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Original Article

EVALUATION OF VITAMIN-C AND SPIRULINA (ARTHROSPIRA PLATENSIS) SUPPELENTATION DIET ON GROWTH PERFORMANCE, ANTI-OXIDANTS AND CARCASS COMPOSITION OF ROHU, LABEO ROHITA (HAMILTON, 1822)

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ABSTRACT

An experiment was conducted for 60 days to evaluate the growth performance, anti-oxidants and carcass composition of rohu, *Labeo rohita,* under different dietary treatments. The study comprised fourtreatments with three replicates each, along with a control group. This study prepared 4 experimental feeds, which included Control (0), T1 (10 g), T2 (15 g) and T3 (20 g) of spirulina powder-containing diet. The proximate composition of vitamin-C enriched spirulina included crude protein (57.63 ± 0.66%), lipid (8.24 ± 0.35%), moisture (7.27 ± 0.30%), ash (10.20 ± 0.15%), and Nitrogen-Free Extract (NFE) (16.65 ± 0.57%) was observed. The nutritional profile of experimental diets was observed, such as crude protein (30.36-30.73%), lipids (5.17-5.57%), moisture (8.26-8.64%), ash (15.17-15.87%), crude fibre (9.92-11.27%), and nitrogen-free extract (27.92-30.73%). The highest growth performance such as length gain (9.26±0.057), weight gain (12.80±0.10), percentage weight gain (304.76±2.38), specific growth rate (0.67±0.002), protein efficiency ratio (2.43±0.014), hepatosomatic index (0.039±0.001), and intestinal somatic index (0.48±0.005) were observed in T3, whereas the highest feed conversion ratio (1.67±0.028) was in the control group compared to the other treatment groups. The highest anti-oxidant activities including catalase(256.34±1.98), glutathione s-transferases (176.64±0.31) and superoxide dismutase (45.37±0.80) was observed in T3 compared to the other treatment groups. The highest carcass composition was observed such as crude protein (16.64±0.049), crude lipid (6.35±0.036), moisture (70.46±0.088), ash (2.64±0.041), and NFE (6.50±0.14) compared to the other treatment groups. The acceptable range of water quality parameterswas observed during the experiment.The results suggest that the specific dietary T3 group enhanced the overall growth, anti-oxidantsand carcass composition of *L. rohita.*

Keywords: *Labeo Rohita*, Spirulina, Growth Performance, Anti-oxidants, Carcass Composition, Water Quality

INTRODUCTION

Aquaculture, the farming of aquatic organisms, has become a crucial sector for meeting global seafood demand and ensuring sustainable food production. The industry has seen significant growth and transformation over recent years, driven by technological advancements, changing consumer preferences, and environmental considerations. The global aquaculture market was valued at approximately $204 billion in 2020 and is projected to reach $262 billion by 2026, reflecting a compound annual growth rate of about 4.5%. This growth is primarily attributed to the increasing demand for seafood amid declining wild fish stocks. Fish farming

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remains the largest segment, accounting for 66% of the market, with species such as salmon, trout, and sea bass being the most popular FAO (2024). Asia dominates global aquaculture, accounting for over 90% of total production. China alone contributes about 56.7% of global aquatic animal production and 59.5% of algal production. Other regions like the Americas, Europe, and Africa contribute significantly less, at 3.6%, 2.7%, and 1.9%, respectively FAO (2024). Generally, fish species show relatively high protein demand in the diet. Fish meal and soybean meal are the main protein ingredients in fish diets. These protein sources are the most expensive feeds and are not always available Kristofersson and Anderson (2006). Therefore, the need to search for alternative protein sources enhances the scientific community to find viable and accessible solutions Thum et al. (2022). Novel proteins are of major concern in the aquaculture feed industry. Due to the continuous increase in the cost of fishmeal, many studies have started evaluating the economic feasibility and optimum use of these novel proteins as fishmeal substitutes Zhang et al. (2020).

Macro- and microalgae have been used as dietary supplements to improve the nutritional performance and health status of farmed fish species Güroy et al. (2011). S. platensis, a fast-growing cyanobacterium of large size (0.5 mm), has been considered as a potential alternative protein source for cultured fish Abdel-Latif et al. (2022). The reason for this is that it is thought to be a good source of protein, essential amino acids, vitamins, minerals, gamma-linolenic acid, antioxidants like carotenoids (C-phycocyanin), antimicrobial properties, and anticancer activity Wan et al. (2021).Spirulina has been produced commercially for about 20 years and is sold mostly as a human food additive, medicine, and food coloring agent. Nonetheless, about 30% of the current world algal output is sold for animal feeding applications, and over 50% of the current global production of Spirulina is used as a feed supplement Rando and Rene (2020).

Spirulina, a blue-green microalga, is increasingly recognized as a valuable component in fish feed due to its rich nutritional profile, including high protein content, essential fatty acids, vitamins, minerals, and bioactive compounds such as phycocyanin and carotenoids. Its inclusion in fish diets promotes improved digestion by enhancing the breakdown of otherwise indigestible feed components, thereby increasing nutrient absorption and feed efficiency. Additionally, spirulina stimulates enzyme production that converts fats into energy rather than storage, leading to better growth performance and more uniform weight gain in fish. The natural pigments in spirulina also enhance fish coloration, an important factor in aquaculture marketing, while its immune-boosting properties reduce disease incidence and reliance on antibiotics, thereby contributing to more sustainable aquaculture practices Spínola et al. (2024). Hematologically, spirulina has been shown to improve blood parameters such as hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and hematocrit (HCT), which are indicative of enhanced red blood cell production and overall health status in fish. These effects are attributed largely to the bioactive pigment phycocyanin, which supports bone marrow function and immune responses. Spirulina's antioxidant compounds, including phycocyanin, carotenoids, and chlorophyll, confer potent free radical scavenging activity that mitigates oxidative stress in fish tissues. This antioxidant capacity helps protect fish from cellular damage caused by reactive oxygen species (ROS), improving their resilience to environmental and pathogenic stressors Selmi et al. (2011).

Ascorbic acid is an essential micronutrient for fish. Many fish species cannot synthesize vitamin C. The inability to synthesize vitamin C is owing to a lack of the enzyme L-gulonolactone oxidase (GLO, EC 1.1.3.8), which catalyzes the conversion of L-gulonolactone to AA in liver and kidney Roy and Guha (1958). Vitamin C is an indispensable nutrient required for growth, immune response and maintenance of the physiological process in different animals including fishes Al-Amoudi et al. (1992). Several studies have demonstrated the individual effects of *Spirulina* and vitamin C on the growth, survival, skeletal deformities, reproduction and immune responses in various organisms James et al. (2006).Vitamin C is essential for fish growth, reproduction, and health Xie et al. (2006), adverse stress, minimize toxicity by water contaminants, and exert an immunomodulatory effect Tewary and Patra (2008). Furthermore; it acts as a metabolic antioxidant, detoxifying numerous peroxide metabolites, thus protecting cell membranes and other intracellular componentsandprocesses that are sensitiveto oxidation Sandell and Daniel (1988). It is also a cofactor in the hydroxylation of praline and lysine in the synthesis of collagen, a component of connective tissues, blood vessels, bone matrix, and scartissue in wound repair Chaterjee (1978).

L. rohita, referred to as rohu, is a freshwater carp species that is widely dispersed in South Asian rivers and ponds. Rohu is a huge omnivore fish known for its characteristic arched head and silvery colouration. It can reach astonishing sizes of up to 45kg and about 2m in length, however most individuals are much smaller. Its natural range includes India, Pakistan, Bangladesh, Nepal, Myanmar, and Vietnam, with introductions into other regions for aquaculture purposes. Rohu thrives in temperate and tropical water bodies and is known for its flexibility, making it an important component of riverine ecosystems and inland fisheries Arifuzzaman (2018). *L. rohita* is a major freshwater fish species that belongs to the Cyprinidae family. This species is mostly found in rivers and ponds in temperate and tropical climates, including India, Bangladesh, Nepal, Myanmar, and Pakistan. Rohu thrives in freshwater habitats, particularly rivers and ponds. It loves regions with plenty of foliage, which provides both refuge and food. During the southwest monsoon season, the species breeds in shallow waterways and spawns on fertile floodplains Ahasan et al. (2020). Adult *L. rohita* can grow to be two meters long and weigh up to 45 kilogrammes. The fish has huge, overlapping cycloid scales that help with taxonomic classification Yadav and Paul (2023). *L. rohita*, as a column feeder, is important to the aquatic ecology because it consumes plant material and contributes to nutrient cycling in its habitat. It is frequently farmed alongside other carp species, such as *Cirrhina mrigala* (a bottom feeder) and *Catla catla* (a surface feeder), to assist optimise space and resources in aquaculture Gopikrishna (2023). Rohu is one of three principal carps in India that are widely cultivated in aquaculture systems, therefore it has

outstanding economic and nutritional value. It is highly prized for its quick growth, appetising flesh, and use as a principal species in polyculture practices, where it is raised with other carps to maximise productivity. Rohu's feeding habits change throughout its life cycle: the species consumes zooplankton in its early stages before transitioning to a diet dominated by phytoplankton and submerged vegetation as it matures.*L. rohita*is ease of multiplication and tolerance to a variety of water conditions have made it a cornerstone of Asian aquaculture Nandeesha et al. (2013). There has been no previous research on the nutritional profile, growth, anti-oxidant activity, and carcass composition of *L. rohita* following feeding with spirulina.

MATERIALS AND METHODS

LOCATION OF THE WORK

The study were conducted in the L.S.P.N. College of Fisheries' Live Fish Laboratory Department of Aquaculture in Kawardha, Chhattisgarh.

EXPERIMENTAL FISH

The experimental fish will collect from the local state-owned private hatcheries at Pondi, Kabirdham (C.G.), India.

FEED FORMULATION

The nutritional needs required to prepare the experimental meals were taken into consideration when choosing the materials for fish feed. Every feed ingredient was purchased from the Kawardha local market. In order to guarantee quality and nutritional sufficiency, S. platensis was also bought from the market and its proximate composition was examined before feed formulation.Three experimental diets with 30% crude protein were created after the proximate analysis by adding varying amounts of S. platensis meal: 10 g (T1), 15 g (T2), and 20 g (T3) per 100 g of feed. Additionally, a control diet (T0) was made without S. platensis meal. Standard feed ingredients including fish meal, vitamin C, rice bran, groundnut oil cake, tapioca flour, and a vitamin/mineral premix made up the control diet Table 1.

Table 1

Table 1 Ingredient Composition for Experimental Fish Feed.

Ingredients	Control (g)	T1 (g)	T2 (g)	T3 (g)
Spirulina platensis meal	0	10	15	20
Fish meal	25	15	10	5
Vitamin-C	1	1	1	1
Rice bran	40	40	40	40
Groundnut oil cake	23	23	23	23
Tapioca flour	10	10	10	10
Vitamin/Mineral premix	1	1	1	1

Note: Vitamin-C tablet Celin trade name- 500mg.

EXPERIMENTAL DESIGN

In accordance with a completely randomised design (CRD), 120 fingerlings of L. rohita with initial average weights ranging from 4.30±0.11g and initial average lengths ranging from 5.32±0.06 cm will be randomly assigned to four distinct experimental groups, including control, T1, T2, and T3, each with three replicates.

FEEDING TRIAL

Fish were fed all of the prepared meals in pellet form. After being acclimated in aquarium tanks (12 × 18 × 24 cm) for five days, L. rohita fingerlings with an average weight of 4.30±0.11 g and an average length of 5.32±0.06 cm were taken from the fish nursery at Khairbana (Kala). They were then split up into four groups, three of which were given experimental diets, and the fourth group was kept on a control diet (without spirulina). Three duplicates of each food therapy, including the control, were used.Fish weight and length measurements were made at the start of the experiment, and every two weeks after that, the rise in fish weight and length was recorded. Twice daily, at 9 AM and 4 PM, each fish received 5% of their body weight in food. The remaining feed and excrement were taken out of the water prior to feeding. All of the fish were collected at the conclusion of the trial, weighed, and measured. A few fish were also dried in order to determine their body composition. Every day, aquarium water quality indicators like temperature, pH, and dissolved oxygen (DO) were measured.

NUTRITIONAL PROFILE OF SPIRULINA AND EXPERIMENTAL FEED (CARCASS COMPOSITION)

The proximate composition of the fish under trial was ascertained at the conclusion of the trial using the APHA (2005) for the contents of moisture, protein, fat, and ash, respectively.

PROTEIN ANALYSIS

Five grams of dried spirulina, feed and fish sample was taken in a flask and mixed with digestion mixture (potassium sulphate + copper) and transferred to a flask containing 200 mL of concentrated H2SO4. This flask was placedon a heating block, the heaters were turned on and the flask was kept there until white fumes stopped appearing and the solution became clear, indicating completion of the digestion process. The solution was removed away from the heater and then cooled. The solution was diluted with the addition of 60 mL of distilled water and its pH was raised to 6.5–7 by adding 45% NaOH solution. Then five to six drops of indicator solution was added and the flask was connected with a condenser with the tipimmersed in standard acid and heated until NH3 was evaporated. The final solution mixture was then titrated against NaOH. Protein contents were then determined applying thefollowing mathematical formula:

$$Protein = \frac{(A - B)\ X\ N\ X\ 14\ X\ 6.25}{W}$$

LIPID EXTRACTION

The soxhelt apparatus was set and 5 g of sample was placed in the extraction thimble and transferred to the condenser. Petroleum ether was filled in a flask and the apparatus was switched on. This process was continued for 16 hours. Then turned the heaters wereswitched off, and the flask was removed and gently dried on the same heater. When thecontents of the flask smelled oily, they were removed and weighed and the fat content in the test sample was calculated using the following formula.

$$Ash\ (\%) = \frac{Weight\ of\ extraction\ thimble}{Weight\ of\ sample} X\ 100$$

MOISTURE CONTENT

Feed sample was weighed, placed in Petri dish and then dried in oven overnight at 105°C for overnight. Petri dish was taken out the next day and weighed again. The loss in weight represented the moisture contents and was determined. The percentage is determined by the following formula:

$$Moisture = \frac{\sqrt{W1 - W2}}{W1} X\ 100$$

Where W1 = initial weight of the sample

W2 = final weight of the sample

ASH DETERMINATION

Ten grams of sample was taken in a crucible and weighed. Crucible with sample was placed in muffle furnace at a temperature of 550°C for 5–6 hours. When the sample turned white, it was taken out and weighed again. White-coloured contents remaining at the bottom of the crucible represented ash, which was carefully weighed and its percentage present in the feed was calculated by the following formula.

$$Ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} X\ 100$$

GROWTH PERFORMANCE PARAMETERS

After 60 days of the experimental period, the weight (grams) and length (centimeters) of every fish in the aquariums was measured separately Lal et al. (2022), Lal et al. (2023a). Growth performance was evaluated using the formulae given below.

LENGTH GAIN (CM)

$$Body\ length\ gain = \text{Final average length gain} - \text{Initial average length gain}$$

WEIGHT GAIN (G)

$$Body\ weight\ gain = \text{Final average weight gain} - \text{Initial average weight gain}$$

PERCENTAGE WEIGHT GAIN (%)

$$\%\ weight\ gain = \frac{\text{Final average weight gain} - \text{Initial average weight}}{\text{Final average weight gain}} X\ 100$$

SPECIFIC GROWTH RATE (%)

$$SGR = \frac{\text{In final weight} - \text{In initial weight}}{No.of\ days} X\ 100$$

SURVIVABILITY (%)

$$\text{Survivability (\%)} = \frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} X\ 100$$

MORTALITY (%)

$$\text{Mortality (\%)} = \frac{\text{Number of fish that died during the experiment}}{\text{Total number of fish stocked}} X\ 100$$

FEED CONVERSION RATIO (FCR) (G)

$$\text{FCR (g)} = \frac{\text{Feed given(g dry weight)}}{\text{Body weight gain (g wet weight)}}$$

PROTEINEFFICIENCY RATIO (PER) (G)

$$\text{PER (g)} = \frac{\text{Net weight gain}}{\text{Protein fed}}$$

HEPATO-SOMATIC INDEX (HSI) (G)

$$\text{HSI (g)} = \frac{\text{Liver weight (g)}}{\text{Weight of fish (g)}} X\ 100$$

INTESTINAL SOMATIC INDEX (ISI) (G)

$$\text{ISI (g)} = \frac{\text{Intestine weight (g)}}{\text{Weight of fish (g)}} X\ 100$$

ANTI-OXIDANTS PARAMETERS

The catalase activity was measured according to the protocol outlined by (Claiborne, 1985). 50 mM Na3PO4 buffer at neutral pH and 19 mM H2O2 made with a Na3PO4 buffer make up the reaction mixture. To a cuvette in a 3 ml reaction mixture, 300 µl of H2O2,

50 µl of samples, and 2.65 ml of Na3PO4 buffer were added. The consumption of H2O2 at 240 nm was used to measure the reaction at 25 °C. According to Lal et al. (2025), the CAT activity was measured as nmol H2O2 vanished/min/mg protein (ε240nm = 0.0436 m/M/cm).

The Superoxide Dismutase activity was calculated using the methodology of Taufek et al. (2016). 0.005 mM xanthine oxidase, 0.05 mM xanthine, 0.01 mM cytochrome c, 0.1 mM EDTA, and 50 mM sodium phosphate buffer make up the reaction mixture's final concentration. When xanthine oxidase was added to the enzyme extract at 25 °C and 550 nm absorbance, the reaction started. SOD activity, which is a measurement of its capacity to stop 50% cytochrome c reduction, was expressed as nmol/min/mg protein.

Glutathione S-transferase activity was evaluated by measuring its response to 1- chloro-2,4-dinitrobenzene (CDNB) at 340 nm Taufek et al. (2016). The assay mixture contains 60 mM CDNB (dissolved in ethanol), 60 mM glutathione (GSH), and 100 mM sodium phosphate buffer (pH 6.5). GST activity was measured as the amount of enzyme that catalysed the GSH conjugate per minute and 1 µmol of CDNB at 25 °C (ε340nm = 9.6 mM−1 cm−1), expressed as nmol/min/mg protein.

WATER PARAMETER ANALYSIS

The water temperature, dissolve oxygen (DO), pH, alkalinity, and total hardness of the tank water were measured and recorded biweekly prior to sampling using conventional methods APHA (2005). DO and temperature are taken in the early morning, between 7-8:30 AM. Water temperature and dissolve oxygen levels were measured using the APHA (2005) standard approach. The pH was determined using a digital pH meter 335 (Systronics). The total alkalinity and hardness of water were tested using APHA (2005) standard methods Damle et al. (2023), Lal et al. (2023b).

TEMPERATURE

A mercury thermometer with a minimum count of 0.1°C was used to record the water temperature from each experiment-reared tank every day at midday. A glass thermometer set at degrees Celsius was used to measure the water's temperature. The thermometer was lowered into the water tank and left there for two to four minutes in order to measure the water's temperature.

DISSOLVED OXYGEN

A 100 ml DO bottle was used to collect water samples from the experimental tanks. Following collection, 1 ml of MnSO4 & KI solution was added via pipette and slowly sank into the DO bottle's bottom. The bottle was firmly sealed and shaken to thoroughly mix. When 1 millilitre of concentrated H2SO4 was added, the precipitate that had formed dissolved. Now, 250 ml of this solution was placed in a conical flask, and 50 ml of it was titrated against freshly prepared 0.025 N Na2S2O3 until the blue starch indicator turned colourless at the end.After that the dissolve oxygen was calculated by the following formula:

$$\text{Dissolve oxygen (ppm)} = \frac{Ml\ of\ 0.025\ N\ Sodium\ Thiosulphate\ x\ 1000\ x\ 8}{50\ x\ 40}$$

PH

The water's pH was measured using a pH meter (HM Digital, PH 80). A pH 7 buffer solution was used to calibrate the pH meter before to use. Next, a buffer solution with known pH values of 4.0 and 10 was used to check the pH meter. The water sample from the experimental aquarium tank was then subjected to a direct pH measurement.

ALKALINITY

The total alkalinity of water was determined using APHA (2005). A 100 ml water sample was obtained from an experimental reared tank and placed in a 250 ml conical flask with 3-4 blobs of phenolphthalein index, which turned the water pink. The water was titrated with 0.02 (N) H2SO4 until the rose hue faded. The volume (ml) of acid used was recorded. Then 2-3 blobs of the methyl orange index were assembled in the same water. If the water turns yellow, it is titrated with the same acid until a faint orange endpoint appears.The volume (ml) of acid used in the second titration was also recorded, and the alkalinity was estimated by taking the total volume of acid used into account. Total alkalinity was estimated using the following formula:

$$Totalalkalinity(\text{mgl}^{-1}) = \text{Volume of } H_2SO_4 \text{ (N/50) consumed}$$

TOTAL HARDNESS

The total hardness of water was assessed using APHA's standard methodology (2005). In a conical flask, combine 50 ml of sample water with 5 mi of buffer. Add a sprinkle of Eriochrome black-T indicator and tritrate against a standard EDTA solution until the

wine red turns blue, indicating the end point. Keep track of how much standard EDTA you use. The total hardness was estimated using the following formula:

$$Total\,hardness(mgI^{-1}) = \frac{VolumeofEDTA}{Volumeofsample} X1000$$

STATISTICAL ANALYSIS

The data obtained were analysed by SAS (1999; statistical package; version 22.0 for Windows). Data obtained from studies based on completely randomized experimental design were subjected to one-way analysis of variance. Results were considered significant at $P < .05$. Means of each treatment including control then were compared using Duncan's multiple range test for level of statistical significance among treatments.

RESULTS

THE PROXIMATE COMPOSITION OF SPIRULINA

The proximate composition analysis of Spirulina dry powder revealed a high nutritional profilepresented in the Table 2 and Figure 1. The protein content was found to be 57.63 ± 0.66%, indicating that Spirulina is a rich source of protein. The lipid content was recorded as 8.24 ± 0.35%, while the moisture content was 7.27 ± 0.30%, suggesting low water content suitable for storage and shelf life. The ash content, representing the total mineral composition, was 10.20 ± 0.15%. Additionally, the Nitrogen Free Extract (NFE), which includes carbohydrates and other soluble components, was observed to be 16.65 ± 0.57%. These results confirm that Spirulina is a nutrient-dense microalga with significant protein and mineral content, making it a valuable supplement for aquaculture and human nutrition.

Table 2

Table 2 Proximate Composition of Spirulina Dry Powder.

S.No.	Parameters	%
1	Protein	57.63±0.66[a]
2	Lipid	8.24±0.35[d]
3	Moisture	7.27±0.30[e]
4	Ash	10.20±0.15[c]
5	Nitrogen Free Extract (NFE)	16.65±0.57[b]

Figure 1

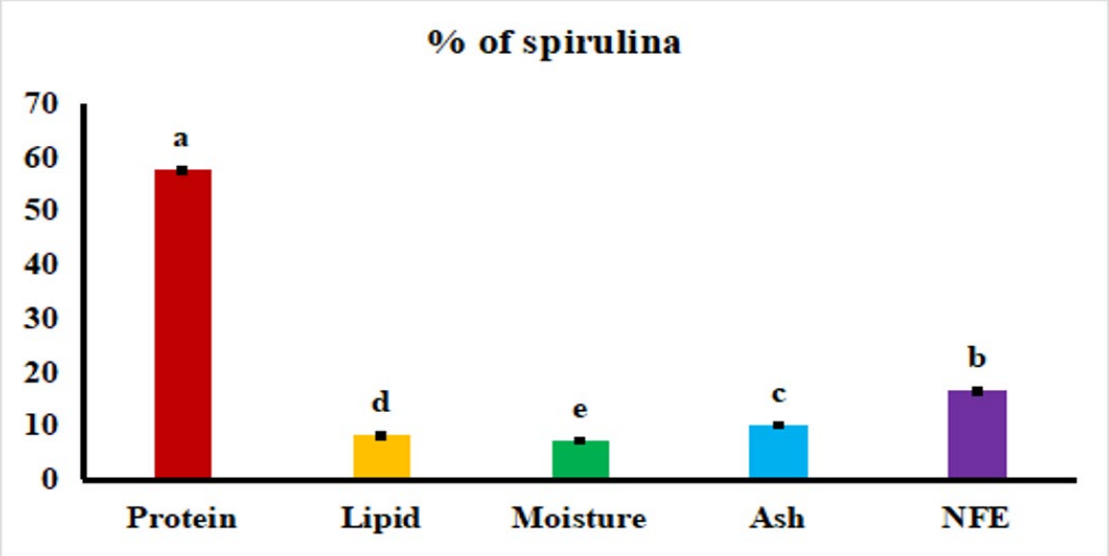

Figure 1 Proximate Composition of Spirulina (dry weight). *Data are Presented as Mean±SE. There is a Statistically Significant Difference (p<0.05) Among the Differentparameters.

THE PROXIMATE COMPOSITION OF EXPERIMENTAL FEED

The proximate composition of experimental feed is shown in Table 3. The proximate composition of the experimental feed was determined by measuring nitrogen-free extract (NFE), ash, moisture, crude protein, fat, and crude fibre. There were no statistically significant differences between treatment groups ($p<0.05$). The T3 group had the highest crude protein content (30.73±0.115), while the control groups had the lowest value (30.36±0.152). The T3 groups had the lowest result (5.17±0.015), whereas the control group had the highest crude lipid (5.57±0.020).The moisture content was highest (8.64±0.020) in the control group and lowest (8.26±0.025) in the T3 group. The control group had the most crude ash (16.23±0.020), whereas the T3 group had the lowest (15.17±0.020) among the treatment groups. The control group had the highest crude fibre level (11.27±0.020) and the T3 group had the lowest (9.92±0.055) among the treatment groups. The T3 group had the highest nitrogen free extract (30.73±0.165) and the control group had the lowest value. Despite slight variations between treatments, there were no significant differences ($p < 0.05$) in the proximate composition of the experimental meals.

Table 3

Table 3 Proximate Composition of Different Experimental Feeds.

Treatment	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	Fibre (%)	Nitrogen free extract (%)
Control	30.36±0.152	5.57±0.020	8.64±0.020	16.23±0.020	11.27±0.020	27.92±0.192
T1	30.36±0.208	5.43±0.020	8.45±0.015	15.87±0.020	10.87±0.020	29.00±0.251
T2	30.53±0.208	5.36±0.025	8.35±0.020	15.63±0.020	10.36±0.025	29.75±0.232
T3	30.73±0.115	5.17±0.015	8.26±0.025	15.17±0.020	9.92±0.055	30.73±0.165
F-Value	2.95	195.64	186.9	1450.95	925.87	93.14

Values are means ± SD, n = 3 per treatment group. Means in a column without a common superscript letter differ ($P < 0.05$) as analyzed by one-way ANOVA and the DUNCAN test.

THE GROWTH PERFORMANCE

The growth performance of L. rohita is presented in Figure 2. Fish growth performance can be measured using length increase, weight gain, percentage weight gain, specific growth rate, protein efficiency ratio, food conversion ratio, hepatosomatic index, and intestinal-somatic index. The T3 group had the highest survivorship of 70% when compared to the other treatment groups. When compared to the other therapy groups, the control group had the greatest mortality rate, at 40%. There is a significant difference ($p>0.05$) between all treatment groups. The T3 group had the most length growth (9.26±0.057a), whereas the control group had the smallest (6.40±0.10d) among the treatment groups.In comparison to the other treatment groups, the T3 group experienced the most weight gain (12.80±0.10a), while the control group experienced the lowest (10.83±0.30c). In comparison to the other treatment groups, the T3 group experienced the largest percentage weight gain (304.76±2.38a), while the control groups experienced the lowest (246.62±16.07c). In comparison to the other treatment groups, the T3 group showed the highest specific growth rate (0.67±0.002a), whereas the control groups showed the lowest (0.59±0.022c). The difference between all treatment groups is statistically significant ($p>0.05$).The control group had the highest food conversion ratio (1.85±0.15a), while the T3 group had the lowest (1.33±0.007c) when compared to the other treatment groups. The T3 group had the highest protein efficiency ratio (2.43±0.014a), while the control groups had the lowest (1.78±0.14c) when compared to the other treatment groups. A statistically significant ($p>0.05$) variation in length increase was seen between the groups. The T2 group had the greatest hepato-somatic index (0.040±0.003a), whereas the control groups had the lowest (0.036±0.004a) in comparison to the other treatment groups. The T3 group had the greatest intestinal-somatic index (0.48±0.005a), whereas the control group had the lowest (0.43±0.06a) among the treatment groups. A statistically significant ($p>0.05$) difference in length gain was reported between the groups.

Figure 2

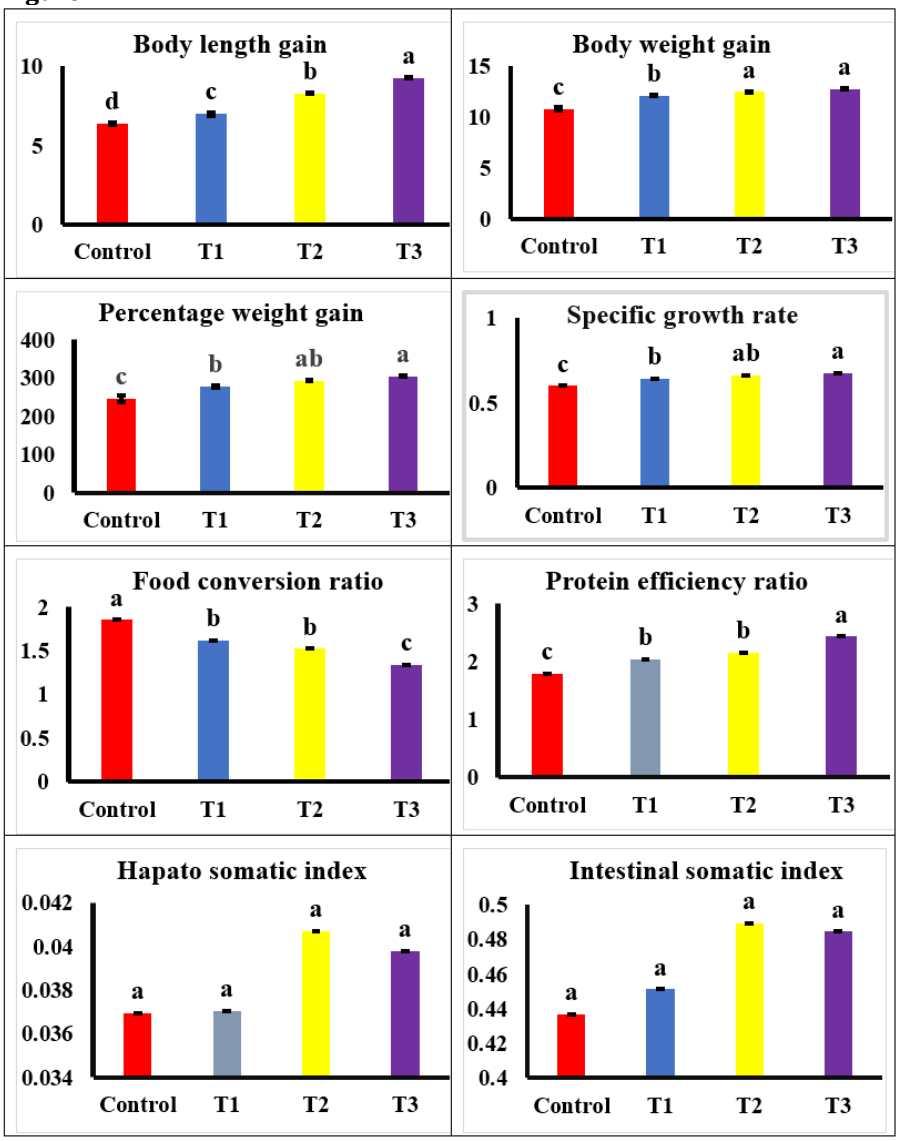

Figure 2 Growth Performance of L. Rohita; A.Body Length Gain; B.Body Weight Gain; C.Percentage Weight Gain; D.Specific Growth Rate (SGR); e.Food Conversion Ratio (FCR); f.Protein Efficiency Ratio (FCR); g.Hapato Somatic Index (HSI); h.Intestinal Somatic Index (ISI) *Data are Presented as mean±SE. Different Superscripts Indicate Statistically Significant Difference ($p<0.05$) Among the Experimental Groups.

ANTI-OXIDANTS ACTIVITIES PARAMETERS

The antioxidant activity parameters of L. rohita during the experimental period are presented in Table 4 and Figure 3.The results showed notable statisticallysignificantly ($p>0.05$)differences among the treatment groups, indicating the influence of dietary treatments on the antioxidant defense system of the fish.The highest catalase activity (256.34 ± 1.98^{a}) was recorded in the T3 group, while the lowest (200.73 ± 2.51^{d}) was observed in the control group. Similarly, glutathione S-transferase (GST) activity was significantly higher in the T3 group (176.64 ± 0.31^{a}) compared to the control group, which showed the lowest value (173.95 ± 0.83^{c}). These findings suggest that the T3 treatment enhanced the enzymatic antioxidant response in L. rohita.Furthermore, superoxide dismutase (SOD) activity followed a similar trend, with the highest activity (45.37 ± 0.80^{a}) observed in the T3 group and the lowest (33.40 ± 0.95^{d}) recorded in the control group. Overall, the results indicate that fish in the T3 treatment group exhibited significantly improved antioxidant enzyme activities compared to the control, reflecting enhanced oxidative stress resistance and better physiological status.

Table 4

Table 4 Anti-oxidant Parameters of L. Rohita During the Experimental Period.

Treatment	CAT (nmol mg–¹ protein)	GST (nmol mg–¹ protein)	SOD (nmol mg–¹ protein)
Control	200.73±2.51^{d}	173.95±0.83^{c}	33.40±0.95^{d}
T1	232.90±1.54^{c}	174.50±0.27^{c}	36.93±0.18^{c}
T2	243.76±1.77^{b}	175.60±0.19^{b}	42.05±0.30^{b}
T3	256.34±1.98^{a}	176.64±0.31^{a}	45.37±0.80^{a}
F-Value	432.201	18.906	201.227

Values are means ± SD, n = 3 per treatment group. Means in a column without a common superscript letter differ (P < 0.05) as analyzed by one-way ANOVA and the DUNCAN test.

Figure 3

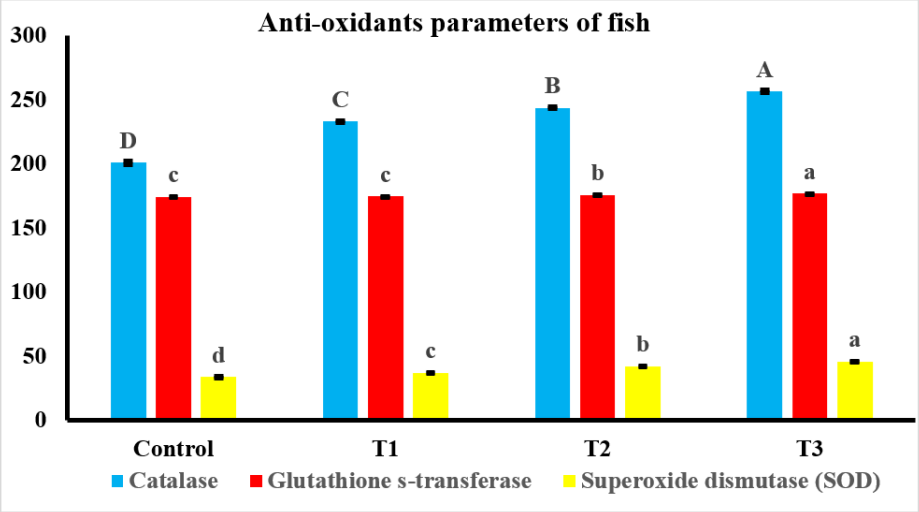

Figure 3 Anti-oxidants Parameters of L. rohita. *Data are Presented as Mean±SE. Different Superscripts Indicate Statistically Significant Difference (p<0.05) Among the Experimental Groups.

THE CARCASS COMPOSITION OF L. ROHITA

The carcass composition of experimental fish was observed at end of the experiment are presented in Table 5 and Figure 4. The nutritional composition of the experimental fish such as analyses of crude protein, lipid, moisture, ash, and nitrogen-free extract (NFE). There is a statisticallysignificantly (p>0.05)difference all among the treatemnt groups. The highest crude protein (16.64±0.049a) was observed in the T3 group whereas the lowest value (15.81±0.14d) was observed in the control groups compared to the other treatment groups.The highest crude lipid(6.35±0.036a) was observed in the T3 group whereas the lowest value (4.88±0.08d) was observed in the control groups compared to the other treatment groups.The highest moisture content(70.46±0.088a) was observed in the control group while the lowest (68.66±0.10d) was observed in the T3 groups, although it differ significantly from the fish reared on diets T2 and T1.The highest crude ash(2.64±0.041a) was observed in the T3 group while the lowest (2.34±0.015d) was observed in the control groupscompared to the other treatment groups.The highest nitrogen free extract(6.50±0.14a) was observed in the control group and lowest (5.69±0.032d) in the T3 groupscompared to the other treatment groups. There is a significantly significantly (p>0.05)difference in between all among the treatment groups.

Table 5

Table 5 The Carcass Compositionl. Rohita at the End of the Experiment 60th days (Wet basis).

Treatment	Crude protein (%)	Crude lipid (%)	Moisture (%)	Ash (%)	Nitrogen free extract (%)
Control	15.81±0.14^{d}	4.88±0.08^{d}	70.46±0.088^{a}	2.34±0.015^{d}	6.50±0.14^{a}

T1	16.16±0.047^{c}	5.73±0.025^{c}	69.77±0.047^{b}	2.43±0.025^{c}	5.88±0.025^{b}
T2	16.44±0.05^{b}	5.95±0.015^{b}	69.20±0.037^{c}	2.52±0.015^{b}	5.87±0.077^{c}
T3	16.64±0.049^{a}	6.35±0.036^{a}	68.66±0.10^{d}	2.64±0.041^{a}	5.69±0.032^{d}
F-Value	53.346	529.270	300.670	68.094	50.665

Values are means ± SD, n = 3 per treatment group. a-dMeans in a column without a common superscript letter differ (P < 0.05) as analyzed by one-way ANOVA and the DUNCAN test.

Figure 4

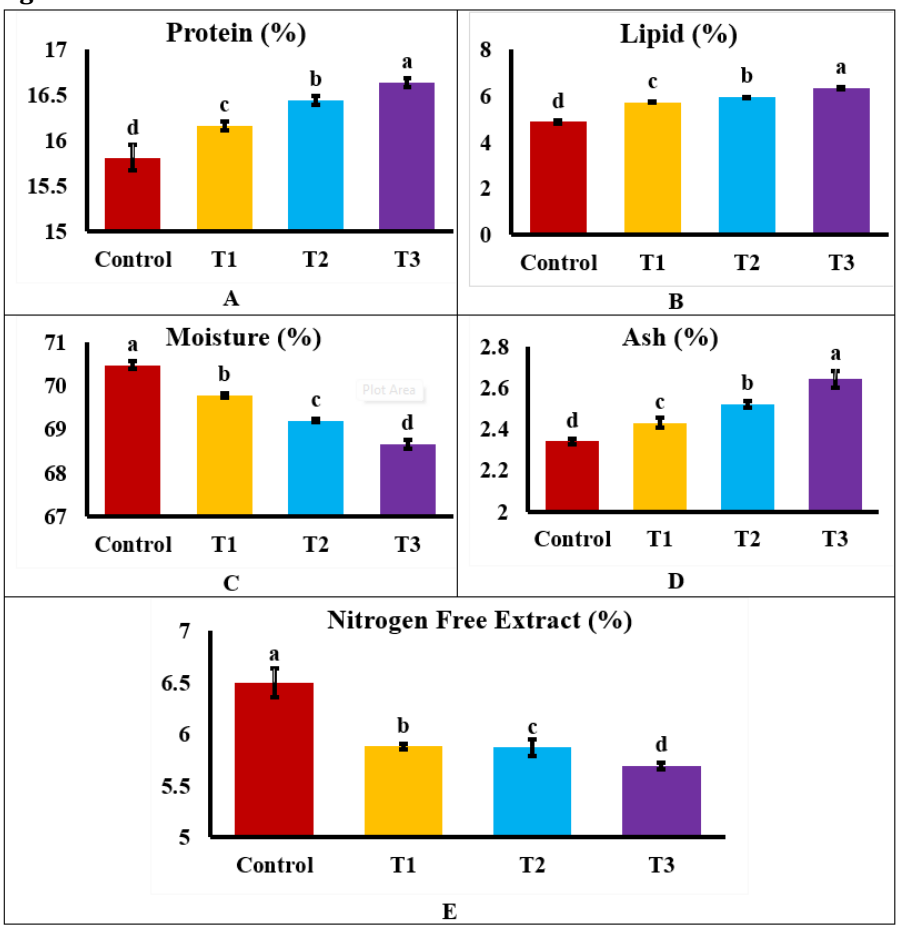

Figure 4 Carcass Composition of L. rohita. *Data are Presented as Mean±SE. Different Superscripts Indicate Statistically Significant Difference (p<0.05) all Among the Groups.

THE WATER QUALITY PARAMETERS

Table 6 shows the water quality parameters of the experimental fish that were recorded during the experiment. Temperature, dissipated oxygen, pH, hardness, and alkalinity were all found to be within the permissible range of water quality parameters. Fish survival, growth, and health depend on several aspects of water quality. A fish's capacity to carry out basic tasks like breathing, feeding, and reproduction is directly impacted by variables like dissolved oxygen, pH, temperature, and the presence of contaminants. Healthy fish populations and aquaculture success depend on maintaining ideal water quality. In the control group, the water temperature ranged from 24.85 to 27.9, while in the T1 groups, the temperature ranged from 23 to 27.4. Throughout the trial period, the T1 group had the largest dissolved oxygen range (5.31–5.9) and the T2 group had the lowest (5.2–6.52). The T2 group had the largest pH range (7.45–7.8) during the trial period, while the control group had the lowest (6.09–6.67). Throughout the testing period, the control group had the highest water hadness (63.4-64.31), whereas the T3 group had the lowest (61.1-63.8). During the testing period, the T2 group had the greatest water alkalinity range (77.5-78.7), whereas the control group had the lowest (76.2-77.7).

Table 6

Table 6 The Water Quality Parameters of L. Rohita During the Experimental Period.

Treatment	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Hardness (mg/L)	Alkalinity (mg/L)
Control	24.85-27.9	5.3-6.1	6.09-6.67	63.4-64.31	76.2-77.7
T1	23-27.4	5.31-5.9	6.7-7.83	63.4-64	76.3-77.7
T2	24.4-26.2	5.2-6.52	7.45-7.8	62.6-63.7	77.5-78.7
T3	25.5-27.8	5.21-6.95	6.5-7.35	61.1-63.8	77.2-78.6

Values are represented as a range of water quality parameters during the experimental periods.

DISCUSSIONS

PROXIMATE COMPOSITION OF SPIRULINA AND FEED

Including spirulina in fish feed significantly enhances fish performance by providing high-quality protein and nutrients, which improves overall growth, survival, and feed utilization, as demonstrated by studies on various species. The proximate composition of fish fed spirulina-supplemented diets shows increased protein retention and desirable carotenoid content, leading to better growth parameters, pigmentation, and potentially a more robust immune system, although the optimal inclusion level varies by species and target outcome Jana et al. (2014). Studies on incorporating spirulina into fish feed consistently report positive impacts on fish growth and health metrics, such as weight gain, length increase, and improved survival rates, often attributing these benefits to the increased feed intake and nutrient digestibility facilitated by the dietary spirulina. The proximate analysis of fish carcasses reveals enhanced protein retention and desirable carotenoid deposition, which boosts fish color and market appeal, making spirulina a valuable functional feed additive for aquaculture. While spirulina can act as a protein and carotenoid supplement, its high nutrient content, including essential minerals and vitamins, can also promote overall fish well-being, improve growth, and potentially boost immune responses, thereby supporting sustainable aquaculture practices Al-Deriny et al. (2020)

Fish diets with 30% spirulina have significantly higher protein content (about 55%) in fish body composition than diets with lower inclusion levels, as well as improved fatty acid profiles with elevated omega-3 levels such as EPA and DHA. Protein concentration in spirulina-based fish feed pellets ranges from 35% to nearly 39%, depending on the formulation, with injectable spirulina protein efficiently substituting fishmeal protein without sacrificing growth performance. Thus, spirulina-containing fish feed is high in protein, healthy fats (omega-3s), vitamins, and minerals, making it a nutritious and sustainable alternative or addition to traditional fishmeal-based diets Soma et al. (2024). In several marine fish species, such as snubnose pompano and sobaity seabream, spirulina has a high digestibility of protein and amino acids; the sobaity seabream has superior digestibility of both proteins and amino acids. According to Siddik et al. (2025), adding spirulina to feed did not decrease intake and demonstrated promise as a sustainable feed ingredient that might replace traditional proteins. Spirulina can up to 30% replace fishmeal protein in young Nile tilapia feeds without affecting mortality, feed intake, or growth performance. According to Soma et al. (2024), n-3 LC-PUFA (EPA and DHA), which are crucial for fish and consumer nutrition, are also preserved by spirulina.Spirulina feeding improves fish health by boosting immunity, decreasing illness mortality, and perhaps reducing the need for antibiotics and other treatments. According to Al Mamun et al. (2023), spirulina promotes development and nutritional utilisation by stimulating good gut flora and enzyme activities. According to a meta-analysis of several studies, adding Spirulina meal (SPM) to the diet considerably increases fish growth (final body weight, specific growth rate), feed efficiency (feed conversion ratio), and protein utilisation (protein efficiency ratio) without having an adverse effect on the hepatosomatic index or fish condition.Fishmeal can be replaced with up to 22-25% or supplemented with 1.5-2.3% at optimal inclusion rates Li et al. (2022). Antioxidants and immune stimulants are among the nutraceutical qualities of spirulina that enhance sustainable aquaculture techniques by enhancing fish welfare and stress resistance Ujjwal et al. (2025).

GROWTH PERFORMANCE

The current study's growth and feed utilisation parameters for L. rohita showed an upward trend up to 20% of fish meal replaced with S. platensis. This could be due to vital vitamins, minerals, and amino acids, as well as higher feed intake and nutrient digestibility. Teimouri et al. (2013) found that rainbow trout fed 5% S. platensis performed significantly worse in terms of growth than those fed 7.5% and 10% S. platensis. They also found that the percentage of weight gain increased from 113.1 ± 4.8% to 131.4 ± 7.7% when fed a diet containing 2.5–10% S. platensis. However, Akter et al. (2023) found that using S. platensis in place of fishmeal in the diet of Ompok pabda produced the highest growth performance at the 15% level when compared to the control. High-quality protein and bioactive substances that are crucial for promoting growth are found in S. platensis Da Silva et al. (2021). According to earlier research, fish that were fed diets supplemented with algae grew more effectively Riano et al. (2012). Abdel-Tawwab and Ahmad

(2009) found a lower FCR value (1.22 ± 0.02) in the 5% level of S. platensis replacement and a higher FCR value in the control diet. They also observed a maximum PER value (2.91 ± 0.08) in the 5% level of S. platensis incorporation and a minimum PER value in the control diet. Roohani et al. (2018) observed comparable results in terms of survival rate. According to James et al. (2006), S. platensis improved the intestinal flora in fish by breaking down indigestible feed components to extract more nutrients and encouraging the creation of enzymes that transport lipids for metabolism rather than storage.The statement also supports the current study's increased feed utilisation pattern. According to earlier studies, the high levels of vitamins, minerals, essential amino acids, linoleic acid, and linolenic acid that S. platensis provides in the diet enhance feed utilisation and growth performance Cao et al. (2018), Roohani et al. (2018).

The growth performance of fish is positively impacted when spirulina is added to their diet. Research indicates that adding spirulina to fish feed increases the length, weight, and survival rate of a variety of fish species. For instance, a meal containing 5% spirulina produced greater length (13.07 cm), average weight growth (60.4 g), and survival (94%) in Pangasius sutchi than control feeds devoid of spirulina. According to Jana et al. (2014), spirulina increases feed intake, nutrient digestibility, and provides vital vitamins and minerals that support growth promotion. When compared to fish fed the control diet, fish fed diets containing Spirulina (5 g/kg) exhibited noticeably superior development and feed utilisation.The current study demonstrated that adding spirulina to the diet improved fish growth. These outcomes might be the consequence of increased feed intake and nutrient digestibility. Additionally, spirulina contains a number of nutrients, particularly vitamins and minerals, which may aid in promoting fish growth. These findings concur with those of a number of researchers who showed that feeding fish spirulina increased their survival and growth rates Belay et al. (1993), Hayashi et al. (1998), Hirahashi et al. (2002). Accordingly, diet supplemented with spirulina powder increased the feed conversion ratio and growth rates for striped jacks, Pseudocaranx Dentex Watanabe et al. (1990). The fish given 5% spirulina showed the largest length gain (28.3 cm), whereas the control group showed the lowest (20 cm). The fish fed 5% spirulina showed the maximum weight gain, whereas the control group (20 grammes) showed the lowest. The control group's survival rate was nearly 100%, whereas the fish given 5% spirulina had the highest survival rate. During a 90-day culture trial, Nandeesha et al. (2001) investigated the effects of S. platensis meal on the growth of two important carps from India, catla (Catla catla) and rohu (L. rohita).Higher levels of Spirulina inclusion in rohu resulted in improvements in the specific growth rate and protein efficiency ratio, however in catla, these metrics did not differ significantly from the control treatment. In our investigation, however, we found that adding spirulina to the feed greatly improved the Pangasius sutchi's length, weight gain, and survival.Dietary spirulina meal supplementation dramatically increases fish final body weight, specific growth rate, protein efficiency ratio, and lowers feed conversion ratio, indicating improved feed utilisation, according to a thorough meta-analysis. For fish diets, spirulina supplementation amounts should be between 1.5% and 2.3%. Spirulina can replace fishmeal in fish diets up to about 22%–25% without having an adverse effect on growth Li et al. (2022). Spirulina inclusion up to 7.5–10% has been shown in other research to boost growth in certain fish species while also improving feed utilisation and immunity. According to Al Mamun et al. (2023), spirulina is a sustainable simproving feedhows promise for enhancing fish development performance in aquaculture.

ANTI-OXIDANTS PARAMETERS

The anti-oxidants parameters were represented in the Table 4. Adding Spirulina to fish feed enhances antioxidant parameters in fish by providing natural antioxidants like phycocyanin, beta-carotene, and superoxide dismutase (SOD). These compounds scavenge free radicals, improving the fish's antioxidant status, immune response, and resistance to stress and disease. Specific antioxidant parameters measured often include superoxide dismutase (SOD) activity, catalase (CAT) activity, and levels of glutathione peroxidase (GPx), all of which are elevated in fish fed Spirulina-supplemented diets Eissa et al. (2024). Similarily, Faheem et al. (2022) reported the hepatic lipid peroxidation decreased significantly in fish fed with a 1 and 5% Spirulina supplemented diet. The activity of catalase, glutathione-S-transferase, and glutathione levels increased significantly in the livers of fish fed with 1% Spirulina supplemented diets while no significant differencewas observed for hepatic superoxide dismutase levels when compared to the control.

Several antioxidant compounds (glutathione) and enzymes (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, etc.) make up the body's anti-oxidant defense system that detoxifies reactive oxygen and nitrogen species through a series of reaction cascades. Recently, a great deal of research focused on supplementing the fish diet with additives that can enhance the natural anti-oxidant level and alleviate oxidative stress. In the present study, fish were not challenged with any stress but it is generally accepted that an enhanced and better anti-oxidant system will provide better resistance against oxidative stress. In the present study, the level of lipid peroxidation decreased in the fish fed with 1 and 5% Spirulina diets. The activity of catalase, glutathione-S-transferase, and glutathione levels increased significantly ($p < 0.05$) in fish livers fed with 1% Spirulina-supplemented diets. Spirulina is a rich source of bioactive compounds such as catechins, phycobiliproteins, allophycocyanin, and phycocyanins Takeuchi et al. (2002). Catechins have the ability to chelate metal ions, scavenge reactive oxygen species, and produce antioxidant enzymes Bernatoniene and Kopustinskiene (2018). Similarly, phycocyanin and allophycocyanin have the properties of antioxidants Esteban (2012). The presence of these bioactive components in Spirulina may be responsible for the improved antioxidant status of grass carp.

The association between fish immunity and antioxidant properties is well-established. Antioxidative enzymes like SOD, GPx, and CAT reduce oxidative stress by lowering reactive oxygen species (ROS) levels Xavier et al. (2021). MDA, a marker for lipid peroxidation, reveals oxidative damage to lipids. Our study showed significant escalations in the activity of CAT, SOD, and GPx enzymes and a decline in MDA contents in fish fed with SP and CUR-NPs, indicating the antioxidant effect of these additives. Similar findings were observed in White leg shrimp supplied with CURNPs and in Nile tilapia supplemented with CUR-NPs Bhoopathy et al. (2021), Moghadam et al. (2021). Recent investigations confirmed the positive role of CUR-NPs additions on the antioxidant status of Nile tilapia Abdel-Tawwab et al. (2022). Various studies have recognized the beneficial impacts of S. platensis on the antioxidant ability of several fish species Mahmoud et al. (2018), Awad et al. (2022), Teimouri et al. (2019), Mohammadiazarm et al. (2021). Curcumin's antioxidant properties are linked with the activation of antioxidative enzymes and the nuclear transcription factor erythroid 2 (Nrf2) signaling pathways, which removes free radicals Xu et al. (2018). Polyphenols found in these additives also promote antioxidant activity by scavenging ROS and preventing oxidative deterioration Bishayee et al. (2011), Moskaug et al. (2005). Spirulina's antioxidant properties can be attributed to its chemical composites, including vitamins, C-phycocyanins, β-carotene, and minerals, particularly phycocyanin, which alters cyclooxygenase-2 and guards against oxidative deterioration Awad et al. (2022), Karadeniz et al. (2009).

CARCASS COMPOSITION OF L. ROHITA

The carcass composition of L. rohita, an Indian major carp widely cultured across South Asia, is aindicator of its nutritional quality and commercial value. The primary components assessed in carcass analysis are moisture, crude protein, lipid (fat), and ash on a wet weight basis. At the start of growth trials, the carcass typically contains high moisture (around 79.28%), moderate protein (approximately 11.93%), low fat (about 2.45%), and ash contents near 3.90%. These values change noticeably with dietary variations and as culture duration progresses, largely reflecting metabolic processes and growth patterns in the species Joshi (2018). The proximate composition of fish varied significantly between the treatments. Protein percentage in carcass was found maximum at 10% levels of spirulina substututions followed by 5% and control (0%). An inverse relationship between fat, moisture and ash deposition were observed. This finding agreed with earlier findings of Takeuchi et al. (2002) and Nandeesha et al. (1998) in tilapia, Oreochromis niloticus and rohu, L. rohita respectively. Similarly, Chandra and Saxena (2012) reported the effectiveness of S. platensis as a source of protein in rohu diets. There was significant difference in the average carcass composition of the fish among treatments. Some studies indicated althoughsomeprotein sources can replace or spare fish meal without impairing growth, fat composition might increase unacceptably Esmaeili et al. (2017). Based on our results, spirulina increased protein and decreased fat content in whole body. It can be due to improvements in protein and fat metabolism by spirulina due to some mechanismsmentioned in the section above. Similarly, other studies reported significant effects of spirulina in increasing whole-body protein content Promya and Chitmanat (2011), Velasquez et al. (2016) and decreasing whole-body fat content Cao et al. (2018), Teimouri et al. (2016). However, in our research ash and moisture were not affected by dietary spirulina, findings which are incompatible with other research reporting increased or decreased ash content in whole body Tongsiri et al. (2010), and decreased whole body moisture content Promya and Chitmanat (2011), Tongsiri et al. (2010), Velasquez et al. (2016). Spirulina mass production methods andconditions might affect its nutritional composition, including proximal composition (fat and protein content), amino acid and fatty acid profiles, and this may explain the distinct findings of research done in different species Takeuchi et al. (2002).

In present research, lipid content in whole body increased only in fishfed the highest supplementation level of spirulina (8% FMS), unlike other spirulina-based diets.The incorporation of Spirulina into fish diets has been widely reported to influence body composition, particularly by enhancing lipid deposition in fish tissues. Spirulina is rich in essential fatty acids, including linoleic and gamma-linolenic acids, which can be directly utilized for lipid synthesis and storage in the fish body Becker (2007). The high digestibility and superior nutrient profile of Spirulina improve lipid absorption and metabolism, leading to increased fat content in muscle tissues Olvera-Novoa et al. (1998). Furthermore, bioactive components such as carotenoids, vitamins, and antioxidants present in Spirulina may modulate metabolic enzymes associated with lipid biosynthesis, thereby enhancing lipid accumulation Kumar et al. (2022). Several studies have demonstrated that dietary supplementation with Spirulina significantly elevates the lipid content of fish. For instance, Ibrahem and Ibrahem and Ibrahim (2014) reported a notable increase in whole-body lipid levels of Nile tilapia (O. niloticus) fed diets supplemented with S. platensis. Similarly, Teimouri et al. (2013) observed enhanced lipid deposition in rainbow trout (Oncorhynchus mykiss) following dietary inclusion of Spirulina, attributing this to improved feed utilization and altered lipid metabolism. These findings suggest that Spirulina not only serves as a high-quality protein source but also acts as a functional feed additive promoting lipid synthesis and storage, ultimately improving the energy reserves and nutritional quality of fish flesh.

As L. rohita is fed formulated diets with increasing protein levels such as 28%, 31%, 34%, or 37% protein there is typically a reduction in carcass moisture and ash, accompanied by a marked increase in crude protein and fat content. For instance, after a 60-day feeding experiment, moisture drops to as low as 74.10%, protein content can rise to nearly 14–15%, and fat content may reach up to 4.58% in fingerlings provided with a 34% protein diet. This shift demonstrates that higher dietary protein and lipid enhance the proximate body composition, particularly boosting muscle protein and fat reserves, which are vital for fish growth and market

quality Salam et al. (2020). Environmental factors, fish age, and soil or pond pH also influence carcass composition. Studies reveal that older L. rohita tend to accumulate higher fat and protein levels in their muscles, while juvenile fish have relatively higher moisture. Alkaline pond conditions (high soil pH) can modulate protein synthesis and lipid accumulation compared to neutral conditions, underlining the importance of culture environment in determining final fish quality. Such age- and environment-dependent biochemical profiles help optimize aquaculture practices for higher quality yields Dwivedi et al. (2025).

Comparative assessments show that L. rohita possesses slightly lower average muscle protein (around 39%) and water content (roughly 74%) than some other carps like Cirrhinus mrigala, but has comparable or higher dry matter and fat content (around 13-14%). This makes L. rohita a reliably nutritious food fish, well-suited for consumers seeking protein-rich and moderately low-fat diets. Ash content, indicating mineral deposition, usually remains stable but can reflect dietary mineral intake and environmental conditions Sikandar et al. (2020). Feed additives can further influence carcass composition. For example, the inclusion of functional ingredients or supplements like poultry waste biochar in the diet has been shown to enhance crude protein and lipid deposition in L. rohita, as well as improve mineral content and feed conversion. This indicates that targeted feed interventions can not only optimize growth but also upgrade the fish's nutritional value for human consumption, making it a strategic tool in value-added aquaculture Khalid et al. (2024). Nutritional indices derived from carcass analysis not only help determine optimal feeding strategies for maximum growth, but also offer insights into nutrient retention and conversion efficiencies. The shift in carcass composition with age, feeding rates, and diet formulation provides practical information to farmers and researchers aiming for high-quality production with enhanced protein and oil content, which are crucial traits for both domestic and export markets Dwivedi et al. (2025)

WATER QUALITY PARAMETERS OF FISH

The temperature of the water varied between 24°C and 30°C during the experiment. Other water quality metrics that were within the optimum ranges for carp growth included pH (7.29-7.46) and dissolved oxygen (7.73-8.06 ppm) Jhingran (1991). The culture and production of L. rohita depend heavily on water quality factors since they have a direct impact on the survival, growth, and health of the fish. The following are important factors to keep an eye on for effective aquaculture management: temperature, pH, dissolved oxygen (DO), total alkalinity, ammonia concentration, and hardness. While water temperatures between 25°C and 36°C are generally favourable for L. rohita, 28°C to 30°C is excellent.The ideal metabolic and enzymatic processes necessary for growth and development are facilitated by this temperature range. Growth performance may be adversely affected by deviations from this range since they may result in metabolic stress and decreased feeding efficiency. To preserve the stability of physiological processes and enzymatic activity in the fish tissues, the pH of the rearing water should be kept close to neutral or slightly alkaline, usually between 7.0 and 8.5 Mahamood et al. (2021).

A crucial factor in L. rohita culture is dissolved oxygen, which should be at least 6 to 7 mg/L to ensure proper respiration and metabolic activity. Hypoxic conditions caused by low dissolved oxygen levels make fish more vulnerable to illness, experience stress, and develop anaemia. To keep these dissolved oxygen levels within the ideal range, frequent aeration and efficient water movement are necessary. Since too much CO2 lowers pH and causes respiratory problems, free carbon dioxide levels must also be managed.Total alkalinity and hardness are two aspects of water chemistry that affect the water's buffering capabilities and ionic equilibrium; alkalinity levels between 20 and 300 mg/L and hardness levels around 150 mg/L are ideal for preserving fish health and water stability Biswal et al. (2020). Among chemical contaminants, ammonia, particularly in its unionised form (NH3), is extremely hazardous to L. rohita even at low doses. Ammonia is produced by fish waste as well as the decomposition of uneaten feed and organic debris, and it has an effect on gill function, oxygen intake, and immunological defence processes. To reduce physiological stress, keep unionised ammonia concentrations at 0.02 mg/L. Proper feeding management, regular water exchange, and/or the use of biofloc or probiotic systems can assist reduce ammonia accumulation and enhance water quality.Heavy metals like cadmium, chromium, and nickel that are present in culture water in excess of allowable levels can bioaccumulate in L. rohita organs, interfering with biochemical pathways and producing harmful consequences that manifest as changed haematological and enzymatic parameters Tabrez et al. (2022).

The physiological well-being and biochemical makeup of L. rohita are likewise impacted by the quality of the water. Different environmental factors, such as temperature and dissolved oxygen, have an impact on the metabolism of proteins, lipids, and carbohydrates in the fish's head, trunk, and tail. Stress brought on by low water quality impairs tissue repair and causes oxidative damage, as seen by alterations in biochemical markers and antioxidant enzyme activity. Changes in biochemical content are correlated with seasonal and regional variations in water quality, highlighting the significance of ongoing water monitoring to maximise feeding and growth tactics and lower mortality Kaur (2020). Haematological parameters are also linked to fish health and water quality, and they can be indicators for environmental stress in L. rohita. Anaemia, immunosuppression, and a reduced ability to transport oxygen are all indicated by decreases in red and white blood cells as well as haemoglobin levels brought on by exposure to less-than-ideal water conditions, such as wastewater or sewage contamination. Thus, routine monitoring of these blood parameters in conjunction with physicochemical water testing can measure fish health indirectly, giving a thorough picture of the cultural environment and directing prompt adjustments Rout et al. (2017). Efficient management of water quality parameters necessitates the use of integrated techniques such as regular water testing, proper oxygenation, balanced feeding regimens, and waste collection. Culture systems with adequate water depth, flow rates, and natural or artificial aeration maintain consistent

temperature and oxygen levels. The use of probiotics and biofloc technology improves water quality by lowering harmful nitrogen compounds and improving fish gut health. Collectively, these approaches improve feed conversion ratios, accelerate growth, minimise mortality, and ensure long-term production yields in L. rohita aquaculture Nesara and Sheethal (2020).

CONCLUSIONS

The 60-day feeding trial revealed that dietary inclusion of S. platensis significantly enhanced the growth performance, antioxidant activity, and carcass composition of L. rohita. Among all dietary treatments, fish fed with the T3 diet containing 20 g spirulina powder per kg feed exhibited the highest growth parametersincluding length gain, weight gain, percentage weight gain, and specific growth ratealong with an improved protein efficiency ratio and lower feed conversion ratio, indicating efficient nutrient utilization.The antioxidant enzyme activities, including catalase, glutathione S-transferase, and superoxide dismutase, were markedly higher in the T3 group, suggesting strengthened oxidative defense mechanisms and improved physiological health. Additionally, the carcass composition of L. rohita in the T3 group showed higher crude protein and lipid content, reflecting superior muscle development and nutritional quality of the fish.Throughout the experiment, water quality parameters remained within the optimal range, ensuring a stable rearing environment. Overall, the study concludes that supplementation of 20 g spirulina powder per kg feed (T3) effectively improves growth, antioxidant capacity, and carcass quality of L. rohita, highlighting its potential as a beneficial natural feed additive in aquaculture nutrition.

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