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Blood Hematological and Biochemical Parameters of *Osteochilus vittatus* with *Spirulina platensis* Supplementation in Biofloc System

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Abstract. *Spirulina platensis* is a microalgae that contains nutrients such us iron and phycocianin higher than other microalgae. Research on suplementation of *S. platensis* in *Osteochilus vittatus* cultured in biofloc system has never been done. The aim of this study was to determine the blood hematological and biochemical parameters of *O. vittatus* fed with *S. platensis* supplementation and maintained in biofloc system. The study was conducted experimentally with Completely Randomized Design, four treatments and five replications. *O. vittatus* were fed with *S. platensis* level 0, 2, 4, and 6 $g.kg^{-1}$ for 56 days. Blood hematological and biochemical parameters were measured on days 0 and 56. Values of those parameters were then analyzed using ANOVA with a confidence level of 95%. The result showed that the highest red blood cells, white blood cells, and hemoglobin counts were $1.57x10^6$ $cell.mm^{-3}$, $2.37x10^5$ $cell.mm^{-3}$, and 6.77 $g.dl^{-1}$ respectivley, while the highest hematocrit value was 17.5 %. The highest total protein, albumin, and globulin in blood were 7.96 $g.dL^{-1}$, 4.31 $g.dL^{-1}$, and 3.79 $g.dL^{-1}$ respectively, and the best for ratio A/G was 1.14. *S. platensis* suplementation level of 4$g.kg^{-1}$ feed was the most optimum level ($P<0.05$). Cultivation of fish with *S. platensis* supplementation in feed can increase fish health indicated by the hematological and biochemical parameters of the blood and the fish maintenance in biofloc systems can improve water quality. The benefit of this research is to make a good condition for cultivation and efficiently used not only water but also feed.

Key words: biofloc; biochemical; hematological; *Ostechilus vittatus; Spirulina platensis*

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INTRODUCTION

Nilem fish (*Osteochilus vittatus*) is a family of Cyprinidae that are widely cultivated and become superior commodities. According to the Banyumas Fisheries and Animal Husbandry Office, the number of *nilem* fish production has increased every year. In 2011 the amount of production increased by 12% from the previous year (Yusuf et al., 2014). Utilization of *nilem* starts from eggs, seeds, juvenile size, and consumption size (100-150gr/ind) (Sumarma et al., 2010). *Nilem* fish (*O. vittatus*) native habitat is freshwater which includes rivers, lakes, and reservoirs. This fish is classified as an endemic animal in Indonesia with quite high diversity. There are 33 species of this fish, 12 of them are endemic species of Indonesia, while the rest are scattered in other Asia (Sumarma et al., 2010). One of the efforts to improve the quality and productivity of *O. vittatus* is by using supplemented feed. The material that can be used in the supplementation process is *Spirulina platensis*.

Spirulina platensis (*S. platensis*) is a species of freshwater microalga. This microalga contains many nutrients such as vitamins, phycocyanin, anti-oxidant pigments, essential amino acids, proteins, and minerals. Macronutrients and micronutrients including proteins, amino acids, saturated fatty acids, minerals, and vitamins are contained in *S. platensis* (Cifferri & Tiboni, 1985; Sitorious & Sotiroudis, 2013). In general, spirulina contains 55-70% protein, 15-25% polysaccharide, 6-13% nucleic acid, 5-6% fat, and 2-5% minerals (Hosseini et al., 2013). These microalga are often used as an ingredient in animal feed supplementation and fishery feed to improve the health. *S. platensis* grows in waters and is very easy to harvest. Some researchers use dried *S. platensis* as a supplement in feeds that can increase the hematological value in African catfish (*Clarias gariepinus*) (Raji et al., 2018) and the growth in guppy fish

(Dernekbasi et al., 2010). Leftover from feed and waste from fish metabolic process can be toxic and make bad influence for water quality. Therefore, one of cultivation system that can maintain the water quality without water changes is biofloc system.

Biofloc technology is a technique in intensive aquaculture systems that can control pond water quality. The principle of biofloc is to balance carbon and nitrogen in pond water. This system is gaining attention in aquaculture because of its potential to get high production from excess feed and metabolic waste. Biofloc works with the addition of carbon as an organic material for microbiology or aerobic bacteria to decompose and maintain the population of floc-forming bacteria. According to Anand et al. (2014), adding carbon to the system will increase the C/N ratio. This increase in the C/N ratio will enhance the conversion of inorganic nitrogen or ammonia (NH_3) by biomass from bacteria in the pond. Some types of floc-forming bacteria that are often used in biofloc systems include *Bacillus* sp., *Bacillussubtilis*, *Pseudomonas* sp., *Bacillus lichenoformis*, *Bacillus pumilus*, and *Lactobacillus* sp. (Anand et al., 2014). The excess of nitrogen will be utilized by phytoplankton as a source of carbohydrates, an energy source for growth. Increased biomass from microorganisms and flocks (algae) that arise will be an alternative source of food in the pond (Kheti et al., 2017).

Research on suplementation of *S. platensis* in *O. vittatus* cultured in biofloc system has never been doneThe purposes of this research was to determine blood hematological and biochemical parameters of *O. vittatus* fed with *S. platensis* supplementation in biofloc system. This research also determined the most effective level of *S. platensis* supplementation in improving blood hematological and biochemical parameters of *O. vittatus* on biofloc system. This research was expected to make a good condition for cultivation and efficiently used not only water but also feed.

METHOD

Preparation of biofloc systems and feed supplemented with *S. platensis*

The research was conducted experimentally with Completely Randomized Design. Formulation of supplemented *S. platensis* as treatment were contain 0, 2, 4, 6 g/kg of commercial pellets, each treatment was replicated five times. The treatments were tested, namely: P0=control (commercial feed, without supplementation *S. platensis*); P1=supplementation of *S. platensis* 2 g/kg of feed; P2=supplementation of *S. platensis* 4 g/kg of feed; P3=supplementation of S. platensis 6 g/kg of feed.

Preparation of feed supplemented with *S. platensis* for the treatment group was as follows, 2 g of dried *S. platensis* were put into a beaker glass then added with 100 mL of distilled water and stirred until homogeneous. Furthermore, 1 kg of commercial pellets LP1 combined with *S. platensis* solution placed on a plastic tray. Feeds that have been supplemented with *S. platensis* were sun-dried. Dried feeds were stored in tightly closed containers and labeled. The same procedures were carried out for *S. platensis* level of 4 $g.kg^{-1}$ and 6 $g.kg^{-1}$ feed.

Fish maintenance was carried out in a fiber tank (20 pieces) measuring 60x40x60 cm^3, each with an aeration and a recirculation system. The biofloc system preparation was carried out with the following procedure. In the fiber tank, 10mL of probiotics EM4 and 40mL of molasses were added, then stirred for seven days using strong aeration. Fish was included on the eighth day to utilize the remaining feed and feces as the source of N. Environmental factors (pH, temperature. and dissolved oxygen content) were routinely controlled every week to maintain optimal maintenance media conditions.

Treatment

The *nilem* fish used were 260 juvenile size weighing 9-15 gr. Fish were originated from the village of Cipaku, Mrebet Subdistrict, Purbalingga Regency. *Nilem* fish were put into a fiber tank with a density of 13 individuals/tank. Before being treated, the fish was acclimated for 7 days. Feeding according to treatment with 5% feeding rate (FR) of the weight of the test fish biomass was given twice a day, for 56 days.

Data retrieval

Fish body weight measurements and hematological analysis were carried out on day 14, 28, 42, and 56 for adjustments to feeding. For biochemical parameters, at the beginning of the study (day 0) and the end of the experimental period (day 56). Blood was taken by syringe (1cc). The syringe for hematological analysis was coated with EDTA, while the one for biochemical analysis was not. The blood taken were then kept at the storage at 4°C for 30 minutes, then sentrifuged at 4000 rpm for 20 minutes. Blood serum were kept frozen at -18°C. Then, measurements of total protein and albumin were carried out with Human Diagnostics Worldwide kit for total protein and albumin. Globulin was measured by the result of Total Protein minus Albumin value, while ratio A/G obtained by deviding the Albumin and Globulin value.

Data analysis

Data of blood hematological and biochemical parameters were analyzed using variance analysis

(ANOVA) with a confidence level of 95%. The significant results were then proceed with the Duncan test.

RESULTS AND DISCUSSIONS

Observation of fish hematological responses can reflect the health status of the fish. Fish blood can be used as an indicator of nutritional status, conditions of environmental influence, and the effect of stress on fish (Yageneh et al., 2014). Fish infected by a disease will experience a decrease in the number of red blood cells, white blood cells, hemoglobin, and hematocrit values (Simanjuntak et al., 2018). Data on blood hematological and biochemical parameters are shown in tables 1, 2, 3, 4, and 5.

Table 1. Red blood cells count of *nilem* (*Osteochilus vittatus*)

Treatment	Red blood cells (sel/mm3) (x106)				
	Day-0	Day-14	Day-28	Day-42	Day-56
P0	0.71±0.11a	0.88±0.23ab	1.04±0.24a	0.94±0.13a	1.12±0.72a
P1	0.47±0.19a	0.96±0.11b	1.12±0.94a	1.02±0.16a	1.21±0.72a
P2	0.76±0.95a	0.67±0.16a	1.16±0.11ab	1.16±0.21ab	1.31±0.13ab
P3	0.69±0.84a	0.79±0.12ab	1.27±0.12b	1.31±0.11b	1.57±0.31b

Different superscript letters in each column show significant differences between treatments (X ± SD, n = 5> 0.05); P0: Supplementation of *S. platensis* dose of 0 g/kg of feed in the biofloc system, P1: Supplementation of *S. platensis* dose of 2 g/kg of feed in the biofloc system, P2: Supplementation of *S. platensis* dose of 4 g/kg of feed in the biofloc system, P3: *S. platensis* supplementation dose of 6 g/kg of feed in the biofloc system.

Table 2.White blood cells count of *nilem* (*Osteochilus vittatus*)

Treatment	White blood cells (Cell/mm^3) (x10^5)				
	Day 0	Day 14	Day 28	Day 42	Day 56
P0	1.04±0.17^a	1.08±0.11^{ab}	1.68±0.18^b	1.55±0.45^a	1.93±0.35^a
P1	0.89±1.25^a	1.18±0.91^b	1.41±0.10^a	1.56±0.11^a	1.98±0.44^a
P2	1.01±0.34^a	0.99±0.11^a	1.66±0.19^b	1.80±0.31^a	1.96±0.29^a
P3	1.08±0.21^a	1.19±0.60^b	1.73±0.23^b	1.89±0.27^a	2.37±0.44^a

Different superscript letters in each column indicate significant differences between treatments (X ± SD, n = 5> 0.05); P0: Supplementation of S. platensis dose of 0 g/kg of feed in the biofloc system, P1: Supplementation of S. platensis dose of 2 g/kg of feed in the biofloc system, P2: Supplementation of S. platensis dose of 4 g/kg of feed in the biofloc system, P3: S. platensis supplementation dose of 6 g/kg of feed in the biofloc system.

Table 3. Hemoglobin level of *nilem* (*Osteochilus vittatus*)

Treatment	Hemoglobin (g/dL)				
	Day 0	Day 14	Day 28	Day 42	Day 56
P0	4.67±0.58^a	4.35±0.47^a	5.12±0.51^a	6.20±0.11^{ab}	5.52±0.38^a
P1	4.67±1.15^a	4.50±1.25^a	5.02±0.76^a	6.00±0.27^a	6.17±0.52^{ab}
P2	5.00±1.41^a	4.60±0.81^a	5.35±0.17^a	6.75±0.47^b	6.77±0.52^b
P3	6.50±0.71^a	4.65±0.78^a	5.47±0.49^a	6.77±0.63^b	6.77±0.65^b

Different superscript letters in each column indicate significant differences between treatments (X ± SD, n = 5> 0.05); P0: Supplementation of *S. platensis* dose of 0 g/kg of feed in the biofloc system, P1: Supplementation of *S. platensis* dose of 2 g/kg of feed in the biofloc system, P2: Supplementation of *S. platensis* dose of 4 g/kg of feed in the biofloc system, P3: *S. platensis* supplementation dose of 6 g/kg of feed in the biofloc system.

Table 4. Hematocrit value of *nilem* (*Osteochilus vittatus*)

Treatment	Hematocrit (%)				
	Day 0	Day 14	Day 28	Day 42	Day 56
P0	11.00±0.87a	9.62±4.14a	11.62±2.75a	14.87±0.47a	13.12±1.18a
P1	10.00±2.65a	11.25±1.32a	12.62±0.85ab	15.12±1.54a	17.50±2.88b
P2	12.00±1.41a	13.00±2.48a	15.50±1.58ab	16.87±2.01a	17.25±2.59b
P3	9.75±0.35a	14.12±2.71a	16.62±4.71b	17.00±2.44a	18.00±2.94b

Different superscript letters in each column indicate significant differences between treatments (X ± SD, n = 5> 0.05); P0: Supplementation of *S. platensis* dose of 0 g/kg of feed in the biofloc system, P1: Supplementation of *S. platensis* dose of 2 g/kg of feed in the biofloc system, P2: Supplementation of *S. platensis* dose of 4 g/kg of feed in the biofloc system, P3: *S. platensis* supplementation dose of 6 g/kg of feed in the biofloc system.

The results of the analysis of ANOVA test on the number of red blood cells (erythrocytes) showed a significant difference between treatments (P> 0.05). Based on the results of Duncan's test analysis with a confidence level of 95%, P3 treatment (*S. platensis* supplementation dose 6g.kg^{-1}) had the highest number of red blood cells compared to P0 (without supplementation) and P1 treatment (*S. platensis* supplementation dose 2g.kg^{-1}) (Table 1.)

The results of the study revealed an increase in the number of blood cells. The increase in the number of red blood cells is thought to be caused by the iron (Fe) contained in *S. platensis*. Research conducted by Satyantini et al. (2014) revealed that red blood cells can be increased by the addition of feed containing high iron content. Iron and protein is a constituent of erythrocyte cells in the body. The iron (Fe) and C-phycocyanin can improved the hematopoiesis and secretion of the hormone Erythropoeitin (EPO). EPO hormone is produced by the kidneys and plays a role in regulating erythrocyte production (Satyantini et al., 2014). An increase in the number of marrow cells can induce red blood cells production (Guardiola et al., 2018).

Research by Adel et al. (2016) stated that supplementing *S. platensis* in feed by 10% in great sturgeon fish (*Huso huso*) can increase the number of red blood cells. In addition, Simanjuntak et al. (2016) also stated that the *S. platensis* supplementation dose of 6g.kg^{-1} could increase the number of red blood cells in gouramy fish (*Osphronemus gouramy*). Promya and Chitmanat (2011) in their research using catfish (*Clarias gariepinus*) showed that the supplementation of a dose of 5g.kg^{-1} can increase the number of red blood cells to $2.87 \pm 0.06x10^6$ cells.mm^{-3} from the previous number of $2.65 \pm 0.03x10^6$ cells.mm^{-3}

The results of the analysis of ANOVA test on the number of white blood cells (leucocytes) showed a significant difference between treatments (P> 0.05). Based on the results of Duncan's test analysis with a confidence level of 95%, in day 14, P1 (*S. platensis* supplementation dose 2g.kg^{-1}) and P3 treatment (*S. platensis* supplementation dose 6g.kg^{-1}) had the highest number of white blood cells and significant difference compared to P0 (without supplementation) and P2 treatment (*S. platensis* supplementation dose 4g.kg^{-1}). While, in day 28, P0, P2 and P3 treatment had significant difference compared to P1 treatment (Table 2.) *S. platensis* were contains phycocianis can improve blood parameter of white blood cells (Liu et al., 2016).. According to Simanjuntak et al. (2018) shown that level suplementation level 6g.kg^{-1} had improve level of hematological significanlly.

In day 56, there was no significant difference in each treatment. This was because the use of supplementation doses is relatively low. Research carried out by Yu et al. (2018) showed an increase in the number of white blood cells in coral fish (*Plectropomus leopardus*) fed with *S. platensis* supplementation at a dose of 10% in feed. This supplementation dose will increase the value of the active ingredient β-carotene and phycocyanin-polypeptide which can increase the number of white blood cells. Another study conducted by Adel et al. (2016) stated that there was no significant difference in the increase in the number of white blood cells (leukocytes) in great strugeon fish (*Huso huso*) which were fed with 5% supplementation dose in the feed. White blood cell count can be used as an indicator of an increase in the fish's immune system both humoral and mucosal (Adel et al., 2016; Yu et al., 2018).

The results of the analysis using ANOVA on the blood hemoglobin value of *nilem* fish (*O. vittatus*) at 95% confidence level, showed a significant difference between treatments (P> 0.05). According to Satyantini et al. (2014) hemoglobin values in freshwater teleost fish are 4.00–8.33 g.dl^{-1}, so the hemoglobin value during the study is still within the normal range for fish

The level of hemoglobin is very closely related to erythrocyte cells. The analysis results in table 3 shows an increase in the number of erythrocytes. This increase in hemoglobin value is thought to be due to an increase in red blood cells count. The increase was due to the phycocyanin and iron (Fe) contained in *S. platensis* that increasing the number of red blood cells and hemoglobin level (Satyantini et al., 2014) Hemoglobin is one of the blood pigments that has the function of binding oxygen. The low value of hemoglobin can be used as an indicator of low oxygen levels in the blood, and vice versa the high value of hemoglobin is an indicator of high oxygen values in the blood. Low hemoglobin values can cause anemia in fish (Simanjuntak et al., 2018).

The results of the analysis using ANOVA on the blood hematocrit value of *nilem* fish (*O. vittatus*) at 95% confidence level, showed a significant difference between treatments (P> 0.05). Duncan's test results with the same level showed the treatment P2 and P3 (supplementation dose 4g.kg^{-1} and 6g.kg^{-1}) had the highest hematocrit value when compared with P0 (without *S. platensis* supplementation) (Table 4.).

These results are in accordance to Rani et al. (2018), that used Grouper fish feed with supplementation of *Dunaliella salina* at dose 6g.kg^{-1} had increase hematocrit value and showed hematocrit examination can be used as a benchmark to determine fish health. Hematocrit in the world of aquaculture can be used as parameters to check the condition of anemia in fish. Low hematocrit values indicate anemia and high hematocrit values also show high blood cell counts and high appetite in fish (Norousta & Mousavi-Sabet, 2013).

ANOVA analysis results for blood protein levels of *nilem* fish (*O.vittatus*) did not show any significant differences between treatments (P> 0.05). The results of Duncan's analysis of the test with the same confidence level also showed that there were no significant differences between treatments during the study (P > 0.05).

Table 5. Blood biochemical parameters of *nilem* (*O. vittatus*)

Treatment	Total protein (g.dL^{-1})	Albumin (g.dL^{-1})	Globulin (g.dL^{-1})	Ratio A/G
P0	7.88±0.70^{a}	4.24±0.38^{a}	3.63±0.91^{a}	1.24±0.38
P1	7.89±0.28^{a}	4.09±018^{a}	3.79±0.40^{a}	1.09±0.15
P2	7.96±1.25^{a}	4.31±0.70^{a}	3.64±1.18^{a}	1.30±0.53
P3	7.63±0.22^{a}	4.07±0.06^{a}	3.55±0.19^{a}	1.14±0.06

Different superscript letters in each column indicate significant differences between treatments (X ± SD, n= 5> 0.05); P0: Supplementation of *S. platensis* dose of 0 g/kg of feed in the biofloc system, P1: Supplementation of *S. platensis* dose of 2 g/kg of feed in the biofloc system, P2: Supplementation of *S. platensis* dose of 4 g/kg of feed in the biofloc system, P3: *S. platensis* supplementation dose of 6 g/kg of feed in the biofloc system.

According to Rehulka et al. (1993), the normal range of total protein, albumin, globulin, and A/G ratio values are 6-8 g.dL^{-1}, 3.5 - 5 g.dL^{-1}, 1.5 - 2.5 g.dL^{-1}, and 0.7 - 1.18 g.dL^{-1} respectively. The optimum value of A/G ratio was in the range of 0.7-1.18 g.dL^{-1}. The results of the A/G ratio (Table 5.) on day 56 show the supplementation dose of *S. platensis* 6g.kg^{-1} of the feed reached the ratio of 1.14 ± 0.06 g.dL^{-1}. It indicates the A/G ratio of P3 had normal range of the A/G ratio.

Ahmed and Ali (2013) said that A/G ratio values that are too high indicate a low value of globulin, which can correlate to low immune levels and inhibit the immune response. The content of protein in the blood can maintain the concentration of hydrogen ions and osmotic pressure. The concentration of globulin in the blood can be used as a good indicator of physiological standards. A/G ratio index can be an index in fish health.

Measurement of blood protein can be used as an indicator of fish health. Albumin is a protein that plays a role in the transportation of nutrients in the circulatory system and osmotic regulation (Guardiola, et al., 2018). Globulin can also be used as an indicator of fish immunity (Kumar et al., 2017). An increase in total protein, albumin and globulin can increase non-specific immune responses. Increases in total protein and albumin doses in studies may indicate an increase in non-specific immune responses. This can be caused by the content of *S. platensis* which contains active ingredients β-carotene and phycocyanin which can increase the fish's immune response. The content of phycocyanin and β-carotene will increase the value and number of blood cells, the increase will give effect to the increase in total protein, albumin, and globulin (Yageneh et al., 2014).

This research is beneficial for fish farming system. Biofloc system can improve the quality of water by providing natural food from degradation of leftover feed and fish metabolic waste. Feed supplemented with *S. platensis* can increase fish health indicated by the hematological and biochemical parameters of the blood. Therefore, aquaculture using biofloc system and feed supplemented with *S. platensis* is very benificial for fish farmers because it can reduce feed cost, toxicity, and improve the imunity of the fish.

CONCLUSION

The highest red blood cells, white blood cells, and hemoglobin count were 1.57x10^{6} cell.mm^{-3} (P3), 2.37x10^{5} cell.mm^{-3} (P3), and 6.77 g.dl^{-1} (P2 and P3) respectively, while the highest hematocrit was 17.5 % (P1). The highest total protein, albumin, and globulin in blood were 7.96 g.dL^{-1} (P2), 4.31 g.dL^{-1} (P2), and 3.79 g.dL^{-1} (P1) respectively, while the best A/G ratio was 1.14 P3. *S. platensis* suplementation level of 4g.kg^{-1} and 6g.kg^{-1} feed were the most optimum level in improving the performance of hematological and biochemical blood (P<0.05).

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