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Impact of *Spirulina platensis* Supplementation on Growth, Health, and Antioxidant Defence in Giant Gourami (*Osphronemus goramy*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A 90-day trial investigated the effects of dietary *Spirulina platensis* (SP) supplementation on growth performance, haematology, serum biochemistry, digestive enzymes, and hepatic antioxidants in giant gourami (*Osphronemus goramy*). Fish (6.96 ± 0.011 g) were distributed into six treatments with 3 replicates each (15 fish per replicate) across 18, 60 L aquaria. Six isonitrogenous diets with 0% (control), 1% (SP1), 2% (SP2), 3% (SP3), 4% (SP4), and 5% (SP5) SP were provided *ad*

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libitum twice daily. Results showed dose-dependent improvements in final weight, weight gain, weight gain rate, protein efficiency ratio, and specific growth rate, with the highest values at 5% SP ($P<0.05$). Feed conversion ratio decreased with higher SP, indicating enhanced efficiency. Body indices increased significantly with higher SP ($P<0.05$). Haematological parameters, including WBC (white blood cells), HB (hemoglobin), HCT (hematocrit), monocyte %, and neutrophil %, improved with increasing SP significantly ($P<0.05$). Serum biochemistry showed markedly reduced cholesterol and triglycerides concentration, and increased total protein, albumin, and globulin levels with higher SP ($p<0.05$). Intestinal amylase and lipase activities increased significantly with SP ($p<0.05$), while protease remained consistent. Hepatic antioxidants revealed decreased MDA (malondialdehyde) and increased SOD (Superoxide dismutase) with higher SP ($P<0.05$). Carcass analysis showed increased protein, fibre, and fat content, and decreased ash and carbohydrates with higher SP. These findings suggest that 5% SP enhances growth, biochemical parameters, haematology, digestive, and antioxidant mechanisms in *O. goramy*, supporting its potential as a dietary supplement in aquaculture.

Keywords: Spirulina; giant gourami; growth; haematology; immune response; digestive enzyme.

1. INTRODUCTION

In this modern era, the decline in the production of capture fisheries contrasts with the escalating demand for fish products for human consumption. Consequently, the Food and Agriculture Organization anticipates that aquaculture activities will contribute 60% of future fisheries production, a trend projected to persist and increase [1]. The expansion of aquaculture to include a diverse range of fish species has amplified the requirement for commercial feed [2,3,4,5]. Approximately 70% of animal production in aquaculture relies on commercial feeds rich in protein [2,3,4,5,6].

The giant gourami, *Osphronemus goramy*, stands out as a favoured species for both pisciculture [7] and the ornamental industry, primarily owing to its impressive size, reaching nearly 2 feet in length, its adaptability to freshwater as well as brackish water, and its suitability for live fish trade [8]. This fish exhibits omnivorous behaviour with a strong inclination towards vegetarianism, demonstrating relative robustness and adaptability to adverse environmental conditions such as aerial respiration, which renders it highly appealing for low-input aquaculture [9]. While the production of giant gourami fry relies on the natural spawning of captive broodfish held in ponds, efforts to standardize commercial-scale propagation of the species are still underway [10–12]. It is noteworthy that the growth rate of gourami is relatively slow, requiring 18 months to attain a weight of 500 grams [13]. Native to Southeast Asia, particularly from Indonesia, the Malay Peninsula, Thailand, and the Mekong basin, the giant gourami *Osphronemus goramy* Lacépède, 1801, has been introduced to numerous other countries including India, Pakistan, Sri Lanka, Mauritius, Seychelles, Madagascar, Uganda, Philippines, Australia, New Zealand, Papua New Guinea, Japan, Hawaii, Colombia, France, Italy, among others, for aquaculture purposes [14,15]. In India, the giant gourami was first introduced from Mauritius and Java (Indonesia) to Chennai during 1865–66 and subsequently distributed to various regions of the country [8]. The cultivation of this species for food is particularly well-established in the state of Kerala, situated on the south-west coast. Furthermore, the National Fisheries Development Board, Government of India, is actively promoting this fish as one of the potential candidate species for both aquaculture and the ornamental fish trade in India [16].

Spirulina platensis is a type of blue-green algae characterized by its filamentous, spiral shape and remarkable nutritional value. Its cell wall possesses a soft texture due to a coating of complex sugars and proteins, facilitating easy digestion. This alga boasts an impressive nutrient composition, with approximately 62% protein content, as well as vitamins and antioxidants such as β-carotene and xanthophyll [17]. Henrikson et al. [18] highlighted the presence of Gamma Linolenic Acid (GLA) and iron in *S. platensis,* alongside chlorophyll, glycogen, minerals [19], and phycocyanin and porphirin [20]. Furthermore, it is rich in essential amino acids, vitamins B12 and β-carotene, phosphorus, iron, and calcium [21]. *Spirulina platensis* is also known for its bioactive compounds with antioxidant properties, making it a valuable supplement for enhancing food [22] and serving as a significant source of micronutrients due to its high protein content [23].

Spirulina, renowned for its phycocyanin content [24], particularly C-phycocyanin, demonstrates antioxidant properties and may serve as a viable alternative to heparin due to its anticoagulant effects [25]. Phycocyanin has been utilized for its antioxidative, antifungal and antitumor characteristics [26,27], as well as its immunostimulatory and hepatoprotective benefits [28]. Multiple studies on Nile tilapia have indicated that incorporating *Spirulina* into their diet significantly enhances growth rates, immune response, and disease resistance [29–32]. Li et al. demonstrated that phycocyanin augments the activities of SOD and GPx enzymes in pancreatic beta cells [32]. The inclusion of *S. platensis* in diets can modulate the immune system [33] facilitate large-scale fish farming [34], and serve as a pivotal component for animal feed [35]. Moreover, supplementing fish feed with *S. platensis* has been shown to enhance growth and survival rates [36,37] and can serve as a primary ingredient for feed production [38].

The rise in giant gourami production can be attributed to advancements in production inputs such as feed, seed, disease management, and optimal culture techniques within giant gourami aquaculture. However, a significant challenge in culturing this fish lies in the inefficiency of production systems, largely due to their slow growth rate [39,40]. In terms of feed technology development, numerous studies have been undertaken, particularly focusing on identifying alternative protein sources capable of promoting optimal growth performance in giant gourami [41,42]. The utilization of chemicals for controlling bacterial or parasitic populations presents drawbacks such as bioaccumulation, pollution, and potential human health risks. Furthermore, the excessive reliance on antibiotics in aquaculture has led to issues of resistance, immunosuppression, and disruption of beneficial bacterial populations.

The utilization of *Spirulina*, known for its nutritional value and bioactive properties, offers a promising avenue for enhancing fish growth performance, immune response, and overall health, thereby contributing to the advancement of sustainable aquaculture practices. By investigating the effects of *S. platensis* supplementation on the growth and health parameters of *Osphronemus goramy*, a commercially important fish species, this study aims to identify potential strategies for improving aquaculture efficiency and reducing reliance on conventional feed additives and antibiotics.

2. MATERIALS AND METHODS

2.1 Water Quality

Water quality analysis was conducted three times throughout the maintenance period (on days 1, 30, and last days also taken). A thermometer calibrated in Celsius was utilized to measure water temperature. Dissolved oxygen (O2; mg L-1) levels were measured using an oxygen meter (YSI Model 52, Yellow Instrument Co, Yellow Spring, OH USA). The pH values of the water were determined using a digital pH meter (Mini 0–14 pH IQ, Scientific Cemo Science, Thailand). The concentrations of nitrite (mg/L), nitrate (mg/L), TAN (mg/L), alkalinity (mg L-1), and hardness (mg L-1) were determined following standard procedures outlined by APHA [58].

2.2 Experimental Fish

A collection of 270 superficially robust giant gourami (*Osphronemus goramy*), averaging 6.96 ± 0.011g in weight, was procured from a seed farm. These fish were subjected to a one-week acclimatization period in laboratory conditions before being randomly distributed into six dietary groups. Each group of juvenile gouramis was accommodated in aquariums 60L, with a stocking density of 15 fish per aquarium. To maintain optimal conditions, each aquarium was equipped with a heater set to maintain a temperature of 30°C and aeration equipment. Before introducing the juveniles, the aquariums underwent sterilization using 30 ppm chlorine. Throughout the one-week acclimatization period, the juveniles were provided with a commercial diet containing 30% protein ad libitum. Body weight measurements were taken individually at the commencement and conclusion of the maintenance period for each aquarium. To sustain water quality, 25% of the total aquarium water volume was replaced before the morning feeding. Over the 90-days experimental period, fish were weighed every 15 days to adjust the daily feed ration, following the recommendation of the National Research Council [43], which was administered twice daily at 9:00 am and 5:00 pm.

2.3 Experimental Diets

Six different diets were prepared with varying levels of *S. platensis* (SP) ranging from 0% (C), 1% (SP2), 2% (SP2), 3% (SP3), 4% (SP4) and 5% (SP5). Detailed information regarding the formulation of the feeds and their proximate

compositions presented in Table 1. Each diet was carefully prepared by weighing the ingredients according to the specified proportions and thoroughly mixing them for a period of 10 minutes. Subsequently, fish oil and plant oil were incorporated into the mixture, which was then passed through a 425 µm sieve. Further mixing was carried out for components containing fish oil for an additional 10 minutes. Afterward, distilled water was added and stirred for 5 minutes before being cooked in an autoclave. Pellets of 2.0 mm to 2.5 mm diameter was produced using a pelletizer through wet-extrusion after autoclave, followed by air-drying. The resulting pellets were then packaged in plastic bags and stored at room temperature.

2.4 Growth Performance

The daily feed intake (FI) of the fish was monitored consistently throughout the entire experiment. Before being weighed, the fish were carefully dried using fresh filter paper to eliminate any excess moisture. They were then weighed using a digital balance. The parameters were computed using the following formulas:

1) Weight gain rate (WGR, %) = 100 × (Final body weight - initial body weight)/Initial body weight [45]
2) WG (g fish-1) = Final body weight - Initial weight of the fish (g) [45]
3) Specific growth rate (SGR, % day−1) = 100 × [(ln final individual weight - ln initial individual weight)/number of days
4) Average daily growth ADG (g fish-1 day-1) = (Final body weight - Initial weight of the fish (g))/Day
5) Feed conversion ratio (FCR) = Total diet fed/ total wet weight gain [45]
6) Feed efficiency ratio (FER) = 1/FCR
7) Survival rate (SR, %) = 100 × (Final fish number/Initial fish number).
8) Protein efficiency ratio (PER) = Final biomass (g) - initial biomass (g) /Protein intake [46]

Three fish from each tank were dissected to determine the Viscerosomatic index (GSI%), Hepatosomatic index (HSI%), Visceral fat-somatic indexes (VFSI%), and Bilesomatic index (BSI) utilizing the prescribed formulas:

GSI = 100 [viscera weight (g) / whole body weight (g)]
HSI = 100 [liver weight (g) / whole body weight (g)]
VFSI = 100 [visceral fat weight (g) / whole body weight (g)]
BSI = 100 [Bile weight (g) / weight of liver (g)]

2.5 Proximate Analysis

The fish body samples collected before infection, comprising three fish from each replicate (totalling nine from each group), underwent chemical analysis for moisture, protein, fat, and ash using the standard method recommended by the Association of Official Analytical Chemists [47]. Approximately nine fish per treatment were included in the analysis. Dry matter (DM) content was determined by drying the samples in an oven at 105°C for 24 hours. Crude protein was quantified using the micro-Kjeldhal method, with nitrogen content multiplied by 6.25 (utilizing a Kjeltech autoanalyzer, Model VELP Scientifica, UDK 127). Crude lipids were assessed using the Soxhlet method with ether extraction (Model VELP Scientifica, SER 148), while ash content was determined by burning samples at 550°C for 16 hours. Crude fiber (CF) analysis was conducted after digestion with 5% sulfuric acid and 5% sodium hydroxide for 15 minutes; the residues were subsequently dried and ashed. The nitrogen-free extract (NFE) was calculated using the formula: NFE = 100 – (CP+EE+CF+ash).

2.6 Sample Collection

At the end of 90-day experiment period, all fish underwent a 24-hour fasting period before being subjected to the final sampling. Fish samples were randomly collected from each treatment group and anesthetized using Tricaine methanesulfonate. Blood samples (2ml/replica) were then obtained from the caudal blood vessel using a 1 ml syringe (MDI Europa GmbH, Germany). After blood collection, the blood samples were immediately divided into two equal parts. One part was transferred to a tube containing an anti-coagulant (heparin) for hematological analysis, while the other part was transferred to non-heparinized tubes for biochemical and other analyses. The samples were then centrifuged at 4°C with 3000 rpm for 10 minutes and stored at −80 °C until further use, following the protocol outlined by Adel et al [44]. Additionally, carcass composition analysis was conducted on three fish samples, which were stored at −20°C.

Table 1. Feed ingredients and the proximate chemical composition (% of dry weight) in the experimental diets

Treatment	Control	T1	T2	T3	T4	T5
Spirulina p.	0	1	2	3	4	5
Soybean meal	21	21	21	21	21	21
Fish meal	10.5	10.5	10.5	10.5	10.5	10.5
Poultry by-product meal	16.5	16.5	16.5	16.5	16.5	16.5
Rice bran	21.3	20.3	19.3	19.3	18.3	18.3
Wheat flour	17.7	17.7	17.7	17.7	17.7	17.7
Tapioca powder	5	5	5	4	4	3
Plant oil	2	2	2	2	2	2
Fish oil	3	3	3	3	3	3
Vitamin mineral mix[1]	3	3	3	3	3	3
Total	100	100	100	100	100	100
Moisture	8.74	8.84	8.86	8.82	8.76	8.88
Dry matter	91.26	91.16	91.14	91.18	91.24	91.12
Protein	30.399	30.289	30.179	30.179	30.069	30.069
Ether extraction	5.24	5.33	5.27	5.38	5.28	5.3
Ash	6.34	6.25	6.25	6.38	6.38	6.4
Fiber	4.2	4.26	4.24	4.23	4.36	4.3
CHO	43.85065	43.66796	43.7554	43.29211	43.28394	43.00126
NFE** (%)[2]	44.98	44.93	44.83	44.74	44.52	44.46
Gross energy (kkal/g)[3]	405.26	404.8	403.95	403.08	402.61	401.58

[1]Composition of vitamin mineral mixture of 1 kg: Vitamin A - 50,00,000 IU; Vitamin D3 - 10,00,000 IU; Vitamin B2 - 2.0 g; Vitamin E - 750 units; Vitamin K - 1.0 g; Calcium pantothenate 2.5 g; Nicotinamide - 10.0 g; Vitamin B12 - 6.0 g; Choline Chloride - 150.0 g; Calcium - 750.0 g; Manganese - 27.5 g; Iodine - 1.0 g; Ion - 7.5 g; Zinc - 15.0 g; Copper - 2.0 g; Cobalt - 0.45 g.

[2]NFE: Nitrogen free extract calculated using the following equation: NFE = 100- (crude protein + ether extract + crude fiber + ash).

[3]GE: Gross energy calculated on the basis of 23.6, 39.4 and 17.2 k joule gross energy g-1 protein, ether extract and NFE, respectively.

P/E ratio: Protein energy ratio (mg crude protein kJ-1 gross energy) = CP/GE × 1000.

2.7 Hematological Parameters Analysis

Red blood cell (RBC, 10^6 mm^3) and white blood cell (WBC, 10^3 mm^3) counts, hemoglobin (Hb, g dL-1), and hematocrit (Ht %) were measured according to the method detailed by Li et al. [54]. Differential leukocytic counts (neutrophils, lymphocytes, and monocytes) were conducted using an automated blood cell counter.

2.8 Biochemical Measurements

Superoxide dismutase (SOD Umg/prot) and malondialdehyde (MDA µg/g) levels were determined following the methods outlined by Nishikimi et al. [48] and Mihara & Uchiyama [49], respectively. The concentration of total protein (g dL-1) in plasma samples from the various experimental groups was measured using the Biuret method as described by AG [50]. Albumin concentration (g dL-1) was assessed using the bromocresol green method [51], while globulin concentration (g dL-1) was computed by subtracting albumin from total protein. The Albumin to Globulin ratio (A/G ratio) was obtained by dividing albumin by globulin. Total cholesterol (TC) and triglycerides (TG) levels were determined using colorimetric diagnostic kits provided by spectrum-bioscience (Egyptian Company for Biotechnology, Cairo, Egypt), following the protocols of Allain et al. [52] and McGowan et al. [53], respectively.

2.9 Enzyme Activity

Enzyme activity, such as protease, amylase, and lipase, was determined using the methodologies described by [55, 56,57].

2.10 Statistical Analysis

The values for each parameter were expressed as the arithmetic mean ± standard error (SE). The effects of enriched diets on growth performance, hematological, biochemical, digestive enzyme activity, and water quality parameters were assessed using one-way ANOVA. Comparison of the mean values was conducted using Duncan's multiple range tests at a significance level of 0.05%. The software

program SPSS (Version 14.0; SPSS) for Windows was employed for the analysis.

3. RESULTS

3.1 Water Quality

The water quality of the aquarium where giant gourami juveniles were reared was assessed. Parameters including water temperature, dissolved oxygen (DO), total alkalinity, ardness, pH, and nitrates were measured and found to be within the typical range recommended by WHO/FAO standards, as outlined in Table 2.

3.2 Growth Performance

The growth performance of fish fed with various experimental diets is presented in Table 3. At the conclusion of the 90-day experiment, the results indicated a dose-dependent increase in Final Weight (FW), Weight Gain (WG), Weight Gain Rate (WG%), Feed Efficiency Ratio (FER), Protein Efficiency Ratio (PER), Average Daily Gain (ADG) (g fish-1 day-1), and Specific Growth Rate (SGR). The highest values for FW, WG, WG%, PER, FER, ADG, and SGR were observed in the T5 (5% SP) diet group, showing statistical significance ($P<0.05$). These parameters were notably higher in the T5 (5% SP) diet group compared to the control group (Control) and other treatments ($P < 0.05$). Conversely, a dose-related decrease was observed in Feed Conversion Ratio (FCR), with the highest decrease recorded in the T5 (5% SP) diet group ($P<0.05$). No significant variations were detected in Survival Rate (SR) among all treatment groups ($P>0.05$). According to the broken line regression model, the optimal SPC supplementation level was determined to be 5% based on the survival rate and growth parameters.

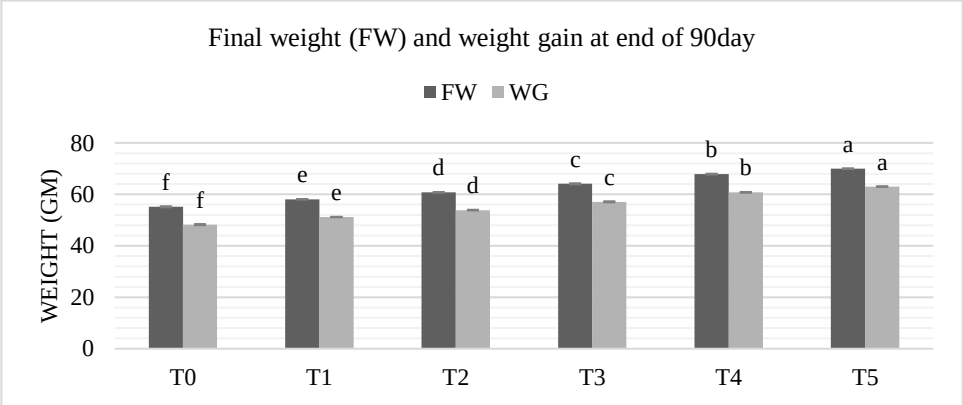

Fig. 1. Body weight and weight gain of *Osphronemus goramy* during the 90-day experiment after feeding trial of Spirulina platensis.

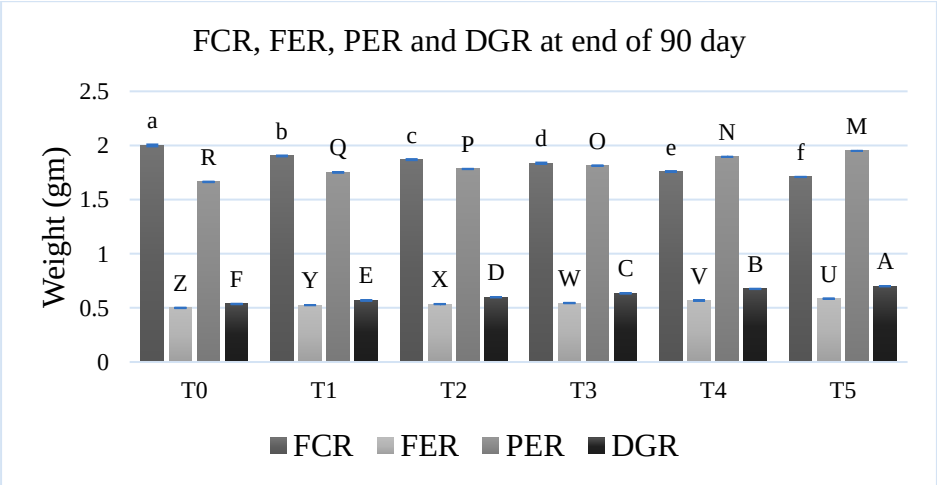

Fig. 2. FCR, FER, PER and DGR of *Osphronemus goramy* during the 90-day experiment after feeding trial of Spirulina platensis.

Table 2. Water quality parameter observed during 90 days of experiment

	Control (0% SP)	T1 (1% SP)	T2 (2% SP)	T3 (3% SP)	T4 (4% SP)	T5 (5% SP)
Temperature (°C)	28.56±0.088^{e}	28.93±0.088^{d}	28.6±0.057^{e}	30.1±0.057^{b}	30.7±0.057^{a}	29.3±0.057^{c}
PH	7.833±0.008^{a}	7.396±0.012^{f}	7.496±0.008^{e}	7.533±0.008^{d}	7.716±0.008^{c}	7.793±0.008^{b}
DO (mg/L)	5.813±0.012^{e}	6.096±0.012^{b}	6.24±0.011^{a}	5.963±0.012^{c}	5.863±0.008^{d}	6.266±0.012^{a}
Total Alkalinity (mg/L as CaCO3)	55.33±0.088^{d}	56.46±0.088^{c}	59.53±0.088^{b}	54.73±0.088^{e}	60.06±0.088^{a}	59.33±0.088^{b}
Hardness (mg/L as CaCO3)	66.56±0.088^{e}	68.56±0.088^{d}	69.23±0.088^{c}	65.56±0.088^{f}	69.86±0.088^{b}	70.23±0.088^{a}
NO3 (mg/L)	0.054±0.001^{a}	0.031±0.001^{c}	0.046±0.001^{b}	0.035±0.001^{c}	0.047±0.001^{b}	0.057±0^{a}
NO2 (mg/L)	0.027±0.002^{e}	0.069±0.001^{b}	0.021±0.001^{f}	0.063±0.001^{c}	0.041±0.001^{d}	0.079±0^{a}
TAN (mg/L)	0.606±0.008^{e}	0.773±0.008^{d}	0.48±0.005^{f}	1.506±0.008^{b}	1.806±0.008^{a}	0.896±0.008^{c}

Table 3. Mean (SE) value growth parameter of *Osphronemus goramy* during the 90-day experiment after feeding trial of Spirulina platensis.

	Control (0% SP)	T1(1% SP)	T2(2% SP)	T3(3% SP)	T4(4% SP)	T5(5% SP)
Initial weight (IW)	6.926±0.012^{d}	6.893±0.008^{d}	6.896±0.008^{d}	7.01±0.015^{b}	7.1±0.011^{a}	6.973±0.012^{c}
Final weight (FW)	55.16±0.202^{f}	58.07±0.12^{e}	60.76±0.145^{d}	64.1±0.208^{c}	67.87±0.145^{b}	69.98±0.06^{a}
Weight gain (WG) gm	48.24±0.214^{f}	51.173±0.111^{e}	53.87±0.143^{d}	57.09±0.202^{c}	60.766±0.156^{b}	63.01±0.05^{a}
Weight gain Rate (WG%)	696.45±4.29^{f}	742.35±0.676^{e}	781.10±2.163^{d}	814.41±2.794^{c}	855.88±3.594^{b}	903.58±1.102^{a}
Total feed consumption (TFC) gm	96.5±0^{f}	97.4±0^{e}	100.7±0^{d}	104.8±0^{c}	106.9±0^{b}	107.7±0^{a}
Feed conversion ratio (FCR)	2±0.008a	1.903±0.004^{b}	1.869±0.004^{c}	1.835±0.006^{d}	1.759±0.004^{e}	1.709±0.001^{f}
FER	0.499±0.002^{f}	0.525±0.001^{e}	0.534±0.001^{d}	0.544±0.001^{c}	0.568±0.001^{b}	0.585±0^{a}
Protein efficiency ratio (PER)	1.663±0.009^{f}	1.751±0.006^{e}	1.783±0.008^{d}	1.813 ±0.007^{c}	1.895±0.008^{b}	1.950±0.002^{a}
ADG (g fish-1 day-1)	0.536±0.002^{f}	0.568±0.001^{e}	0.598±0.001^{d}	0.634±0.002^{c}	0.675±0.001^{b}	0.7±0^{a}
Specific growth rate (SGR) (%)	2.305±0.005^{f}	2.367±0^{e}	2.417±0.002^{d}	2.459±0.003^{c}	2.508±0.004^{b}	2.562±0.001^{a}
Survival rate (SR%) (%)	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}

3.3 Body Indices

The body indices of fish fed with varying *spirulina* concentration diets are listed in Table 4. The hepatosomatic index (HSI) exhibited a significant increase from Control (0% SP) to T5 (5% SP) over the experimental period (P<0.05). Similarly, the viscerosomatic index (GSI%) showed a significant upward trend from Control (0% SP) to T5 (5% SP), indicating an increase in visceral mass relative to body weight (P<0.05). The visceral fat-somatic indexes (VFSI%) displayed a significant rise from Control to T5 (5% SP), signifying an augmentation in visceral fat deposition over time (P<0.05). The bile somatic index (BSI%) demonstrated a statistically significant increase from Control to T5 (5% SP), indicating an elevation in bile accumulation relative to body weight (P<0.05).

3.4 Carcas Composition

Protein content exhibited a significant increase (P<0.05) as *spirulina* content increased, from 17.07% ± 0.03 at Control to 18.6% ± 0.01 at T5 (Table 5). Fat content showed a similar trend, with significant differences observed between each treatment (P<0.05). Fiber content also increased significantly with higher SP content, ranging from 0.96% ± 0 at Control to 1.2% ± 0.01 at T5. ASH (Ash content) decreased significantly (P<0.05) with increasing SP content, with values ranging from 5.13% ± 0.01 at Control to 4.23% ± 0.01 at T5. Carbohydrate (CHO) content displayed a significant decrease (P<0.05) as SP content increased, with values ranging from 1.98% ± 0.01 at Control to 1.21% ± 0 at T5. Dry matter content showed significant variability across SP levels, and Moisture content exhibited an inverse relationship with *spirulina* content. Overall, the results indicate that increasing SP content in the body composition led to significant changes in nutritional composition, particularly in protein, fat, fibre, ash, and carbohydrate contents.

3.5 Hematological Parameters

As indicated in Table 6, no significant differences were observed in RBC levels among all treatments (P>0.05). However, WBC, Hb, HCT, Monocyte, and Neutrophil levels exhibited a significant increase from Control to T5 (5% SP) as *spirulina* concentration increased (P<0.05). Lymphocyte levels demonstrated significant fluctuations across different concentrations of *spirulina*, with the highest level observed at T5 (5% SP) and the lowest in Control (P<0.05).

3.6 Serum Biochemical Parameters

The serum biochemical parameters of fish fed with *Spirulina* are showed in Table 7. Fish fed the control diet exhibited the highest levels of cholesterol and triglyceride, with these levels decreasing as the dietary *S. platensis* levels increased (P<0.05). Total protein (g dL-1), albumin (g/dL), and globulin (g/dL) levels increased with the increase in *spirulina* concentration. The significantly highest levels of protein (g dL-1), albumin (g/dL), and globulin (g/dL) were observed in the T5 (5% SP) diet group (P<0.05). The A:G ratio (g/dL) was significantly highest in the Control (0% SP) diet group and lowest in the T1 (1% SP) diet group (P<0.05).

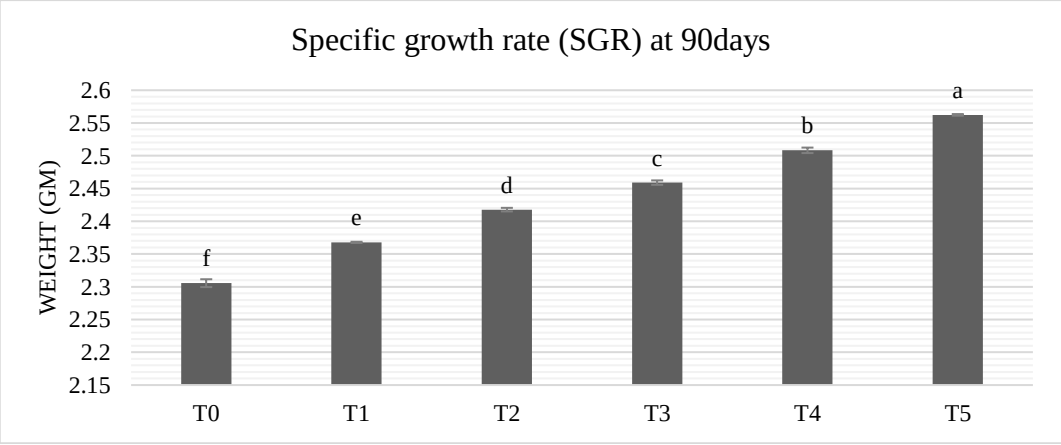

Fig. 3. Specific growth rate (SGR) of *Osphronemus goramy* during the 90-day experiment after feeding trial of Spirulina platensis.

Table 4. Mean (SE) value condition factor and body indices of *Osphronemus goramy* during the 90-day experiment after feeding trial of Spirulina platensis

	Control (0% SP)	T1 (1% SP)	T2 (2% SP)	T3 (3% SP)	T4 (4% SP)	T5 (5% SP)
Hepatosomatic index (HSI) (%)	0.926±0.012^{e}	0.956±0.008^{d}	0.976±0.008^{d}	0.993±0.008^{c}	1.1±0.011^{b}	1.29±0.005^{a}
Viscerosomatic index (GSI%)	2.706±0.029^{f}	2.78±0.017^{e}	2.853±0.014^{d}	2.983±0.014^{c}	3.216±0.02^{b}	3.493±0.02^{a}
Visceral fat-somatic indexes (VFSI%)	2.09±0.005^{f}	2.133±0.008^{e}	2.17±0.005^{d}	2.21±0.005^{c}	2.29±0.005^{b}	2.41±0.005^{a}
Bilesomatic (BSI%)	9.916±0.014^{f}	10±0.025^{e}	10.206±0.008^{d}	10.296±0.014^{c}	10.343±0.008^{b}	10.443±0.008^{a}

Table 5. Effects of dietary *S. platensis* on Body composition of *Osphronemus goramy*.

	Control (0% SP)	T1 (1% SP)	T2 (2% SP)	T3 (3% SP)	T4 (4% SP)	T5 (5% SP)
Crude Protein%	17.07±0.03^{f}	17.36±0.01^{e}	17.51±0.01^{d}	17.79±0.01^{c}	18.19±0^{b}	18.6±0.01^{a}
Crude Fat	4.2±0.01^{d}	4.29±0.01^{c}	4.28±0.01^{c}	4.51±0.02^{b}	4.53±0.02^{b}	4.61±0.01^{a}
Crude Fiber	0.96±0^{d}	0.98±0.01^{d}	1.04±0^{c}	1.08±0.01^{b}	1.11±0.01^{b}	1.2±0.01^{a}
ASH	5.13±0.01^{f}	4.91±0.01^{e}	4.81±0.01^{d}	4.51±0.01^{c}	4.3±0.01^{b}	4.23±0.01^{a}
CHO	1.98±0.01^{a}	1.96±0^{a}	1.95±0.01^{a}	1.97±0^{a}	1.52±0^{b}	1.21±0^{c}
Dry matter	29.35±0.06^{d}	29.52±0.02^{c}	29.61±0.04^{c}	29.87±0.03^{a}	29.66±0.02^{b}	29.87±0.05^{a}
Moisture content	70.64±0.06^{a}	70.48±0.02^{b}	70.39±0.04^{bc}	70.12±0.03^{d}	70.33±0.02^{c}	70.13±0.05^{d}

Table 6. Effects of dietary *S. platensis* on hematological parameters in *Osphronemus goramy*

	Control (0% SP)	T1(1% SP)	T2(2% SP)	T3(3% SP)	T4(4% SP)	T5(5% SP)
RBC ($mm^3.10^6$)	3.216±1.608^{a}	4.933±0.008^{a}	4.996±0.008^{a}	5.123±0.012^{a}	5.263±0.008^{a}	5.413±0.008^{a}
WBC (mm^3. 10^4)	8.886±0.02^{f}	9.316±0.02^{e}	9.686±0.012^{d}	10.516±0.02^{c}	11.203±0.017^{b}	12.303±0.024^{a}
(Hb, g dL-1)	5.526±0.012^{e}	5.67±0.011^{d}	5.793±0.008^{c}	5.766±0.014^{c}	5.906±0.008^{b}	6±0.011^{a}
HCT (%)	33.513±0.008^{f}	33.813±0.008^{e}	34.1±0.005^{d}	34.423±0.008^{c}	34.846±0.008^{b}	35.216±0.008^{a}
Monocyte (%)	2.51±0.011^{f}	2.776±0.017^{e}	2.916±0.014^{d}	3.33±0.015^{c}	3.79±0.015^{b}	4.21±0.011^{a}
Lymphocyte (%)	76.233±0.12^{a}	73.066±0.12^{d}	75.166±0.088^{b}	72.733±0.088^{d}	74.333±0.12^{c}	70.733±0.12^{e}
Neutrophil (%)	10.116±0.012^{f}	11.423±0.014^{e}	12.663±0.008^{d}	12.983±0.017^{c}	13.65±0.015^{b}	14.306±0.008^{a}

Table 7. The effects of dietary *S. platensis* (SP) on the blood biochemical parameters of *Osphronemus goramy*.

	Control (0% SP)	T1(1% SP)	T2(2% SP)	T3(3% SP)	T4(4% SP)	T5(5% SP)
Total protein (g dL-1)	3.53±0.015^{f}	3.94±0.017^{e}	4.186±0.012^{d}	4.36±0.011^{c}	4.663±0.008^{b}	4.833±0.014^{a}
Albumin (g/dL)	1.843±0.012^{f}	1.91±0.011^{e}	1.97±0.005^{d}	2.093±0.008^{c}	2.153±0.008^{b}	2.27±0.011^{a}
Globulin(g/dL)	1.823±0.014^{f}	2.033±0.02^{e}	2.093±0.008^{d}	2.176±0.008^{c}	2.256±0.014^{b}	2.33±0.015^{a}
A:G ratio (g/dL)	1.011±0.003^{a}	0.939±0.015^{c}	0.941±0.006^{c}	0.961±0.001^{bc}	0.954±0.005^{bc}	0.974±0.007^{c}
Triglyceride (Mg/dl)	315.366±0.384^{a}	300.3±0.115^{b}	293.633±0.12^{c}	289.7±0.115^{d}	283.733±0.12^{e}	280.066±0.12^{f}
Cholesterol (mg/dl)	191.9±0.115^{a}	187.9±0.057^{b}	181.1±0.115^{c}	178±0.057^{d}	172.933±0.088^{e}	168.566±0.145^{f}

Table 8. The effects of dietary *S. platensis* (SP) on Intestinal digestive enzymes activities and Hepatic antioxidant activities of *Osphronemus goramy*.

	Control (0% SP)	T1(1% SP)	T2(2% SP)	T3(3% SP)	T4(4% SP)	T5(5% SP)
Amilase (U/mg substrate)	2.11±0.011^{f}	2.326±0.014^{e}	2.25±0.011^{d}	2.456±0.008^{c}	2.656±0.02^{b}	2.873±0.012^{a}
Lipase (U/mg substrate)	6.803±0.017^{f}	7.346±0.014^{e}	7.126±0.017^{d}	7.833±0.02^{c}	7.553±0.017^{b}	7.973±0.008^{a}
Protease (U/mg substrate)	0.019±0^{c}	0.022±0^{b}	0.019±0^{c}	0.02±0^{c}	0.023±0^{b}	0.025±0^{a}
MDA (µg/g)	2.02±0.017^{a}	1.96±0.011^{b}	1.906±0.008^{c}	1.836±0.014^{d}	1.496±0.017^{e}	1.356±0.012^{f}
SOD (Umg/prot)	6.406±0.008^{f}	7.25±0.011^{e}	7.73±0.011^{d}	8.03±0.011^{c}	8.36±0.011^{b}	8.576±0.008^{a}

3.7 Intestinal Digestive Enzymes and Hepatic Antioxidant Activities

Table 8 illustrates the digestive enzymes activities and antioxidant activities of fish fed with *Spirulina*. Both Amylase and Lipase activities experienced a noteworthy increase as the concentration of *spirulina* increased from 0% to 5% ($P<0.05$). These enzymatic activities demonstrated a gradual elevation with the increasing *spirulina* concentration in the diet. However, there was no significant variation observed in Protease activity across different concentrations of *spirulina* ($P > 0.05$). MDA levels showed a significant decrease from Control (0% SP) to T5 (5% SP) with the increasing concentration of *spirulina* ($P < 0.05$). Conversely, SOD levels exhibited a significant increase from Control (0% SP) to T5 (5% SP) with the increasing *spirulina* concentration ($P<0.05$).

4. DISCUSSION

4.1 Water Quality

In The present study found that water quality parameters, including temperature (°C), dissolved oxygen (mg/L), total alkalinity (mg/L as CaCO3), hardness (mg/L as CaCO3), Ph and nitrates (mg/L), were all within acceptable limits according to WHO/FAO standards (Table 9).

4.2 Growth Performance

During feeding trial, observed a significant increase in the growth performance of *O. goramy* when the *Spirulina* concentration in the feed increased from 1% to 5% over a span of 90 days. The inclusion of *Spirulina* in aquafeed led to an increase in hunger and notably elevated feed intake, resulting in improvements in parameters such as Final weight (FW), weight gain (WG), weight gain% (WG%), feed efficiency ratio (FER), average daily growth (ADG), and specific growth rate (SGR). These findings are consistent with previous research [29,36,59,60]. Studies by Hassanpour et al. [61] and Zeinab et al. [62] found that the supplementation of *Spirulina* in the diets of rainbow trout and Nile Tilapia, respectively, resulted in higher body weights compared to control groups. Similarly, research by Jana et al. [37], Simanjuntak et al. [13], and Abu-Elala et al. [63] demonstrated significant growth enhancement in fish fed with *Spirulina*, without any adverse effects [64] and with potential as a feed material [38]. Additionally, the inclusion of Spirulina in aquafeed improved survival, growth rate, and feed utilization in *Macrobrachium rosenbergii prawn* culture at concentration level from 5% to 20%, as observed in similar studies on *Litopenaeus vannamei* [65]. Overall, *Spirulina* shows promise as a natural supplement for enhancing growth and health in aquaculture.

S. platensis enhances growth in fish due to its highly digestible protein content, abundant essential fatty acids, vitamins (such as vitamin B12), and minerals [66,67]. Its cellular structure lacks cellulose, making it easily digestible by fish digestive enzymes, aided by the presence of mucopolymer murein [64]. Additionally, *Spirulina* promotes the growth of beneficial intestinal flora in fish and aids in the breakdown of indigestible feed components [36,64]. Parameters like protein efficiency ratio (PER), survival rate (SR%), and FCR significantly improve with increased *Spirulina* concentration.

4.3 Body Indices

At the end of experiment result was found that the body indices (HIS%, VFSI%, GSI% and BSI%) was increased with increase in concentration of *spirulina* in diet from (0% to 5%). Significant highest Hepatosomatic index (HSI) (%), Viscerosomatic index (GSI%), Visceral fat-somatic indexes (VFSI%) and Bilesomatic (BSI%) observed in T5 (5% SP) diet ($P<0.05$). A higher hepatosomatic index (HSI) reflects increased glycogen levels and favorable environmental conditions. This relationship was observed in separate studies conducted by Velasquez et al. [68] on Nile tilapia and Tulli et al. [69] on European sea bass juveniles (*Dicentrarchus labrax*), where diets containing 20% *Tetraselmis suecica* microalgae resulted in lower HSI compared to control diets without microalgae. This connection between the level of microalgae inclusion and improved lipid mobilization efficiency in fish liver has also been noted by [70] and [71]. The viscerosomatic index (VSI) serves as an indicator of fish digestive capacity. Consistent findings were reported by [68] in Nile tilapia, where the highest VSI was observed in fish fed diets with 0% to 30% microalgae inclusion, showing a downward trend as the level of *Arthrospira* inclusion increased. Nevertheless, it's essential to recognize that VSI represents just one aspect of fish digestive capacity, as other factors likely play a role.

Table 9. Standard water quality parameter range according to WHO/FAO limits of fish culture

Water quality parameters	WHO/FAO limits	References
Water temperatures (°C)	25–33	[108]
Dissolved oxygen (mg/L)	3-5	[109]
Total alkalinity (mg/L as CaCO3)	120	[110]
Hardness (mg/L as CaCO3)	168	[110]
pH	6.5–9.0	[110]
Nitrates (mg/L)	0.2–219	[110]

4.4 Body Composition

In this study, findings revealed that protein deposition in the body increased with higher *spirulina* content, notably peaking at 18.6±0.01a in the T5 group (5% SP) (P>0.05). Likewise, lipid (4.61±0.01a) and fibre (1.2±0.01a) content rose with escalating *spirulina* concentration, while carbohydrate (CHO) (1.21±0c) and ash (ASH) (4.23±0.01a) levels declined in *O. goramy* bodies with increasing *spirulina* content. Overall, the body composition exhibited optimal results in the high 5% (*spirulina*) concentration diet, indicating efficient utilization of *spirulina*-infused feed by *O. goramy* bodies. These results are consistent with past research; Moreira et al. [105] proposed that *spirulina* LEB-18 is a valuable dietary addition for addressing nutritional deficiencies due to its rich mineral and nutritional content. Changes in body fat and protein content are linked to variations in synthesis and deposition levels within muscle tissues [29,106]. Bhavan et al. [107] noted significant enhancements in the biochemical composition of *Macrobrachium rosenbergii* PL bodies when fed *Spirulina*-enriched Artemia nauplii. Feeding larvae of *C. carpio* with 20% *Spirulina* led to considerable improvements in body composition, particularly in protein composition and body fat [59].

4.5 Hematological Parameters

In present study, we observed a dose-dependent increase in blood parameters, including, WBC, HCT, Hb, Monocyte (%) and Neutrophil (%), in fish fed with 5% *S. platensis* compared to the control group. But there is no significant difference was found in the RBC count among fish fed different concentrations of *Spirulina* (P>0.05) and Lymphocyte (%), count is significantly decrease with increase in the concentration of *spirulina* (P<0.05). the RBC count Similar findings were reported by [72] and [34]. Promya and Chitmant [73] demonstrated higher WBC and RBC counts in African sharp tooth catfish fed diets containing 5% *A. platensis* + basal. [44] found increased RBC and Hb levels in Great Sturgeon fed with *S. platensis*. *Spirulina*'s high iron content significantly affects erythropoiesis, as seen in studies on anaemic rats [74]. Yu et al. [75] observed the highest levels of RBC, WBC, and Hb in Nile tilapia with 10% *Spirulina* supplementation. Similar increases in WBC were reported in large yellow croaker and carp with *Spirulina* supplementation [54,74]. This finding was also supported by [76] and [77]. This is attributed to polypeptide-phycocyanin, enhancing WBC production [78]. Zeinab et al. [62] noted increased hemoglobin levels in Nile tilapia fed *Spirulina*, while [36] found 10% *S. platensis* supplementation increased hematocrit values. However, carp RBCs were unaffected by 3.0–5.0 g/kg *Spirulina* [79].

4.6 Blood Biochemical Parameters

In fish, the levels of total protein, albumin, and globulin in serum provide valuable diagnostic insights into overall nutritional status, vascular system integrity, and liver function [80]. The current study observed that supplementation with 5% *spirulina* resulted in higher levels of total protein, albumin, and globulin compared to the control group. Conversely, previous research by [13] found that diets containing 2.0–6.0% *Spirulina* significantly increased serum levels of total protein, globulin, and the A/G ratio. Similar findings were reported by [81], where feeding with 10.0% *Spirulina* significantly elevated serum albumin levels, as also observed by [29]. Additionally, [62] noted that increased levels of *S. platensis* correlated with higher serum total protein and globulin, although there was no significant difference in albumin levels (P>0.05). The rise in serum albumin levels could be attributed to Spirulina's various active compounds, including carotenoids, polysaccharides, vitamins, minerals, and linoleic acid, which act as immunostimulants, enhancing the immune system [82]. Spirulina's potent antioxidant properties contribute to its ability to

improve immune capacity and health status in *Osphronemus gourami*, similar to findings in *O. niloticus* [22,63]. Furthermore, [83] demonstrated that different levels of *S. platensis* improved total serum protein, albumin, globulin, and biochemical responses in Mugil cephalus. Including *S. platensis* and *Sargassum ilicifolium* extract in diets for *Huso huso* and *O. niloticus* also increased serum total protein and globulin levels [62,84]. The serum albumin level plays a crucial role in regulating metabolism, maintaining osmotic pressure in the blood, and facilitating the transport of both exogenous and endogenous metabolites [85]. Globulin serves as a prominent carrier/transporter in living organisms Simanjuntak et al. [13].

In the present result cholesterol and triglyceride level decrease with increase in S*pirulina* concentration. Similarly, [86] discovered that incorporating 1.0 g/kg *Spirulina* into tilapia diets did not impact the serum levels of total cholesterol and triglycerides. However, diets containing 10.0% *Spirulina* significantly raised the serum levels of cholesterol and triglycerides [29]. *S. platensis* contains α-linolenic acid, linoleic acid, and β-carotene, which have the potential to lower cholesterol and triglyceride levels and prevent hypercholesterolemia and atherosclerosis [87,88].

4.7 Digestive Enzyme

Algae are abundant in both macro and micro elements, which have the potential to stimulate digestive enzyme activity [89]. Low concentrations of microalgae in the gut may trigger the secretion of endogenous digestive enzymes and promote fish growth [90,91]. Lu et al. [92] noted that *Isochryisis galbana* algae enhanced pancreatic and intestinal enzyme production in sea bass (*Dicentrarchus labrax*) larvae. While fish typically struggle to digest plant matter due to the cellulose cell wall, *S. platensis* is known to have a thin cell wall composed primarily of 80% pectin and 20% cellulose, facilitating improved digestion and assimilation [93]. Consequently, *S. platensis* is easily digested and may enhance the assimilation of other nutrients, including antioxidants.

In the current experiment, the activity of amylase (U/mg substrate), lipase (U/mg substrate), and protease (U/mg substrate) significantly increased with increase the concentration of *spirulina* in diet compared to the control group ($P>0.05$). Similarly, Biabani Asrami et al. observed that dietary supplementation of phycocyanin extracted from *Spirulina* enhanced the activities of amylase, protease, and lipase in Guppy fish (*Poecilia reticulata* [27]). Additionally, Teimouri et al. [94] found that *S. platensis* enhanced digestive enzyme activity, thereby improving nutrient assimilation. Therefore, increasing the inclusion of *spirulina* in the diet improved the digestive enzyme activity in *Osphronemus gourami*.

4.8 Antioxidant Activity

Superoxide dismutase (SOD) play crucial roles in scavenging free radicals and protecting essential cellular macromolecules and organelles from oxidative damage [95]. Previous studies have indicated that dietary supplementation of *spirulina* can reduce antioxidant activities in Nile tilapia (*Oreochromis niloticus*) [96] and goldfish (*Carassius auratus*) [97]. Additionally, Li et al. [93] demonstrated that phycocyanin enhances the activities of SOD enzymes in pancreatic beta cells. Tayag et al. [98] and Lin et al. [99] reported a direct increase in SOD enzyme activity with *spirulina* extract in white shrimp. Abdelkhalek et al. [100] illustrated that *spirulina* supplementation enhances SOD enzyme activity in *Oreochromis niloticus*. Furthermore, Yu et al. [75] observed that rainbow trout fed with 10% *spirulina* exhibited the highest SOD activity.

MDA serves as the end product of lipid peroxidation, leading to cellular toxicity and accelerating damage to cells and tissues [101–103]. In the current study, MDA enzyme activity decreased with an increase in *spirulina* concentration, consistent with previous findings in Nile tilapia (*O. niloticus*) [96], and Carp (*Cyprinus carpio var*) [104]. Similarly, Abdelkhalek et al. [100] reported that dietary supplementation with *S. platensis* reduced MDA levels in the liver, kidney, and gills of Nile tilapia.

5. CONCLUSIONS

In conclusion, recent experiment showed that *Osphronemus gourami* fed a diet supplemented with *S. platensis* at 5% for 90 days improved fish growth performance (FW, WG, WG%, PER, FCR, FER, ADG, SGR, and SR%), increased feed and protein utilization, improve the body deposition (Protein, fat, ASH, CHO, fiber), elevated body indices (HIS%, VFSI%, GSI%, and BSI%), enhanced hematological parameters

(RBC, WBC, HCT, Hb, Monocyte %, and Neutrophil %), improved biochemical responses (Total serum protein, Albumin, and Globulin), decreased levels of cholesterol and triglyceride, and increased digestive (Amylase, Lipase, and Protease) and antioxidant (SOD & MDA) enzyme activity in fish and improve the immunity.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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