Dietary impact of *Spirulina platensis* powder supplementation on the growth and immunity of *Clarias gariepinus*

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Abstract. North African catfish (*Clarias gariepinus*) is the second most consumed commodity, having a good nutritional and a high economic value. The increased consumption of *C. gariepinus* and the lack of availability on the market requires an intensive cultivation. However, the intensive cultivation can reduce water quality which can interfere with fish growth and immunity. To overcome this problem, it is necessary to add immunostimulants and growth promoters to increase fish productivity. One of the natural ingredients that can be used is *Spirulina platensis* flour, which is given through feed. This research was carried out from February to May 2022 at the Aquaculture Wet Laboratory, Karangsari Biofloc Pond, using an experimental method of Completely Randomized Design with 3 treatments and 1 control, each repeated 4 times. The treatments were a supplementation of spirulina powder mixed into the feed with different doses, namely T1 20 g kg^{-1} feed, T2 30 g kg^{-1} feed, and T3 40 g kg^{-1} feed, and T0 as a control. The observed parameters were the final weight and length growth, the immunity, consisting of haematocrit and leukocrit percentages, and the visceral organs, including HSI and VSI. The data obtained were analysed using ANOVA, at the 95% confidence level, to determine whether the difference was significant or not. If there was a significant difference, then it was proceeded with the DMRT test (Duncan Multiple Range Test). The results of data analysis showed that the addition of spirulina flour through feed had a significant effect ($p<0.05$) on the final weight and length growth and the percentage of haematocrit with the optimum dose at 40 g kg^{-1} feed (T3). This shows that *S. platensis* has the potential to be a natural supplement when it is mixed into feed for growth and immunity in African catfish cultivation.

Key Words: North African catfish, macroalgae, immunostimulants, protection.

Introduction. The need for food derived from fish continues to increase every year, in line with the increasing consumer demand. The government targets an average consumption of 19.6 kg of fish by the Indonesian population, by 2021. Of the various types of freshwater fish, catfish is the species the most often consumed and used as a source of food from fish origin, in Indonesia (KKP 2017). Increase the productivity of catfish is carried out by an intensification of cultivation. However, improperly managed, this intensification can lead to the appearance of diseases as well as of a slow growth of the cultured fish. Catfish is one of the freshwater fishery commodities that ranks at the top of production volumes, but the diseases, such as Motile Aeromonas Septicaemia (MAS), affect the productivity of catfish farming. MAS disease is caused by the *Aeromonas hydrophila,* a pathogenic bacteria that often infects freshwater fish including catfish (*Clarias gariepinus* L.) and causes the MAS disease (Rofiani et al 2017). This disease can lead to crop failure and inflicts significant losses to the fish farming. External symptoms of MAS disease are the presence of red (hemorrhagic) patches on the body, ulcers (ulcers), pale gills, skin depigmentation, excessive mucus production, and thinning fins (Mulia & Vauziyyah 2021). Antibiotics are administered to cope with catfish infection with *A. hydrophila*. However, improper use of antibiotics can cause pathogenic organisms to become resistant, to accumulate residues on the body of fish and to harm consumers (Lengka et al 2013). Currently, the use of antibiotics is limited so it needs other solutions that are environmentally friendly. One of the natural ingredients that are environmentally friendly is the addition of herbal supplements including *Spirulina plantesis*.

Previous research has proven that the administration of *S. plantesis* supplements containing polysaccharide compounds is known to increase immunity in fish and shrimp. Besides being able to increase immunity, spirulina administration can be applied through injection and immersion methods that can increase resistance to bacteria (Zulmi et al 2018). Similarly reported by Resmawati et al (2016), hot water extract of spirulina at a dose of 30 ppm may increase the total leukocytes and phagocytosis index on the fourteenth day, and the TNFα concentrations on day seven compared to controls in gourami fish. This suggests the hot water extract of spirulina functions as a non-specific immune system. Spirulina is known to contain lipopolysaccharides found in its cell walls. *S. platensis* microalgae include cyanobacterium which is often used as a natural feed enrichment ingredient because it has a variety of immunostimulant nutri (Widyaningrum et al 2017). The administration of hot water extract of *S. platensis* at a dose of 30 ppm is able to increase (1) the total leukocytes of carp at 409,292 x 105 cells mm^{-3}, (2) the phagocytosis index of carp fish at68% and (3) the concentration of TNF-α carp at 38,851 ng L^{-1}, on the fourteenth day (Resmawati et al 2016).

S. platensis biomass contains compounds needed by the human body including 55-70% proteins, 4-6% lipids, 17-25% carbohydrates, unsaturated fatty acids such as linoleic acid (LA) and gamma linolenic (GLA), several vitamins, for example nicotinic acid, riboflavin (vitamin B2), thiamin (vitamin B1), cyanocobalamin (vitamin B12), minerals, amino acids, carotenoids, chlorophyll and phycocyanin (Christwardana & Nur 2013). *S. platensis* is known as a green-blue microalgae that is classified into cyanobacteria, single-celled and spiral-shaped. Positive phytochemical test results contain flavonoids, phenols, steroids and saponins (Afriani et al 2018). Until now, there is not much information about the addition of *S. plantesis* supplement extract through feed to increase immunity, growth and visceral organ profile in catfish. The study aimed to study the effect of the addition of spirulina flour supplements and the optimal dose given through feed for improving the profile of visceral organs, growth and immunity of *C. gariepinus*.

Material and Method

This study used as many as 96 North African catfish (*Clarias gariepinus*) specimens with a length between 12 and 15 cm. The feed used is a commercial pellet with the brand PF 1000, obtained from the place of sale of fish. The method used in this study is an experimental method using a Complete Randomized Design (CRD). The study consisted of 4 treatments and 4 replications. The treatments consisted of different doses of spirulina flour in each treatment:
T0: control (feed without spirulina supplements);
T1: feed + spirulina powder 20 g (kg^{-1} of feed);
T2: feed + spirulina powder 30 g (kg^{-1} of feed);
T3: feed + spirulina powder 40 g (kg^{-1} of feed).

Catfish were placed in 4 different plastic boxes, each as a control (plastic box 1), treatment 1 (plastic box 2), treatment 2 (plastic box 3) and treatment 3 (plastic box 4). Each plastic box was divided into 2 bulkheads, each of which containing 6 catfish, used for the experiment replication. Feed was given at 3% of the weight of total biomass, with 2x a day in the morning at $\pm$08.00 and afternoon at $\pm$17.00. Pelleted feed and spirulina flour were weighed according to the prescribed dosages of 20, 30 and 40 g (kg^{-1} of feed). Spirulina powder and pelleted feed are mixed until it became homogeneous, by adding sterile aquades. Furthermore, the feed was air-dried and after drying, placed into a jar and stored in a refrigerator, until use.

HIS and VSI. The observed parameters include Visceral Somatic Index (VSI) and Hepatosomatic Index (HSI). Measurement of HSI and VSI by means of fish dissection and examination of the liver and visceral organs, for each treatment.
The HSI was calculated using the formula (Sari et al 2017):

$$HSI = Hw/W \times 100$$

Where:
Hw - weight of heart (g);
W - body weight.

The VSI is calculated using the formula (Sari et al 2017):

$$VSI = Vw/W \times 100$$

Where:
Vw - weight of visceral organs;
W - body weight.

Statical analysis. The data obtained were analyzed statistically using ANOVA, with a Complete Randomized Design (RAL), and test F, at a confidence level of 95%. The data obtained are expressed in the form of Mean and Stdv (Standard deviation). If the results of the analysis showed a noticeable difference, it was continued with the DMRT (Duncan Multiple Range Test) test at a 95% confidence level.

Results and Discussion. *S. platensis* is a green alga that can stimulate growth, improve visceral organ performance and increase immunity. The results of the study of supplementation of *S. platensis* diet in *C. gariepinus* were able to improve the growth, performance of visceral organs and immunity. Growth can be seen from the achievement of weight and final length; the improvement in visceral organ performance is derived from the values of HSI and VSI (Table 1). Meanwhile, the increase in immunity can be seen from the increase in the percentage of haematocrit and leukocrit (Table 2).

Table 1

Weight and final length, hepatosomatic index (HSI), visceral somatic index (VSI) of catfish for 60 days of spirulina supplement administration through feed

	HSI	*VSI*	*Weight (g)*	*Length (cm)*
T0	2.3^{ab}±0.28	8.73^{a}±1.95	35.81^{a}±6.819	18.30^{ab}±1.294
T1	1.75^{a}±0.12	5.85^{a}±3.580	35.37^{a}±4.335	17.94^{a}±0.888
T2	1.88^{a}±0.18	7.28^{a}±0.651	51.47^{b}±4.793	20.63^{c}±0.723
T3	2.56^{b}±0.72	9.63^{a}±2.533	57.26^{b}±11.048	20.23^{bc}±1.934

Values are represented as the mean ±stdv. Within-row different superscript letters show significant difference (p<0.05).

Final weight and length achievements. Based on the results of statistical analysis test (ANOVA) with a confidence level of 95%, it shows that feeding with mixed with *S. platensis* flour has a real effect (P<0.05) on the weight and final length of catfish. The results of the analysis showed that T2 and T3 differed significantly from T0 and T1, but T0 did not differ significantly from T1. The addition of *S. platensis* flour through feed is proven to increase the weight and final length of catfish. This is reinforced by the results of another research (Puspitarani et al 2019), stating that the addition of *S. platensis* flour through feed had a significant effect on the weight and final length of catfish seeds, with the best results found at a concentration of 3 g kg^{-1} feed. As reported by Abd-El Alim et al (2018), dried *S. platensis* in diet for 3 months could have an excellent effect, by increasing the final weight and weight gain, with a dose of 10 g kg^{-1} feed of *Oreochromis niloticus*. Nazhiroha et al (2019) also reported that supplementation of *S. platensis* flour can have a significant effect on the growth of length and weight, as well as on the feed efficiency in carp (*Carassius auratus*). Research conducted by Simanjuntak et al (2019) also reported that the addition of flour supplements *S. platensis* to feed had a significant influence on the weight and length gain of *Osphronemus gourami*. Spirulina flour contains a high protein content, of about 65%, so it can stimulate not only fish growth, but also chicken growth, according to the research. *S. platensis* administered through drinking water at a percentage of 2% leads to a significant improvement in the productive performance, immune response and quality of broiler meat (Afriani et al 2018).

Research in dietary supplementation using spirulina powder in Oscar fish, *Astronotus ocellatus*, reported by Mohammadiazarm et al (2021), showed that the feed conversion rate, percentage of relative weight gain, daily specific growth rate and final body weight significantly increased ($p<0.05$), compared with other treatments. A similar report by Şahan et al (2015) stated that feed efficiency and specific growth rate increased after adding a supplementation spirulina diet in *Oreochromis niloticus*. A similar study by Baksi et al (2017) showed that an enhancement of the growth of fish by increasing the feed efficiency was caused by the supplementation spirulina diet at a dose 2-4 g kg^{-1} of feed, recommended in fish farming. The review of Zhang et al (2020) showed that spirulina has a broad spectrum function and benefits in aquaculture. Spirulina is a highly important component of the diet of fish, with a number of benefits, such as promoting the growth of *O. niloticus* and *Labeo rohita*. The inclusion of spirulina in the diet of fish has a positive effect on the carcass composition. Dietary with spirulina flour could enhance the weight gain, total weight gain and daily growth rate in *O. niloticus* fingerlings (Sherif et al 2012). Diet spirulina can also improve carcass quality and utilization efficiency.

HSI and VSI. The results of a research conducted by Putra et al (2020) using sembilang fish in Bintan Island Bay showed that there was a relationship between the liver growth and gonadal development. This happens because the metabolism that occurs in the liver is channeled to the growth and development of the gonads. The heavier and longer the body of the fish, the greater the liver and gonadal organs, as indicated by the HSI and GSI values. But in this study, data on the development of gonads are excluded, because the gonads were not yet formed, so what can be discussed is the HSI value.

The increase in Hepatosomatic Index (HSI) occurs rapidly during the development of ovary and decreases in the late maturity period. In the liver of fish there are different colors and chewiness of the texture. This can happen due to differences in the amount of fat stored in the liver (Sari et al 2017). HSI is an indicator to determine the changes that occur in the heart, quantitatively. Factors that affect HSI values include temperature, food, vitellogenesis activity and maturity level of the gonads. Supplementary spirulina may gain weight by increasing the mobilization of lipid reserves in rainbow trout, as evidenced by the reduction of HSI and VSI (Güroy et al 2011).

Growth leads to the occurrence of morphological and anatomical changes. Morphological changes concern the length and weight, while anatomical changes include liver and other visceral organs (Jobling 2010). Growth can be studied through the approach of morphological index variables in the form of hepatosomatic index (HSI) and visceral somatic index (VSI). Therefore, growth-related research has always been related to HIS and VSI. A research conducted by Sari et al (2017) reported that VSI values tend to decrease, due to changes in fat content in the visceral organs, transferred to the gonads during reproductive development. During the reproductive development, the HSI increases because the liver's metabolic process has increased for the vitellogenesis process. Fats contained in the visceral organs will be transferred in the development of the gonads, especially for the process of oocyte maturation (Sari et al 2017). The value of VSI tends to decrease during the reproductive growth and development, followed by an increase of HSI during the vitellogenesis process. Increased HSI values during the period of vitellogenesis have also been reported in some species of *O. gourami*. Arief et al (2012) stated that there is a relationship between the energy from fat in the visceral or abdominal cavity and the vitellogenesis in the liver, showing that VSI tends to decrease while HSI increases during the vitellogenesis gonadal development. Arief et al (2012) stated that changes in fat deposits in the liver cause the liver color to become paler and oily. The pale color of the liver indicates the addition of fat deposits (the HSI value increases) transferred from the visceral organs (VSI decreases). The fats and proteins present in the liver are used for the vitellogenesis, along with the development of the gonads. Vitellogenesis is the process of forming vitellin in the liver which will then be deposited on the egg as its reproductive development progresses. The accumulation of fat in the liver from the visceral organs, as the HSI value increases from H7 to H35, causes the texture of the fish liver to become chewier. As further reported by Şahan et al (2015), some tilapia growth patterns were a VSI mild decrease and HSI mild increase, but not significant, after diet supplementation with spirulina.

Immunity. The results of the study on dietary supplementation of *Spirulina platensis* flour in *C. gariepinus* showed an increase of the catfish immunity. The increase in immunity can be seen from a significant increase in the percentage of hematocrit while the percentage of leukocrit also increased but not significantly.

Table 2

Percentage of hematocrit and leukocrit catfish dumbo for 60 days of spirulina supplementation through feed

Treatment	*Haematocrit*	*Leukocrit*
T0	$1.40^{a}\pm0.20$	$0.01^{a}\pm0.01$
T1	$2.27^{b}\pm0.69$	$0.10^{a}\pm0.03$
T2	$2.70^{b}\pm0.37$	$0.16^{a}\pm0.00$
T3	$2.70^{b}\pm0.47$	$0.30^{b}\pm0.31$

Based on the statistics analysis test of variety (ANOVA) with a confidence level of 95%, showing that the introduction of a feed diet mixed with *S. platensis* flour has a real effect ($P<0.5$) on the catfish hematocrit. The percentage of hematocrit in all treatments (P1, P2 and P3) differed markedly from that of controls (P0). At the percentage of leukocrit, the addition of dietary supplement of spirulina flour through feed can increase the percentage of leukocrit but not significantly ($p>0.05$). A paper conducted by Mohammadiazarm et al (2021) showed that supplemented spirulina powder diet could increase in blood hematological parameters such as red blood cells, hemoglobin and hematocrit compared to control in Oscar fish *Astronotus ocellatus*. In tilapias, a research by Şahan et al (2015) using spirulina diets showed that the immune parameter of hematocrit and leukocrit increased significantly, compared with control. A raise of leukocrit was indicated by the increased percentage of lymphocyte and monocyte. This means spirulina can be used as a protective agent against diseases, by supporting the fish immune system.

Hematocrit is a method used to determine the percentage or volume of red blood cells against the volume of total red blood cells. Hematocrit examination aims to determine the health status and immunity of *C. gariepinus*. Haematocrit levels show the proportion of the number of erythrocytes in the blood of fish (Payung et al 2017). The addition of *S. platensis* flour has been shown to increase the percentage of haematocrit which indirectly affects the immunity of fish. The number of blood cells in shrimp, namely haemocytes, also increased in number after being given a diet of spirulina flour. As reported by Puspitarani et al (2019), the administration of hot water extract of *S. platensis* through feed can significantly increase the survival and haemocytes count of vanamei shrimp after being infected by *Vibrio harveyi*. Shrimp body defences also increased, as evidenced after *V. harveyi* infection, the survival percentage of vanamei shrimp remaining high at an optimal dose of 800 mg kg^{-1} feed. Another study, reported by Zulmi et al (2018), stated that the addition of *S. platensis* flour can improve the survival and development of giant prawn larvae. On the contrary, a decrease in the percentage of haematocrit in fish can be used as an indicator that the fish has anaemia. The fluctuating percentage of haematocrit can also be used as an indicator of a low intake of nutrients given to fish, vitamin deficiency, age, and infection (Jasmanindar et al 2020). A research conducted by Satyantini et al (2014) stated that spirulina can increase the concentration of the hemoglobin (HB) and white and red blood cells in animals, increasing immunity, due to the presence of phycocyanin and polysaccharides contained in spirulina. This phycocyanin functions as an antioxidant, protects liver function, and removes radical compounds. A study conducted by Rosid et al (2019), showed that spirulina can be a natural feed because it contains a high protein of 39.63%, as well as micronutrients such as B12, β carotene and xanthophyl phytopigment for comet fish larvae. It is known that *S. platensis* can boost the immune system with increased leukocrit, which reflects an increase in the number of leukocytes. Leukocytes are immune system cells that can protect the body from disease attacks. The increase in leukocytes is a mechanism of protection of the body against foreign cells, including bacterial infections, that will attack the immune system. The formation and production of leukocytes in the body will be directed towards the infected part as a defence. The increase in the percentage of leukocytes is one of the indicators that has occurred in the body of fish (Masduqi et al 2014). The administration of flour supplement *S. platensis* containing 1.5% polysaccharides, from the total dry weight, increased the immunity. Polysaccharides can improve the immune system by inducing and spurring leukocyte-forming cells to produce more leukocytes (Rustikawati 2011).

The addition of *S. platensis* flour to the feed has been shown to increase the percentage of leukocytes that affect the fish's immune system. This is reinforced by a research conducted by Widyaningrum et al (2017), by demonstrating that the addition of *S. platensis* flour can increase the leukocytes of gourami, with the most effective dose being 4 g kg^{-1}. Another study conducted by Sedjati et al (2022), on the administration of *S. platensis* in nauplii artemia, has reported an increase in the resistance to the infection by the bacterium *V. harveyi*. A similar report, realized by Abd-El Alim et al (2018), showed that the immunity of *O. niloticus* increased after a dietary supplementation with spirulina (1%) and enhanced fish protection against the *A. hydrophila* infection. Furthermore, the survival rate increased in fish fed on a diet supplemented with 1% spirulina. The increase in leukocrit is one of the causes due to the stimulation of macrophage cells to produce interleukin. Lymphocytes divide into T lymphocytes and B lymphocytes. T lymphocytes stimulate the formation of interferon which activates and increases the ability of macrophages to eat and kill bacteria, viruses, and other particles(Wahjuningrum et al 2014). Spirulina powder enhances immune responses and disease resistance (Zhang et al 2020). Similar report results revealed that *S. platensis* used as feed additives in *O. niloticus* diets improve of non-specific immune response of fish (Sherif et al 2012). The addition of phycocyanin 250 mg kg^{-1} feed provided an increase in total leukocytes, phagocytic activity and growth of juvenile duck grouper (Satyantini et al 2014). The results showed that the addition of *Spirulina* sp. to the diet improved the growth and color brightness of the comet fish. The best behavior was obtained by applying 2.1 g of *Spirulina* sp. flour in a 100 g pellet feed, which produced a weight of 4.33 g, a length of 1.95 cm, and an average color brightness level of 25.183 (Rosid et al 2019).

Conclusions. The results demonstrated that *S. platensis* powder diet was able to increase significantly ($p<0.05$) the final weight and length growth and the percentage of haematocrit of catfish, with the optimum dose at 40 g kg^{-1} feed (T2). Macroalgae *S. platensis* has the potential to be used for the cultivation of *C. gariepinus* and other freshwater fish.

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

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