

Alginate from *Sargassum* sp. Improve the Hematology Performance and Oxygen Tolerance Exposure of *Lates calcarifer*

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Abstract

Indonesia's fish production is abundant, especially in aquaculture. *Lates calcarifer* is farmed fish species. The failure of *L. calcarifer* cultivation due to disease problems. We are utilizing a natural compound derived from tropical *Sargassum* sp. extracts, namely alginate. This study aims to determine and analyse the supplementation of alginate in the diet by oral administration to improve the fish's hematological performance and oxygen stress tolerance. There were one control and three treatments (2 g.kg⁻¹, 4 g.kg⁻¹, and 8 g.kg⁻¹ alginate/feed). The experiment was a completely randomized design with three replications. Fish were reared in a 350 L fiber tank for 12 days at a density of 20 ind.tank⁻¹. Stress oxygen tolerance was applied by rearing the 10 fish in 12 L fully plastic-wrapped containers. The parameters test analyzed were phagocytic activity and phagocytic index, red blood parameters which are hematocrit and hemoglobin. The survival rate after 5 hr anoxic exposure was also recorded. The best treatments were achieved at a dose of 6 g.kg⁻¹ and 8 g.kg⁻¹ in all parameters, except the phagocytic index. The alginate addition at all oxygen stress treatments also performed a better survival rate compared to the control. Adding alginate to feed as feed supplementation by oral administration can boost hematological performance and higher tolerance from oxygen stress exposure.

Keywords: alginate, *Lates calcarifer*, hematology, stress oxygen

INTRODUCTION

Global population development and escalating per capita fish consumption have increased the demand for fish and seafood (FAO, 2020). Global fish production is estimated to have reached about 179 million tonnes in 2018, with a total first sale value estimated at USD 401 billion, of which 82 million tonnes, valued at USD 250 billion, came from aquaculture production. Of the total, 156 million tonnes were used for human consumption, equivalent to an estimated annual supply of 20.5 kg per capita (FAO, 2020). Fish production in Indonesia was 4.401 tonnes in 1996 and 2935 tonnes in 2005 (FAO, 2007). Viral nerve necrosis is a disease by which the virus can infect *L. calcarifer* (Azad *et al.*, 2006; Azad *et al.*, 2005; Hick *et al.*, 2011), *Hyporthodus septemfasciatus* grouper (Krishnan *et al.*, 2020), sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) (Volpe *et al.*, 2020).

In 1988, Seabass disease was firstly reported by Zafran *et al.* (1988) in East Java and Bali, Indonesia. Infected fish were characterized by abnormal behaviour, such as swimming upside down or sinking to the bottom. These findings revealed that the mortalities among seabass larvae were due to viral nervous necrosis (VNN) caused by nodavirus. Another research in Southeast Asia and Australia showed that the partial nucleotide sequence of the affected larvae's nervous necrosis virus coat protein gene showed 94.0–96.1% homology to the nucleotide sequences of the coat protein gene from the nervous necrosis virus. In Sabah, the mass mortality in seabass larvae is associated with viral nervous necrosis (Ransangan & Manin, 2010). Moreover, alginate can decrease mortality as an immunostimulant (Santos *et al.*, 2019) and has a good potency against viral disease (Yudiati *et al.*, 2019).

Modification on feed formulation has reported by Goodrich *et al.* (2022). The researchers stated that acidified fish feeds in feed formulation of *L. calcarifer* juvenile by oral administration indicate the improvement in the condition for endogenous acid secretion to the stomach, avoided

a post-prandial blood alkaline tide, and cut the total energetic cost of digestion by 45%. Recent information reported that the application of biofloc managed to improve the blood serum of *L. calcalifer* (Nayak *et al.*, 2023).

Sargassum sp. is a brown alga found in alginate at the cell wall (Liu *et al.*, 2019). Alginate is composed of mannuronic acid (M-block) and guluronic acid (G-block) (Liu *et al.*, 2019; Yudiati & Isnansetyo, 2017). Sodium alginate (40.34% + 0,21) was the highest extract of alginate from *Sargassum* sp, followed by acid alginate (11.51% + 0,15) and calcium alginate (4.8% + 0,09) (Yudiati & Isnansetyo, 2017). Some research approves that raw (Zeynali *et al.*, 2020), extract (Talpur, 2014), and Alginate (Ashouri *et al.*, 2020) from *Sargassum* sp. were managed to increase the health status of *L. calcarifer* with oral administration. This study investigates, and analyses feed supplemented with alginate to enhance hematology performance namely phagocytic activity/index, red blood hematocrit and hemoglobin, and oxygen tolerance exposure of *L. calcarifer*.

MATERIALS AND METHODS

Alginate was prepared via the sodium alginate pathway, as described by Yudiati and Isnansetyo (2017). Using a commercial blender, brown algae (*Sargassum* sp.) that had been air-dried was crushed. Twenty gr of *Sargassum* sp. powder were weighed and combined with 500 ml of aquadest, 25 gr of Na₂CO₃, and 9.307 gr of EDTA. Then, HCl was added to adjust the pH to 8.5. The alginate solution was stirred for twenty-four hours before the extract was filtered. The filter was subsequently treated with 0.13 M potassium chloride (KCl) (Yudiati *et al.*, 2016). The 1:1 ratio of ethanol 96% was added to the filter volume. The solution was centrifuged at 4000 revolutions per minute for six minutes to separate the precipitate from the liquid. The pellet dried for twenty-four hours in a drying cabinet. In our study, alginate was fit with the standard alginate (Sigma®, USA) (Yudiati *et al.*, 2016), which has a molecular weight of 217.5 kDa and an acetylation degree of 89.95% (Yudiati *et al.*, 2018).

L. calcarifer was prepared, referred to Talpur (2014), reared for 13 days, and applicated with some feeding regime. This research used 240 ind of *L. calcarifer* with a size of 10-12 cm and a weight of 6.10-7.64 g. Fish was purchased from the Main Brackishwater Aquaculture Development Center (MBADC), Jepara, Indonesia nursery pond. Megami GR-2 commercial feed was applied. Feed and alginate were mixed to make doses of 2, 4, and 8 g.kg⁻¹ feed. Alginate was diluted to sterile aqua dest, and sprayed all over the feed, and let dry at room temperature (Yudiati *et al.*, 2016). We administered a completely randomized design (CRD) with 3 times replication. The 350 L of seawater was sand filtered and filled to the tank's capacity of 500 L. The density of fish was 20 individuals/tank. The feed was given at a daily rate of 5% body weight, three times per day at 08.00, 12.00, and 16.00. To manage good water quality, the water system was managed by flow-through technics. During the experimental period, water temperature ranged 26.5-28.5°C, pH 7.4-7.8, salinity 33 ppt, and dissolved oxygen (DO) concentration 4.47-4.84 mg.L⁻¹.

The blood sample was collected from the caudal vein between the fish scales near the tail using a 1 mL syringe. Previously, the injection syringe was coated with a 10% EDTA (Ethylene Diamine Tetra Acid) solution. The syringe was inserted from the back of the anal to the spine until the needle touched the bone. The blood will come out slowly. When 1 mL has been taken, the syringe is removed, and the blood sample is transferred to a microtube and ready for hematological observation. Next, hematological parameters were carried out (Talpur, 2014; Yudiati *et al.*, 2016).

The methods of phagocytic activity (PA) and phagocytic index (PI) were referred to Siwicki *et al.* (1994), with modified bacteria, *Bacillus subtilis*. Fifty microliters of white blood cells were transferred to a 96-well plate and added with the formalin-killed *B. subtilis* and incubated at 30°C for 30 min. Samples were then smeared and stained with 10% Giemsa, followed by rinsing with 70% alcohol. Slides were examined microscopically at 1,000 x magnitude. PA and PI were observed and counted from 100 phagocytes on each slide. PI was defined from 100 phagocytes showing the leftovers of *B. subtilis* cells engulfed.

The hematocrit levels test was done basically from methods by Isnansetyo *et al.* (2014) by filling the blood samples into hematocrit capillary tubes. Followed by centrifugation at 1,500 g for 5 min. The hematocrit observed by measuring the length of red blood in the capillaries was then counted and expressed as a percentage. Hemoglobin was assessed using a Sahli tube. The Sahli tube was filled with 0.1 N HCl solution on a scale of 10. Fish blood samples were taken at 0.02 mL, put into the Sahli tube, and waited for 3 minutes. 0.1 N HCl was added, and stirred to cleanse the red blood. The solution was then compared with the standard colour on the haemometer tube. The hemoglobin level is then expressed in G% (Fazio *et al.*, 2017)

The stress tolerance method refers to Supamattaya *et al.* (2005) by modifying the test animal. During rearing treatment, the fish were kept in a fiber tank for up to 14 days and fully aerated to provide complete oxygen circulation. On the 14th day, ten *L. calcarifer* from each treatment were transferred to the media in 12 L closed plastic containers without aeration and kept in this stress condition for 5 hours without oxygen aeration. Fish was observed and recorded and the survival rate after 5 hrs exposure. It was repeated three times.

The obtained data were tested for variance using analysis of variance (ANOVA). If the analysis of the variance test revealed a significant difference, it was further tested using Least Significant Difference (LSD) multiple comparisons of means with a 95 % family-wise confidence level ($p < 0.05$). Phagocytic Activity/Index, red blood performance namely Hematocrit/Hemoglobin Percentage, and the stress tolerance test were among the variables examined.

RESULT AND DISCUSSION

The results of the phagocytic activity are presented in Figure 1. The significant increase in phagocytic activity reached at doses of 4 g.kg⁻¹ and 6 g.kg⁻¹ on the 8th and 2th days against control ($p < 0.05$). At 8th days of rearing, compare to control, the PA at doses of 4 g.kg⁻¹ is 26.4 % and at doses of 6 g.kg⁻¹ is 26.1%, respectively. The highest PA is reached at doses 6 g.kg⁻¹ at 8th days of rearing (27.3%) against control.

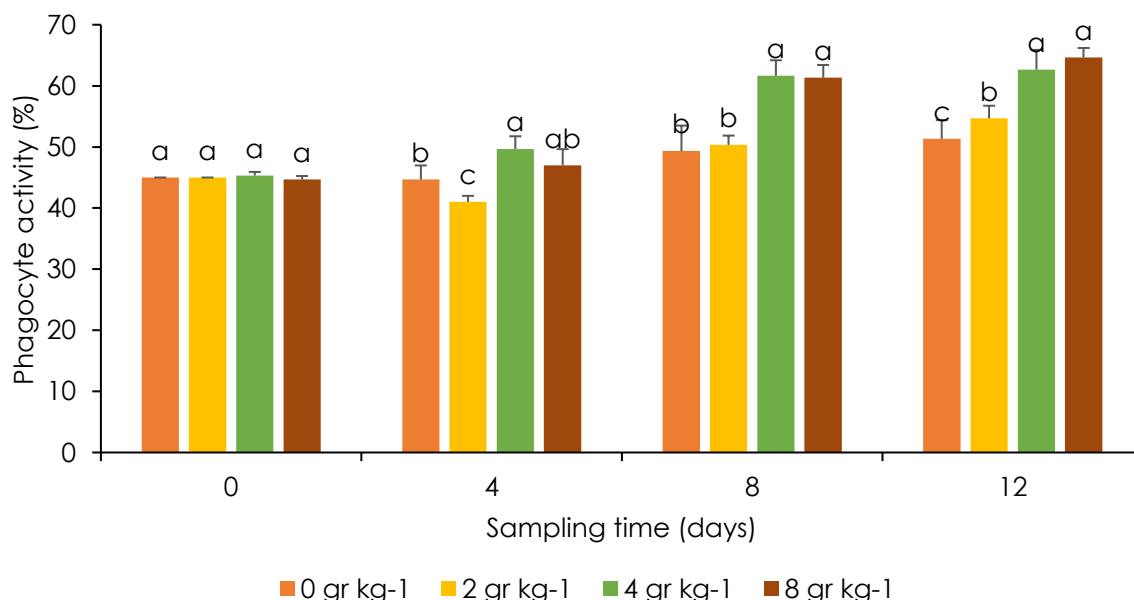


Figure 1. Phagocytic activity of alginate supplementation by oral administration of *L. calcarifer* at 12 days of rearing. Values in bar appointed with different letterings denote the significant differences ($p < 0.05$)

Alginate is found in brown macroalgae species (Phaeophyta) as the main components of the cell wall. Alginate is composed of two unit monomers, which are β -D-mannuronic acid and α -L-guluronic acid. The high guluronic acid can be taken from the outer cortex of *Laminaria hyperborea* (Draget *et al.*, 2011). The mechanism of non-specific defense can be assessed by hematologic examination (Anderson & Siwicki, 1994). So, therefore, the effectiveness of oral administration of alginate supplementation to improve the hematology performance of *L. calcarifer* has been examined. Parameters assessed are, phagocytic activity (PA), phagocytic index (PI), red blood performance by counting the percentage of hematocrit and hemoglobin. The resistance of unoxdised tolerance on fish was also applied. The capability of phagocytic cells to devastate is known as phagocytic activity (PA). The results show that supplementation of alginate by oral administration significantly increases phagocytic activity on the eighth day of the feeding trial. When compared to the control treatment, alginate at a dose of 4 and 8 g.kg⁻¹ of feed significantly increases phagocytic activity by 26.4 26.1 and 26.1 %, respectively. Mononuclear cells (macrophages and monocytes) and polymorphonuclear cells (granulocytes) are the cells responsible for PA (Baratawidjaya, 2002).

Ale *et al.* (2011) reported that polysaccharide, including alginate, the activation of macrophages is intermediated by the existence of specific membrane receptors. Polysaccharide receptors such as Cluster of Differentiation-14 (CD-14), Toll-Like Receptor 4 (TLR-4), Scavenging Receptor (SR), and Competent Receptor-3 (CR-3) can recognize alginate. The effect of polysaccharide compounds from *Kappaphycus alvarezii* seaweed on Asian sea bass (*L. calcarifer*) got the highest activity at 0.5% feeding on the 15th day (Sakthivel *et al.*, 2015).

The research of Harikrishnan *et al.* (2011) with *Epinephelus bruneus* showed that the phagocytic activities of infected fish fed with sodium alginate containing diets at 1.0 g.kg⁻¹ on week 2 and 1.0 and 2.0 g.kg⁻¹ diets on week 4 were significantly higher when compared to the control. In the research of Chiu *et al.* (2015), *L. calcarifer* with *Daphnia similis* meal had the highest phagocytic activity in the 5%, followed by the 10%. In another biota, such as *Epinephelus coioides*, Phagocytic activities of fish fed the 1.0 and 2.0 g.kg⁻¹ sodium alginate-containing diets were significantly higher than those of fish fed the control diet. This dose had increased to 1.35- and 1.50-fold, respectively, compared to those of fish fed the control diet after 12 days (Yeh *et al.*, 2008). Cheng and Yu (2013) found that supplementation alginate at the concentrations of 1, 2, and 3 g.kg⁻¹ of diet enhanced abalone phagocytic activity for 14 days of rearing. Research on other animals, such as the shrimp *Litopenaeus vannamei* by Yudiati *et al.* (2019) showed that at a dose of 6.0 g.kg⁻¹ alginat, extracted from *Sargassum siliquosum*, could enhance the phagocytic activity/index, and survived against White Spot Syndrome Virus (WSSV).

Figure 2 shows that supplementation of alginate by oral administration of alginate did not influence the phagocytic index (PI) of fish macrophage ($p > 0.05$). The percentage in all treatments and control around 1.01% (control) - 1.22% (alginate at 8 g.kg⁻¹). The supplementation by oral administration of alginate able to increase the phagocytic activity, significantly. On the other hand, the phagocytic index is similar in all treatments and control. Isnansetyo *et al.* (2015) and Purbomartono *et al.* (2019) reported the similar results for alginate and fucoidan food supplementation in *Clarias* sp. Baratawidjaya (2002) noted, that the phagocytic index reflects the immune system in relation to the function of cells phagocytes. Phagocytosis index may also induce by complement. Compliment plays as the role of cells stimulator function which migrates toward the site of infection. Furthermore, complement also serves either to attach to the receptor of phagocytes cells or boost the incidence of antibody-antigen complexes (Irianto, 2005).

Hematocrit and hemoglobin

The results of the hematocrit are presented in Figure 3. The hematocrit is fluctuated over the time of rearing. At 8th day of rearing, the dose of 4 g.kg⁻¹ (24.4%) and 8 g.kg⁻¹ (25.2%) is significantly different from the control. Haemoglobin exceeded control at all doses (Figure 4). At all dose, it significant increased over time against control (7.5 – 7.7%) ($p < 0.05$).

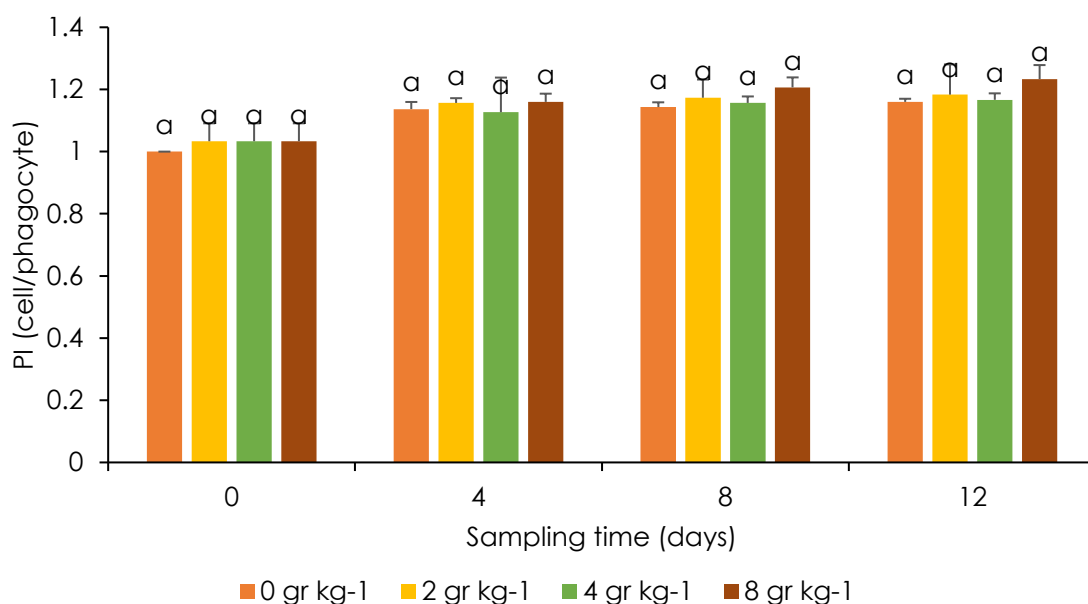


Figure 2. Phagocytic index of alginate supplementation by oral administration of *L. calcalifer* at 12 days of rearing. Values in bar appointed with different letterings denote the significant differences ($p < 0.05$)

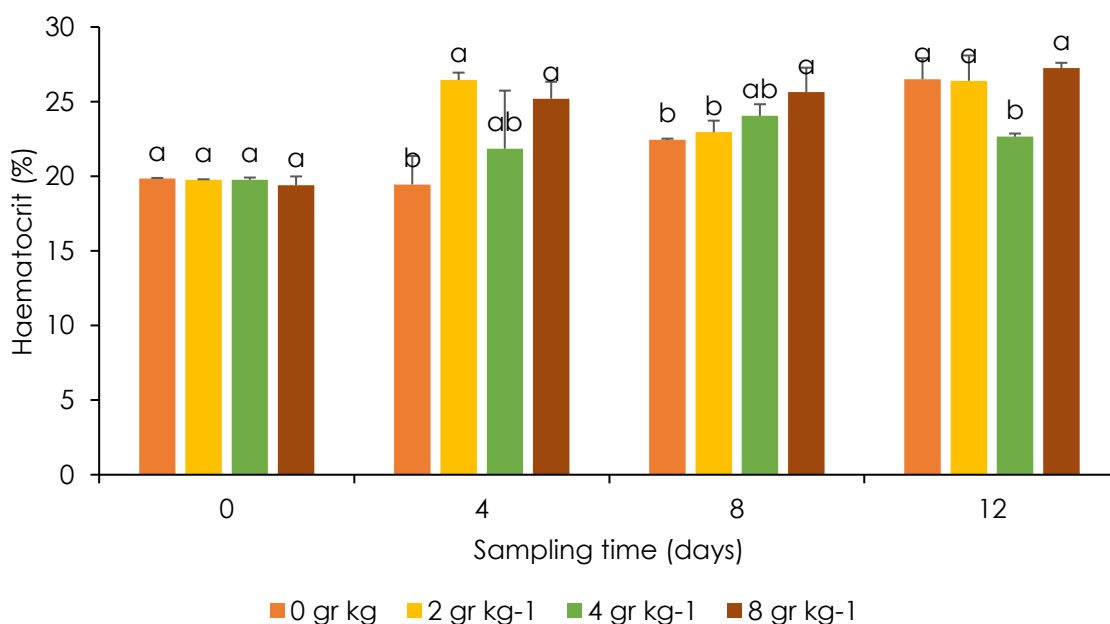


Figure 3. Percentage of hematocrit of alginate supplementation by oral administration of *L. calcalifer* at 12 days of rearing. Values in bar appointed with different letterings denote the significant differences ($p < 0.05$)

Evaluation of reed blood parameters such as hematocrit and hemoglobin has been widely applied in aquaculture systems to determine the health status of fish (Fazio *et al.*, 2017; Isnansetyo *et al.*, 2015). Percentage hematocrit in this present research is tend to fluctuate over rearing time, but at 8th day of rearing, the best hematocrit is achieved by alginate supplementation. Percentage hemoglobin in all treatments is significantly different from control in all days if rearing. Supplementation of alginate indicates that alginate is able to improve the hematology performance, so, therefore, improve the health status of *L. calcalifer*.

The results of the stress tolerance test of fish without dissolved oxygen exposure are presented in Figure 5. The stress tolerance results show that the survival rate of all alginate supplementation treatments was significant against control ($p < 0.05$). The best survival rate reach from alginate 8 g.kg⁻¹ treatment (33%). The treatment of unoxygen exposure in *L. calcalifer* exhibits that oral supplementation of alginate in feed resulted better survival rate compared to the control. Yudiati et al. (2020) reported the similar result with low molecular weight sodium alginate in Zebrafish (*Danio rerio*), and Sudaryono et al. (2018) with *Sargassum* sp. powder feed supplementation in *Litopenaeus vannamei*. At around 0.3 ppm DO level, *L. calcalifer* fed with alginate was still survived.

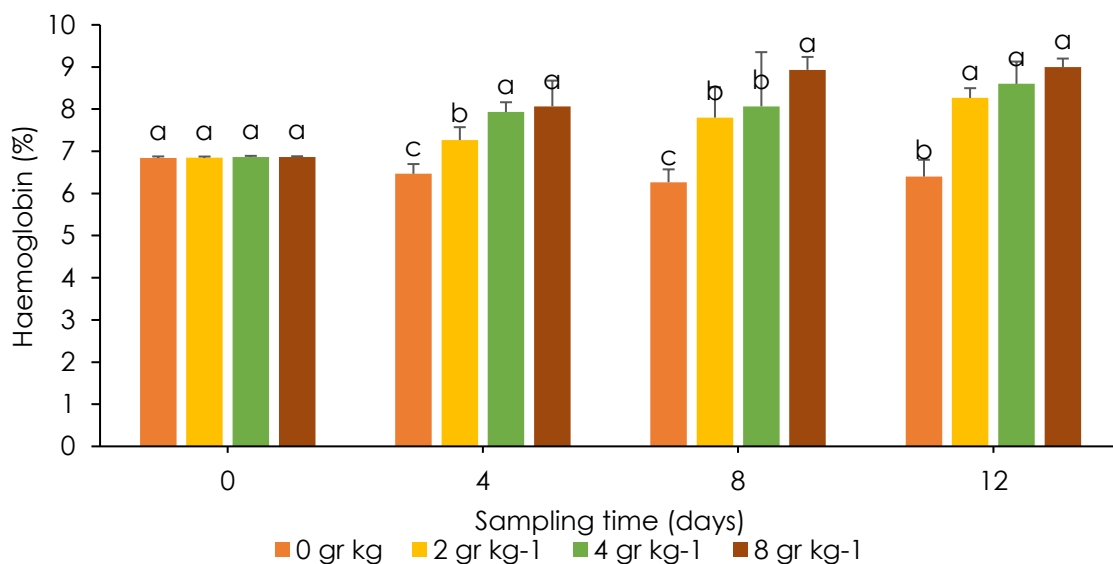


Figure 4. Percentage of hemoglobin of alginate supplementation by oral administration of *L. calcalifer* at 12 days of rearing. Values in bar appointed with different letterings denote the significant differences ($p < 0.05$)

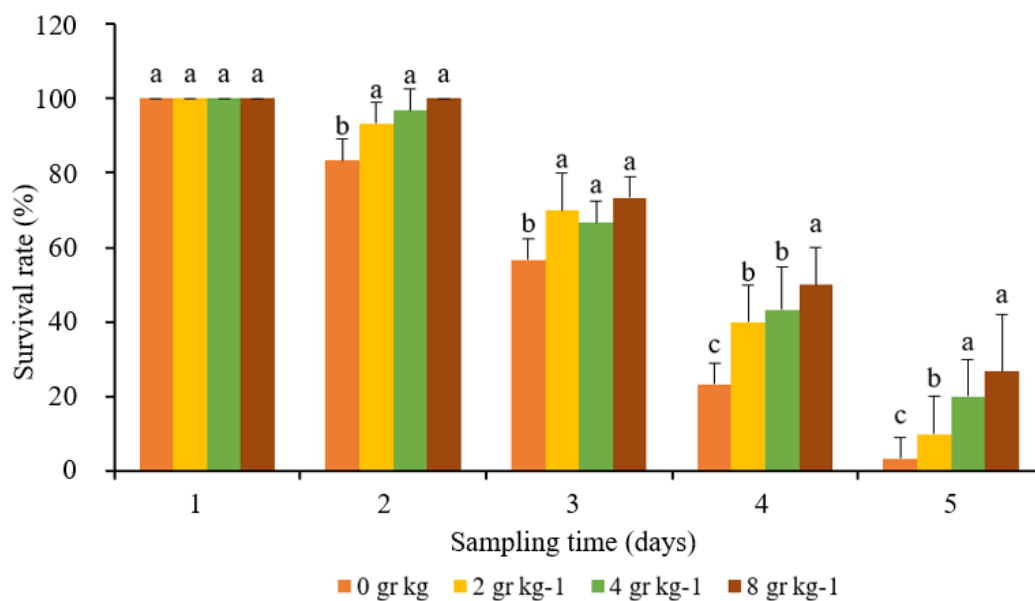


Figure 5. Survival rate of alginate supplementation by oral administration of *L. calcalifer* at 5 hrs of unoxygen exposure. Values in bar appointed with different letterings denote the significant differences ($p < 0.05$)

Alginate performance can be improved by combining low molecular weight sodium alginate and *Pediococcus acidilactici* bacteria separately or in symbiotic form, with promising results as functional feed additives in juvenile Asian sea bass, *L. calcarifer* (Ashouri *et al.*, 2020). Due to the sustainable on the availability of seaweed, especially *Sargassum* sp. in the nature, people have to aware of the over-utilization and over-exploitation of *Sargassum* sp. and so, therefore, the management risks in the seaweed supply chain is needed (Mulyati & Geldermann, 2017). Another solution to manage the supply of *Sargassum* sp. is cultivation (Aaron-Amper *et al.*, 2020; Han *et al.*, 2018; Largo *et al.*, 2020).

CONCLUSION

The best treatments were observed at a dose of 4 g.kg⁻¹ and 8 g.kg⁻¹ in all parameters, except in phagocytic index. Adding alginate to feed by oral administration can boost the hematological performance, tolerance to oxygen stress and *Lates calcarifer* health status.

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REFERENCES

- Aaron-Amper, J., Largo, D.B., Handugan, E.R.B., Nini, J.L., Alingasa, K.M.A., & Gulayan, S.J. (2020). Culture of the tropical brown seaweed *Sargassum aquifolium*: From hatchery to field out-planting. *Aquaculture Reports*, 16, p.100265. doi: 10.1016/j.aqrep.2019.100265
- Ale, M.T., Mikkelsen, J.D., & Meyer, A.S. (2011). Important determinants for fucoidan bioactivity: a critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds. *Marine Drugs*, 9(10), 2106-2130. doi: 10.3390/md9102106
- Anderson, D.P., & Siwicki, A.K. (1994). Simplified Assay for Measuring Nonspecific Defence Mechanism in Fish. Fish Health Section/American Fisheries Meeting. Seattle. Washington, 1-26.
- Ashouri, G., Soofiani, N.M., Hoseinifar, S.H., Jalali, S.A.H., Morshedi, V., Valinassab, T., Bagheri, D., Van Doan, H., Mozanzadeh, M.T., & Carnevali, O. (2020). Influence of dietary sodium alginate and *Pediococcus acidilactici* on liver antioxidant status, intestinal lysozyme gene expression, histomorphology, microbiota, and digestive enzymes activity, in Asian sea bass (*Lates calcarifer*) juveniles. *Aquaculture*, 518, 734638. doi: 10.1016/j.aquaculture.2019.734638
- Azad, I., Jithendran, K., Shekhar, M., Thirunavukkarasu, A., & De la Pena, L. (2006). Immunolocalisation of nervous necrosis virus indicates vertical transmission in hatchery produced Asian sea bass (*Lates calcarifer* Bloch)—A case study. *Aquaculture*, 255(1-4), 39-47. doi: 10.1016/j.aquaculture.2005.04.076
- Azad, I., Shekhar, M., Thirunavukkarasu, A., Poornima, M., Kailasam, M., Rajan, J., Ali, S., Abraham, M., & Ravichandran, P. (2005). Nodavirus infection causes mortalities in hatchery produced larvae of *Lates calcarifer*: first report from India. *Diseases of Aquatic Organisms*, 63(2-3), 113-118. doi: 10.3354/dao063113
- Baratawidjaya, K.G. (2002). *Imunologi Dasar*. Balai Penerbit Fakultas Kedokteran Universitas Indonesia. Jakarta, 3-69.
- Cheng, W., & Yu, J.S. (2013). Mar. Effects of the dietary administration of sodium alginate on the immune responses and disease resistance of Taiwan abalone, *Haliotis diversicolor supertexta*. *Fish Shellfish Immunology*, 34(3), 902. doi: 10.1016/j.fsi.2012.12.020
- Chiu, S.T., Shiu, Y.L., Wu, T.M., Lin, Y.S., & Liu, C.H. (2015). Improvement in non-specific immunity and disease resistance of barramundi, *Lates calcarifer* (Bloch), by diets containing *Daphnia similis* meal. *Fish Shellfish Immunology*, 44(1), 172-179. doi: 10.1016/j.fsi.2015.02.002
- Draget, K.I., Moe, S.T., Bræk, G.S. & Smidsrød, O. (2011). Alginates. In : Stephen . A. M.. G.O. Phillips and P.A. Williams. 2006. *Food Polysaccharides and Their Applications*. Second Edition. Taylor and Francis Group, USA, 290–328.

- FAO. (2007). Yearbook of fishery statistics 2005. Rome, Italy.
- FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in Action; The State of World Fisheries and Aquaculture (SOFIA). FAO: Rome, Italy. doi: 10.4060/ca9229en
- Fazio, F., Ferrantelli, V., Saoca, C., Giangrosso, G., & Piccione, G. (2017). Stability of haematological parameters in stored blood samples of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). *Veterinary Medicine*, 62, 401-405. doi: 10.17221/51/2017-VETMED
- Goodrich, H.R., Wilson, R.W., Smullen, R., Barnes, A.C., & Franklin, C.E. (2022). Acidified fish feeds reduce the energetic and physiological costs of digestion in juvenile barramundi (*Lates calcarifer*). *Aquaculture*, 546, 737400. doi: 10.1016/j.aquaculture.2021.737400
- Han, T., Qi, Z., Huang, H., Liao, X., & Zhang, W. (2018). Nitrogen uptake and growth responses of seedlings of the brown seaweed *Sargassum hemiphyllum* under controlled culture conditions. *Journal of Applied Phycology*, 30(1), 507-515. doi: 10.1007/s10811-017-1216-1
- Harikrishnan, R., Kim, M.C., Kim, J.S., Han, Y.J., Jang, I.S., Balasundaram, C., & Heo, M.S. (2011). Immunomodulatory effect of sodium alginate enriched diet in kelp grouper *Epinephelus brneus* against *Streptococcus iniae*. *Fish Shellfish Immunology*, 30(2), 543-549. doi: 10.1016/j.fsi.2010.11.023
- Hick, P., Schipp, G., Bosmans, J., Humphrey, J., & Whittington, R. (2011). Recurrent outbreaks of viral nervous necrosis in intensively cultured barramundi (*Lates calcarifer*) due to horizontal transmission of betanodavirus and recommendations for disease control. *Aquaculture*, 319(1-2), 41-52. doi: 10.1016/j.aquaculture.2011.06.036
- Irianto, A. (2005). Patologi Ikan Teleostei. Gadjah Mada University Press. Yogyakarta, 83-94.
- Isnansetyo, A., Irpani, H., Wulansari, T., & Kasanah, N. (2015). Oral Administration of Alginate From A Tropical Brown Seaweed, *Sargassum* sp. To Enhance Non-Specific Defense In Walking Catfish (*Clarias* sp.). *Aquacultura Indonesiana*, 15, 73-80. doi: 10.21534/ai.v15i1.29
- Krishnan, R., Kim, J.O., Qadiri, S.S.N., Kim, J.O., & Oh, M.J. (2020). Early viral uptake and host-associated immune response in the tissues of seven-band grouper following a bath challenge with nervous necrosis virus. *Fish Shellfish Immunology*, 103, 454-463. doi: 10.1016/j.fsi.2020.05.012
- Largo, D.B., Diola, A.G., & Rance, G.M.S. (2020). Culture of the brown seaweed *Sargassum siliquosum* J. Agardh (Phaeophyceae, Ochrophyta): from hatchery to out-planting. *Journal of Applied Phycology*, 32(6), 4081-4098. doi: 10.1007/s10811-020-02228-8
- Liu, J., Yang, S., Li, X., Yan, Q., Reaney, M.J., & Jiang, Z. (2019). Alginate oligosaccharides: production, biological activities, and potential applications. *Comprehensive Reviews in Food Science and Food Safety*, 18(6), 1859-1881. doi: 10.1111/1541-4337.12494
- Mulyati, H., & Geldermann, J. (2017). Managing risks in the Indonesian seaweed supply chain. *Clean Technologies and Environmental Policy*, 19(1), 175-189. doi: 10.1007/s10098-016-1219-7
- Nayak, S., Yogev, U., Kporzaxor, Y., Zhu, Z., Gur, N., Gross, A., & Zilberg, D. (2023). From fish excretions to high-protein dietary ingredient: Feeding intensively cultured barramundi (*Lates calcarifer*) a diet containing microbial biomass (biofloc) from effluent of an aquaculture system. *Aquaculture*, 562, 738780. doi: 10.1016/j.aquaculture.2022.738780
- Purbomartono, C., Isnansetyo, A., Murwantoko, & Triyanto. (2019). Dietary Fucoidan from *Padina boergesenii* to Enhance Non-specific Immune of Catfish (*Clarias* sp.). *Journal of Biological Sciences*, 9(2), 173-180. doi: 10.3923/jbs.201
- Ransangan, J., & Manin, B.O. (2010). Mass mortality of hatchery-produced larvae of Asian seabass, *Lates calcarifer* (Bloch), associated with viral nervous necrosis in Sabah, Malaysia. *Veterinary Microbiology*, 145(1), 153-157. doi: 10.1016/j.vetmic.2010.03.016
- Sakthivel, M., Deivasigamani, B., Rajasekar, T., Kumaran, S., & Alagappan, K. (2015). Immunostimulatory effects of polysaccharide compound from seaweed *Kappaphycus alvarezii* on Asian seabass (*Lates calcarifer*) and its resistance against *Vibrio parahaemolyticus*. *Journal of Marine Biology and Oceanography*, 4(2), p.1-9. doi: 10.4172/2324-8661.1000144
- Santos, H.M., Tsai, C.Y., Yanuaria, C.A.S., Tayo, L.L., Vo, D.D., Mariatulqabtiah, A.R., & Chuang, K.P. (2019). Effects of sodium alginate-fed Pacific white shrimps, *Litopenaeus vannamei*, on Toll-like receptors and *Vibrio alginolyticus* infection. *Aquaculture Research*, 50(4), 1384-1392. doi: 10.1111/are.13989
- Siwicki, A.K., Anderson, D.P. & Rumsey, G.L. (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology*, 41(1), 125-139. doi: 10.1016/0165-2427(94)90062-0

- Sudaryono, A., Sukardi, P., Yudiarti, E., Hardi, E.H., Hastuti, S., & Susilowati, T. (2018). Potential of using tropical brown macroalgae *Sargassum cristaefolium* meal in the diets for juvenile white shrimp (*Litopenaeus vannamei*). *IOP Conference Series: Earth and Environmental Science*, 144(1), p.012049. doi: 10.1088/1755-1315/144/1/012049
- Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M., & Borowitzka, L. (2005). Effect of a *Dunaliella* extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). *Aquaculture*, 248(1-4), 207-216. doi: 10.1016/j.aquaculture.2005.04.014
- Talpur, A.D. (2014). *Mentha piperita* (Peppermint) as feed additive enhanced growth performance, survival, immune response and disease resistance of Asian seabass, *Lates calcarifer* (Bloch) against *Vibrio harveyi* infection. *Aquaculture*, 420, 71-78. doi: 10.1016/j.aquaculture.2013.10.039
- Volpe, E., Gustinelli, A., Caffara, M., Errani, F., Quaglio, F., Fioravanti, M.L., & Ciulli, S. (2020). Viral nervous necrosis outbreaks caused by the RGNNV/SJNNV reassortant betanodavirus in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). *Aquaculture*, 523, 735155. doi: 10.1016/j.aquaculture.2020.735155
- Yeh, S.P., Chang, C.A., Chang, C.Y., Liu, C.H., & Cheng, W. (2008). Dietary sodium alginate administration affects fingerling growth and resistance to *Streptococcus* sp. and iridovirus, and juvenile non-specific immune responses of the orange-spotted grouper, *Epinephelus coioides*. *Fish Shellfish Immunology*, 25(1-2), 19-27. doi: 10.1016/j.fsi.2007.11.011
- Yudiati, E., Ginzal, F.I., Hidayati, J.R., Rizfa, M.S., Azhar, N., Djarod, M.S.R., Heriyati, E., & Alghazeer, R. (2020). Oral Administration of Alginate Oligosaccharide from *Padina* sp. Enhances Tolerance of Oxygen Exposure Stress in Zebrafish (*Danio rerio*). *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 25(1), 7-14. doi: 10.14710/ik.ijms.25.1.7-14
- Yudiati, E., & Isnansetyo, A. (2017). Characterizing the Three Different Alginate Type of *Sargassum siliquosum*. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 22(1), 7-14. doi: 10.14710/ik.ijms.22.1.7-14
- Yudiati, E., Isnansetyo, A., Murwantoko, Ayuningtyas, Triyanto, & Handayani, C.R. (2016). Innate immune-stimulating and immune genes up-regulating activities of three types of alginate from *Sargassum siliquosum* in Pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunology*, 54, 46-53. doi: 10.1016/j.fsi.2016.03.022
- Yudiati, E., Isnansetyo, A., Murwantoko, Triyanto, & Handayani, C.R. (2019). Alginate from *Sargassum siliquosum* simultaneously stimulates innate immunity, upregulates immune genes, and enhances resistance of Pacific white shrimp (*Litopenaeus vannamei*) against white spot syndrome virus (WSSV). *Marine Biotechnology*, 21(4), 503-514. doi: 10.1007/s10126-019-09898-7
- Yudiati, E., Pringgenies, D., Djunaedi, A., Arifin, Z., & Sudaryono, A. (2018). Free Radicals Scavenging Activities of Low Molecular Weight Sodium Alginate (LMWSA) from *Sargassum polycystum*, Produced by Thermal Treatment. *Aquacultura Indonesiana*, 19(1), 21-27. doi: 10.21534/ai.v19i1.121
- Zafran, Z., Harada, T., Koesharyan, I.K.I., Yuasa, K., & Hatai, K. (1988). Indonesian hatchery reared seabass larvae (*Lates calcarifer*), associated with viral nervous necrosis (VNN). *Indonesian Fisheries Research Journal*, 4(1), 19-22. doi: 10.15578/ifrj.4.1.1998.19-22
- Zeynali, M., Nafisi Bahabadi, M., Morshedi, V., Ghasemi, A., & Torfi Mozanzadeh, M. (2020). Replacement of dietary fishmeal with *Sargassum ilicifolium* meal on growth, innate immunity and immune gene mRNA transcript abundance in *Lates calcarifer* juveniles. *Aquaculture Nutrition*, 26(5), 1657-1668. doi: 10.1111/anu.13111