E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2022; 10(1): 29-32

www.fisheriesjournal.com
Received: 10-11-2021
Accepted: 12-12-2021

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A review-the effect of dietary supplementation of Ulva on the growth performance and haematological parameters of Nile tilapia (*Oreochromis niloticus*)

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Abstract
Green seaweed Ulva has a fast growth rate and spreads in along the coastal zone of Indonesian waters. This species has a great bioactive compound that made it possible as one of natural sources to be used as an alternative fish feed ingredient phenolics, saponins, flavonoids, alkaloids, steroids. This potentiality could answer the problem of tilapia farmers from suffering the high cost of feed. Besides the ingredients, the utilization of Ulva-based fish feed could help increase the immune system. This review is specifically discuss the effect of green seaweed Ulva as fish feed ingredient to improve the growth performance and the immune system of Tilapia. Based on the literature review of proximate analysis, different species of Ulva have an additional nutrient content. From 4 species found *U. fasciata*, *U. clathrata*, *U. lactuca*, and *U. rigida*, the highest crude protein content was *U. rigida* as 27.11 %. Moreover, adding 5-25% Ulva into the fish feed could increase the growth performance, but supplementation higher than 30% could decrease the body weight of Tilapia. For additional information, Ulva has secondary metabolites that boost fish's immune system and resistance to pathogenic infections. This review is highlighted the utilization of Ulva in tilapia feed ingredients to growth performance and immune system based on its nutrient compounds and hematological parameters.

Keywords: Ulva, green seaweed, tilapia, fish feed, growth performance, hematology

1. Introduction
Based on data from the worldometer [1], the global human population as of January 5, 2022, reached 7,918,159. The average increase in world population per year is estimated to increase by 81 million people. The world's population is estimated to reach 9 billion in 2037 and 10 billion in 2057. The increase in the world's population causes the demand for food to meet human nutritional needs to increase. In 2020, hunger will become one of the global problems where the number of malnourished people will reach 770 million people, 118 million higher than in 2019 [2].

Fish is one of the food sources that can meet protein needs. Based on Fao (2020) [3], the total fishery production in 2018 reached 179 million tonnes. The production value is estimated at 401 billion USD. The aquaculture sector contributes 82 million tonnes with 250 billion USD [3]. The need for fish as food is also expected to double by 2050.

One of the most popular and most cultivated consumption fish commodities globally is Tilapia. Tilapia has a wide tolerance to the environment, is easy to cultivate, grows relatively fast, has a rather large body size, has an efficient ability to form high-quality Protein from organic materials, domestic and agricultural waste [4]. The delicious, savory, cheap taste and high nutritional content make this fish famous for consumption. In 2020, the number of tilapia production globally will reach 6 million. The growth rate reaches more than 7% [5]. The value of Indonesia's production of this fish achieved 1,265,201 tons, with a value of around 27.8 billion in 2017. This value was ranked third out of 10 primary aquaculture commodities in Indonesia in 2017 after Snapper and Shrimp [6]. Production in the third quarter of 2018 was 579,688 tons [7]. Not only in Indonesia, but Tilapia is also popular among people in the world. Indonesia has exported this fish to other countries with an export value of 60,487,000 US dollars in 2018. The export price per kilo is 5.53 US dollars [8]. However, the development of tilapia cultivation also has its own challenges, especially disease agents that cause the amount of fish production to decrease.

It was reported in 1997, the presence of Streptococcus that attacked the Tilapia caused a loss of $150 million[9]. Losses that have occurred due to Streptococcus infection. Economic loss at the Thai site reached 16,270 tonnes with 26.57 million USD. Apart from Thailand, Bangladesh has experienced mass mortality of Tilapia, estimated at >26% or around 20.910 tonnes with a value of 36.67 million USD [10].

In addition to pathogens, the availability of quality feed is also an essential factor for growth. The number of nutrients in the meal must be sufficient to meet the nutritional needs of fish for basal metabolic activities and for growth. Low feed prices are also a factor in the success of the aquaculture business because 60-80 % of the costs in cultivation activities come from feed costs [11]. Therefore, it is necessary to find alternative materials that can positively affect fish growth and have a positive effect on the immune system of Tilapia.

Ulva species are green algae that contain many bioactive compounds. The nutritional content of the Ulva is Protein, carbohydrates, fat, vitamins, fiber, and minerals [12, 13]. Ulva also contains phenolics, saponins, flavonoids, alkaloids, steroids [14]. Some researches have been conducted to learn about the effect of some species of genus Ulva on Nile tilapia (*Oreochromis niloticus*) growth and hematological parameters. So, this article aims to describe the effects of supplementation of some Ulva species on growth and hematological parameters of Nile tilapia (*Oreochromis niloticus*).

2. Ulva Species

The genus Ulva is a green alga that belongs to the family *Ulvaceae.* Approximately 50 species have been identified [15]. Ulva has bright green sheets in color and is strongly influenced by environmental conditions [16]. Some of the species discussed in this article are *Ulva fasciata, Ulva clathrata, Ulva lactuca*, and *Ulva rigida*.

Ulva fasciata's thallus varies from bright green to dark green, with lengths varying from 35-60 cm. In intertidal pools, *Ulva fasciata* can grow well on the middle to a low intertidal rigid substrate on beaches with moderate waves. This alga can grow well in an environment rich in nutrients, wave forces are low, and herbivory is reduced. Because this alga is tolerant of stress conditions, it can be used as a pollution bioindicator [17].

Meanwhile, *Ulva clathrata* has a bright-light green thallus with 0.5-4.5 cm height. The thallus is tubular, soft, flaccid. Grows on medium and low intertidal hard substrates [18]. *Ulva clathrata* is distributed in tropical to temperate latitudes of the Atlantic, Indian, and Pacific Oceans. Antarctic and subantarctic Islands.

Thallus from *Ulva lactuca* is bright green with a rounded or oval shape. The color can change into black or white when dry. It lives free-floating in marine water at low-high intertidal zone at depths of 75 feet or more. This algae measures 6 inches to 6 feet [19]. Thallus from *Ulva rigida* is thin, sheet-like as turfs, tufts, or solitary blades in bright grass green to dark green, or colorless when stressed. This alga is found in nutrient-rich waters, intertidal rocks, and tidepools. This alga can also be used as a bioindicator because it has tolerance in a stressful environment [20].

The four Ulva species have good nutritional content to be used as fish feed ingredients. The proximate analysis can be seen in Table 1. Apart from carbohydrates, fat, fiber, and protein, the four algae also contain various secondary metabolites. They also have a lot of minerals (Table 2).

Table 1: Proximate Analysis of *Ulva species*

Composition	*U. fasciata*	*U. clathrata*	*U. lactuca*	*U. rigida*
Crude Protein (% dw)	3.89	20.1 ± 0.1	19.34 ± 0.2	27.11 ± 0.62*
Crude fat (% dw)	0.1878	2.2 ± 0.1	3.46 ± 0.1	2.71 ± 0.70
Ash (% dw)	20.48	27.5 ± 0.2	44.1 ± 0.1	19.63 ± 0.63
Dietary fibre	6.3567	40.6 ± 3.0	25.81 ± 0.2	18.65 ± 0.78
reference	[21]	[12]	[22]	[23]

Table 2: Minerals content of Ulva species

Mineral	*U. clathrata* (mg/kg dw)	*U. fasciata* (µg/g dw)	*U. lactuca* (mg/kg dw)	*U. rigida (mg/g dw)*
Iron	4172 ± 77.1	2.54 ± 0.999	228.6	0.70 ± 0.13
Copper	13.80 ± 3.68	1.46±0.092	5.7	0.02 ± 0.01
Calcium	18.80 x (10^3) ± 6.7 (10^3)	6.698 ± 0.7	1892	2.39 ± 0.17
Zinc	16.66 ± 0.67	NT	42.16	NT
Cadmium	1.32 ± 0.02	0.297 ± 0.008	0.27	NT
Chromium	0.80 ± 0.55	NT	0.87	0.003 ± 0.00
Nickel	5.72 ± 0.11	NT	5.51	NT
Sodium	NT	146.4 ± 9.99	2065	NT
Potassium	NT	170.6 ± 0.95	1570	0.27 ± 0.03
Magnesium	NT	198.9 ± 2.11	3334	6.07 ± 0.49
Mangan	NT	0.382 ± 0.04	37.7	0.02 ± 0.00
Lead	NT	0.297 ± 0.099	1.0	NT
Reference	[12]	[24]	[22]	[23]

3. The Effect of Ulva Species on The Growth of *Oreochromis niloticus*

Several researchers have reported the effect of Ulva species on the growth of *Oreochromis niloticus.* Giving *Ulva lactuca* at 0.0, 2.5 and 5% significantly increased the growth performance of nile tilapia fingerlings. The most optimal results were obtained from the administration of 5% of *Ulva lactuca* into the feed [25].

However, the addition of 5, 10, and 15% *Ulva rigida* to the juvenile nile tilapia diet did not have a significant effect on the growth performance of the fish. The smallest weight gain value was obtained in the experiment of adding 15% *Ulva rigida* to the feed [26]. The same results were also obtained in research conducted by Azaza et.al (2008) [27]. *Ulva rigida* was added to the feed as much as 10, 20, 30% to replace soybean meal on the growth of *Oreochromis niloticus.* The results

showed that *Ulva rigida* did not give a significant difference in the growth performance of tilapia. Giving 30% *Ulva rigida* actually reduced body weight compared to control. Non-significant results were also obtained when giving Ulvan of *Ulva clathrata* to Juvenile of *Oreochromis niloticus*. Although Ulvan from *Ulva clathrata* did not give significantly different results from the control, fish weight gain continued to increase when given ulvan [28]. The administration of *Ulva fasciata* methanol extract as a dietary supplement in tilapia with concentrations of 50, 100, 150 mg/kg increased the body weight of tilapia, but it was also not significantly different from the control [29].

4. The Effect of Ulva Species on The Hematological Parameters of *Oreochromis niloticus*

Hematological and biochemical parameters can be used to monitor fish health [30]. The use of natural ingredients contains many metabolites that can act as immunostimulants. Immunostimulants can improve the performance of the immune system thereby increasing fish resistance to disease [28].

Several studies have tested the effect of administration of Ulva species on the hematological and biochemical parameters of nile tilapia. Abo-Raya et.al (2021) [29] tested the effect of giving *Ulva fasciata* methanol extract (UFME) at concentrations of 50, 100 and 150 mg/kg in feed on the haemato-biochemical profile and immune response of Nile tilapia (*Oreochromis niloticus*). The results showed that administration of 100 mg/kg UFME significantly increased the number of hemoglobin, red blood cells, and hematocrit nile tilapia compared to control and other trials. Another research is giving ulvan of *Ulva clathrata* as much as 0.1, 0.5, 1%/kg into the feed of (*Oreochromis niloticus*). The research was conducted for 90 days (60 days of treatment + 30 days of suspended treatment). The results showed that there was an increase in white blood cells on day 30 in fish with diets containing 0.1, 0.5, and 1% ulvan; which maintained for 15 days in 0.5 and 1% ulvan after their dietary suspension [28].

Supplementation *Ulva lactuca* on nile tilapia fingerlings had no significant (P>0.05) on blood serum white blood cell, red blood cell and haemoglobin concentrations with different levels and strain. There were slight increases with supplemented Ulva for the pervious parameters compared to other experimental diets. Also, blood protein fractions as albumin, globulin and total protein were not affected significantly (P>0.05). These results indicate the potential of Ulva species to enhance immune response in tilapia. Ulva contains various secondary metabolites such as carotenoids that have a positive effect on fish health and increase resistance to pathogenic infections. This ability is also supported by the presence of minerals and vitamins in Ulva spp. [25].

Table 3: Review of Hematological Parameters of Nile tilapia Fed Different Ulva species

Parameters	***Ulvan from Ulva clathrata* (90 days experimental to juvenile of Nile tilapia)**				***Ulva lactuca (12 weeks experimental to* Nile tilapia fingerlings)**			***Ulva fasciata***			
	0	0.1%	0.5%	1 %	0	2.5%	5%	0	50	100	150
Hematocrit (%)	29.66 ± 2.33	29.2 ± 1.09	28.66 ± 3.26	30.4 ± 2.60	-	-	-	20.00	21.33	29.67	22.17
Red blood cell (per mm^3)	1.64 x 10^6	1.62 x 10^6	1.66 x 10^6	1.52 x 10^6	3.54 x 10^6	3.65 x 10^6	3.47 x 10^6	2.112 x 10	2.22 x 10	3.063 x 10	2.313 x 10
Hb (g/dl)	8.08 ± 1.41	7.95 ± 1.28	8.18 ± 1.22	8.73 ± 2.47	6.70	7.21	7.24	-	-	-	-
Total protein (g/dl)	2.18 ± 0.31	2.21 ± 0.32	1.87 ± 0.60	2.00 ± 0.26	3.21	2.89	3.14	3.515	3.652	3.573	3.548
Albumin (g/dl)	1.00 ± 0.11	1.05 ± 0.18	0.98 ± 0.14	1.04 ± 0.14	1.34	1.45	1.39	1.405	1.442	1.373	1.403
Globulin (g/dl)	1.18 ± 0.22	1.15 ± 0.22	0.88 ± 0.51	0.96 ± 0.1	1.87	1.44	1.75	2.110	2.210	2.200	2.145
reference	[28]				[25]			[29]			

5. Conclusion

In conclusion, based on literature review green seaweed Ulva has a high protein contents such as protein, carbohydrates, fiber, vitamins, and minerals. The highest protein content was found on species *U. Rigida* with the percentage of 27.11%. Ulva could be added as supplement in tilapia feed with range 5-25%, it could increase the growth performance, but supplementation higher than 30% could decrease the body weight of Tilapia. Based on hematological analysis, Ulva has the secondary metabolites which increase the fish health and resistance to pathogenic infections. There are no significant effect (P>0.5) found in previous research which mentioned the effect of Ulva in tilapia fingerlings.

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