

Lipase Activity, Hematological and Blood Biochemistry of *Osphronemus gouramy* Fed with Supplementation of *Spirulina platensis* and *Chlorella vulgaris*

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ABSTRACT. *Spirulina platensis* and *Chlorella vulgaris* are algae that contain high nutrients, such as protein, vitamins, and minerals. The purpose of this study was to determine the lipase activity, hematological, blood biochemistry and to obtain the most effective supplementation of *Spirulina platensis* and *Chlorella vulgaris* in feed to enhance lipase activity, hematological parameter, blood biochemistry of gourami. The study was conducted experimentally in which 100 gourami fish were assigned randomly to the following treatments: P1 = *Spirulina platensis* 6 g kg⁻¹ feed; P2 = *Chlorella vulgaris* 4 g kg⁻¹; P3 = *Spirulina platensis* 3 g kg⁻¹ + *Chlorella vulgaris* 2 g kg⁻¹; P4 = *Spirulina platensis* 2 g kg⁻¹ + *Chlorella vulgaris* 3 g kg⁻¹; and C = feed without supplementation as control, in four replicates. Lipase activity was measured in various digestive organs at pH 2, 5, 7, 8 and 10. The results showed that supplementation of *Spirulina platensis* and *Chlorella vulgaris* affected lipase activity; hematological parameter and blood biochemistry of gourami. The combination *Spirulina platensis* + *Chlorella vulgaris* supplementation in feed showed the highest increased of the lipase activity, hematological parameter and blood biochemistry of gourami. *Spirulina platensis* + *Chlorella vulgaris* supplementation in feed might improve growth and immunity since the increase of digestive enzyme functioning which enhances feed utilization and the increase of biochemical parameters of blood, respectively.

Keywords: Blood biochemistry, *Chlorella vulgaris*, hematological, lipase activity, *Spirulina platensis*.

INTRODUCTION

Spirulina platensis (*S. platensis*) and *Chlorella vulgaris* (*C. vulgaris*) live in fresh water and some are in sea water. These algae contain diverse bioactive compounds, such as polysaccharide and β carotene. *Spirulina platensis* is also known as blue-green algae, with very high nutritional profile. According to Kozlenko & Henson (2010), *S. platensis* cell wall consists of protein and soft complex sugars which is so that is easily digested. Commercial feed supplemented with *Spirulina platensis* is safe for humans to eat, including fish without significant side effects (Kumari et al., 2011).

Spirulina platensis contains nutrients such as vitamins, antioxidant pigment, minerals, essential amino acids and proteins (Raji et al., 2018), C-phycocyanin (Kumar, Dhar, Pabbi, Kumar, & Walia, 2014). Furthermore, Augustin, Kuzina, Andersen, & Bak (2011), reported that *S. platensis* contains abundant fatty acids, gamma – linolenic acid (GLA), minerals, vitamins, essential amino acids, protein and antioxidant pigments like carotenoids.

Chlorella vulgaris is a green algae which is rich in nutrients, small in size, and round in shape

(Ponnuswamy, Madhavan, & Shabudeen, 2013) with nutritional composition consisting of proteins, fats, polysaccharides, minerals and vitamins (Xu et al., 2014), chlorophyll (Job, Kaveriammal, & Elayarasi, 2014), lutein and other micro-nutrients (Jeon, Kim, Im, Oh, and Lim, 2012; Buono, Langellotti, Martello, Rinna, & Fogliano, 2014), pigments and essential amino acids (Becker, 2007). This microalgae also contains chlorophyll, intracellular protein, carbohydrates, lipids, vitamin C, β -carotene and vitamin B therefore it can be used as a dietary supplement (Coronado-Reyes, Salazar-Torres, Juárez-Campos, & González-Hernández, 2020).

Chlorella vulgaris also contains Chlorella Growth Factor that plays a role in growth (An, Kim, Jeon, & Lee, 2016). Allen, Ten-Hage, & Leflaive (2018), said that *Chlorella vulgaris* contains Chlorellin which is formed from a mixture of C18 fatty acids, especially stearic acid, oleic, linoleic, and linolenic acid. Chlorellin content in *C. vulgaris* allows this microalgae to be used as a natural antibiotic against pathogenic infections (Ahmad, Shariff, Yusoff, Goh, & Banerjee, 2018). Liu, Pohnert, & Wei (2016), reported that *C. vulgaris* contains a compound of C 18 fatty acids that

is able to stimulate growth but it is also inhibit growth of *Chlorella pyrenoidosa*. In addition, Becker. (2007) found that *C. vulgaris* contains 51-58% protein and contains various essential amino acids so that *C. vulgaris* can be used as a source of protein for animal feed, including fish. Simanjuntak, Soedibya, & Wibowo (2014), have also reported that dry *C. vulgaris* can be supplemented in feeds and increased the growth of gourami juvenile.

Fish growth is closely related to the ability of the fish to use available feed. Feed which contains high nutrients and fed in accordance to fish's age might increase its growth. Feed utilization correlates with the fish digestion enzymes functioning in such effective metabolic process that the feed provided is utilized efficiently. The feed utilization efficiency in fish might be indicated by the effect of feeding rate on growth performance. It has been found that feeding rate affected growth performance of snow trout (*Schizothorax zarudnyi*) juvenile (Khandan, Dahmardeh, Miri, & Rigi, 2019).

Digestive enzyme activity is an important indicator of digestive physiology in fish species. The digestive physiology of skipjack tuna and Atlantic bonito enhance knowledge on the subject of feeding ecology, feeding behavior and physiology diversity as a result of adaptation to specific habitat conditions and certain seasons (Dias, Dardengo, Engrola, & Navarro-Guille'n, 2021). Feeding that has been supplemented with algae can enhance feed nutrition and this will enhance the activity of digestive enzymes.

Gourami physiological and pathological changes can be monitored by measuring haematological characters. The biochemical composition of blood is among other factors regarding fish health. Exogenous factors, such as disease (Chen, Jin, & Wang, 2005), stress (Cnaani, Tinman, Avidar, Ron, & Hulata, 2004), and management (Svobodova et al., 2008), are the most influential factors that cause major changes in the biochemical composition of blood.

Fish immunity can be determined by measuring the biochemical composition of blood including the percentage of total protein, albumin, globulin and albumin: globulin (A / G) ratio. Biochemical blood parameters can also be used to detect fish health (De Pedro, Guijarro, Lopez-Patino, Marinez-Alvarez, & Delgado, 2005). Analysis of blood parameters has been proven to be a valuable approach for analyzing animal health status because blood parameters provide reliable information about metabolic disorders, deficiency and chronic stress status (Bahmani, Kazemi, & Donskaya, 2001). For instance, albumin involved in plastic metabolism and plays an important role in the function of exogenous transport of chemicals and endogenous metabolites (Baker, 2002). Albumin has been used as a diagnostic tool that adequately reflects animal health, liver function, metabolic status and stress conditions.

Research on gourami nutrition and cultivation management has been carried out (Simanjuntak, Indarmawan, & Wibowo, 2018), but studies with *Spirulina platensis* and *Chlorella vulgaris* supplementation in feed on lipase enzyme activity, hematological and blood biochemistry of gourami have not been done. The purpose of this study was to determine the increase of the digestive enzymes activity, hematological and blood biochemistry of gourami supplemented with *Spirulina platensis* and *Chlorella vulgaris* in feed and to find the most appropriate composition of *Spirulina platensis* and *Chlorella vulgaris* supplementation in feed to enhance the digestive enzyme activity, hematological and blood biochemistry of gourami.

EXPERIMENTAL SECTION

Materials, Equipments and Tools

The materials used in this study were 100 *Osphronemus gouramy* Lac., 4-nitrophenylpalmitate (Sigma Aldrich), 4-nitrophenol (Merck), isopropanol (Merck), NaOH (Merck), Na₂CO₃ (Merck), Tris (hydroxymethyl) aminomethane (Merck), Hayem solution, Turk solution, HCl (Merck), EDTA (Merck), NaH₂PO₄.H₂O (Merck), Na₂HPO₄ (Merck), Glycine (Merck), asam asetat (Merck), Sodium asetat (Merck), KCl (Merck), Akuabidestilata steril (Generik), fish pellets PF 1000, *Chlorella vulgaris* powder, *Spirulina platensis* powder, tissue. kit diasys albumin, and kit diasys protein total.

The equipments and tools used in this research include fiber tubs measuring 60x40x60 cm³ equipped with aerators, 1 mL syringe (Terumo), glass beakers, plastic trays, plastic containers, scales with an accuracy of 0.01 grams, seser, surgical instruments, ice box, electric homogenizer (Heidolph Diax 900), centrifuge (Hitachi, Himac CT 15 E), test tube, test tube rack, petri dish, preparation tray, dropper, micro pipette (Soccoro) and pipette tip, Eppendorf tube, waterbath, refrigerator (Glacier -86°C, ultraflow, temperature freezer), spectrophotometer (Hitachi U-2900), hemocytometer, hemometer, hematocrite tube and microhematocrit centrifuge (KHT-410).

Methods

The study was conducted experimentally by applying 4 different treatment of supplementation compositions of *S. platensis* and *C. vulgaris* and a control, in four replicates. The treatments P1 = *Spirulina platensis* supplementation 6 g kg⁻¹ commercial feed; P2 = *Chlorella vulgaris* supplementation 4 g kg⁻¹ commercial feed; P3 = *Spirulina platensis* supplementation 3 g kg⁻¹ + *Chlorella vulgaris* 2 g kg⁻¹ commercial feed and P4 = *Spirulina platensis* supplementation 2 g kg⁻¹ + *Chlorella vulgaris* 3 g kg⁻¹ commercial feed, while C as control was commercial feed without supplementation of algae. The experiment was carried out for 56 days.

Experimental Diets. Supplementation *Spirulina platensis* and *Chlorella vulgaris* on feed (Simanjuntak, Yuwono and Rachmawati, 2006)

Supplementation of *S. platensis* in feed with dose of 6 g.kg⁻¹ commercial pellets was carried out with the following procedure: 6 g *Spirulina platensis* were placed in a beaker glass, 150 mL aquadest was poured into a beaker glass, containing *S. platensis* as was stirred until homogeneous. An homogeneous *S. platensis* suspension was supplemented to 1 kg of commercial pellets which have been placed on the tray, being gently reversed so that the supplementation is evenly distributed in all parts of the pellet. Furthermore, the supplemented feed was sun dried for three hour. As soon as it was dried, the feed was then cooled in room temperature and placed in a clean glass container sealed. Feed supplementation 6 g.kg⁻¹ *S. platensis* in commercial pellets was ready to be tested on experimental specimen of gourami. The same procedure was performed for treatment P2, P3 and P4.

Experimental Fish

One hundred individuals of gourami, *Osphronemus gouramy*, were obtained from the spawning of a pair of parents. The gourami broodstock was obtained from Purbalingga Regency, Central Java Province, Indonesia. Experimental fishes were placed in a fiber glass aquaria with a size of 40 cm x 60 cm x 60 cm, each of which hold 20 individuals. Fiber glass aquaria were equipped with a heater and electric water pump devices. The fish was acclimated to the laboratory condition for one week prior to the use in experiment. Gourami was fed twice a day at 08.00 and 16.00 as much as 3% of the total weight of gourami per aquarium, for 56 days.

Measurement of Lipase Activity of Digested Organ

Lipase activity of various organs digested at several buffered pHs (2, 5, 7, 8 and 10) was measured by spectrophotometric method (Markweg et al., 1995 according to Klahan, Areechon, Yoonpundh, and Engkagul, 2009). Measurements were made using 0.1 M tris-HCl buffer solution (pH 8) Substrate manufacturing is done by making p-nitrophenylpalmitate 0.01 M solution into isopropanol, enzyme activation is started by mixing buffer (1750 µL) and enzyme extract (100 µL) and incubated for 10 minutes at waterbath at 37 °C, then enzyme reaction followed by enzyme reaction. Start with mixing 100 mM pNPP substrate as much as 300 µL was added to the reaction and incubated for 15 minutes at a water temperature of 37 °C. Then, 0.1 M Na₂CO₃ reagent was added to the sample to stop the reaction. Control was measured by adding 0.1 M Na₂CO₃ and the enzyme extract after the solution was incubated. The standard p-nitrophenol was measured by the same method. The whole reaction mixture was centrifuged at 10,000 rpm for 15 minutes. The supernatant was removed as much as 2500 µL and

was then vortexed. The absorbance was read at a wavelength of 410 nm using a spectrophotometer. Lipase enzyme activity was calculated using a standard p-nitrophenol curve and one unit of enzyme activity was defined as the amount of 1 µg p-nitrophenol released per minute per mg of supernatant protein.

Measurement of Hematological Parameters

The hematological data of gourami (number of erythrocyte, number of leukocyte, hemoglobin concentration and hematocrit value) calculated at the end of the study. Blood was drawn from the heart using a 1 mL syringe that has been moistened with anti-coagulant (EDTA). Calculation of the total number of erythrocytes and leukocytes using hemocytometer "Improved Double Naubauer's", measurement of hemoglobin levels using hemometer "Assistant" and measurement of hematocrit value used microhematocrit "Hawkskey hematocrit reader" (Chairlan & Lestari, 2011).

Measurement of Blood Biochemistry Parameters

Blood biochemistry parameters measured were total protein levels, albumin levels, globulin levels and albumin: globulin ratios. Total protein levels and albumin levels were measured by using the kit diasys and were analyzed by the color change method (spectrophotometer). Serum blood globulin was calculated by subtracting the total protein concentration with albumin concentration. Albumin / globulin ratio (A / G ratio) is calculated by dividing albumin and globulin concentrations.

Statistical Analysis

One way analysis of variance using the SPSS software programs and Duncan least significance difference test were applied to compare the differences among the treatments. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The results showed that feeding commercial pellet supplemented with *Spirulina platensis* and *Chlorella vulgaris* supplementation significantly affect on lipase activity, hematological and blood biochemistry parameters of gourami ($P < 0.05$).

Initial Lipase Activity (before treatment)

Lipase activity in digestive organ of gourami was examined prior to administration of the treatment using a buffer solution at a different pH, namely pH 2; 5; 7; 8 and 10. It was found that lipase activity in digestive organ of gourami was influenced by differences in buffer pH (Figure 1, $p < 0.05$).

Differences in buffer pH play a major role in the lipase activity of the liver, stomach, foregut, midgut and hindgut (Figure 1-6). Lipase activity of gastric organs was observed at all pH buffers and the highest lipase activity was detected in that of gourami fed commercial pellet supplemented with combination of *S. platensis* and *C. vulgaris*.

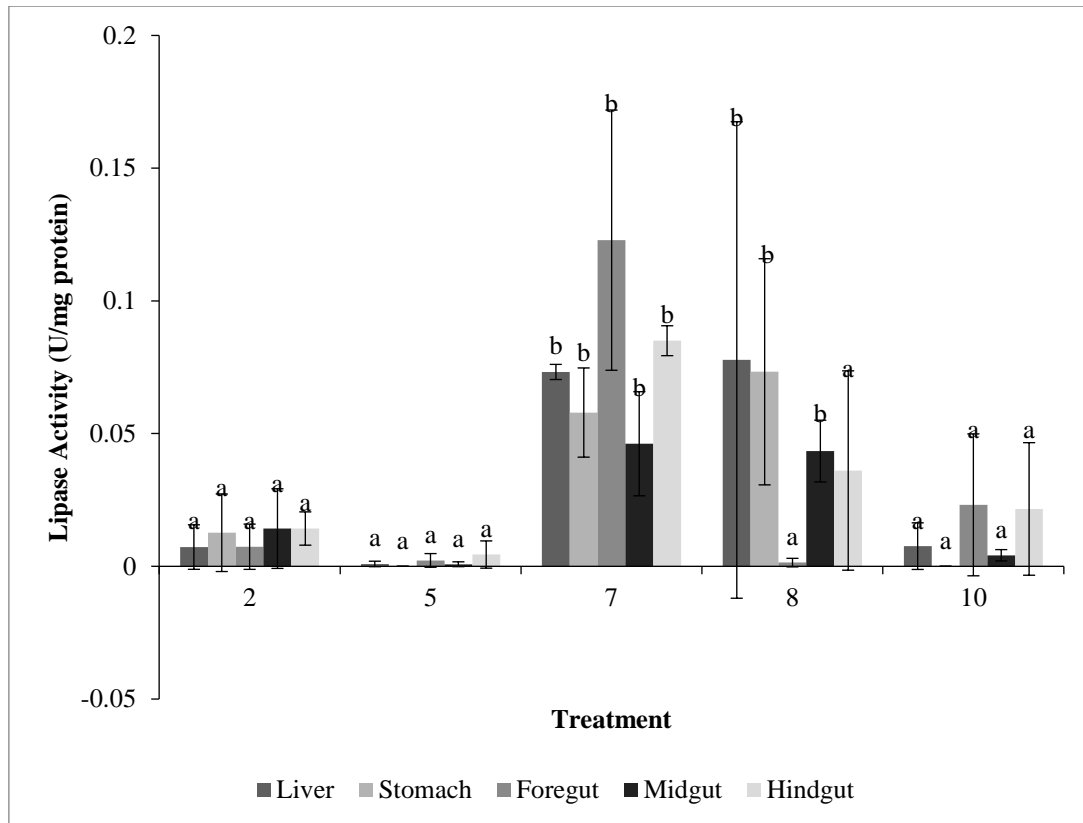


Figure 1. Initial Lipase Activities Value with the different superscript in the figure showed there was significant differences between treatments (Mean±SD, n=P<0.05).

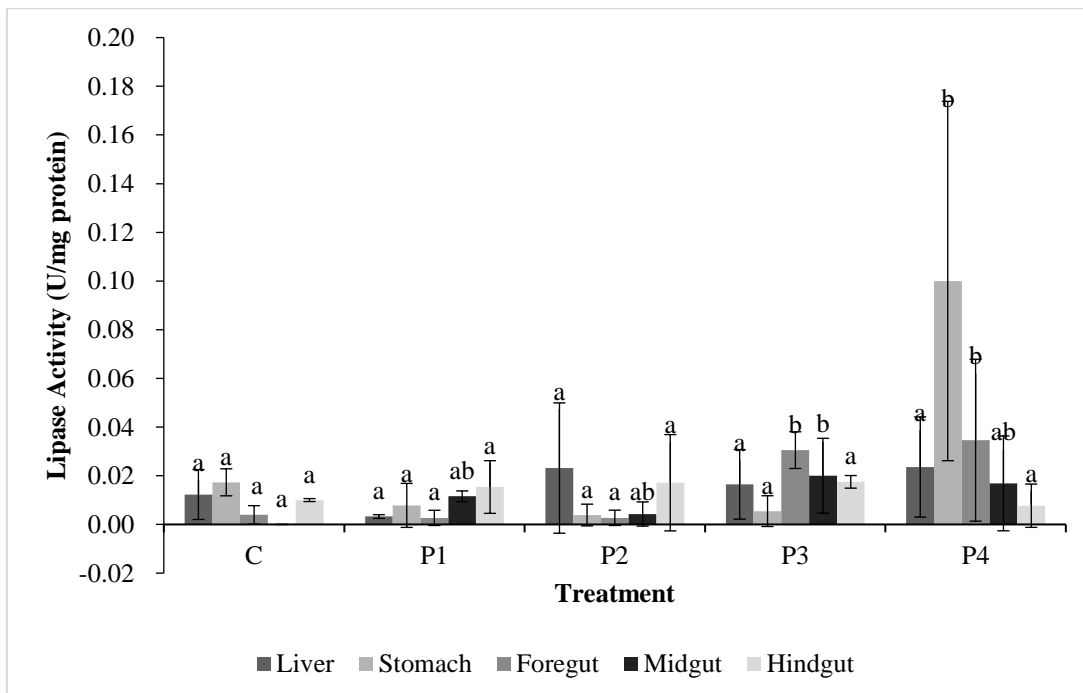


Figure 2. Lipase Activity of Gourami at pH 2 Value with the different superscript in the figure showed there was significant differences between treatments (Mean±SD, n=P<0.05).

Lipase Enzyme Activity in Digestive Organ of Gourami (after treatment).

Lipase enzyme activity in the gourami digestive organ was examined following the accomplishment of feeding treatment using a buffer solution with a

different pH, namely pH 2, 5, 7, 8 and 10.

Lipase Activity at pH 2

The result of data analysis showed that lipase activity of gourami at pH 2 was significantly different

between treatments ($P < 0.05$). The highest lipase activity was in the stomach of gourami that was given combination supplementation of *Spirulina platensis* 2 g.kg⁻¹ + *Chlorella vulgaris* 3 g.kg⁻¹ feed (Figure 2).

Lipase Activity at pH 5

The data showed a significant difference ($P < 0.05$) of lipase activity at pH 5. The highest lipase activity was in the stomach of gourami that was given combination supplementation with *Spirulina platensis* 3 g.kg⁻¹ + *Chlorella vulgaris* 2 g.kg⁻¹ feed (Figure 3).

Lipase Activity at pH 7

The data showed that lipase activity of gourami at

pH 7 was significantly different between treatments ($P < 0.05$, Figure 4.).

Lipase Activity at pH 8

The results of the study on the lipase activity of gourami at pH 8 showed that the lipase activity was significantly different between treatments ($P < 0.05$, Figure 5.).

Lipase Activity at pH 10

The analysis showed that at pH 10, lipase activity was significantly different between treatments ($P < 0.05$, Figure 6.).

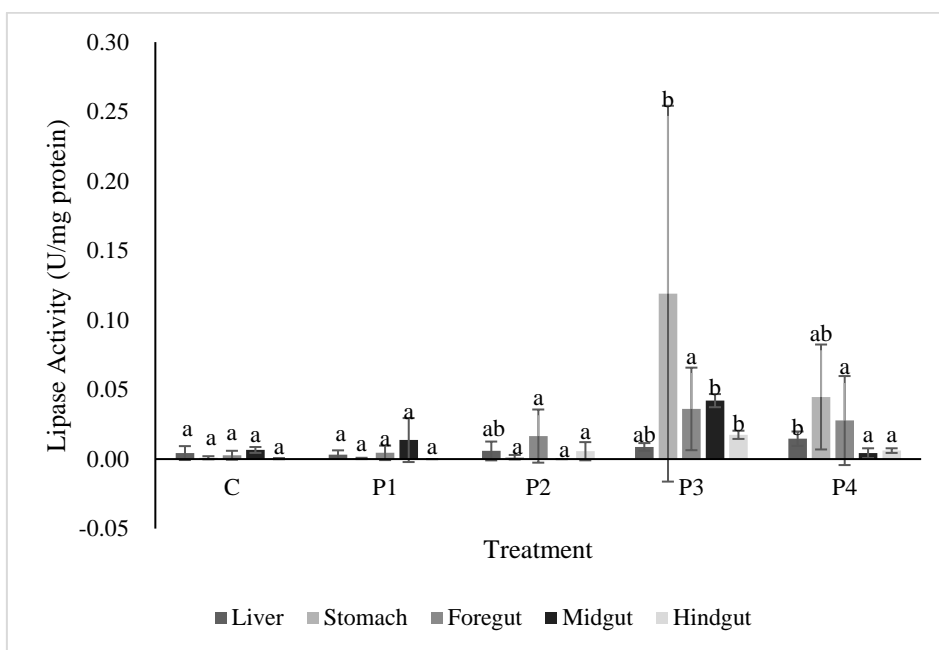


Figure 3. Lipase Activity of Gourami at pH 5 Value with the different superscript in the figure showed there was significant differences between treatments (Mean±SD, n=P<0.05).

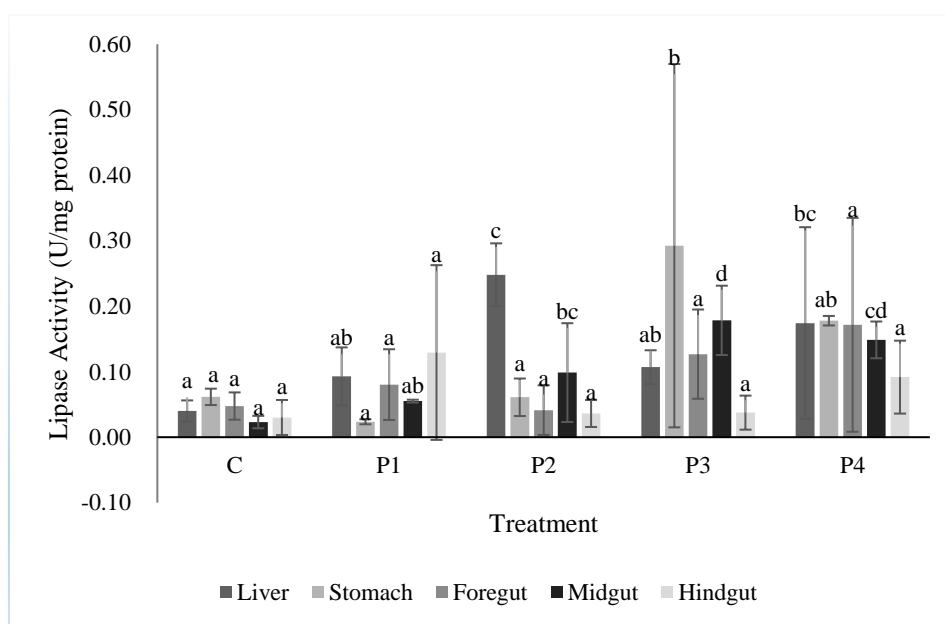


Figure 4. Lipase Activity of Gourami at pH 7 Value with the different superscript in the figure showed there was significant differences between treatments (Mean±SD, n=P<0.05).

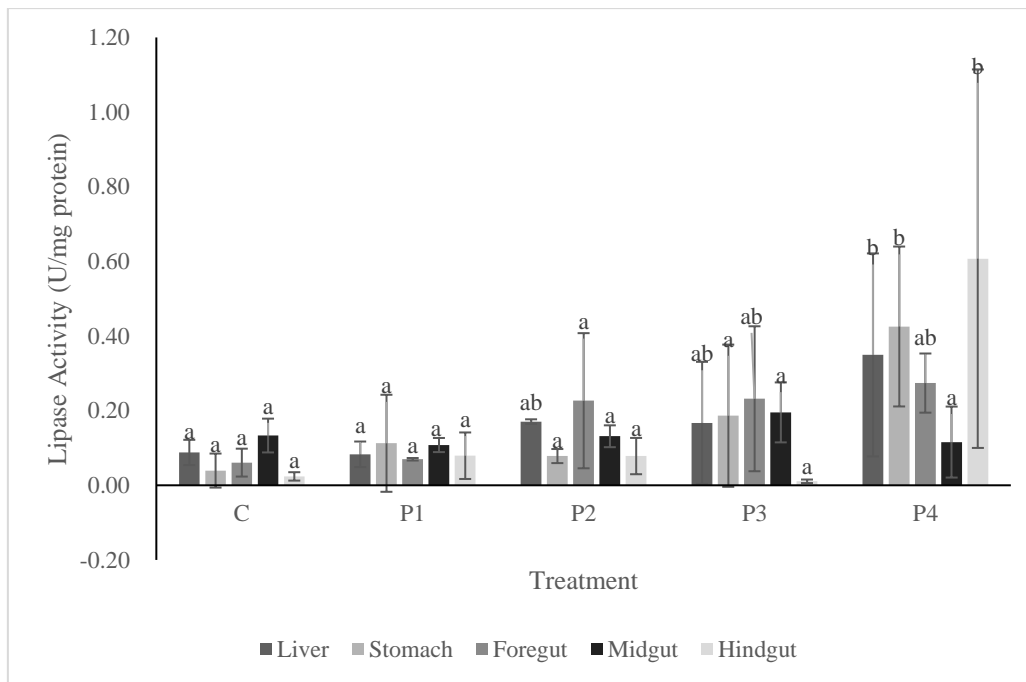


Figure 5. Lipase Activity of Gourami in pH 8 Value with the different superscript in the figure showed there was significant differences between treatments (Mean±SD, n=P<0.05).

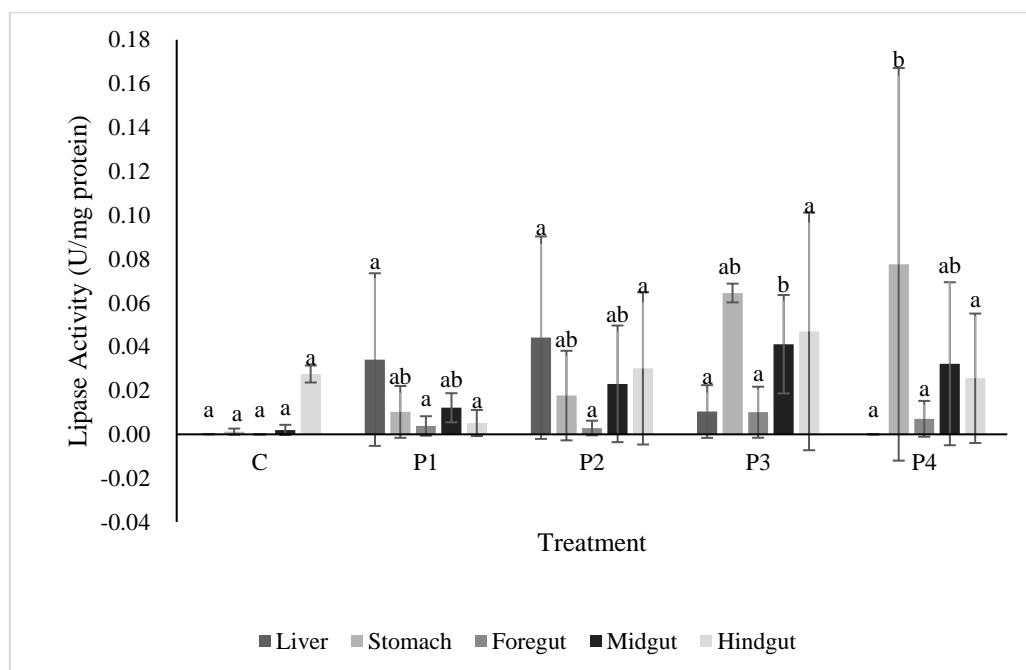


Figure 6. Lipase Activity of Gourami in pH 10 Value with the different superscript in the figure showed there was significant differences between treatments (Mean±SD, n=P<0.05).

Digestive membranes of gourami might undergo changes due to disruption of synthesis and translocation of enzymes on the mucosal surface owing to the influence of harmful chemical and physical factors (Gera, Kiran, & Mahmood, 2009). Enzyme synthesis systems from various parts of the intestine were influenced by exogenous and endogenous factors. The digestion of food was accelerated by a catalyst that is the digestion enzyme.

With increased lipase activity in some digestive organs compared to control, it can be said that supplementation of *S. platensis* and *C. vulgaris* in gourami improves the performance of digestive enzymes in digestive tract. This confirms the findings reported by Liu et al. (2016.).

German, Sung, Jhaveri, & Agnihotri (2015), reported that the activity of herbivorous fish enzymes is closely related to digestive physiology. Wu et al.

(2012), reported that long term (56 days) consumption of probiotic *Bacillus subtilis* Ch9 did not significantly increase fish digestive enzyme activity. However, the present study showed that lipase activity performed effectively in the liver, stomach, foregut, midgut and hindgut (pH 7) whereas at pH 8 it also highly effective in all organs but midgut (**Figure 1**. $P < 0.05$).

Supplementation of green blue algae and green algae in the diet enhance the performance of the lipase enzyme (**Figures 2-6**). In controls, the buffer pH 2-10 did not improve lipase activity (**Figure 2-6**, $P > 0.05$). At pH 7 (**Figure 4**.), *C. vulgaris* 4 g.kg⁻¹ supplementation treatment result in the highest lipase activity in the liver compared to other treatments. This is because lipase has not been secreted to other organs. In the treatment of *S. platensis* supplementation 6 g.kg⁻¹ feed + *C. vulgaris* 4 g.kg⁻¹ feed, lipase has been used to digest feed and has arrived at midgut. When viewed from the lipase activity at each pH shows that the highest lipase activity at pH 7 was in the stomach (**Figure 4**), the highest lipase activity at pH 8 was in the hindgut (**Figure 5**) and the highest lipase activity at pH 10 was in the stomach (**Figure 6**).

The results showed that supplementation of *S. platensis* and *C. vulgaris* in feed improved the performance of the lipase enzyme. Lipase activity perform excellently at pH 7 and pH 8. This dissimilarity most probably caused by differences of the composition of feed supplementation consumed by the experimental fishes. According to the study of Al-Saraji & Nasir (2013), on *Cyprinus carpio* fish, the differences in composition of proteins, fats and carbohydrates resulted in significant differences in the activity of digestive enzymes.

Spirulina platensis contains long-chain unsaturated fatty acids (PUFAs) (Lin et al., 2007), Gamma Linoleic Acid (GLA) and enzymes (Demir & Tukul, 2010) which evidently improve the performance of digestive enzymes. Improved digestion enzyme activity presumably also affect the growth and immunity of fish. Chlorella hot water extract contains Chlorella Growth Factor (CGF) which is rich in amino acids, peptides, vitamins, minerals and nucleic acids. Diets containing CGF might promote growth (Merchant & Andre, 2001), and probably controlling body weight comparable to that observed in rats (Hidaka, Okamoto, & Arita, 2004.).

For fish that do not have a stomach such as the Halfbeak fish (*Zenarchopterus buffonis*), which are grouped into herbivores, α -amylase digestion shows the highest enzymatic activity, followed by lipase and lowest proteases along the digestive tract (Diana et al., 2016). Gourami has a stomach, high lipase activity at various pH buffers and at various feed supplementation compositions.

Liu, Zhang, and Wang (2010), from a study reported that the activity of various digestive enzymes

of larvae of sheatfish (*Silurus soldatovi*) was influenced by age. Foods containing cottonseeds are effective in increasing the activity of digestive enzymes in juvenile *Labeo rohita* (Iqbal et al., 2016). In carnivorous fish, lipase enzyme activity is higher than that of herbivorous fish (Langeland, Lindberg, and Lundh, 2013).

Hematological Parameters

The average results of the calculation of the hematological parameters of gourami fed experimental are shown in **Table 1**. ($p < 0.05$). Hematology of animals are influenced by several things, including: feed, age, sex, temperature and others. Feeding strategy also affects the fish hematological aspect. In this study, differences in the composition of feed supplemented with *S. platensis* and *C. vulgaris* affected the hematological gourami. Supplemental feeding with *S. platensis* and *C. vulgaris* in gourami improved hematological parameters compared to gourami fed without supplementation (control). The combination of supplementation of *S. platensis* 2 g.kg⁻¹ + *C. vulgaris* 3 g.kg⁻¹ feed enhanced all hematological parameters (erythrocytes, leukocytes, Hb, Hct) of gourami (**Table 1**.).

This is in accordance with the results of study conducted by Simanjuntak et al. (2018), that showed the differences in the level of *S. platensis* supplementation in feeds influenced hematological parameters of gourami. Other studies on gourami which were given a starvation treatment with *S. platensis* supplemented feed showed that increase hematological was obtained in gourami fed *S. platensis* supplementation every day (Simanjuntak, Wibowo, & Indarmawan, 2016).

Research Rahmati, Falahatkar, & Khara, 2019 reported that hemoglobin content and hematocrit did not seem to be affected by starvation. This authors applied short and long term period of starvation: 2 week starvation, 3 weeks starvation and 6 weeks starvation. Six weeks starvation without refeeding significantly increase red blood counts & white blood counts. The longer the starvation the lower the lipid contents since this is mobilized to produce energy during lack of nutrient input. Hemoglobin counts also influenced by feed protein content, while hematocrit contents affected by moisture content, this shall increase when the fish is dehydrated (Yanuhar, Raharjo, Caesar, & Junirahma, 2021). However, these authors observed that 6 weeks (long term) starvation without refeeding throughout the experiment increased red blood counts and white blood counts. The RBCs change might be associated with metabolic level and immune status. Inconsistent results occur in the scientific literature concerning the effects of starvation on blood hemoglobin content and hematocrit values. These could be due to different method applied on experiment regarding period of feed deprivation.

Table 1. Hematological of gourami fed with experimental diets.

Parameters	Treatment				
	C	P1	P2	P3	P4
RBCs (cells/mm ³)x 10 ⁶	1.57 ± 0.06 ^a	1.75 ± 0.03 ^{ab}	1.85 ± 0.05 ^{bc}	1.98 ± 0.05 ^{cd}	2.17 ± 0.16 ^d
WBCs (cells/mm ³)x10 ⁵	1.00 ± 0.04 ^a	1.39 ± 0.21 ^a	1.47 ± 0.16 ^b	1.88 ± 0.11 ^b	2.01 ± 0.09 ^c
Hemoglobin (Hb, g/dl)	7.3 ± 0.88 ^a	7.8 ± 0.55 ^{ab}	7.8 ± 0.46 ^b	7.9 ± 0.39 ^b	9.2 ± 0.48 ^b
Hematocrit (Hct, %)	36.87 ± 1.89 ^a	41.62 ± 1.93 ^b	43 ± 1.08 ^b	42.5 ± 0.58 ^b	47.5 ± 2.48 ^c

Note : C = (control, commercial feed); P1 = *Spirulina platensis* supplementation 6 g kg⁻¹ feed; P2 = *Chlorella vulgaris* supplementation 4 g kg⁻¹ feed; P3 = *Spirulina platensis* supplementation 3 g kg⁻¹ feed + *Chlorella vulgaris* 2 g kg⁻¹ feed and P4 = *Spirulina platensis* supplementation 2 g kg⁻¹ feed + *Chlorella vulgaris* 3 g kg⁻¹ feed. RBC: Red blood cells, WBC: White blood cells
Different superscripts in the same column signify statistical differences (P<0.05) (mean ± S.D.).

Rainbow trout (*Oncorhynchus mykiss*) fed with probiotic, *Lactobacillus plantarum* and immunized with the bivalent streptococcosis / lactococcosis vaccine did not show significant differences (P> 0.05) in red blood cell (RBC), Hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) levels, but white blood cell count (WBC) in vaccinated as well as in that fed with probiotic and vaccinated were significantly higher than in both fed with feed supplemented with probiotic and unvaccinated fish fed normal diet (Soltani, Kane, Taheri-Mirghaed, Pakzad, & Hosseini-Shekarabi, 2019).

The number of red blood cells and white blood cell counts indicate the health status of animals including gourami. The types of leukocytes have an important role in fish immunity. Supplementation of ingredients that contain high nutritional value also increase fish immunity. *Chlorella vulgaris* is rich in nutrients, contains chlorellin which might function as an antibiotic and antimicrobial. Feeding with high protein can increase the number of red blood cells (Nasir & Al-Sraji, 2013).

Chlorella sp. has been reported as the most suitable photoautotrophic microalgae for biofuel production due to its high productivity of fatty acids relevant to transesterification reaction (Hempel, Petrick, & Behrendt, 2012). These species are photosynthetic single cell green algae which are also used for body detox and human nutrition. Juvenile *Labeo rohita* which is given a non protein diet is effective in increasing hematological (Iqbal et al., 2016).

Supplementation of *S. platensis* in feed African catfish (*Clarias gariepinus*) has been reported to improve fish haematology (Raji et al., 2018). *Spirulina*

platensis supplementation at 10% by weight of feed Rainbow trout (*Oncorhynchus mykiss*) increases hematology (Yeganeh, Teimouri, and Amirkolaie, 2015) and increases the number of leukocytes of Great sturgeon fish (*Huso huso* L.) (Adel, Yeganeh, Dadar, Sakai, & Dawood, 2016). Hematology of *Clarias gariepinus* fed with *Spirulina platensis* supplementation increased compared with stress and control fish (Sayed & Fawzy, 2014).

Blood Biochemistry

The average results of the calculation of the blood biochemistry of gourami fed experimental are shown in **Table 2.** (p <0.05). The result above showed that supplementation of *S. platensis* and *C. vulgaris* in feed gave significant differences in the biochemistry of blood serum levels (P<0.05). Total protein levels, globulin levels, and albumin: globulin ratio of gourami fed with supplemented feed enhanced, but albumin levels were not affected. **Table 2.** shows that the highest enhancement in total protein levels, globulin levels, and albumin: globulin ratio of gourami has the highest increase when using combination supplementation of *S. platensis* 2 g.kg⁻¹ feed + *C. vulgaris* 3 g.kg⁻¹ feed.

Biochemical of blood serum (total protein, albumin and globulin) has an important role in detecting fish health (Yang, Guo, Ye, Zhang, & Wang, 2015). Serum biochemical range varies from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal patterns, food, age and sex of fish (Jawad, Al-Mukhtar, & Ahmed, 2004). Increased plasma protein concentrations can be caused by structural changes in the liver that reduce aminotransferase activity, with a concomitant reduction in deamination capacity (Kavadias, Castritsi-Catharios, & Dessypris, 2003).

Table 2. Blood biochemical of gourami fed with experimental diets.

Treatments	Parameters (g/dL)			
	Total Protein	Albumin	Globulin	A : G ratio
C	6.18 ± 0.76 ^a	2.73 ± 0.51 ^a	3.45 ± 0.20 ^a	0.78 ± 0.12 ^a
P1	5.83 ± 0.62 ^{ab}	2.79 ± 0.44 ^a	2.86 ± 0.46 ^b	0.97 ± 0.03 ^b
P2	5.68 ± 0.19 ^{ab}	2.91 ± 0.10 ^a	2.72 ± 0.12 ^{bc}	1.08 ± 0.03 ^c
P3	5.20 ± 0.08 ^{ab}	2.89 ± 0.12 ^a	2.32 ± 0.06 ^{cd}	1.24 ± 0.03 ^c
P4	5.30 ± 0.14 ^b	3.08 ± 0.12 ^a	2.19 ± 0.03 ^d	1.42 ± 0.07 ^d

Note : C = Control, commercial feed; P1 = *Spirulina platensis* supplementation 6 g kg⁻¹ feed; P2 = *Chlorella vulgaris* supplementation 4 g kg⁻¹ feed; P3 = *Spirulina platensis* supplementation 3 g kg⁻¹ feed + *Chlorella vulgaris* 2 g kg⁻¹ feed and P4 = *Spirulina platensis* supplementation 2 g kg⁻¹ feed + *Chlorella vulgaris* 3 g kg⁻¹ feed. Different superscripts in the same column signify statistical differences (P<0.05) (mean ± S.D.).

This study found that supplementation of *S. platensis* and *C. vulgaris* in feed increased blood biochemical. This increase in blood biochemical is due to *S. platensis* and *C. vulgaris* containing high protein. This is in accordance with Nasir and Al-Sraji (2013), who said that feeding with a high protein diet will increase total blood protein. *Spirulina platensis* supplementation 5 g.kg⁻¹ increases total protein levels, albumin, and globulin compared to control (Abdel-Tawwab & Mohammad, 2009). Increased blood protein levels due to antioxidants and enzyme activity that improve fish health (Hoseini, Yousefi, Hoseinifar, & Doan, 2019). Increased blood biochemistry indicates an increase in non-specific immune responses (El-Asely, Abbass, & Austin, 2014).

Chlorella vulgaris can regulate the innate immune system and adaptive immune system in the gible carp (Zhang, Gao, Qiu, Shao, Xu, & Qi, 2014). Green algae *C. vulgaris* also contains polysaccharide compounds that function in resistance to toxins (Liu *et al.*, 2016) and *Spirulina platensis* contain C-phycoyanin which can be used as functional food (Liu, Huang, Zhang, Cai, & Cai, 2016). The administration of *C. vulgaris* extract as an exogenous antioxidant in white rats induced with Carbon tetrachloride (CCl₄) can protect from oxidative stress. This is indicated by decreased Malondialdehyde (MDA) activity and increased Super oxide dismutase (SOD) and Glutathione Peroxidase (GPx) activity (Hernayanti & Simanjuntak, 2019).

Albumin/Globulin ratio is an indicator of fish health. Albumin / Globulin ratio between 0.7-1.18 g.dL⁻¹ is the normal ratio in teleostei fish (Rehulka, 1993). The results showed that supplementation of *S. platensis* and *C. vulgaris* in gourami feed increased the A / G ratio (Table 2, P <0.05). This can be caused by the antioxidant β-carotene contained in *S. platensis*

and chlorellin contained in *C. vulgaris*. Fish feed containing good fatty acids can affect immune cell function and disease resistance (Wang, Pan, Sheng, Xu, & Hu, 2007; Lin *et al.*, 2007). Diets containing CGF can stimulating immune system (An *et al.*, 2010) and serum lipid (Hidaka *et al.*, 2004). However, information concerning the effect of *S. platensis* and *C. vulgaris* supplementation in feed on immunity of some aquaculture species including gourami (*Osphronemus gouramy* Lac.) remains limited.

CONCLUSIONS

In conclusion, our results indicate that supplementation of *Spirulina platensis* and *Chlorella vulgaris* has a significant physiological effect on gourami (*Osphronemus gouramy* Lac.). The main effects are increased activity of the lipase enzyme and increased immunity. Therefore, it is necessary to optimize the feeding strategy during maintenance conditions to enhance gourami growth and immunity.

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