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Body Composition and Digestive Enzyme Activity of Hard-Lipped Barb (*Osteochilus vittatus*) Feed with Supplementation of *Spirulina platensis* and *Chlorella vulgaris*

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ABSTRACT. Cultivation of hard-lipped barb (*Osteochilus vittatus*) increased in the district of Banyumas, Indonesia. Supplementation *Spirulina platensis* and *Chlorella vulgaris* has lots utilized in field aquaculture as high nutritious food to increase fish production. Evaluation digestibility to *S. platensis* and *C. vulgaris in* hard-lipped barb fish can done with measurement composition body and activity enzyme digestion. The purpose of this study was to know the influence of supplementation of *S. platensis* and *C. vulgaris* and to find the most appropriate composition of supplementation to enhance body composition and digestive enzyme activity of hard-lipped barb. The study was conducted experimentally with completely randomized design (CRD) and consisting of five Treatments: P0 = without supplementation as control; P1 = *S. platensis* 6 g.kg^{-1}; P2 = *C. vulgaris* 4 g.kg^{-1}; P3 = (*S. platensis* 3 g + *C. vulgaris* 2 g).kg^{-1}; and P4 = (*S. platensis* 2 g + *C. vulgaris* 3 g).kg^{-1}, in four replicates. The results showed *S. platensis* and *C. vulgaris* increase hard-lipped barb protein, fat and fiber levels. *C. vulgaris* and combinations of *S. platensis* + *C. vulgaris* enhance activity *trypsin-like,* lipase, amylase, and alkaline phosphatase in foregut, hindgut, and hepatopancreas. *C. vulgaris* and *S. platensis* can be given as supplement of hard-lipped barb feed to enhance enzyme activity that contributing digestion to enhance body composition.

Keywords: body composition*, Chlorella* vulgaris, enzyme, *Osteochilus vittatus*, *Spirulina platensis*

INTRODUCTION

One of the native species of Indonesia, the hard-lipped barb (*O. vittatus*), was begin to increase significantly in Banyumas (BPS, 2022). Hard-lipped barb fish are lots of interest by the public because their high nutrients and relatively affordable prices. Giving the fish high-nutrient feed will promote fish growth and development of hard-lipped barb fish cultivation (Sa'adah et al., 2023).

Few study about inclusion microalgae in fish feed has carried out (Galal et al., 2018). *Spirulina platensis* (*S. platensis*) and *Chlorella vulgaris* (*C. vulgaris*) are the microalgae that become base chain food in waters and become source food for any fish (Simanjuntak et al., 2022; Galal et al., 2018). Component nutrients contained in *S. platensis* and *C. vulgaris* can digested by the fish, can be used as feed supplement to promote fish growth (Raji et al., 2020).

Spirulina platensis is a Cyanobacteria, autotrophic, spiral shaped, and can grows in fresh water, lakes, and land (Christwardana et al., 2013). *Spirulina platensis* is abundant cultivated because the content of high nutrition (Ansari et al., 2021), including protein (55-70%), minerals (3-7%), essential amino acids (1.3-15%), fatty acids (6-6.5%) such as Gamma Linoleic Acid (GLA), palmitic acid, and oleic acid. *S. platensis* also contain chlorophyll (0.8%), phycocyanin (6.7-11.7%), carotenoids (0.43%), zeaxanthin (0.1%), water (3-6%) (Christwardana et al., 2013), iron, glycogen, rhamnose, vitamins, more of 2000 active enzymes, energy-rich flour, and active components such as flavonoids, steroids, saponins and phenols hydroquinone (Raji et al., 2018).

Chlorella vulgaris is a green algae, unicellular, growing in waters bid (Galal et al., 2018), round in shape with a diameter of around 2-10 μm, and has a cell wall (Safi et al., 2014). *Chlorella vulgaris* in class Chlorophyceae and already cultivated as natural feed of fish (Ansari et al., 2021) because the content of macronutrients such as protein (37.5%), fat (14.4%), and carbohydrates (26.6%) (Viegas et al., 2021) and micronutrients like polysaccharides, α-carotene, β-carotene, minerals (calcium, zinc, magnesium, manganese, copper, and iron), vitamins (B, C, D, E, and K), pro-vitamins, chlorophyll, lutein (Galal et al., 2018), amino acid essentials, fatty acids (DHA, EPA, ALA), and compounds bioactive *Chlorella Growth Factor* (CGF) (Ansari et al., 2021).

Spirulina platensis and *C. vulgaris* have many benefits in aquaculture such as promoting growth and body composition in various fish such as gourami (*Osphronemus gouramy*), catfish (*Clarias gariepinus*), and nile tilapia (*Oreochromis niloticus*). (Simanjuntak et al., 2018; A. Raji et al., 2020; Peng et al., 2020).

The nutrients contained in *S. platensis* and *C. vulgaris* can be digested and utilized by fish, thus encouraging growth which characterized by increased body composition. The digestive process is carried out by enzymes (Faheem et al., 2022).

Enzyme digestion is one of important indicator to give information about digestive physiology status as response to feed. Jiao et al., (2023) show that activity enzyme digestion can influenced by feeding habit because adaptation to availability food in the environment. Enzymes that play a role in digestive system such as trypsin, amylase, lipase (Faheem et al., 2022), and alkaline phosphatase (Faccioli et al., 2016). Supplementation of algae into fish feed can improve the nutritional content of the feed and this will enhance the activity of digestive enzymes (Simanjuntak et al., 2022).

Information about evaluation of the digestibility of *S. platensis* and *C. vulgaris* in hard-lipped barb fish (*O. vittatus*) to body composition and digestive enzyme activity, especially trypsin, lipase, amylase and alkaline phosphatase are not available. The purpose of this study to determine the effect of *S. platensis* and *C. vulgaris* in supplementation to body composition and enzyme activity of hard-lipped barb and to find the most appropriate composition of *S. platensis* and *C. vulgaris* supplementation to enhance body composition and digestive enzyme activity of hard-lipped barb (*O. vittatus*).

EXPERIMENTAL SECTION

Materials, Equipment and Tools

Materials used in this research were hard-lipped barb fish (*O. vittatus*) seruni varieties within size 9-12 cm and 3 months old, dried *S. platensis* and *C. vulgaris* (source: Center for Brackish Water Aquaculture Development, Jepara), pellets commercial pf 1000, clean water, label, ice cubes, distilled water, aquabides, silica gel, bovine serum albumin, reagent A (2% Na_2CO_3 in 0.1 N NaOH), reagent B (0.5% $CuSO_4.5H_2O$ in 1% Na-K tartate (Merck)), reagent C (copper alkaline), tris (hydroxymethyl) aminomethane 50 mM pH 7.6 (Merck), casein, trichloroacetic acid (TCA)(Merck), p-nitrophenylpalmitate (p-NNP)(Merck), sodium carbonate (Na_2CO_3), NaOH, isopropanol (Merck), starch, 3,5-dinitrosalicylic acid (DNS)(Merck), phosphate buffer ($NaH_2PO_4.H_2O$), HCl 1 N, HCl 37 %, glycine, magnesium chloride ($MgCl_2$), Folin-Ciocalteu (Merck) and nitrophenyl phosphate.

The equipment and tools used in this research were cement fish pond size 1.5 m^3 , fishing net, aerator, thermometer, pH indicator, tray, covered-container plastic, analytical scale, millimeters block, surgical instruments, petri dish, beaker glass, measuring cylinder, bar stirrer, pipette, stationery, marker, eppendorf, centrifuge, vortex, tube reaction, rack tube, micropipette, blue tip, yellow tip, refrigerator, freezer –80 °C, gas stove, cuvette, and spectrophotometer.

Methods

Study done in a way experimental use design random complete consisting of five treatments with four replicates. Treatment for 84 days. The treatments consist:

P0 = Commercial pellets without supplementation (control)
P1 = Supplementation of *S. platensis* 6 $g.kg^{-1}$ feed
P2 = Supplementation of *C. vulgaris* 4 $g.kg^{-1}$ feed
P3 = Supplementation of (*S. platensis* 3 g + *C. vulgaris* 2 g)$.kg^{-1}$ feed
P4 = Supplementation of (*C. vulgaris* 3 g + *S.platensis* 2 g)$.kg^{-1}$ feed

Supplementation of *S. platensis* and *C. vulgaris*

Procedure supplementation referring to Simanjuntak et al., (2022). A total of 6 g of dried *S. platensis* (P1) was placed into beaker glass, then add 100 mL of distilled water and stir until homogeneous. 1 kg commercial pellets placed in the tray, supplement solution added to the pellets gradually and mixed until homogenous. Feed already supplemented then dried below the sun. After dried, cool to room temperature, then stored inside receptacle and given silica gel, then closed tightly. Feed is ready given to fish. Same procedure carried out in treatments P2, P3, and P4.

Experimental Fish

Hard-lipped barb fish were obtained from breeders in Beji village, Banyumas Regency, Central Java Province, Indonesia. Hard-lipped barb fish arrested in condition life as many as 200 with size range 9-12 cm. The total length and weigh of body of every fish measured, then prepared 10 each fish for every pool. Aerator in each pool used for supply oxygen in the water. Fish are acclimated inside pool for 7 days under laboratory conditions. Fish were given feed supplementation every days at 08.00 and 16.00 WIB as much as 5% of the total fish biomass in each pool for 84 days.

Measurements of Body Composition

Body composition was assessed by measuring the wet weight of fish. At 60 °C, fish samples without visceral (carcass) were dried in the oven for 7 days, weighed, and crushed. Approximately 10 g of each sample (P0-P4) were measured for body proximate (Sudarmadji et al., 1996). The proximate analysis followed the Maienthal (1974) guidelines and included the evaluation of moisture, protein, fat, ash, and Nitrogen Free Extract (NFE) content. Moisture content was determined using the warmup method in the oven at 105 °C for 4-6 hours. Protein and fat content were measured using semi-micro Kjedahl and Soxhlet methods, respectively. Ash content was measured with the heating method at 450 °C for 4-6 hours, while NFE levels were determined using the following formula.

$$\text{NFE (\%)} = 100\% - (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Fiber} + \text{Ash})\%.$$

The composition body consists of protein, fat, fiber, ash, and NFE. The composition body is calculated with the formula as follows:

$$\text{Protein (\%)} = \frac{\text{protein proximate (\%) X dry weigh (\%)}}{100}$$

$$\text{Fat (\%)} = \frac{\text{fat proximate (\%) X dry weigh (\%)}}{100}$$

$$\text{Fiber (\%)} = \frac{\text{fiber proximate (\%) X dry weigh (\%)}}{100}$$

$$\text{Ash (\%)} = \frac{\text{ash proximate (\%) X dry weigh (\%)}}{100}$$

$$\text{NFE (\%)} = \frac{\text{NFE proximate (\%) X dry weigh (\%)}}{100}$$

Measurments of Digestive Enzyme Activity

The preparation of the enzyme followed the method described by Susilo et al. (2013). Digestive organs were isolated using surgical instruments on a petri dish placed over ice cubes. The organs were partitioned into three parts namely the foregut, hindgut, and hepatopancreas, with each section homogenized in a cold 50 mM Tris-HCl solution at a 1:4 mass-to-solution ratio. The homogenate was poured into Eppendorf, and centrifuged at 12000 rpm for 10 minutes, and the resulting supernatant was stored in the freezer at –80 °C after labeling.

Protein content in the supernatant was quantified using Lowry's method (1951) before enzyme activity analysis. The trypsin-like activity was evaluated with the Folin-Ciocalteu method (Rick, 1974) using a 1% casein substrate. Amylase activity was assessed through the starch hydrolysis method (*3,5*-dinitro salicylic acid) following (Areekijseree et al., 2006) with 1% starch substrate. Lipase activity was measured using the hydrolysis method of para-nitrophenyl palmitate (p-NNP) (Markweg-Hanke et al., 1995). Alkaline phosphatase activity was analyzed based on p-nitrophenyl phosphate hydrolysis (Walter and Schutt, 1974).

Data Analysis

Body composition body and digestive enzyme activity analyzed with one way analysis of variance (ANOVA) with error level 5% (P<0.05). Duncan analysis was using to compare the differences among the treatments. Data analysis using device SPSS 23.0 version for Windows.

RESULTS AND DISCUSSION

Body Composition of *Osteochilus vittatus*

The results showed that supplementation of *S. platensis* and *C. vulgaris* significantly affected the body composition of *O. vittatus* (P<0.05), especially in protein, fat, and fiber levels compared to control (**Table 1**).

Supplementation of *S. platensis* 6 g.kg^{-1} and *C. vulgaris* 4 g.kg^{-1} increased the protein and fat levels of the body of hard-lipped barb compared to the control in this study (**Table 1**). Supplementation of *S. platensis* has also been shown to increase the protein and fat levels of the body of gourami (*Osphronemus gouramy*) (Simanjuntak et al., 2018) and catfish (*Clarias gariepinus*) (Raji et al., 2020).

Similar results were also shown by Raji et al. (2020) and Eissa et al., (2024) that *C. vulgaris* supplementation also increased protein levels in catfish (*Clarias gariepinus*) and Pacific white shrimp (*Litopenaeus vannamei*). *C. vulgaris* has been reported to increase body fat levels in tilapia (*Oreochromis niloticus*) and sea bass (*Micropterus salmoides*) (Peng et al., 2020; Xi et al., 2022).

The fiber content in the combination supplementation (*S. platensis* 3 g + *C.vulgaris* 2 g).kg^{-1} and (*S. platensis* 2 g + *C. vulgaris* 3 g).kg^{-1} were significantly different compared to the control (**Table 1**). Simanjuntak et al. (2018) reported that *S. platensis* can improve fiber levels of gourami. *Spirulina platensis* and *C. vulgaris* contain protein and essential amino acids, fat, and carbohydrates. Protein assimilation in the body depends on the amino acid content derived from the breakdown of protein and essential amino acids contained in *S. platensis* and *C. vulgaris*. The fat and fatty acid content in *S. platensis* and *C.vulgaris* also contributes to increasing body fat levels, while the carbohydrate, starch, and other polysaccharide content contributes to the assimilation of fiber levels in the body of hard-lipped barb (A. Raji et al., 2020; Alagawany et al., 2021; Xi et al., 2022). This shows that the nutrients contained in *S. platensis* and *C. vulgaris* can be digested and utilized by hard-lipped barb, thereby contributing to improving body composition.

Table 1. Body composition of *O. vittatus* after treatment

Parameters (%)	Treatments				
	P0	P1	P2	P3	P4
Moisture	78.09 ± 2.36	76.2 ± 1.34	77.25 ± 2.29	77.73 ± 0.76	78.29 ± 2.36
Protein	10.88 ± 0.06^{b}	12.07 ± 0.03^{c}	12.62 ± 0.33^{c}	11.04 ± 0.01^{b}	10.25 ± 0.04^{a}
Fat	3.31 ± 0.08^{a}	3.82 ± 0.01^{b}	4.49 ± 0.03^{c}	4.42 ± 0.11^{c}	5.55 ± 0.01^{d}
Fiber	0.51 ± 0.05^{a}	0.48 ± 0.01^{a}	0.63 ± 0.13^{ab}	0.80 ± 0.01^{b}	0.51 ± 0.07^{a}
Ash	4.02 ± 0.10^{d}	3.88 ± 0.00^{d}	3.13 ± 0.01^{b}	3.48 ± 0.10^{c}	2.66 ± 0.12^{a}
NFE	3.20 ± 0.09^{cd}	3.55 ± 0.04^{d}	1.88 ± 0.17^{a}	2.53 ± 0.21^{b}	2.73 ± 0.23^{bc}

*superscripts indicate significant differences between treatments in the column, $\bar{x}$±SD, n=4, (P<0.05)

Enzyme Activity of Hard-lipped barb

The results showed that supplementation of *S. platensis* and *C. vulgaris* significantly affected on trypsin-like, lipase, amylase, and alkaline phosphatase activity of hard-lipped barb (P<0.05). Trypsin-like activity in this study has found in the foregut, hindgut, and hepatopancreas. Trypsin-like activity is also found in various digestive organs in other fish such as Atlantic salmon (*Salmo salar*) (Martínez-Llorens et al., 2021), grass carp (*Ctenopharyngodon idellus*), common carp (*Carassius auratus*), mandarin fish (*Siniperca chuatsi*), and cunming trout (*Schizothorax grahami*) (Jiao et al., 2023).

Supplementation of *C. vulgaris* 4 g.kg^{-1} significantly different compared to control and other treatments in trypsin-like activity (**Figure 1**). Pakravan et al., (2018) and Peng et al., (2020) also reported that *C. vulgaris* supplementation increased trypsin activity in the intestines of white shrimp (*Litopenaeus vannamei*) and tilapia (*Oreochromis niloticus*). The increase of trypsin-like activity in this study was a response to food sources and nutrient concentrations contained in the feed, especially protein. Supplementation of *S. platensis* and *C. vulgaris* can enhance protein content in feed. Trypsin will break down protein into amino acids. These amino acids then used to produce more endogenous digestive enzymes such as trypsin, so that activity can be increased (Hasanein et al., 2018; Peng et al., 2020).

The low trypsin-like activity is not comparable to the high protein content of the tilapia fish body in the treatment of *S. platensis* of 6 g.kg^{-1}. Arslan et al., (2013) showed similar results that high protein levels in South American catfish (*Pseudoplatystoma* sp.) were not followed by high trypsin activity. The digestion process does not only depend on digestive enzymes, but also on brush border enzymes that play a role in absorbing nutrients such as creatine kinase and Na-K ATPase. Research by Jiang et al., (2015) on Jian carp (*Cyprinus carpio* var. Jian) fed with different protein and fat compositions revealed that the ability to digest protein will gradually decrease along with the increase in body weight of fish.

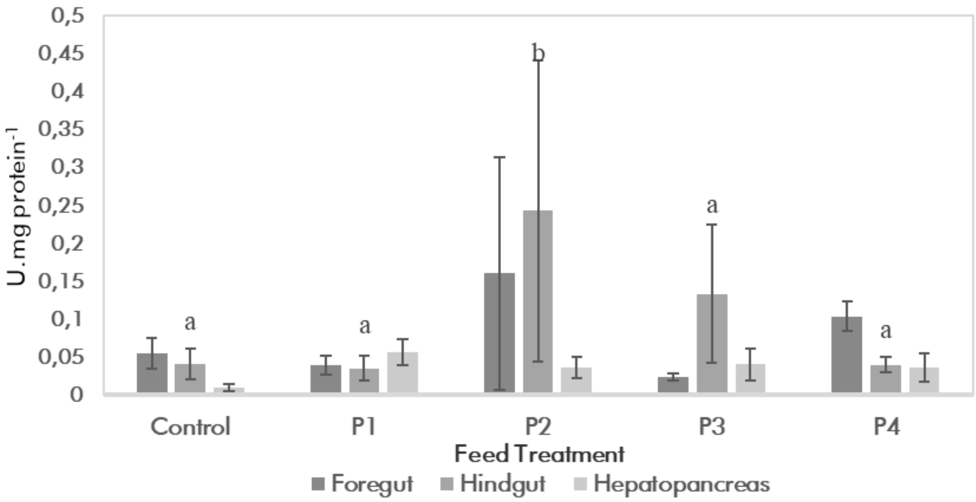

Figure 1. Trypsin-like Activity of Hard-lipped barb (*O. vittatus*). Superscripts indicate significant differences between treatments ($\bar{x}$±SD, n=4, P<0.05)

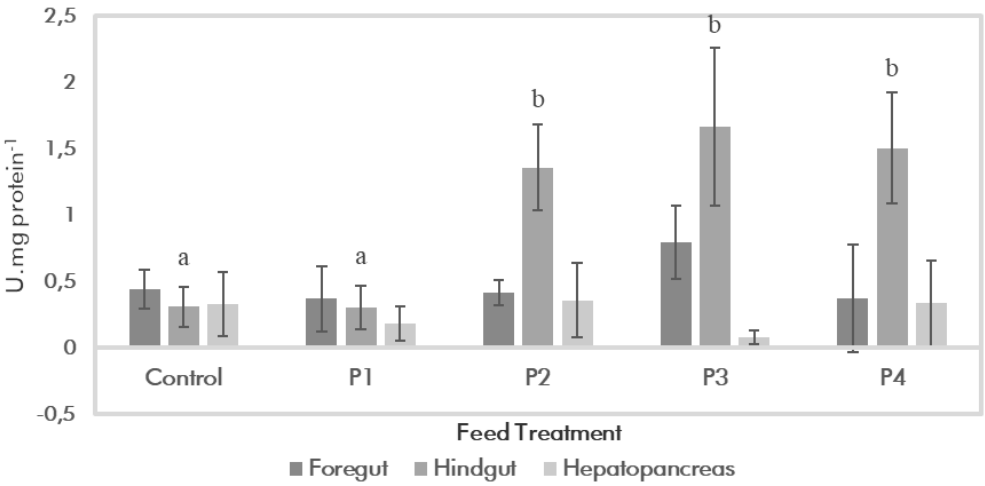

Figure 2. Lipase activity of hard-lipped bard (*O. vittatus*). Superscripts indicate significant differences between treatments ($\bar{x}$±SD, n=4, P<0.05)

This study also showed that supplementation of *S. platensis* and *C. vulgaris* had a significant effect on lipase activity of hard-lipped barb (P<0.05). Lipase activity in this study was found in all digestive organs. Simanjuntak et al. (2022) and Jiao et al., (2023) also found lipase activity in various digestive organs in several fish species. Supplementation of *C. vulgaris* 4 g.kg^{-1} feed and a combination of (*S. platensis* 3 g + *C. vulgaris* 2 g).kg^{-1} and (*S. platensis* 2 g + *C. vulgaris* 3 g).kg^{-1} showed significant differences compared to the control (**Figure 2**). Similar results were also reported by Simanjuntak et al. (2022) that supplementation of a combination of *S. platensis* and *C. vulgaris* with similar dose had higher lipase activity compared to the control and single supplementation of *S. platensis* 6 g.kg^{-1} feed. Research by Akbary & Malek Raeisi, (2020) and Peng et al., (2020) reported that single supplementation of *C. vulgaris* also increased lipase activity in flathead grey mullet (*Mugil cephalus*) and tilapia (*Oreochromis niloticus*).

Lipase activity supplemented with *S. platensis* and *C. vulgaris* can vary due to differences in nutrition in the supplemented feed consumed by fish (Simanjuntak et al., 2022; Alagawany et al., 2021). Different nutrient contents in feed, especially fat, cause differences in digestive enzyme activity, especially lipase (Asrami et al., 2020; Peng et al., 2020). Feeding habits also affect lipase activity in fish. Herbivorous and omnivorous fish generally have higher lipase activity than carnivorous and plankton-eating fish (Jiao et al., 2023). *O. vittatus* are herbivorous fish in their natural habitat and generally become omnivorous in cultivation (Pratiwi et al., 2011).

Amylase activity in hard-lipped barb fed with *S. platensis* and *C. vulgaris* supplementation showed significant results (P<0.05). The combination treatment of (*S. platensis* 3 g + *C. vulgaris* 2 g).kg^{-1} and (*S. platensis* 2 g + *C. vulgaris* 3 g).kg^{-1} showed significant differences compared to the control and single treatments (**Figure 3**). Asrami et al., (2020) and AlMulhim et al., (2023) reported that *S. platensis* supplementation in feed increased amylase activity in gourami (*Trichogaster lalius*) and tilapia (*O. niloticus*). The provision of *C. vulgaris* as feed has also been reported to increase amylase activity in carp (*Carassius auratus*) (Shi et al., 2019), flathead grey mullet (*Mugil cephalus*) (Akbary & Malek Raeisi, 2020), and tilapia (*O.niloticus*) (Peng et al., 2020).

Higher amylase activity in the supplementation treatment indicates that the carbohydrate and polysaccharide content in *S. platensis* and *C. vulgaris* can be digested on hard-lipped barb (Raji et al., 2020; Alagawany et al., 2021). Amylase activity in this study was only found in the foregut and hindgut, while it was not found in the hepatopancreas. Amylase activity in fish can be found in all digestive organs including the hepatopancreas, however mostly amylase activity in the hepatopancreas is lower than in the digestive tract (Khani et al., 2017; Jiao et al., 2023). This confirm that amylase activity in the hepatopancreas in this study was very low.

Alkaline phosphatase activity in this study can be found in the foregut, hindgut, and hepatopancreas with dominant activity in the foregut and hindgut (**Figure 4**). Study of (Martínez-Llorens et al., 2021) showed that alkaline phosphatase activity can be found from the pylorus, foregut, midgut to hindgut in Atlantic salmon (*Salmo salar*) with the highest activity in the pylorus and decreasing towards the posterior (hindgut).

Supplementation of *S. platensis* and *C. vulgaris* also had a significant effect on alkaline phosphatase activity (P<0.05). Supplementation of *C. vulgaris* 4 g.kg^{-1} and a combination of (*S. platensis* 3 g + *C. vulgaris* 2 g).kg^{-1} and (*S. platensis* 2 g + *C. vulgaris* 3 g).kg^{-1} were significantly different compared to the control (**Figure 4**). Research by Lin et al. (2016) reported that supplementation of *S. platensis* in feed increased alkaline phosphatase activity in pompano (*Trachinotus ovatus*). Carneiro et al., (2020) also reported similar results that supplementation of *Chlorella* sp. increased alkaline phosphatase activity in zebrafish (*Danio rerio*).

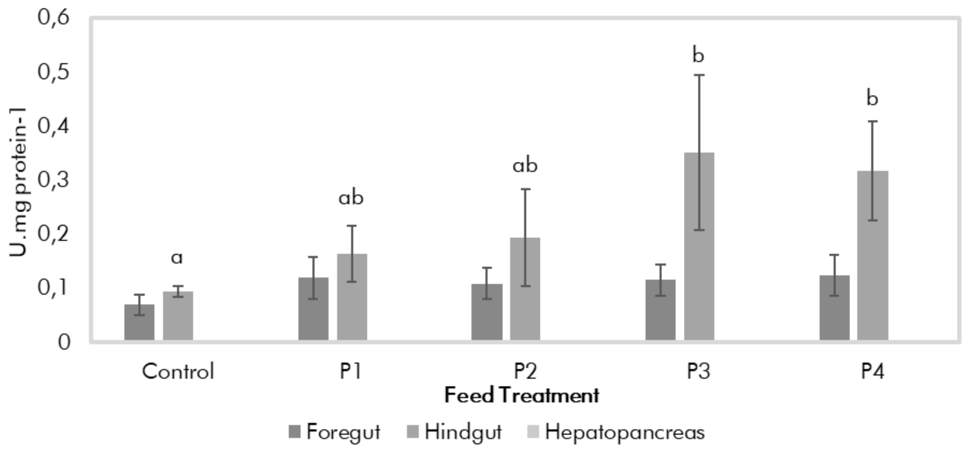

Figure 3. Amilase activity of hard-lipped barb (*O. vittatus*). Superscripts indicate significant differences between treatments ($\bar{x}$±SD, n=4, P<0.05)

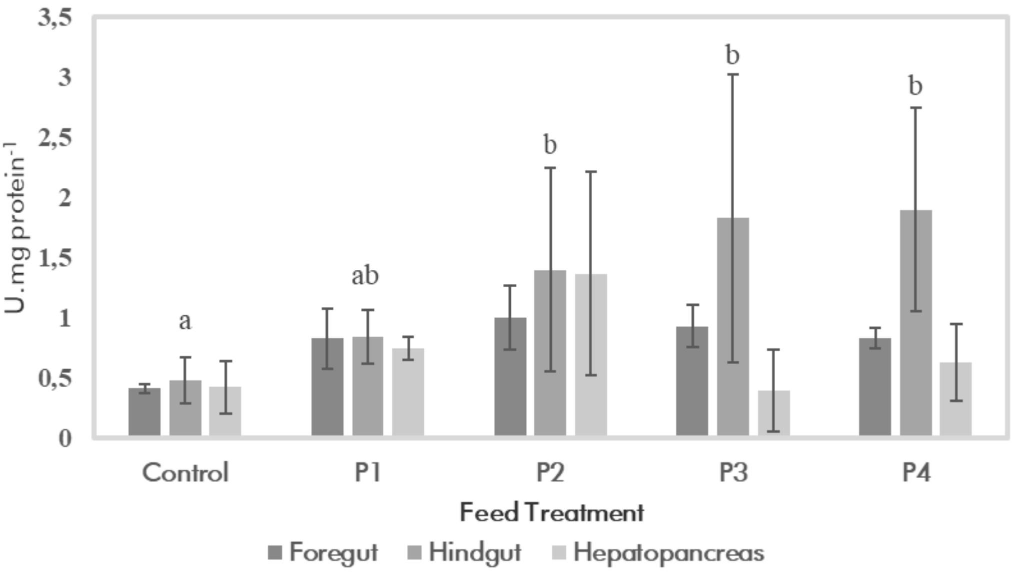

Figure 4. Alkaline phosphatase activity of hard-lipped barb (*Osteochilus vittatus*). Superscripts indicate significant differences between treatments ($\bar{x}\pm$SD, n=4, P<0.05)

The increase of alkaline phosphatase activity in hard-lipped barb fed with *S. platensis* and *C. vulgaris* supplementation indicates that the fish are eating well and their nutritional conditions are well. Alkaline phosphatase is thought to play a role in regulating the absorption of fatty acids and minerals (calcium and phosphorus) (Martínez-Llorens et al., 2021). Microalgae *S. platensis* and *C. vulgaris* contain a lot of fatty acids and minerals, including calcium and phosphorus (Alagawany et al., 2021).

Alkaline phosphatase activity is also influenced by the amount of feed consumed by fish. Fish that consume more feed have higher alkaline phosphatase activity than fish that eat less (Lallès, 2020). Alkaline phosphatase activity is also thought to be influenced by the protein content in the feed. Martínez-Llorens et al. (2021) reported that Atlantic salmon (*Salmo salar*) fed with hydrolyzed protein had higher alkaline phosphatase activity than without hydrolyzed protein. This shows that alkaline phosphatase activity can be stimulated by the protein content in the feed, especially simple proteins with smaller molecular sizes. *S. platensis* and *C. vulgaris* have high protein content (Lallès, 2020; Alagawany et al., 2021).

CONCLUSIONS

Supplementation of *S. platensis* and *C. vulgaris* can improve protein, fat, and fiber levels in the body of hard-lipped barb (*O. vittatus*). Supplementation of *S. platensis* and *C. vulgaris* can be digested, that indicated by an increase in the activity of trypsin-like, lipase, amylase, and alkaline phosphatase. A dose of *C. vulgaris* supplementation of 4 $g.kg^{-1}$ can be used as a feed supplement for hard-lipped barb (*O. vittatus*) to improve body composition, especially protein, and increase the activity of trypsin-like, lipase, amylase, and alkaline phosphatase.

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