunity in tilapia. The addition of herbal supplements is safe for consumption, easy to decompose, has no residue on the fish's body, and does not harm the environment. This study aims to find the effectiveness of the addition of the herbs *Nigella sativa* and *Gracilaria verrucosa* to increase non-specific immunity in tilapia. The study used an experimental method with a completely randomized design, applying 4 treatments and 1 control to tilapia. The dietary doses of *Nigella sativa* were T0 (control, without *Nigella sativa*), T1 (2.5), T2 (5), T3 (7.5), and T4 (10) (g kg^{-1} feed). While the dietary doses of *Gracilaria verrucosa* were T0 (control, without *G. verrucosa*), T1 (0.5), T2 (1), T3 (1.5), and T4 (2) (g kg^{-1} feed). The results showed that the percentage of hematocrite increased significantly with the *Nigella sativa* diet, while on the *Gracilaria verrucosa* diet, the percentage of hematocrit was not significant. Further, the *Nigella sativa* diet and the *Gracilaria verrucosa* diet can significantly increase the percentage of lymphocytes, but not significantly to the percentage of monocytes. In fish farming, the *Nigella sativa* and *Gracilaria verrucosa* diets have the potential to be used to increase non-specific immunity in tilapia, but it is more effective to use the *Nigella sativa* diet at a dose of 5 g kg^{-1} feed.

Keywords: tilapia, *Nigella sativa, Gracilaria verrucosa*, non-specific immunity

INTRODUCTION

Herbal therapy has been widely used in developing countries with tropical climates. The fishing industry has begun to apply herbs to replace the use of antibiotics which have a negative impact. Herbs have advantages over synthetic chemical drugs, have low side effects, and the extraction method is simpler. Black cumin (*Nigella sativa*) is a type of herb that functions as an immunomodulator, as well as being an antioxidant, antibacterial, antifungal, anti-cholesterol, and anticancer (Asniyah, 2009). As an immunostimulant, natural sources of black cumin are safer than synthetic chemical supplements in preventing disease infections (Cheng *et al.*, 2014).

Tilapia fed black cumin diet can increase non-specific immunity at a dose of 3.5%. The addition of black cumin to tilapia fish can inhibit and prevent *Streptococcus agalactiae* infection with a survival rate of 90%, a hematocrit level of 30%, a low feed conversion value, and an increase in the weight and length of the fish after being infected with bacteria (Sa'adah *et al*., 2015). Furthermore, reported by *Silviana et al*. (2022) that a diet of black

cumin flour in tilapia at a dose of 50 g kg^{-1} could increase the percentage of hematocrit by 34%, lymphocytes 84.3%, monocytes 6%, neutrophils 9.7% and survival rate by 95% after being given the *Aeromonas hydrophila* challenge test. In general, fish that were given immunostimulant herbs produced an increase in the number of leukocytes. This is because immunostimulants can stimulate the body to produce more white blood cell production as a non-specific immune respons (Devi *et al.*, 2019). Black cumin diet preparations are not only given in the form of flour which is mixed with the feed, but can be given in the form of black cumin oil which is added to the feed. The addition of black cumin oil mixed into the feed by 30% can increase the immune response and prevent *Aeromonas hydrophila* infection in *Anabas testudines* fish. In addition to increasing the immune response, black cumin oil can reduce mortality and increase survival (Khatun *et al.*, 2015). There are many other types of herbs besides black cumin, including red seaweed which is known to increase non-specific immunity in fish.

Red seaweed *Gracilaria verrucose* is known to increase immunity in shrimp after being challenged with *Vibrio harveyi* bacteria. The results of a study conducted by Jasmanindar *et al.* (2018) proved that vanamei shrimp (*Litopenaeus vanamei*) fed a *Gracilaria verrucosa* extract diet for 42 days could increase the number of haemocyte cells and other activities that strengthen immunity after being challenged with *Vibrio harveyi* bacteria. The indicator for increasing the number of cells is shown by the increase in the total number of haemocytes, the number of hyaline cells, the number of granular cells, the number of semi-granular cells. While the activity that increased was phenol oxidase activity, bacterial and phagocytic activity which resulted in increased immunity. A dose of 2 g kg^{-1} feed is the optimal dose compared to other treatment doses. The results of this study indicate that *Gracilaria verrucosa* can be used as an immunostimulant. Reports of adding red seaweed *Gracilaria verrucosa* to fish are still few, therefore this study was applied to fish.

Utilization of herbs as immunostimulants is safer and more beneficial for fish than antibiotics. Improper use of antibiotics causes residual deposits in fish bodies, harms consumers, environmental pollution and resistance to certain bacteria causing harm. This causes herbal immunostimulants to be widely used to improve immune quality. Black cumin seed is an effective immunostimulant although its activity varies from moderate to optimal. However, the price of black cumin is relatively cheap, so it is recommended to be applied in fish farming in order to reduce mortality and prevent pathogen infection (Dorucu *et al.*, 2009).

Like black cumin, red seaweed is also widely used as an immunostimulant in fish and shrimp. The results of a study conducted by (Zahra *et al.*, 2017) stated that vanamei shrimp fed a *Gracilaria verrucosa* diet of 4 g kg^{-1} could increase non-specific immune responses and be resistant to white spot disease infection (WSSV) with a Relative Percentage Survival (RPS) value by 41.07%. Furthermore, explained by Wongprasert *et al*. (2014), in shrimp there is a toll-like receptor (TLR) which plays an important role in the shrimp immune system. Red seaweed contains sulphated galactan compounds which then bind to TLRs causing regulation and activation of antioxidant enzyme systems. Viruses can die because antioxidant enzymes remove excess reactive oxygen species (ROS), and impair viral replication in host cells. This study aims to find the effectiveness and optimal dose of immunostimulants from *Nigella sativa* black cumin and *Gracilaria verrucosa* red seaweed in increasing the non-specific immunity of tilapia.

METHOD

1. Reseach Materials

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The main ingredients of this research are black cumin (*Nigella sativa*), red seaweed *Gracilaria verrucosa* and tilapia (*Oreochromis niloticus*). The tilapia used has a length of 10-13 cm. Fish feed uses commercial pellets, produced by PT Mataram Sakti, Surabaya, East Java. The nutritional composition consisted of 33% protein, 5% fat, 4% crude fibre, 12% ash content, and 10% water content. Feed is given 2 times a day, in the morning and evening at a dose of 5% kg^{-1} by weight of fish biomass.

2. Research Design

The study used an experimental method with a completely randomized design, consisting of 4 treatments and 1 control with 3 individual replications. Treatment doses for *Nigella sativa* (g kg^{-1} feed) were: T0 (control, without *Nigella sativa*), T1 (2.5), T2 (5), T3 (7.5), and T4 (10). As for the doses of *G. verrucosa* (g kg^{-1} feed) used were: T0 (control), T1 (0.5), T2 (1), T3 (1.5), and T4 (2). The tilapia was placed in round buckets filled with 60 L of water; each bucket filled with 5 tilapias maintain during 7 days.

3. Parameters

The research parameters observed in each treatment included the percentage of hematocrite, lymphocyte and monocyte percentage. Blood was taken using a 1 mL disposable syringe and put in a microhematocrit tube. The microhematocrit tube was centrifuged and then the length of the volume of hematocrit, lymphocytes and monocytes in the microhematocrit tube was measured in percentage (%).

4. Data analysis

Research data were analyzed using Analysis of Variance (Anova at 5% test level). If the ANOVA results are significantly different, then proceed with the Duncan Multiple Range Test (DMRT) at a test level of 5%.

RESULTS AND DISCUSSION

1. Hematocrite

The results showed that the black cumin *Nigella sativa* diet significantly increased the percentage of hematocrite during the 7 days of treatment. Meanwhile, feeding the red seaweed *Gracilaria verrucosa* diet slightly increased the percentage of hematocrite at a dose of 1.5 g kg^{-1} feed but not significantly (Table 1).

Table 1. *Nigella Sativa* Diet and *Gracilaria Verrucosa* Diet on Tilapia for 7 Days on the Percentage of Hematocrite

Tilapia day-7			
Nigella sativa		***Gracilaria verrucosa***	
g kg^{-1} feed	**Hematocrite**	***g kg^{-1} feed***	**Hematocrite**
Control	43.7±2.3^{a}	Control	38.9±4.9^{a}
2.5	45.4±3.4^{a}	0.5	36.0±1.6^{a}
5	47.6±6.2^{ab}	1	36.4±2.2^{a}
7.5	52.1±1.9^{bc}	1.5	41.6±2.0^{a}
10	57.9±0.7^{c}	2.0	35.8±4.2^{a}

The increase in the percentage of hematocrite in the treatment of the black cumin and the *Gracilaria verrucosa* diet showed an increase in tilapia non-specific immune response. An indicator of increased non-specific immune response is characterized by an increase in the percentage of hematocrite. Based on the results of this study, the black

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cumin extract diet was more effective than the *Gracilaria verrucosa* diet in increasing the percentage of hematocrite.

The results of the study Abd El-Hack *et al*. (2020) proved that the bioactive compounds contained in black cumin such as alkaloids, triterpenoids, steroids, saponins, phenolics and flavonoids function as immunostimulants which can increase the percentage of hematocrit. African catfish fed black cumin diet can increase hematocrite levels (Dontriska, 2014). The black cumin diet at a dose of 50 g kg^{-1} feed on tilapia effectively increased the non-specific immune response which was marked by an increase in the percentage of hematocrite up to 34% *(Silviana et al.*, 2022).

The same thing was reported by Sa'adah *et al*. (2015), the addition of 35 g kg^{-1} black cumin feed on tilapia can effectively increase hematocrite levels by up to 30%. Tilapia fed the black cumin diet and then infected with *S. agalactiae* were able to survive until the survival rate reached 90% without showing symptoms of *S. agalactiae* infection. This shows that the black cumin diet can increase non-specific immunity and prevent bacterial infections. Furthermore, a diet enriched with *Nigella sativa* oil increased the immunity of rainbow trout (Awad, Austin and Lyndon, 2013), increased haemoglobin and tilapia hematocrite (Hussein *et al.*, 2020).

The *Gracilaria verrucosa* diet in this study yielded the same results as the research reported by (Sahfitri *et al*., 2021), that the administration of *Gracilaria* sp. flour on the feed has a significant effect on the hematocrite of white snapper *Lates calcalifer*. Furthermore Puspasari (2010) proved that the addition of 1.0 g kg^{-1} feed *Gracilaria verrucosa* extract to the feed can improve the immune system of African catfish as indicated by an increase in hematocrite levels. In this study, even though the black cumin diet was more effective than the *Gracilaria verrucosa* diet, the percentage of hematocrite increased slightly at a dose of 1.5 g kg^{-1} feed.

2. Percentage Limphocytes and Monocytes

The black cumin *Nigella sativa* diet and the red seaweed *Gracilaria verrucosa* diet had an effect on the leukocyte differential of lymphocytes and monocyte. The percentage of lymphocytes increased significantly with the *Nigella sativa* diet and the *Gracilaria verrucosa* diet for 7 days in tilapia. As for the percentage of monocytes, the *Nigella sativa* diet and the *Gracilaria verrucosa* diet increased but not significantly (Table 2). The results of a study conducted by Hidayat *et al*. (2014) showed that white snapper *Lates calcalifer* fed black cumin diet increased its non-specific immune response. The percentage of lymphocytes and monocytes increased effectively at the dose of black cumin by 7.5%. Increased immunity with black cumin causes white snapper to be able to prevent *Vibrio alginolyticus* infection.

Table 2. *Nigella Sativa* Diet and *Gracilaria Verrucosa* Diet in Tilapia for 7 Days on Lymphocyte and Monocyte Percentages

Tilapia day-7					
Nigella sativa			***Gracilaria verucosa***		
g kg^{-1} feed	**Limphocyte (%)**	**Monocyte (%)**	***g kg^{-1} feed***	**Limphocyte (%)**	**Monocyte (%)**
Control	67.3±1.2[a]	14.0±5.2[a]	Control	66.3±3.1[a]	15.9±2.2[a]
2,5	67.0±2.7[a]	15.7±3.8[a]	0.5	70.2±0.4[b]	14.7±3.4[a]
5	67.3±0.6[b]	16.3±1.5[a]	1	71.4±0.7[b]	19.1±3.1[a]
7,5	71.3±1.2[b]	16,3±4.0[a]	1.5	72.3±2.1[b]	17.9±2.1[a]
10	72.3±0.6[b]	12.0±3.5[a]	2.0	71.4±0.7[b]	14.8±4.2[a]

3. Immunomodulatory Black cumin *Nigella Sativa*

Likewise, according to Yuliana *et al*. (2021), the addition of black cumin through feed can significantly increase the number of monocytes. Furthermore, monocytes differentiate into macrophages and go to the site of inflammation or infection. Apart from fish, the use of black cumin has also been reported in mice. Zikriah (2014) showed the results of his research, giving black cumin ethanol extract to mice was able to affect the total number of leukocytes and the percentage of lymphocytes with significantly different results ($p < 0.05$) but was unable to affect the percentage of monocytes.

Black cumin seed oil is known to be immunomodulatory in fish, but the mechanism is unknown. Whereas in mice (Akrom *et al*., 2015) reported, the ethanol extract of black cumin seeds increased the number of monocyte, then in the tissue the monocytes turned into macrophages. Macrophages carried out peritoneal phagocytosis and secreted reactive oxygen intermediate (ROI) in male mice infected with *L. monocytogenes* with an effective dose of black cumin 5 mg kg^{-1} feed. In rats, black cumin hexane extract can increase the activity and index of phagocytosis of monocytes (Akrom & Fatimah, 2015). Dey *et al*. (2020) noted an increase in immune parameters such as phagocytic activity and respiratory bursts in tilapia after being given black cumin. Apart from black cumin, the results of this study also proved that the *Gracilaria verrucosa* diet could significantly increase the percentage of lymphocytes to 72.3%, while the percentage of monocytes increased to 19.1% but not significantly.

4. Immunomodulatory of Red Seaweed *Gracilaria verrucosa*

Administration of *Gracilaria* sp. flour on feed had a significant effect on leukocyte differential lymphocyte types in white snapper *Lates calcalifer* but did not have a significant effect on monocytes (Sahfitri *et al*., 2021). Research using the immersion of *Gracilaria verrucosa* ethanol extract given to carp showed an increase in the percentage of monocytes before and after infection with *Aeromonas hydrophila* 10^7 cells mL^{-1} bacteria. This happens because at the beginning of the infection there is mobilization of monocytes to the infected tissue area so that monocytes in the blood circulation increase. Then monocytes go to the infected tissue area and turn into macrophages to carry out phagocytosis (Maftuch *et al.*, 2013). The results of the study Rijoly (2018), the addition of *Gracilaria verrucosa* flour through feed can increase the immune response as indicated by an increase in the percentage of monocytes. The increase in monocytes can be seen from the increase in phagocytic activity at the prevention and treatment dose of 2.0 g kg^{-1} feed which is higher than the control group.

Furthermore, white snapper fed *Gracilaria verrucosa* diet increased monocytes but not significantly. This is presumably because the white snapper *Lates calcalifer* had never been infected before so the haematology was still normal and monocytes did not increase significantly (Sahfitri *et al*., 2021). In addition to fish, research on the addition of gracilaria through feed has also been reported. Satyantini *et al*. (2016) reported that giant prawns (*Macrobrachium rosenbergii*) fed a *Gracilaria verrucosa* diet resulted in an increase in total haemocytes. Likewise, for vanamei shrimp (*Litopenaeus vanamei*), the addition of *Gracilaria verrucosa* extract can increase total haemocytes (Jasmanindar *et al.*, 2018).

In this study, the percentage of monocytes increased 15.9-19.1%, but then decreased from 19.1-14.8%. This is thought to be a natural infection so that the monocytes in the blood circulation slightly increase and are not significant. After a slight rise and then monocytes enter the tissue to become macrophages for phagocytosis function so that their percentage decreases in the blood circulation (Maftuch *et al.*, 2013). According to Sitepu (2016), a low percentage of monocytes can be caused by the absence of infection so that they do not need a supply of monocytes. Monocytes are required for conversion to tissue macrophages in areas of inflammation. In inflammation when tissue damage or antigen-

antibody reactions occur, the percentage of monocytes increases, but then decreases due to migration to inflamed tissues to become macrophages (Maftuch *et al.*, 2013).

CONCLUSION

In tilapia, the *Nigella sativa* diet significantly increased the percentage of hematocrite and lymphocytes, whereas in monocytes the percentage increased but not significantly. The *Gracilaria verrucosa* diet was able to significantly increase the percentage of lymphocytes, while the percentages of hematocrite and monocytes increased but not significantly. The *Nigella sativa* diet was more effective than the *Gracilaria verrucosa* diet in increasing tilapia non-specific immunity. The *Nigella sativa* diet more effective than the *Gracilaria verrucosa* diet in increasing immunity in tilapia at a dose 5 g kg^{-1} feed.

REFERENCES

Abd El-Hack, M. E., Mohamed E.A., Sameh A.K., Asmaa F.T., Ayman E. (2020) '*Nigella sativa* Seeds and Its Derivatives in Fish Feed', in *Book chapter*, pp. 297–315. doi: 10.1007/978-3-030-48798-0_19.

Akrom, A. & Fatimah, F. (2015) 'Ekstrak heksan biji jintan hitam (*Nigella sativa* L) meningkatkan aktivitas fagositosisi makrofag tikus betina galur SD (Sprague Dawley) yang diinduksi DMBA (7,12Dimetilbenz(α)antrasen) secara in vitro', *Pharmaciana*, 5(1), pp. 69–76. doi: 10.12928/pharmaciana.v5i1.2288.

Akrom, Widjaya, A. & Armansyah, T. (2015) 'Ethanolic Extract of Black Cumin (*Nigella sativa*) Seed Increases Macrophage Phagocytic Activity of Swiss Mice Infected with *Lysteria monocytogenes*', *Jurnal Kedokteran Hewan*, 9(2), pp. 94–100.

Asniyah (2009) 'Efek Antimikroba Minyak Jintan Hitam (*Nigella sativa*) terhadap Pertumbuhan *Escherichia coli* In Vitro', *Jurnal Biomedika*, 1(1), pp. 25–29.

Awad, E., Austin, D. & Lyndon, A. R. (2013) 'Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum)', *Aquaculture*, 388–391(1), pp. 193–197. doi: 10.1016/j.aquaculture.2013.01.008.

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L. and Yuan, Z. (2014) 'Antibiotic alternatives: The substitution of antibiotics in animal husbandry?', *Frontiers in Microbiology*, 5(MAY), pp. 1–15. doi: 10.3389/fmicb.2014.00217.

Devi, G., Harikrishnan, R., Paray, B.A., Al-Sadoon, M.K., Hoseinifar, S.H., Balasundaram, C. (2019) 'Effects of aloe-emodin on innate immunity, antioxidant and immune cytokines mechanisms in the head kidney leucocytes of *Labeo rohita* against *Aphanomyces invadans*', *Fish and Shellfish Immunology*, 87(2019), pp. 669–678. doi: 10.1016/j.fsi.2019.02.006.

Dey, B. K., Hossain, M. M. M. & Alam, M. E. (2020) 'Effect of black cumin seed oil on growth, innate immunity and resistance against *Pseudomonas fluorescens* infection in Nile tilapia *Oreochromis niloticus*', *Aquaculture International*, 28(4), pp. 1485–1499. doi: 10.1007/s10499-020-00539-8.

Dontriska (2014) 'Efektivitas tepung jintan hitam (*Nigella sativa*) untuk mencegah infeksi *Aeromonas hydrophila* pada ikan patin', *Jurnal Akuakultur Rawa Indonesia*, 2(2), pp. 188–201.

Dorucu, M., Ispir, U., Colak, S., Altinterim, B., Celayir, Y. (2009) 'The Effect of Black Cumin Seeds, *Nigella sativa*, on the Immune Response of Rainbow Trout,

Oncorhynchus mykiss', *Mediterranean Aquaculture Journal*, 2(1), pp. 27–33. doi: 10.21608/maj.2009.2667.

Hidayat, R., Harpeni, E. & Wardiyanto (2014) 'Profil hematologi kakap putih (*Lates calcalifer*) yang distimulasi dengan jintan hitam (*Nigela sativa*) dan efektivitasnya terhadap infeksi *Vibrio alginolyticus*', *e-Jurnal Rekayasa dan Teknologi Budidaya Perairan*, III(1), pp. 327–334.

Hussein, S.Y., Abouelezz, K.F.M., Kellawy, M.I. El., Doa, Y.A. (2020) 'Effect of *Nigella sativa* on Growth Performance and Some Blood Constituents of Nile Tilapia (*Oreochromis niloticus*)', *Assiut Veterinary Medical Journal*, 66(166), pp. 121–135. doi: 10.21608/avmj.2020.167326.

Jasmanindar, Y., Salosso, Y., Dahoklory, N. (2018) 'Dietary administration of *Gracilaria verrucosa* extract on litopenaeus vannamei immune response, growth, and resistance to *Vibrio harveyi*', *AACL Bioflux*, 11(4), pp. 1069–1080.

Khatun, A., Hossain, M., Rahman, M., Alam, M., Yasmin, F., Islam, M., Islam, M. (2015) 'Effect of Black Cumin Seed Oil (*Nigella sativa*) on Enhancement of Immunity in the Climbing Perch, *Anabas testudineus*', *British Microbiology Research Journal*, 6(6), pp. 331–339. doi: 10.9734/bmrj/2015/15330.

Maftuch, Adam, Moh A., Hasan, V., Sanoesi, E., Andayani, S., Marsoedi (2013) 'Pengaruh Pemberian Imunostimulan Ekstrak Kasar *Gracilaria verrucossa* Terhadap Respon Seluler Ikan Mas (*Cyprinus carpio*) Pasca diinfeksi Bakteri *Aeromonas hydrophila*', in *Konferensi Akuakultur Indonesia 2013*, pp. 367–372.

Puspasari, N. (2010) *Efektivitas ekstrak rumput laut Gracilaria verrucosa sebagai imunostimulan untuk pencegahan infeksi bakteri Aeromonas hydrophila pada ikan lele dumbo Clarias sp* . Available at: http://repository.ipb.ac.id:8080/handle/123456789/59490.

Rijoly, S. M. A. (2018) *Pemanfaatan ekstrak rumput laut Gracilaria verrucosa untuk pengendalian infeksi Aeromonas hydrophila pada ikan lele (Clarias sp.)*, *Institute Pertanian Bogor*. Available at: http://repository.ipb.ac.id/handle/123456789/95879.

Sa'adah, R., Sasanti, A. D. and Taqwa, F. H. (2015) 'Aplikasi tepung jintan hitam (*Nigella sativa*) untuk pencegahan infeksi bakteri *Streptococcus agalactiae* pada ikan nila (*Oreochromis niloticus*)', *Jurnal Akuakultur Rawa Indonesia*, 51(3), pp. 295–298.

Sahfitri, I. A. H., Wulandari, R. and Zahra, A. (2021) 'Profil Darah Ikan Kakap Putih *Lates calacalifer* Yang Diberi Pakan Mengandung Gracilaria sp .', *Intek Akuakultur*, 5(2), pp. 59–70.

Satyantini, W. H., Kurniawan, A. and Kusdarwati, R. (2016) 'Penambahan Ekstrak *Gracilaria verrucosa* Terhadap Peningkatan Total Hemosit, Kelangsungan Hidup dan Respon Fisiologi Udang Galah (*Macrobrachium rosenbergii*)', *Jurnal Akuatika Indonesia*, I(2), pp. 120–129.

Silviana, N. R., Rosidah, Pamungkas, Wahyuniar, Grandiosa, R. (2022) 'Utilizing of black cumin (*Nigella sativa*) flour to increase the immunity system of tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila* bacteria attack', *Jurnal Akuakultur Indonesia*, 21(2), pp. 161–177. doi: 10.19027/jai.21.2.161-177.

Sitepu, L. L. E. (2016) 'Efek Perendaman Ekstrak *Spirulina platensis* Sebagai Imunostimulan Terhadap Jumlah Leukosit dan Hitung Jenis Leukosit Ikan Gurame (*Osphronemus gouramy*) yang Di Infeksibakteri *Aeromonas hydrophila*', *Thesis*, p. 79.

Wongprasert, K., Rudtanatip, T. & Praiboon, J. (2014) 'Immunostimulatory activity of

sulfated galactans isolated from the red seaweed *Gracilaria fisheri* and development of resistance against white spot syndrome virus (WSSV) in shrimp', *Fish and Shellfish Immunology*, 36(1), pp. 52–60. doi: 10.1016/j.fsi.2013.10.010.

Yuliana, A., Wulandari, R. & Zahra, A. (2021) 'Pemberian Ekstrak *Sargassum* sp. melalui Pakan Komersil Terhadap Nilai Hematokrit dan Diferensial Leukosit pada Ikan Bawal Bintang *Trachinotus blochii* Provision of *Sargassum* sp. Extract Through Commercial Feed Against Hematocrit Value and Leukocyte Diffe', *Intek Akuakultur*, 5(2), pp. 36–49.

Zahra, A., Sukenda, S. & Wahjuningrum, D. (2017) 'Extract of seaweed *Gracilaria verrucosa* as immunostimulant to controlling white spot disease in Pacific white shrimp *Litopenaeus vannamei*', *Jurnal Akuakultur Indonesia*, 16(2), p. 185. doi: 10.19027/jai.16.2.185-194.

Zikriah (2014) *Uji imunomodulator ekstrak etanol jinten hitam (Nigella sativa* L.*) terhadap jumlah total leukosit, persentase limfosit, persentase monosit dan kadar interleukin-1β pada mencit BALB/c*.