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RESEARCH ARTICLE

Effects of dietary *Chlorella ellipsoidea* on growth performance, body composition, and hematology of stinging catfish, *Heteropneustes fossilis*

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Abstract. Microalgae have emerged as a promising feed supplement in aquaculture diets. Therefore, the aim of this study was to investigate the effect of *Chlorella ellipsoidea* as a feed supplement on the growth performance, body composition, and hematology of stinging catfish, *Heteropneustes fossilis* (Bloch). Five experimental diets were formulated to conduct this experiment by supplementing 0, 2.5, 5.0, 7.5, and 10% *C. ellipsoidea* at the expense of fish meal, and these are referred to as the control, CE 2.5, CE 5, CE 7.5, and CE 10 diets, respectively. A total of 900 fish (mean body weight of 0.50 ± 0.01 g) were divided equally into15 glass aquaria (180 L). Triplicate groups of fish were fed with each experimental diet to satiation twice daily for 10 weeks. At the end of the feeding trial, the weight gain (%) of the fish fed CE 5 and CE 7.5 was significantly ($P < 0.05$) higher than that of fish fed the control diet. The specific growth rate (SGR) of the fish fed CE 5 was significantly higher ($p < 0.05$) but comparable to those fed CE 2.5 and CE 7.5. The feed conversion ratio (FCR) of the fish fed CE 5, CE 7.5, and CE 10 was significantly ($P < 0.05$) lower, and the protein efficiency ratio (PER) of the fish fed CE 7.5 and CE 10 was significantly ($P < 0.05$) higher compared to all the other diets. In comparison to the control, dietary *C. ellipsoidea* of 5.0–10.0% significantly ($P < 0.05$) increased the protein content of stinging catfish, while lowering the lipid and moisture contents. The red blood cells, white blood cells, hemoglobin, hematocrit, and mean corpuscular hemoglobin levels of *H. fossilis* increased significantly ($P < 0.05$) when the fish were fed 5.0–10.0% dietary *C. ellipsoidea* compared with the control. Based on the findings of the study, 5.0–10.0% *C. ellipsoidea* supplementation was optimal in the stinging catfish diet to improve growth performance, body composition, hematology, and immunological response. However, based on regression analysis, the optimum level of *C. ellipsoidea* as a feed supplement was calculated to be 4.9–5% in stinging catfish.

Keywords: *Chlorella ellipsoidea*, Fish meal, Growth performance, *Heteropneustes fossilis*, Hematology.

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Introduction

Aquaculture is characterized by fast expansion and the introduction of new species, with huge potential to produce more fish in the future to feed the world's growing population (Naylor et al. 2021). Stinging catfish, *Heteropneustes fossilis* (Bloch), is an important tropical freshwater fish in the Indian subcontinent and Southeast Asia, which is popular among fish producers because of its high market price and customer demand (Saha et al. 2022). The fish is tasty, simple to digest, high in protein, and low in fat, and it has long been included in the diets of patients in many Asian nations (Hossain et al. 2023, Nandi et al. 2023). Stinging catfish endures high stocking densities and adapts to hypoxic conditions thanks to its auxiliary respiratory organs (Fatma and Ahmed 2020). Consumer demand for stinging catfish has grown rapidly in Bangladesh, but commercial aquaculture farms are suffering from higher production costs from rising prices of aquafeed (Arifa et al. 2022).

Aquafeed supplies fish with their primary source of protein and accounts for 60–70% of production costs (Khan et al. 2018). Fish meal (FM) is a key component as a protein source in aquafeeds because it provides necessary nutrients while also improving digestibility and palatability (Gasco et al. 2018). The rising cost of FM in aquafeed has prompted the search for cost-effective additives. Plant proteins and other alternative protein sources are now used as ecologically sustainable feed additives since they can support fish growth (Jannathulla et al. 2019, Zahan et al. 2024). Additionally, the price of commercial FM has increased steadily, thus, it is vital to develop other feed sources and create a reliable supply for commercial diets (Hua et al. 2019). The long-term sustainability of FM is a major concern facing the aquaculture industry that has prompted the development of good quality, reasonably priced protein supplements such as plant feedstuffs (Cai et al. 2022) and algae (Kiron 2012).

Microalgae are increasingly recognized as valuable and sustainable feed ingredients in aquaculture (Akter et al. 2022). They offer high-value products with fast growth (Dolganyuk et al. 2020), suitable land usage, carbon dioxide reduction, wastewater cultivation capacity (Udayan et al. 2022), and environmental impacts (Ansari et al. 2021). They also provide bioactive metabolites like polyphenols, vitamins, and lipids, making them suitable for sustainable energy, food, and health applications (Azaman et al. 2017). Furthermore, microalgae can serve as a source of long-chain polyunsaturated fatty acids (PUFAs) such as DHA and EPA, which are crucial for fish nutrition (Remize et al. 2021). Microalgae, particularly spirulina, *Spirulina platensis* (Mamun et al. 2023), *Chlorella vulgaris* (Raji et al. 2018) are commonly used in aquaculture feed thanks to their high nutritional values, including proteins, lipids, and essential amino acids (Ansari et al. 2021).

Chlorella ellipsoidea, a single-celled green alga, is a phytoplankton with high protein content and essential nutrients that is used as a protein source when dried, making it a valuable candidate as an aquaculture feed ingredient (Cheirsilp et al. 2016). *C. ellipsoidea* is a nutrient-rich microalgae used as a dietary protein source for marine and freshwater fishes (Xu et al. 2014) because of the benefits is provides for feed utilization, growth, stress response, and disease resistance (Alagawany et al. 2021). The nutritional composition of dried *C. ellipsoidea* consists of protein (42–58%), lipid (5–40%), carbohydrate (12–55%), fiber (5%), minerals (10%), MUFA (6.81%), and PUFA (61.17%) (Baidya et al. 2021, Safi et al. 2014). Dietary supplementation of 2% *C. ellipsoidea* powder in commercial diets is reported to improve growth, feed utilization, serum cholesterol level, and whole-body fat contents in juvenile Japanese flounder, *Paralichthys olivaceus* (Teminck and Schlegel) (Kim et al. 2002). Pradhan et al. (2023) fed fingerlings of rohu, *Labeo rohita* (Hamilton), *a* diet supplemented with *Chlorella vulgaris* and found that chlorella supplementation stimulated immunity and protected *L. rohita* from *Aeromonas hydrophila* infection. Another study found that the inclusion of 5 g *C. vulgaris* powder improved the blood chemical responses and the activity of digestive enzymes in grey mullet, *Mugil cephalus* L. (Akbari and Raeisi 2020). Chlorella mix meal replaced FM in studies on zebrafish, *Danio rerio* (Hamilton) (Carneiro et al. 2020), African catfish, *Clarias gariepinus* (Burchell) (Raji et al. 2020), crucian carp, *Carassius auratus* (L.) (Shi et al. 2017), *L. rohita* (Andrews et al. 2009), and rainbow trout, *Oncorhynchus mykiss* (Walbaum)

(Chen et al. 2021), all of which showed improved growth performance. To the best of our knowledge, no studies have yet reported on the effects of chlorella as feed supplement in the diet of stinging catfish.

Therefore, considering the nutritional features, availability, and potential of *C. ellipsoidea* as a feed supplement, this study was designed to investigate the effects of dietary supplementation of graded levels of *C. ellipsoidea* on the growth performance, body composition, and hematology of stinging catfish. The present study also aimed to determine the optimum level of *C. ellipsoidea* as a feed supplement in stinging catfish.

Materials and Methods

Culture and collection of dried *C. ellipsoidea*

C. ellipsoidea was mass cultured using Bold Basal Medium (BBM) (Da Silva et al. 2020, Akter et al. 2022) in a 200 L tank. It was produced using continuous-harvesting technology that maintains exponential growth, and it was dried in the sun. After proper drying, *C. ellipsoidea* was ground in a grinder (model no-WBL-VK01, Walton, Bangladesh), and the powder was stored in a freezer (–18°C) for further use.

Collection of feed ingredients and experimental diet preparation

Fish meal (FM), soybean meal, rice bran, wheat bran, mustard oil cake, and wheat flour were obtained from a local feed market, and then the ingredients were sun-dried and ground into fish feed. The proximate composition of *C. ellipsoidea* was determined prior to feed formulation. The dry matter content was as follows: protein (44.7%), fat (5.9%), and ash (15.1%). Five experimental diets were formulated containing around 35% protein (Table 1). The control diet contained 40% FM as the primary protein source. Then, graded levels of (0, 2.5, 5, 7.5, and 10%) *C. ellipsoidea* were added as a feed supplement at the expense of FM and were the control, CE 2.5, CE 5, CE 7.5, and CE 10 diets, respectively. The diets were supplemented with 0–10% chlorella powder because a positive impact has been reported on fish growth and immunity when 0.5–5.0% chlorella powder was included in fish diets (Chen et al. 2021, Pradhan et al. 2023). To make the feed, all the components were mixed with enough water to create a dough that was then pelleted using a laboratory-type pellet machine to produce pellets with a 2 mm diameter, which was then sun-dried for two days. Finally, the formulated diets were stored in polyethylene bags in a freezer (–18°C).

Fish collection, rearing, and feeding

The feeding trial of *H. fossilis* fingerlings was conducted using a completely randomized design (CRD). The stinging catfish fingerlings were obtained from the commercial Fresh Water Fish Hatchery in Mymensingh, Bangladesh. Live, healthy fish were collected and brought to the laboratory in oxygenated polythene bags. The fish were acclimatized in 300 L circular tanks with proper aeration for one week prior to the feeding trial. The fish were fed the control diet during their acclimatization. After a 1% salt solution treatment, a total 900 fish (mean body weight of 0.50 ± 0.01 g) were divided equally into15 glass aquaria (180 L). Triplicate groups of fish were fed with each experimental diet to satiation twice daily for 10 weeks. Every day, about 30% of the water in each aquarium was replaced. Air stones connected to a central compressor were used to aerate each aquarium continuously. The experiment was conducted with a natural photoperiod (about 10:14 light:dark). The physicochemical parameters (water temperature, dissolved oxygen: DO, pH, and total ammonia) were measured regularly to monitor the overall culture environment during the experimental period. Water pH, dissolved oxygen (DO), temperature (°C), and total ammonia were measured using a digital pH meter (Hach Co., Colorado, USA), a digital DO meter

Table 1
Ingredients and proximate composition of the experimental diets for stinging catfish

	C. ellipsoidea supplementation (%)				
Ingredients	Control	CE 2.5	CE 5	CE 7.5	CE 10
Fish meal	40.0	37.5	35.0	32.5	30.0
C. ellipsoidea powder	0.0	2.5	5.0	7.5	10.0
Soybean meal	10.0	10.0	10.0	10.0	10.0
Mustard oil cake	10.0	10.0	10.0	10.0	10.0
Rice bran	30.0	30.0	30.0	30.0	30.0
Wheat bran	6.0	6.0	6.0	6.0	6.0
Wheat flour	2.0	2.0	2.0	2.0	2.0
Vitamin premix[1]	1.0	1.0	1.0	1.0	1.0
Mineral Premix[2]	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100
Chemical composition (% dry matter basis)					
Protein	35.28	35.71	35.71	35.93	35.48
Lipid	4.50	4.05	5.80	5.78	6.48
Ash	14.40	15.70	16.42	16.17	16.60
Moisture	13.6	12.57	13.32	13.89	14.53

[1]Vitamin premix supplied the following (mg/g mixture): thiamin hydrochloride, 5 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; nicotinic acid, 6.05 mg; biotin, 0.003 mg; pyridoxine hydrochloride, 0.825 mg; inositol, 10 mg; folic acid, 0.041 mg; L-ascorby1-2-Monophosphate-Mg, 2.025 mg; choline chloride, 44 mg; menadione, 4 mg; alpha-tocopherol acetate, 3.35 mg; *para*-aminobenzoic acid, 5 mg; *myo*-inositol, 20 mg; retinyl acetate, 0.4 mg; cholecalciferol, 0.0004685 mg. All ingredients were diluted with alpha-cellulose to 1 g (Hossain and Furuichi, 1999, 2001).
[2]Mineral premix supplied the following (mg/g mixture): $FeSO_46H_2O$, 2.125 mg; $MgSO_4$, 137 mg; KCl, 75 mg; NaH_2PO_4, 87.2 mg; NaCl, 43.5 mg; $AlCl_3 \cdot 6H_2O$, 0.15 mg; KI, 0.15 mg; $CuCl_2 \cdot 2H_2O$, 0.1 mg; $ZnSO_4 \cdot 7H_2O$, 0.80 mg; $CoCl_2 \cdot 6H_2O$, 1 mg. All ingredients were diluted with alpha-cellulose to 1 g (Hossain and Furuichi 1999, 2001).

(Hach Co., Loveland, Colorado, USA), a Celsius thermometer (Digi-thermo WT-2), and an ammonia measurement kit (HANNA Instrument Test Kit), respectively. The temperature, DO, pH, and ammonia were in the ranges of 27.41 to 28.87°C, 6.38 to 7.32 mg L^{-1}, 6.95 to 7.57, and 0.13 to 0.24 mg L^{-1}, respectively.

Fish sampling and weighing

At the end of the 10-week experimental period, all fish were counted and weighed individually. First, the fish were harvested with scoop nets and 10–15 fish were weighed with a digital electric balance (model-EK600i) to measure growth parameters. During sampling, the fish were handled very carefully. A pooled blood sample (2.0 ml) from 10 fish in each tank was taken using a syringe and a tube filled with EDTA (BD Microtainer®, UK) and stored for hematological analysis. Following the collection of blood samples from 10 fish in each aquarium, the viscera were removed, the carcasses were washed with distilled water, and stored at –18°C for proximate analysis.

Growth parameters, efficiency of feed, and biological metrics

The following metrics were used to evaluate the growth parameters of the fish and feed utilization, which included weight gain (WG), weight gain % (WG %), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), survival (S), and hepatosomatic index (HSI).

$$WG\ (g) = Mean\ final\ weight\ (g) - Mean\ initial\ weight\ (g)$$

$$WG\% = \frac{Mean\ final\ fish\ weight - Mean\ initial\ fish\ weight}{Mean\ initial\ weight} \times 100$$

$$SGR\ (\%day^{-1}) = \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100$$

Here, W_1 = the initial live body weight (g) at time T_1 (day); W_2 = the final live body weight (g) at time T_2 (day); $T_2 - T_1$ = No. of days of the experiment.

$$FCR = \frac{Total\ feed\ consumption}{Total\ body\ weight\ of\ fish} \times 100$$

$$PER\ (\%) = \frac{Total\ weight\ gain\ (g)}{Amount\ of\ protein\ in\ take\ (g)} \times 100$$

$$Survival\ rate\ (\%) = \frac{The\ final\ number\ of\ fish\ survived}{Number\ of\ actual\ fish\ stocked} \times 100$$

$$HSI = \frac{Weight\ of\ liver}{Weight\ of\ the\ body} \times 100$$

Proximate composition analysis

As per guidelines of the Association of Official Analytical Chemists (AOAC 2000), samples of the diets and whole-body fish were taken to analyze proximate composition, which included moisture, crude protein, crude ash, and crude lipid (Table 1). The crude protein content was determined by measuring the nitrogen values using the Kjeldahl systematic method after acid digestion using an auto-Kjeldahl System (UDK 152, VELP); crude lipid content was determined by the ether extraction method using a solvent extractor (SER 148, VELP); crude ash content was determined after combustion at 550°C; and moisture content was determined by oven-drying samples for 24 hours at 105°C.

Hematological parameters

The hematological parameters of leukocyte (WBC), erythrocyte (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined with a fully automatic hematology analyzer (DYMIND, DH36, China).

Statistical analysis

All the data were collected, recorded, and arranged in a computer spreadsheet during the experimental period. Prior to statistical analysis, all data were tested for normality and homogeneity. The data were analyzed statistically with one-way ANOVA with Statistix 10 (2013), and significant differences of means were indicated by the package's Least Significant Difference (LSD) option. The significance level was determined at $P < 0.05$.

Results

Growth parameters, efficiency of feed, and biological metrics

Growth performance (WG, WG %, and SGR), feed utilization (FCR and PER), and biological indices (HSI) of stinging catfish fed the experimental diets are presented in Table 2. In the current study, growth performance of stinging catfish gradually improved with increasing levels of *C. ellipsoidea* supplementation up to 7.5% and then decreased at 10%. The WG and WG % were significantly ($P < 0.05$) higher in fish fed the CE 5 and CE 7.5 diets but not significantly ($P > 0.05$) different in fish fed the CE 2.5 diet. However, the SGR of fish fed the CE 5 diet was significantly ($P < 0.05$) higher than that of fish fed the other diets; however, it was not significantly ($P > 0.05$) different in fish fed the CE 2.5 and CE 7.5 diets. The second-order polynomial regression analysis of weight gain and SGR against the dietary inclusion level of *C. ellipsoidea* showed that optimum *C. ellipsoidea* supplementation was 4.9–5% (Figure 1).

The survival rate of *H. fossilis* ranged from 90.43 to 96.33% in all the treatments, and there were no

Table 2
Growth parameters and biological metrics of *H. fossilis* fed different experimental diets for 10 weeks

	C. ellipsoidea supplementation (%)				
Parameters	Control	CE 2.5	CE 5	CE 7.5	CE 10
Initial weight (g)	0.56 ±0.03	0.56 ±0.05	0.56 ±0.03	0.57 ±0.01	0.57±0.04
Final weight (g)	2.75 ± 0.20^{bc}	2.86 ± 0.03^{ab}	2.94 ±0.02^{a}	2.99 ±0.01^{a}	2.69 ± 0.02^{c}
Weight gain (g)	2.19 ± 0.32^{bc}	2.29 ± 0.33^{ab}	2.39 ± 0.79^{a}	2.42 ± 0.36^{a}	2.12 ±0.37^{c}
Weight gain %	387.34 ±3.04^{b}	408.90±3.26^{ab}	426.40±2.62^{a}	437.85 ± 2.30^{a}	375.61 ±2.78^{b}
SGR (% day^{-1})	2.64 ± 0.05^{b}	2.71 ± 0.06^{ab}	2.80 ± 0.03^{a}	2.76 ± 0.06^{ab}	2.60 ± 0.05^{b}
Survival rate (%)	96.33 ± 1.67^{a}	91.58 ± 1.57^{a}	95.16 ± 0.44^{a}	90.43 ± 1.29^{a}	91.66 ± 1.76^{a}
HSI	1.24 ± 0.18^{b}	1.34 ± 0.29^{ab}	1.46 ± 0.28^{a}	1.45 ± 0.26^{a}	1.40 ± 0.30^{ab}

* Values are mean ± standard deviation (SD, n = 3). Different superscripted means in the same row differ significantly (P < 0.05).

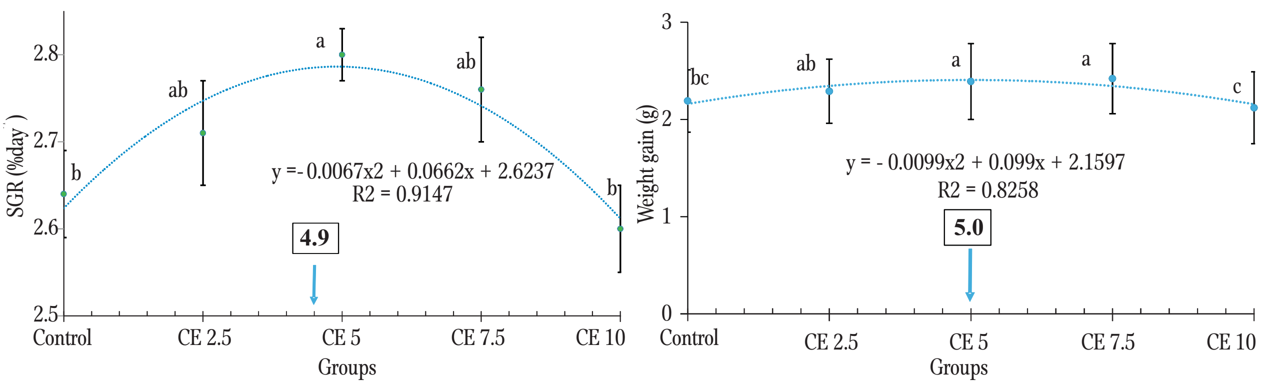

Figure 1. Second-order polynomial regression analysis of WG ($y = -0.0099x^2 + 0.099x + 2.1597$; $R^2 = 0.8258$; $Y_{max} = 5.0$) and SGR ($y = -0.0067x^2 + 0.0662x + 2.6237$; $R^2 = 0.9147$; $Y_{max} = 4.9$) against *C. ellipsoidea* supplementation in the diets of stinging catfish. Different letters indicate significant differences (P < 0.05) among treatments.

significant (P > 0.05) differences among them. The HSI of *H. fossilis* was impacted by *C. ellipsoidea* dietary supplementation. The value of HSI increased with dietary *C. ellipsoidea* supplementation up to CE 5 compared to the control; however, it decreased at CE 10. The FCR value decreased and the PER value increased with *C. ellipsoidea* supplementation in the experimental diets. Significantly (P < 0.05) lower FCR values were recorded in fish fed the CE 5, CE 7.5, and CE 10 diets, whereas significantly (P < 0.05) higher PER values were recorded in the fish fed the CE 7.5 and CE 10 diets (Figure 2).

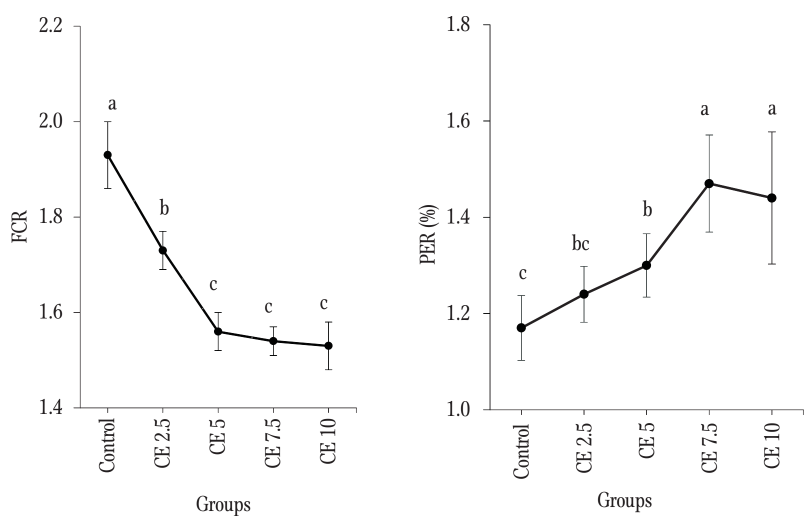

Figure 2. Feed utilization of *H. fossilis* fed different experimental diets for 10 weeks. Different letters indicate significant differences (P < 0.05) among treatments.

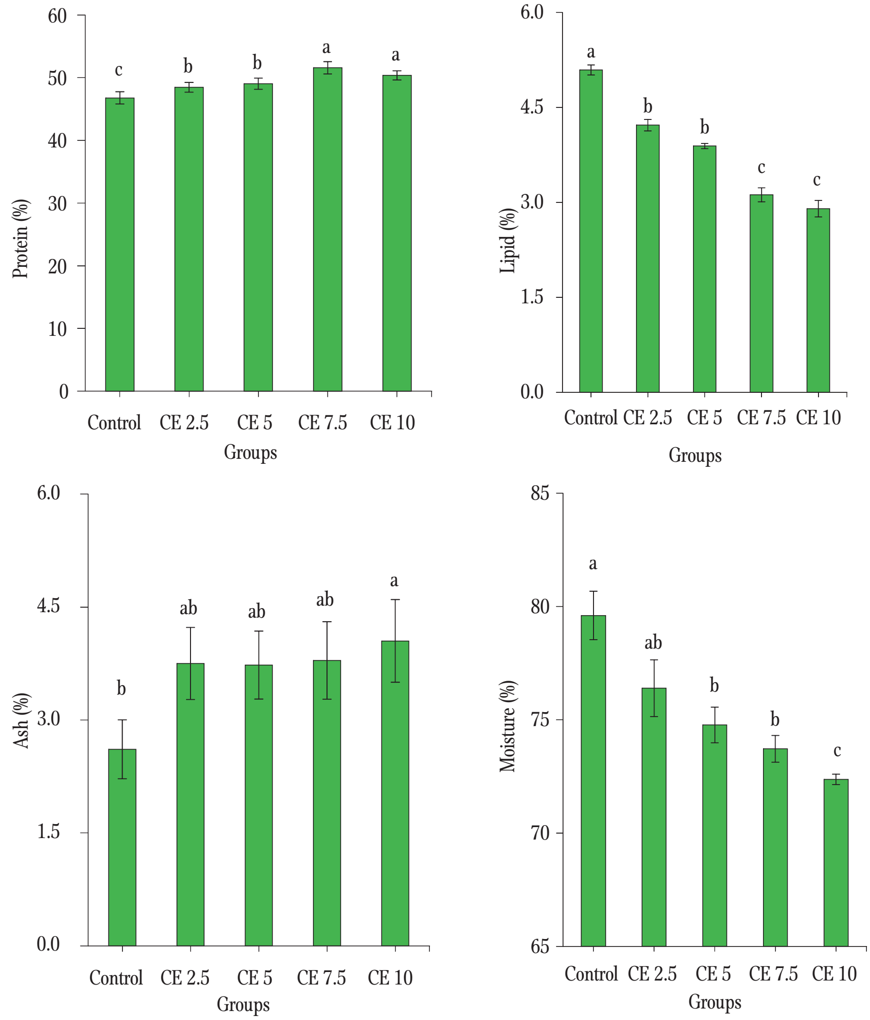

Figure 3. Proximate composition (% dry matter) of *H. fossilis* in different experimental diets for 10 weeks. Different letters indicate significant differences ($P < 0.05$) among treatments.

Proximate composition

After 10 weeks of the experiment, the protein and ash contents of the whole fish body increased whereas the moisture and lipid content decreased with graded levels of *C. ellipsoidea* as a feed supplement (Figure 3). The carcass crude protein content was significantly ($P < 0.05$) higher in fish fed the CE 7.5 and CE 10 diets compared to those fed the other diets. The ash content was significantly ($P < 0.05$) higher in fish fed the CE 10 diet but was comparable in fish fed the CE 2.5, CE 5, and CE 7.5 diets. However, the lipid and moisture content were significantly ($P < 0.05$) lower in fish fed the CE 7.5 and CE 10 and CE 10 diets, respectively.

Hematological parameters

The hematological parameters of stinging catfish are presented in Table 3. The findings of the present study showed that Hb, WBC, RBC, lymphocyte, and HCT gradually increased with the increase of dietary *C. ellipsoidea* supplementation, and, significantly ($P < 0.05$), the higher counts of these parameters were observed in fish fed the CE 7.5 and CE 10 diets compared to all the other diets. However, PCT values decreased significantly ($P < 0.05$) with dietary *C. ellipsoidea* supplementation, and values of this parameter were significantly ($P < 0.05$) higher in the control treatment compared to the other treatments. The MCV, MCH, and MCHC values of fish fed the CE 7.5

Table 3
Hematological parameters of *H. fossilis* fed experimental diets for 10 weeks

	C. ellipsoidea supplementation (%)				
Blood parameters	Control	CE 2.5	CE 5	CE 7.5	CE 10
Hb (gdL^{-1})	4.57 ± 0.94^{c}	5.77 ± 0.77^{c}	6.23 ± 0.74^{bc}	7.39 ± 0.50^{ab}	9.78 ± 1.15^{a}
RBC ($\times10^{6}\mu L^{-1}$)	2.44 ± 1.12^{c}	2.95 ± 0.51^{bc}	3.67 ± 0.77^{b}	4.04 ± 0.99^{ab}	4.77 ± 1.09^{a}
WBC ($\times10^{3}\mu L^{-1}$)	6.32 ± 0.60^{c}	7.33 ± 0.75^{bc}	7.97 ± 0.85^{bc}	8.63 ± 0.76^{ab}	11 ± 1.01^{a}
LYM (%)	93.62 ± 2.07^{b}	94.60 ± 1.70^{c}	95.00 ± 2.41^{c}	95.77 ± 2.04^{d}	97.53 ± 1.42^{a}
HCT (%)	7.41 ± 1.47^{d}	7.97 ± 1.60^{d}	8.57 ± 1.29^{c}	10.47 ± 1.18^{b}	14.80 ± 3.03^{a}
PCT (%)	0.10 ± 0.04^{a}	0.05 ± 0.01^{b}	0.05 ± 0.01^{b}	0.03 ± 0.01^{c}	0.04 ± 0.02^{bc}
MCV (fl)	88.70 ± 2.80^{c}	89.10 ± 3.15^{c}	90.56 ± 6.44^{b}	92.23 ± 2.95^{bc}	100.37 ± 2.85^{a}
MCH (pg)	42.60 ± 2.90^{e}	43.24 ± 3.35^{d}	44.14 ± 3.06^{c}	45.34 ± 2.52^{b}	48.17 ± 3.01^{a}
MCHC (gdL^{-1})	46.20 ± 2.90^{c}	47.49 ± 2.85^{c}	47.40 ± 3.10^{b}	46.13 ± 3.10^{c}	48.20 ± 2.61^{a}

*Values are mean ± standard deviation (SD, n = 3). Different superscripted means in the same row differ significantly ($P < 0.05$). Hb, hemoglobin; RBC, red blood cell; WBC, white blood cell; HCT, hematocrit; PCT, plateletcrit; LYM, lymphocyte; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

and CE 10 diets were higher than those of the control diet.

Discussion

Growth performance, feed efficiency, and biological metrics of stinging catfish

The study found that supplementing stinging catfish diets with *C. ellipsoidea* improved growth metrics (WG and SGR) compared to the control diet. Significantly better growth performance was observed at 5–7.5% supplementation, which indicated that *C. ellipsoidea* had a positive effect on the growth performance of stinging catfish. However, regression analysis indicated that optimum *C. ellipsoidea* supplementation in the stinging catfish diet was 4.9–5%, which induced maximum growth. Khani et al. (2017) found that dietary supplementation of 5% *Chlorella vulgaris* significantly enhanced the growth of koi, *Cyprinus carpio* L. Similarly, dietary spirulina supplementation of 5% resulted in maximum growth in stinging catfish (Rahman et al. 2023). Chen et al. (2021) reported maximum growth of rainbow trout at 5% chlorella when the fish were provided with three diets supplemented with *Chlorella sorokiniana* at 0, 5, and 10% without any negative effects on feed efficiency. Badwy et al. (2008) found increased growth performance of Nile tilapia, *Oreochromis niloticus* (L.), with high *C. ellipsoidea* supplementation. All these research findings indicated the positive impact of *C. ellipsoidea* supplementation on the growth performance of fish, which also supports the findings of the present study.

In the present study, regression analysis revealed that optimum *C. ellipsoidea* supplementation in the stinging catfish diet to obtain the highest SGR was 4.9–5.0%. This result was in agreement with the findings of Khani et al. (2017) which noted higher SGR in 5% *Chlorella* supplemented diet group of koi, *Cyprinus carpio*. Dietary *S. platensis* supplementation at 5% resulted in the best growth performance in Mekong giant catfish, *Pangasianodon gigas* Chevey (Tongsiri et al. 2010). Rahman et al. (2023) found significantly higher SGR in stinging catfish *Heteropneustes fossilis* fed with 5% spirulina supplementation. Sahan et al. (2015) observed higher SGR with 7.5% spirulina supplementation in the diet of tilapia, *Oreochromis niloticus*. Rahimnejad et al. (2017) reported that dietary supplementation of 10–15% *Chlorella vulgaris* significantly enhanced growth performance and SGR in olive flounder, *P.*

olivaceus. The improvement in the growth performance of the stinging catfish in the current study from increased *C. ellipsoidea* supplementation may have resulted from elevated digestive enzyme activity, intestinal micro-biota, vitamin production, breakdown of indigestible components, and possible appetite improvement. Garcia et al. (2022) reported that the increased dietary *C. ellipsoidea* supplementation improved the SGR of thick-lipped grey mullet, *Chelon labrosus* (Risso), which might have been attributed to increased digestive enzyme activity, high protein content, necessary fatty acid synthesis, and vitamin production.

HSI measures an animal's energy reserves, particularly in fish, by comparing liver weight to total body weight (Prakash 2022). In the present study, the maximum HSI values were found at 5.0% dietary *C. ellipsoidea* supplementation. According to Bai et al. (2001), the best HSI value was achieved when adding 2% chlorella powder to the diet of young Korean rockfish, *Sebastes schlegeli* Hilgendorf. Improved HSI from dietary *C. ellipsoidea* supplementation in fish was due to nutrient intake, energy reserves, and protein synthesis (Nugraha et al. 2020, Ahmad et al. 2023). According to Matulic et al. (2020), higher HSI values indicate proper nutrient utilization and energy reserves.

FCR and PER are the two basic metrics used to assess fish feed utilization. Lower FCR values indicate lower production costs and improved fish feed utilization (Fry et al. 2018). The lower FCR and higher PER values obtained with the diets supplemented with *C. ellipsoidea* compared to the control diet in this study indicated increased feed utilization efficiency in stinging catfish and a reduction in production costs. FCR decreases and PER increases when the quality of protein in the diet improves. In the present study, the significantly lowest FCR and highest PER values were recorded at 5–10% *C. ellipsoidea* supplementation. The findings of this study correspond with Xu et al. (2014) who reported the lowest FCR with a higher PER when 7.6% chlorella was incorporated in the diet of gibel carp, *Carassius gibelio* (Bloch). Zahan et al. (2024) reported lower FCR and higher PER in stinging catfish, *Heteropneustes fossilis,* fed diets supplemented with 7.5 and 10% spirulina. Fadl et al. (2017) also observed the lowest FCR value in Nile tilapia, *O. niloticus*, fed a diet supplemented with 15% chlorella. Abdulrahman et al. (2019) reported that chlorella is easily digestible due to its small size and lack of a strong fibrous wall.

Proximate composition of whole-body carcass

Proximate composition including moisture, crude protein, total lipids, and ash, is a conventional index for assessing carcass quality, and it is critical for attaining consumer acceptance (Karmakar et al. 2021). In this study, whole-body protein and ash content of stinging catfish were enhanced; however, fat and moisture content decreased along with *C. ellipsoidea* supplementation. Numerous research studies stated that the inclusion of chlorella in the diets of fish increased the crude protein content of the carcass (Enyidi, 2017, Rahimnejad et al. 2017, Abdulrahman et al. 2018, Pantami et al. 2020). Reduced whole-body lipid content with *C. ellipsoidea* supplementation in fish diets has also been reported in Nile tilapia, *O. niloticus*; Korean rockfish, *S. schlegeli*; olive flounder, *P. olivaceus*; and *Macrobrachium rosenbergii* (De Man) (Bai et al. 2001, Badwy et al. 2008, Radhakrishnan et al. 2015, Rahimnejad et al. 2017). In contrast, dietary supplementation with chlorella for common carp, *C. carpio*, and rainbow trout, *O. mykiss*, resulted in higher lipid contents (Abdulrahman et al. 2019, Chen et al. 2021). The effects of dietary chlorella on fish whole-body protein and lipid contents are correlated with their synthesis and accumulation rates in body muscle and the growth rates of fishes (Abdel-Tawwab et al. 2022). Carcass moisture content also follows the same trend as lipid content. The moisture content of freshwater fish is reported to be between 72.1 and 83.6%, which supports the findings of the present study (Paul et al. 2018). The gradual increase in carcass ash composition with the increase in dietary chlorella is also supported by previous studies (Radhakrishnan et al. 2015, Rahimnejad et al. 2017, Abdulrahman et al. 2018, Chen et al. 2021, Abdel-Tawwab et al. 2022).

Hematological parameters of stinging catfish

Hematological characteristics are crucial for assessing the physiological status, stress tolerance, potential illness, and feed toxicity in fishes (Witeska et al. 2023). Fish hematological parameters indicate their overall health (Abdel-Tawwab et al. 2022). The present study indicated a gradual increase of Hb content in stinging catfish at 5–10% dietary *C. ellipsoidea* supplementation. Raji et al. (2018) reported increased Hb concentration in African catfish, *C. gariepinus*, compared to the control diet when dietary *C. vulgaris* was added to the meal. Arteaga et al. (2021) reported that increased Hb concentrations were observed in rainbow trout, *O. mykiss*, by feeding 0.45% dietary *C. peruviana*. Increasing Hb rates are a useful measure of fish oxygen transportation ability, allowing correlations to be established between oxygen content in the habitat and fish health conditions (Rummer and Brauner, 2015). RBC and WBC counts gradually increased with an increase of *C. ellipsoidea* in the diets. Andrew et al. (2009) reported a higher RBC count ($3.82 \pm 0.04 \times 10^9\ \mu L^{-1}$) in *L. rohita* fingerlings at 0.5% *C. vulgaris* supplementation. Similar results were observed in African catfish, *C. gariepinus*, and common carp, *C. carpio* (Andrews et al. 2009, Abdulrahman et al. 2018, Raji et al. 2018, Abdel-Tawwab et al. 2022). Abbas et al. (2020) reported increased WBC counts in tilapia, *O. niloticus*, when 5% *C. vulgaris* was added to the feed. These findings may be attributed to the enhancement of erythropoietin production, the release of new RBC into the blood, and erythrocytic stability (Yeganeh et al. 2015).

Lymphocytes (LYM) help to make antibodies, kill tumor cells, and control immune responses of fishes (Scapigliati et al. 2018), while HCT measures the volume of red blood cells compared to total blood volume (Erhunmwunse and Ainerua 2013, Galagarza et al. 2017). Lower hematocrit values are indicative of anemia, while high WBC counts indicate long-term illness, infection, or leukemia (Allen et al. 2015). The high LYM and HCT values in stinging catfish with increasing dietary *C. ellipsoidea* supplementation indicated that the fish were in good health. The PCT count gradually decrease increasing dietary *C. ellipsoidea* supplementation. Lower PCT was noted with the CE 7.5 treatment compared to control, which means that the fish fed with 7.5% *C. ellipsoidea* had a lower risk of developing sepsis. MCV, MCH, and MCHC are red blood cell indices that are important indicators in the diagnosis of anemia in most animals (Demir et al. 2014). An improvement in these immune parameters suggested the positive role of *C. ellipsoidea* supplementation (up to 10% of the diet) on the immunity of stinging catfish.

Conclusion

The findings of the study indicates that the inclusion of *C. ellipsoidea* in the diet of stinging catfish positively impacted the growth, feed utilization, carcass composition, and immunity of fish. The higher WG, WG %, and SGR value of fish was recorded in 5–7.5% dietary *C. ellipsoidea* supplementation. Lower FCR and higher PER values were recorded in fish fed diets with 5–10% and 7.5–10% *C. ellipsoidea* supplementation, respectively. The carcass composition and immunity of fish improved with increasing *C. ellipsoidea* supplementation. However, optimum *C. ellipsoidea* supplementation to induce maximum WG and SGR was calculated to be 4.9–5% based on second-order polynomial regression analysis. Investigations on a commercial scale are required to access the practical application of chlorella in the stinging catfish diet. Extending the research to other species with similar dietary requirements could broaden the applicability of chlorella in aquaculture feed.

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References

Abbas, N., El-shafei, R., Zahran, E., Amer, M. (2020). Some pharmacological studies on *Chlorella vulgaris* in tilapia fish. Kafrelsheikh Veterinary Medical Journal, 18(1), 6-9.

Abdel-Tawwab, M., Abdulrahman, N. M., Baiz, A. I., Nader, P. J., Al-Refaiee, I. H. (2022). The using of *Chlorella pyrenoidosa* and *Daphnia magna* as feed supplements for common carp, *Cyprinus carpio*: growth performance, somatic indices, and haemato-biochemical biomarkers. Journal of Applied Aquaculture, 34(1), 64-78.

Abdulrahman, N. M., Abid, S. H., Koyun, M. (2019). Effect of microalgae *Chlorella pyrenoidosa* as feed additive on common carp (*Cyprinus carpio*) nutritional performance, proximate composition and organoleptic evaluation. Iranian Journal of Veterinary Medicine, 3(2), 118-126.

Abdulrahman, N., Abid, S., Khidir, A., Omer, B., Rasheed, D., Alddin, L. (2018). Effect of adding microalgae *Chlorella* sp. on some biological parameters and proximate analysis of common carp *Cyprinus carpio* L. Iranian Journal of Veterinary Medicine, 12, 199-206.

Ahmad, M. T., Shariff, M., Goh, Y. M., Banerjee, S., Yusoff, F. M. (2023). Interaction of low-level dietary supplementation of *Chlorella vulgaris* (Beijerinck, 1890) and feeding duration on growth hormone, growth performance and serum biochemistry of red hybrid tilapia (*Oreochromis mossambicus*× *Oreochromis niloticus*). Journal of Fish Biology, 103(3), 715-726.

Akbari, P., Raeisi, E. M. (2020). Effect of dietary supplementation of *Chlorella vulgaris* on several physiological parameters of grey mullet, *Mugil cephalus*. Iranian Journal of Fisheries Science, 19(3), 1130-1139.

Akter, T., Hasan, M. M., Das, M., Mondal, M. N., Hossain, S., Munir, M. B., Hossain, M. A. (2022). Utilization of fermented wheat bran extract medium as a potential low-cost culture medium for *Chlorella ellipsoidea* . Borneo Journal of Resource Science and Technology, 12(2), 63-73.

Akter, T., Hossain, A., Rabiul Islam, M., Hossain, M. A., Das, M., Rahman, M. M., Aye, A. T., Abdel-Tawwab, M. (2023). Effects of spirulina (*Arthrospira platensis*) as a fishmeal replacer in practical diets on growth performance, proximate composition, and amino acids profile of pabda catfish (*Ompok pabda*). Journal of Applied Aquaculture, 35(1), 69-82.

Alagawany, M., Taha, A. E., Noreldin, A., El-Tarabily, K. A., Abd El-Hack, M. E. (2021). Nutritional applications of species of *Spirulina* and *Chlorella* in farmed fish: A review. Aquaculture, 542, 736841.

Allen, P. J., Wise, D., Greenway, T., Khoo, L., Griffin, M. J., Jablonsky, M. (2015). Using 1-D 1 H and 2-D 1 H J-resolved NMR metabolomics to understand the effects of anemia in channel catfish (*Ictalurus punctatus*). Metabolomics, 11, 1131-1143.

Andrews, S. R., Sahu, N. P., Pal, A. K., Kumar, S. (2009). Hematological modulation and growth of *Labeo rohita* fingerlings: Effect of dietary mannan oligosaccharide, yeast extract, protein *hydrolysate* and *chlorella.* Aquaculture Research, 41(1), 61-69.

Ansari, F. A., Guldhe, A., Gupta, S. K., Rawat, I., Bux, F. (2021). Improving the feasibility of aquaculture feed by using microalgae. Environmental Science and Pollution Research, 28(32), 43234-43257.

AOAC, 2000. Official methods of analysis, 17th ed. Association of Analytical Chemists, Washington, DC.

Arifa, B., Lalon, R. M., Alam, A. S., Rahman, M. S. (2022). Economic feasibility of Pabda and stinging catfish culture in recirculating aquaculture systems (RAS) in Bangladesh. Aquaculture International, 30(1), 445-465.

Arteaga, C. Q., Mariano Astocondor, M., Aquino Ortega, R. (2021). Dietary supplementation with *Chlorella peruviana* improves the growth and innate immune response of rainbow trout *Oncorhynchus mykiss* fingerlings. Aquaculture, 533, 736117.

Azaman, S. N. A., Nagao, N., Yusoff, F. M., Tan, S. W., Yeap, S. K. (2017). A comparison of the morphological and biochemical characteristics of *Chlorella sorokiniana and Chlorella zofingiensis* cultured under photoautotrophic and mixotrophic conditions. PeerJ, 5, 1-12.

Badwy, T. M., Ibrahim, E. M., Zeinhom, M. M. (2008). Partial replacement of fish meal with dried microalga (*Chlorella* spp. and *Scenedesmus* spp.) in Nile tilapia (*Oreochromis niloticus*) Diets. Aquaculture*,* 2004, 801-811.

Bai, S. C., Koo, J. W., Kim, K. W., Kim, S. K. (2001). Effects of chlorella powder as a feed additive on growth performance in juvenile Korean rockfish, *Sebastes schlegeli* (Hilgendorf). Aquaculture Research, 32, 92-98.

Baidya, A., Akter, T., Islam, M. R., Shah, A. A., Hossain, M. A., Salam, M. A., Paul, S. I. (2021). Effect of different wavelengths of LED light on the growth, chlorophyll,

, β-carotene content and proximate composition of *Chlorella ellipsoidea*. Heliyon, 7(12), e08525.

Cai, W., Liu, H., Han, D., Zhu, X., Jin, J., Yang, Y., Xie, S. (2022). Complete replacement of fishmeal with plant protein ingredients in gibel carp (*Carassius auratus gibelio*) diets by supplementation with essential amino acids without negative impact on growth performance and muscle growth-related biomarkers. Frontiers in Marine Science, 8, 759086.

Carneiro, W. F., Castro, T. F. D., Orlando, T. M., Meurer, F., Paula, D. A. de J., Virote, B. do C. R., Vianna, A. R. da C. B., Murgas, L. D. S. (2020). Replacing fish meal by *Chlorella* spp. meal: Effects on zebrafish growth, reproductive performance, biochemical parameters and digestive enzymes. Aquaculture, 528, 735612.

Cheirsilp, B., Mandik, Y. I., Prasertsan, P. (2016). Evaluation of optimal conditions for cultivation of marine *Chlorella* sp. as potential sources of lipids, exopolymeric substances and pigments. Aquaculture International, 24, 313-326.

Chen, W., Luo, L., Han, D., Long, F., Chi, Q., Hu, Q. (2021). Effect of dietary supplementation with *Chlorella sorokiniana* meal on the growth performance, antioxidant status, and immune response of rainbow trout (*Oncorhynchus mykiss*). Journal of Applied Phycology, 33(5), 3113-3122.

Demir, O., Türker, A., Acar, U., Kesbiç, O. S. (2014). Effects of dietary fish oil replacement by unrefined peanut oil on the growth, serum biochemical and hematological parameters of Mozambique tilapia juveniles (*Oreochromis mossambicus*). Turkish Journal of Fisheries and Aquatic Sciences, 14(4), 887-892.

Dolganyuk, V., Belova, D., Babich, O., Prosekov, A., Ivanova, S., Katserov, D., Sukhikh, S. (2020). Microalgae: A promising source of valuable bioproducts. Biomolecules, 10(8), 1153.

Enyidi, U. D. (2017). *Chlorella vulgaris* as protein source in the diets of African Catfish *Clarias gariepinus*. Fishes, 2(4), 17, https://doi:10.3390/fishes2040017.

Erhunmwunse, N., Ainerua, M. (2013). Characterization of some blood parameters of African Catfish (*Clarias gariepinus*). American-Eurasian Journal of Toxicological Sciences, 5(3), 72-76.

Fadl, S. E., ElGohary, M. S., Elsadany, A. Y., Gad, D. M., Hanaa, F. F., El-Habashi, N. M. (2017). Contribution of microalgae-enriched fodder for the Nile tilapia to growth and resistance to infection with *Aeromonas hydrophila*. Algal Research, 27, 82-88.

Fatma, S., Ahmed, I. (2020). Effect of water temperature on protein requirement of *Heteropneustes fossilis* (Bloch) fry as determined by nutrient deposition, hematobiochemical parameters and stress resistance response. Fisheries and Aquatic Science, 23 (1), https://doi.org/10.1186/s41240-020-0147-y.

Fry, J. P., Mailloux, N. A., Love, D. C., Milli, M. C., Cao, L. (2018). Feed conversion efficiency in aquaculture: do we measure it correctly? Environmental Research Letters, 13(2), 024017.

Galagarza, O. A., Kuhn, D. D., Smith, S. A., Hrubec, T. C. (2017). Hematologic and plasma chemistry RIs for cultured striped catfish (*Pangasius hypophthalmus*) in recirculating aquaculture systems. Veterinary Clinical Pathology, 46(3), 457-465.

Garcia-Marquez, J., Galafat, A., Vizcaíno, A. J., Barany, A., Martos-Sitcha, J. A., Mancera, J. M., Abdala-Díaz, R. T. (2022). Dietary use of the microalga *Chlorella fusca* improves growth, metabolism, and digestive functionality in thick-lipped grey mullet (*Chelon labrosus*, Risso 1827) juveniles. Frontiers in Marine Science, 9, 902203.

Gasco, L., Gai, F., Maricchiolo, G., Genovese, L., Ragonese, S., Bottari, T., Caruso, G. (2018). Fishmeal alternative protein sources for aquaculture feeds. In: Feeds for the Aquaculture Sector—Current Situation and Alternative Sources (Ed.) L. Gasco, F. Gai, G. Maricchiolo, L. Genovese, S. Ragonese, T. Bottari, G. Caruso, Springer Briefs in Chemistry of Foods, Cham Switzerland, 1-28.

Hossain, A., Hossain, M. A., Rasul, M. G., Akter, T., Zaman, M. F. U., Islam, M. R. (2023). Efficacy of using sunflower meal as an ingredient, and partial fishmeal-replacer, in practical feed formulated for stinging catfish (*Heteropneustes fossilis*). Aquaculture, Fish and Fisheries, 3(3), 237-246.

Hossain, M. A., Furuichi, M. (1999). Calcium requirement of tiger puffer fed a semi-purified diet. Aquaculture International, 7, 287-293.

Hossain, M. A., Furuichi, M. (2001). Effects of calcium supplement in purified diets on growth and bone mineralization in juvenile red sea bream and black sea bream. Journal of the Faculty of Agriculture, Kyushu University, 45 (2), 481-486.

Hua, K., Cobcroft, J. M., Cole, A., Condon, K., Jerry, D. R., Mangott, A., Praeger, C., Vucko, M. J., Zeng, C., Zenger, K., Strugnell, J. M. (2019). The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth, 1(3), 316-329.

Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M., Dayal, J. S. (2019). Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. Aquaculture Research, 50(12), 3493-3506.

Karmakar, M., Mansur, M. A., Uddin, M. N., Islam, M. R., Mely, S. S., Akter, S., Islam, M. S. (2021). Sensory evaluation and proximate composition analysis of export oriented and locally consumed fishes of Bangladesh. Bangladesh Journal of Fisheries, 33(2), 317-326.

Khan, M. A., Guttormsen, A., Roll, K. H. (2018) Production risk of pan-gas (*Pangasius hypophthalmus*) fish farming. Aquaculture Economics &Management, 22, 192-208.

Khani, M., Soltani, M., Shamsaie Mehrjan, M., Foroudi, F., Ghaeni, M. (2017). Short communication: The effects of *Chlorella vulgaris* supplementation on growth performance, blood characteristics, and digestive enzymes in Koi (*Cyprinus carpio*). Iranian Journal of Fisheries Sciences, 16(2), 832-843.

Kim, K. W., Bai, S. C., Koo, J. W., Wang, X., Kim, S. K. (2002). Effects of dietary *Chlorella ellipsoidea* supplementation on growth, blood characteristics, and whole-body composition in juvenile Japanese flounder *Paralichthys olivaceus*. Journal of the World Aquaculture Society, 33(4), 425-431.

Kiron, V. (2012). Fish immune system and its nutritional modulation for preventive health care. Animal Feed Science and Technology, 173(1–2), 111–133.

Mamun, M. A., Hossain, M. A., Saha, J., Khan, S., Akter, T., Banu, M. R. (2023). Effects of spirulina *Spirulina platensis* meal as a feed additive on growth performance and immunological response of Gangetic mystus *Mystus cavasius.* Aquaculture Reports, 30, 101553.

Nandi, S. K., Suma, A. Y., Rashid, A., Kabir, M. A., Goh, K. W., Seong Wei, L. (2023). The potential of fermented water spinach meal as a fish meal replacement and the impacts on growth performance, reproduction, blood biochemistry and gut morphology of female stinging catfish (*Heteropneustes fossilis*). Life, 13(1), 176-184.

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., Troell, M. (2021). A 20-year retrospective review of global aquaculture. Nature, 591(7851), 551-563.

Nugraha, S., Ekasari, J., Junior, M. Z., Widanarni, W. (2020). Digestive system and growth performance of African catfish larvae *Clarias gariepinus*, (Burchell, 1822) maintained with biofloc technology with the addition of *Chlorella* sp. Jurnal Iktiologi Indonesia, 20(3), 297-306 (in Indonesian).

Pantami, H., Bustamam, M., Lee, S., Ismail, I., Faudzi, S., Nakakuni, M., Shaari, K. (2020). Comprehensive GCMS and LC-MS/MS Metabolite Profiling of *Chlorella vulgaris*. Marine Drugs, 18, 367.

Paul, B. N., Bhowmick, S., Chanda, S., Sridhar, N., Giri, S. S. (2018). Nutrient profile of five freshwater fish species. SAARC Journal of Agriculture, 16 (2), 25-41.

Pradhan, J., Sahu, S. Das, B. K. (2023). Protective effects of *Chlorella vulgaris* supplemented diet on antibacterial activity and immune responses in rohu fingerlings, *Labeo rohita* (Hamilton), subjected to *Aeromonas hydrophila* infection. Life 13(4), 1028.

Prakash, S. (2022). Condition Factor, Hepato-somatic Index and Gonado-somatic Index of Fish, *Channa punctatus* Collected from Sawan Nallaha, Balrampur, UP. The Scientific Temper, 13(01), 46-50.

Radhakrishnan, S., Seenivasan, C., Muralisankar, T. (2015). Effect of dietary replacement of fishmeal with *Chlorella vulgaris* on growth performance, energy utilization and digestive enzymes in *Macrobrachium rosenbergii* post larvae. International Journal of Fisheries and Aquaculture, 7(5), 62-70.

Rahimnejad, S., Lee, S. M., Park, H. G., Choi, J. (2017). Effects of dietary inclusion of *Chlorella vulgaris* on growth, blood biochemical parameters, and antioxidant enzyme activity in olive flounder, *Paralichthys olivaceus*. Journal of the World Aquaculture Society, 48(1), 103-112.

Rahman, M., Mamun, M. A. A., Rathore, S. S., Nandi, S. K., Abdul Kari, Z., Wei, L. S., Kabir, M. A. (2023). Effects of dietary supplementation of natural Spirulina on growth performance, hemato-biochemical indices, gut health, and disease resistance to *Aeromonas hydrophila* of Stinging catfish (*Heteropneustes fossilis*) fingerling. Aquaculture Reports, 32, 101727.

Raji, A. A., Alaba, P. A., Yusuf, H., Bakar, N. H. A., Taufek, N. M., Muin, H., Razak, S. A. (2018). Fishmeal replacement with *Spirulina Platensis* and *Chlorella vulgaris* in African catfish (*Clarias gariepinus*) diet: Effect on antioxidant enzyme activities and haematological parameters. Research in Veterinary Science, 119, 67-75.

Raji, A. A., Jimoh, W. A., Bakar, N. H. A., Taufek, N. H. M., Muin, H., Alias, Z., Milow, P., Razak, S. A. (2020). Dietary use of *Spirulina (Arthrospira*) and *Chlorella* instead of fish meal on growth and digestibility of nutrients, amino acids and fatty acids by African catfish. Journal of Applied Phycology, 32(3), 1763-1770.

Remize, M., Brunel, Y., Silva, J. L., Berthon, J. Y., Filaire, E. (2021). Microalgae n-3 PUFAs production and use in food and feed industries. Marine Drugs, 19(2), 113.

Rummer, J. L., Brauner, C. J. (2015). Root effect hemoglobin in fish may greatly enhance general oxygen delivery relative to other vertebrates. PLoS One, 10(10), e0139477.

Safi, C., Zebib, B., Merah, O., Pontalier, P. Y., Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. Renewable and Sustainable Energy Reviews, 35, 265-278.

Saha, J., Hossain, M. A., Mamun, M. A., Islam, M. R., Alam, M. S. (2022). Effects of carbonnitrogen ratio manipulation on the growth performance, body composition and immunity of stinging catfish *Heteropneustes fossilis* in a biofloc-based culture system. Aquaculture Reports, 25, 101274.

Sahan, A., Tasbozan, O., Aydýn, F., Ozütok, S., Erbas, C., Duman, S., Uslu, L., Ozcan, F. (2015). Determination of some haematological and non-specific immune parameters in Nile tilapia *Oreochromis niloticus* fed with spirulina (*Spirulina platensis*) added diets. Journal of

Aquaculture Engineering and Fisheries Research, 1 (3), 133-139.

Scapigliati, G., Fausto, A. M., Picchietti, S. (2018). Fish lymphocytes: an evolutionary equivalent of mammalian innate-like lymphocytes. Frontiers in Immunology, 9, 971-978.

Shi, X., Luo, Z., Chen, F., Wei, C. C., Wu, K., Zhu, X. M., Liu, X. (2017). Effect of fish meal replacement by *Chlorella* meal with dietary cellulase addition on growth performance, digestive enzymatic activities, histology and myogenic genes' expression for crucian carp *Carassius auratus.* Aquaculture Research, 48(6), 3244-3256.

Tongsiri, S., Mang-Amphan, K., Peerapornpisal, Y. (2010). Effect of replacing fishmeal with spirulina on growth, carcass composition and pigment of the Mekong giant catfish. Asian Journal of Agricultural Sciences, 2 (3), 106-110.

Udayan, A., Pandey, A. K., Sirohi, R., Sreekumar, N., Sang, B. I., Sim, S. J., Kim, S. H., Pandey, A. (2022). Production of microalgae with high lipid content and their potential as sources of nutraceuticals. Phytochemistry Reviews, 22(4), 833-860.

Witeska, M., Kondera, E., Bojarski, B. (2023). Hematological and hematopoietic analysis in fish toxicology—a review. Animals, 13(16), 2625.

Xu, W., Gao, Z., Qi, Z., Qiu, M., Peng, J. Q., Shao, R. (2014). Effect of dietary *Chlorella* on the growth performance and physiological parameters of gibel carp, *Carassius auratus* gibelio. Turkish Journal of Fisheries and Aquatic Sciences, 14(1), 53-57.

Yeganeh, S., Teimouri, M., Amirkolaie, A. K. (2015). Dietary effects of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout *(Oncorhynchus mykiss*). Research in Veterinary Science, 101, 84-88.

Zahan, N., Hossain, M. A., Islam, M. R., Saha, J., Akter, T., Fatema, U. K., Haque, F. 2024. Effects of dietary *Spirulina platensis* on growth performance, body composition, haematology, immune response, and gut microflora of stinging catfish *Heteropneustes fossilis.* Aquaculture Reports, 35, 101997.