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Effect of dietary suppelentation of vitamin-c and spirulina (*Arthrospira platensis*) on growth performance and haematological parameters of rohu, *Labeo rohita* (hamilton, 1822)

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ABSTRACT

An experiment was conducted for 60 days to evaluate the growth performance, feed utilization and haematological parameters of rohu, *Labeo rohita,* under different dietary treatments. The study comprised four experimental treatments 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 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 haematological parameters observed of Nile tilapia include red blood cells (6.83±0.013), white blood cells (62.36±0.80), haemoglobin (6.65±0.036), mean corpuscular volume (136.66±0.20), mean corpuscular haemoglobin (27.64±0.21), mean corpuscular haemoglobin concentration (23.55±0.25) and haematocrit (25.52±0.31) within the healthy physiological range. The acceptable range of water quality parameters was observed during the experiment. The results suggest that the specific dietary T3 group enhanced the overall growth, feed utilization and haematological parameters of *L. rohita.*

1. Introduction

The world population is increasing at a tremendous rate and is expected to hit 10 billion people by 2050 (Hoseinifar *et al.*, 2020). Food supply, especially animal-based protein, will be crucial in the coming decades. The global demand for animal-derived protein will be doubled by 2050 which is expected to intensify pressure on the need to produce more animal-based protein (Henchion *et al*., 2017). Fish and shellfish are the primary sources of protein for approximately 950 million people worldwide (Pradeepkiran, 2019). Aquaculture is expanding faster than any other food-producing sector, and intensive aquaculture has the potential to provide animal-based protein to an exponentially growing human population (Al-Deriny *et al*., 2020). As stocking density is high in an intensive culture system, this may lead to crowding stress. Crowding stress along with poor water quality results in the emergence of disease outbreaks that inflict significant financial losses (Holmes *et al*., 2016).

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 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).

Spirulina platensis is a cyanobacterium (commonly called bluegreen algae) widely recognized for its spiral-shaped, filamentous morphology and adaptability to alkaline, high-salinity environments. Originally described under the genus *Spirulina*, modern taxonomic consensus places it within the genus *Arthrospira*, making the scientifically accepted name *Arthrospira platensis*; however, the term "Spirulina" is still frequently used in commercial and research contexts (Sinetova *et al*., 2024). This microorganism is classified in the phylum Cyanobacteria, class Cyanophyceae, and is notably found in inland alkaline lakes and soda lakes across Africa, Asia, and South America, where environmental conditions often include high carbonate and bicarbonate concentrations and elevated pH levels. The cellular structure of *S. platensis* is filamentous and lacks a true nucleus, reflecting its prokaryotic nature as a member of the kingdom Bacteria. It exhibits vigorous gliding motility and is a photoautotroph, deriving energy from sunlight and using carbon dioxide as a major carbon source. The organism's ecological success in extreme environments is largely attributed to its ability to tolerate high salinity and alkalinity, as well as its robust cellular biochemistry, which allows survival and growth at temperatures around 35°C and pH ranging from 9 to 10 (Mehdizadeh Allaf and Peerhossaini, 2022). Nutritionally, *S. platensis* is highly valued as a food supplement due to its substantial protein content often exceeding 60% dry weight and its richness in essential amino acids, vitamins, minerals, and pigments such as phycocyanin. As a result, it has gained global importance in health and nutrition sectors for both human and animal consumption, and is investigated for potential applications in sustainable food production and biotechnology. Its absence of cellulose in the cell wall makes the nutrients readily bioavailable, further supporting its prominence as a functional food ingredient and dietary supplement (Podgórska-Kryszczuk, 2024).

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 Guma, 1958). 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). 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). Furthermore; it acts as a metabolic antioxidant, detoxifying numerous peroxide metabolites, thus protecting cell membranes and other intracellular componentsandprocesses that are sensitiveto oxidation (Sandel 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). Ascorbic acid, or vitamin C, is an essential nutrient widely used in fish feed to promote optimal health and growth in aquaculture. It functions as a potent antioxidant, helping fish neutralize harmful free radicals that cause cellular damage and oxidative stress, which are common in intensive farming environments. Supplementing fish diets with vitamin C enhances immune function, increases disease resistance, and supports vital physiological processes such as collagen synthesis, which is crucial for maintaining connective tissue, bones, and wound healing in fish (Omoniyi and Ovie, 2018). In addition to its health benefits, ascorbic acid improves feed utilization and growth performance in various fish species. Adequate vitamin C levels contribute to better feed conversion efficiency, faster growth rates, and improved survival by reducing stress caused by handling, environmental changes, and poor water quality. Fish fed with vitamin C-enriched diets also show improved reproductive performance and blood parameters, such as increased red blood cell production and enhanced oxygen transport capacity (Ibrahim *et al*., 2020).

L. rohita, commonly known as rohu, is a significant freshwater fish species belonging to the family Cyprinidae. This species is predominantly found in rivers and ponds across temperate and tropical regions, including countries like India, Bangladesh, Nepal, Myanmar, and Pakistan. Rohu thrives in freshwater environments and is typically located in rivers and ponds. It prefers areas with ample vegetation, which provides both shelter and food. The species breeds in shallow waters during the southwest monsoon season, utilizing fertile floodplains for spawning (Ahasan *et al*., 2020). Adult *L. rohita* can reach lengths of up to 2 meters and weigh as much as 45 kilograms. Their bodies are characterized by a brownish or bluish dorsal surface and a silvery-white underside. The fish features large, overlapping cycloid scales, which are important for taxonomic classification (Yadav and Paul, 2023). As a column feeder, *L. rohita* plays an essential role in the aquatic ecosystem by consuming plant material and contributing to the nutrient cycling within its habitat. It is often farmed alongside other carp species like *Cirrhina mrigala* and *Catla catla*, which helps optimize space and resources in aquaculture settings (Gopikrishna, 2023). There is no earlier study found on the nutritional profile, growth and hematological parameters in the *L. rohita* after the feeding of spirulina.

2. Materials & Methods

2.1 Location of the work

The research work will be carried out at the Live Fish Laboratory Department of Aquaculture, L.S.P.N. College of Fisheries, Kawardha, Chhattisgarh.

2.2 Experimental fish

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

2.3 Feed formulation

The selection of fish feed ingredients was carried out based on the nutritional requirements necessary for the preparation of the experimental diets. All feed ingredients were procured from the local market in Kawardha. *S. platensis* was also purchased from the market and its proximate composition was analyzed prior to feed formulation to ensure quality and nutritional adequacy. Following the proximate analysis, three experimental diets containing 30% crude protein were formulated by incorporating different levels of *S. platensis* meal: 10 g (T1), 15 g (T2), and 20 g (T3) per 100 g of feed. A control diet (T0) was also prepared without the inclusion of *S. platensis* meal. The control diet consisted of standard feed ingredients such as fish meal, Vitamin-C, rice bran, groundnut oil cake, tapioca flour, and a vitamin/mineral premix (Table 1).

Table: 1. Ingredient composition for experimental fish feed.

Ingredients	Control (g)	T1 (g)	T2 (g)	T3 (g)
S. 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.

2.4 Experimental design

One hundred twenty fingerlings of *L. rohita*, initial average weight range from 4.30±0.11g and initial average length range from 5.32±0.06 cm will be randomly stocked in four different experimental groups such as control, T1, T2 and T3 with three replicates, following a completely randomized design (CRD).

2.5 Feeding trial

All the diets were prepared and offered to fish in pellet form. *L. rohita* fingerlings showing average weight (4.30±0.11) g and average length (5.32±0.06) cm were collected from the fish nursery at Khairbana (Kala), and were acclimatized in aquarium tanks (12 × 18 × 24 cm) for 5 days then were divided into four groups; three groups received experimental diets and the fourth group was kept on control diet (without spirulina). All the dietary treatments including control had three replicates. Fish weight and length were taken at the time of initiation of the experiment, and thereafter, the increase in fish weight and length were noted on a fortnightly basis. All the fish were fed at 5% of their body weight twice a day at 9 AM and 4 PM. Before feeding, the leftover feed and faecal matter were removed from water. At the end of the trial, all the fish were harvested, weighed and measured, and some fishes were dried for the determination of body composition. Water quality parameters like temperature, pH and dissolved oxygen of aquarium water were monitored on a daily basis.

2.6 Nutritional profile of experimental feed

At the end of the trial, proximate composition of under trial fish were determined following the standard methods of Association of Official Analytical Chemists (2005) for moisture, protein, fat and ash contents, respectively.

2.6.1 Protein analysis

Five grams of dried fish silage sample was taken in a flask and mixed with digestion mixture (potassium sulphate + copper) and transferred to a flask containing 200 mL of concentrated H_2SO_4. This flask was placed on 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 tip immersed in standard acid and heated until NH_3 was evaporated. The final solution mixture was then titrated against NaOH. Protein contents were then determined applying the following mathematical formula:

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

2.6.2 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 were switched off, and the flask was removed and gently dried on the same heater. When the contents of the flask smelled oily, they were removed and weighed and the fat content in the test sample was calculated using the following formula.

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

2.6.3 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

2.6.4 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.

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

2.7 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;2023a). Growth performance was evaluated using the formulae given below.

2.7.1 Length gain (cm)

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

2.7.2 Weight gain (g)

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

2.7.3 Percentage weight gain (%)

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

2.7.4 Specific growth rate (%)

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

2.7.5 Survivability (%)

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

2.7.6 Mortality (%)

$$\text{Mortality (\%)} = \frac{\text{Number of fish that died during the experiment}}{\text{Total number of fish stocked}} X\ 10$$

2.7.7 Feed conversion ratio (FCR) (g)

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

2.7.8 Protein efficiency ratio (PER) (g)

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

2.7.9 Hepato-somatic index (HSI) (g)

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

2.7.10 Intestinal Somatic Index (ISI) (g)

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

2.8 Haematological parameters

2.8.1 Collection of blood

Each fish was anaesthetized with clove oil (Merck, Germany) at the rate of 50 µl of clove oil per liter of water before collecting blood samples from fish. All the 5 fish from each tank were used for blood collection and pooled samples from each tank were used for analysis. Blood was collected from caudal vein of fish by using 1.0 ml hypodermal syringe and 24 gauge needles. The collected blood (approximately 400- 500µL from each fish) was immediately transferred into vials coated with thin layer of EDTA, as anticoagulant (for blood and plasma collection). Vials having EDTA coats were shaking gently in order to prevent haemolysis and clotting of blood. In the present experiment, mainly the blood in collected and kept with EDTA for the study of different parameters (Lal *et al*., 2025).

2.8.2 Total erythrocyte count (RBC)

TEC was done by the method of Schaperclaus (1991). Blood was drawn in a dry erythrocyte pipette to the 0.5 graduation and then Hayem's solution (Qualigens, India) was drawn up to the 101 mark (dilution 1:200). The RBC pipette was then shaken for 1 minute and counting of the cells was done under trinocular microscope 400 x magnifications. The erythrocytes were counted in 5 group squares (1group square = 16 small squares). All cells lying inside the group squares and also the erythrocytes lying to the left and below the demarcation line were counted. Final TEC was calculated with the formula given below:

$$\text{TEC mm}^{-3} = \frac{\text{No. of cells x Dilution factor x Depth factor x Total ruled area}}{\text{Area count}}$$

Where,

Dilution factor = 200

Depth factor = 10

Total ruled area = 25

Area count = 5

2.8.3 Total leucocyte count (WBC)

TLC was done with the help of Schaperclaus method (1991). Blood was drawn up to 0.5th mark by using a WBC pipette and then the WBC diluting fluid was drawn up to 11th mark (dilution 1:200) of the WBC pipette. The filled WBC pipette was gently revolved for 2-3 minute to mix with the diluting fluid. After efficient mixing, counting chamber was filled with a small drop of diluting blood. The cells were then allowed to settle for 3 minutes and counting was done in the large squares of the four corners of the chamber and is demarcated by triple line (1 mm^3). Final WBC count or TLC was calculated with the formula as follows:

$$\text{TLC mm}^{-3} = \frac{\text{No. of cells x Dilution facto}}{\text{Area count}}$$

Where,

Dilution factor = 20

Depth factor = 10

Area count = 4

2.8.4 Packed cell volume (PCV)

PCV was measured by

Schaperclaus method (1991) with slight modification. Blood was drawn upto the graduation mark 100 on the heparinised capillary tube. The openings of the tube were closed with wax and the tube was centrifuged at 3000 rpm for 3 minutes. After centrifuging, the capillary tube was placed on a reading device and the volume was recorded. The PCV was expressed as the percentage fraction of blood cells in the total volume (volume in %).

2.8.5 Total haemoglobin (Hb)

The Hb Total haemoglobin (Hb) content was estimated by using Sahli Haemometer (Marinefield, Germany). In this method, the measuring tube was first filled with 0.1N hydrochloric acid up to the mark 2, and then 20 µl of blood was added (Schaperclaus, 1991). After proper mixing, the colour of the sample was compared with the standard following dilution of the sample by adding distilled water dropwise. Reading of the Hb content was determined from the scale given in the measuring tube.

2.8.6 Mean corpuscular volume (MCV)

To calculate the MCV, expressed in femtoliters (fl or 10−15 L), the following formula was used:

$$MCV = \frac{Hematocrit\ or\ PCV(\%)\ \times\ 10}{RBC\ count(EC\ in\ million/mm3\ )}$$

2.8.7 Mean corpuscular haemoglobin concentration (MCHC)

To calculate the MCHC, expressed as grams of haemoglobin per 100 mL packed cells, the following formula was used:

$$MCHC = \frac{Hemaglobin(g/dL)\ \times\ 100}{Heamtocrit\ or\ PCV(\%)}$$

2.9 Water quality parameters

The water physio-chemical parameters, viz., water temperature, dissolve oxygen (DO), pH, alkalinity and total hardness of the tank water were estimated and recorded fortnightly prior to sampling by the standard methods (APHA, 2005). DO and temperature is measured in early morning around 7-8:30 AM. The water temperature and dissolve oxygen of water was measured following the standard methodology of APHA (2005). The pH was estimated in a digital pH meter 335 (Systronics). The total alkalinity and hardness of water was measured following the standard methodology of APHA (2005) (Damle *et al*., 2023;Lal *et al*., 2023).

2.9.1 Temperature

Water temperature recorded at the every day in mid noon by used a mercury thermometer with a least count of 0.1°C from each an experiment reared tanks. The water temperature was determined by a glass thermometer calibrated in degrees centigrade. Water temperature was determined by lowering the thermometer into the water tank and retaining it inside for about 2-4 minutes.

2.9.2 Dissolved oxygen

Water sample were collected from the experimental tanks in 100 ml DO bottle. After collection1ml of $MnSO_4$ & KI solution was added through the pipette, gradually sinking into the bottom of the DO bottle. The bottle was tightly closed and mix well by shaking. The precipitate was formed which dissolved by adding 1ml of conc. H_2SO_4. Now, fifty ml of this solution were taking 250ml in a conical flask & than titration contrary freshly prefabricated 0.025 N $Na_2S_2O_3$ till in blue colour of a starch indicator turns colourless at the end point.

After that the dissolve oxygen was calculated by the following formula:

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

2.9.3 pH

A pH meter was used to determine the pH of the water (HM Digital, PH 80). Before usage, the pH meter was calibrated using a pH 7 buffer solution. The pH meter was then checked using a buffer solution with known pH values of 4.0 and 10. After that, the pH of the experimental aquarium tank water sample was measured directly.

2.9.4 Alkalinity

The total alkalinity of water was measured following the standard methodology of APHA (2005). 100 ml water sample were collected from an experimental reared tanks at a 250 ml of conical flask and 3-4 blob of phenolphthalein index were assembled which turn the water pink in colour. The water was titrated with 0.02(N) H_2SO_4unto rosy colour disappeared. The volume (ml) of acid used was noted. Then 2-3 blob of methyl orange index were assembled in the same water. If the water turns yellow then it was again titrated with same acid until a faint orange end point was appeared. The volume (ml) of the acid used in this second titration was also noted and the alkalinity is calculated by taking total volume of acid used in to consideration. Total alkalinity was calculated by the following formula:

$$Total\ alkalinity\ (\text{mgI} - 1)\ = \text{Volume of } H_2SO_4\ (N/50) \text{ consumed}$$

2.9.5 Total hardness

The total hardness of water was measured following the standard methodology of APHA (2005). Take 50 ml of the sample water in a conical flask and add 5 mi of buffer. Add a pinch of Eriochrome black-T indicator and then tritrate against standard EDTA solution until the wine red turns to blue which indicates the end point. Record the amount of standard EDTA. Total hardness was calculated by the following formula:

$$Total\ hardness\ (\mathrm{mgI} - 1) = \frac{Volume\ of\ EDTA}{Volume\ of\ sample} X\ 1000$$

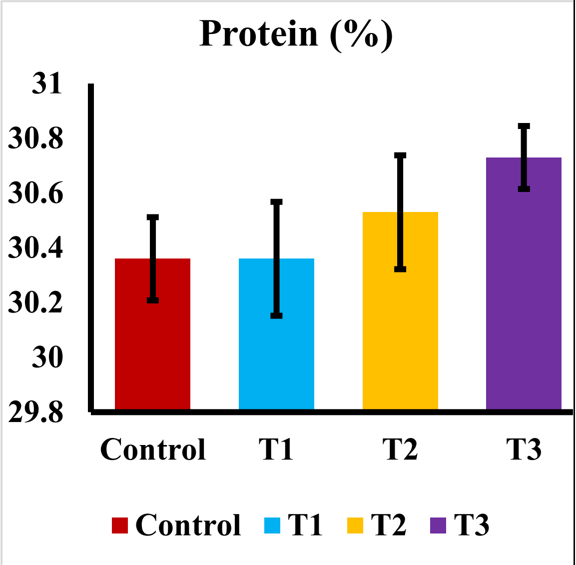
2.10 Statistical analysis

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.

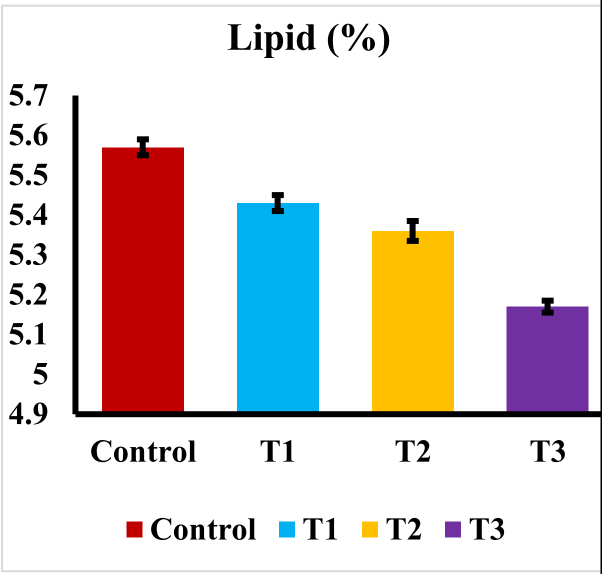
3. Results

3.1 The proximate composition of experimental feed

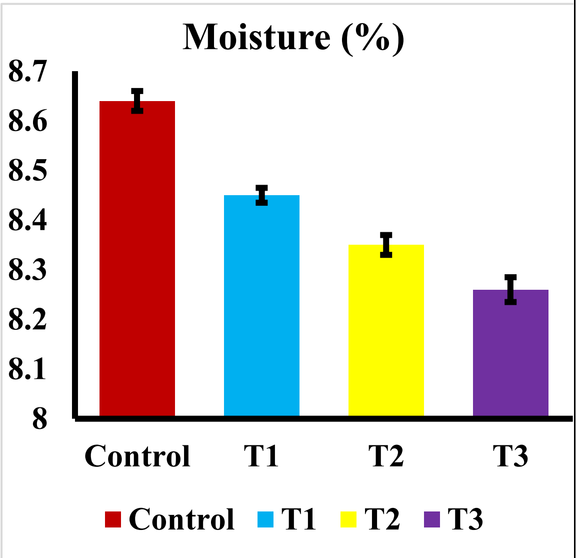
The proximate composition of experimental feed are repersented in the Figure No. 1. The proximate composition of the experimental feed included analyses of crude protein, lipid, moisture, ash, crude fiber, and nitrogen-free extract (NFE). There is a no statistically significantly ($p<0.05$) difference in between all among the treatment groups. The highest crude protein content (30.73±0.115) was observed in the T3 group whereas the lowest value (30.36±0.152) was observed in the control groups. The highest crude lipid (5.57±0.020) was observed in the control group whereas the lowest value (5.17±0.015) was observed in the T3 groups compared to the other treatment groups. The highest moisture content (8.64±0.020) was observed in the control group while the lowest (8.26±0.025) was observed in the T3 groups. The highest crude ash (16.23±0.020) was observed in the control groups, while the lowest (15.17±0.020) was observed in T3 group compared to the other treatment groups. The highest crude fibre (11.27±0.020) was observed in the control group and whereas the lowest (9.92±0.055) in the T3 groups compared to the other treatment groups. The highest nitrogen free extract (30.73±0.165) was observed in the T3 group, while the lowest value was observed in the control group. Overall, although slight variations were observed among treatments, no significant differences ($p < 0.05$) were found in the proximate composition of the experimental feeds.


Protein (%)
31
30.8
30.6
30.4
30.2
30
29.8
Control
T1
T2
T3
Control T1 T2 T3

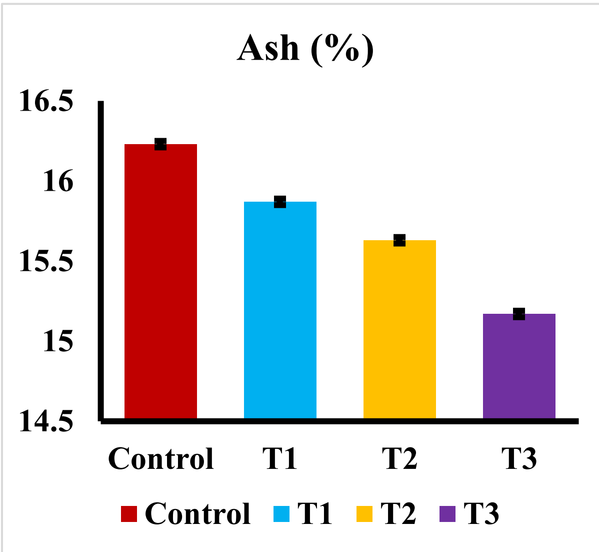
A


Lipid (%)
5.7
5.6
5.5
5.4
5.3
5.2
5.1
5
4.9
Control
T1
T2
T3
Control T1 T2 T3

B


Moisture (%)
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8
Control
T1
T2
T3
Control T1 T2 T3

C


Ash (%)
16.5
16
15.5
15
14.5
Control
T1
T2
T3
Control T1 T2 T3

D

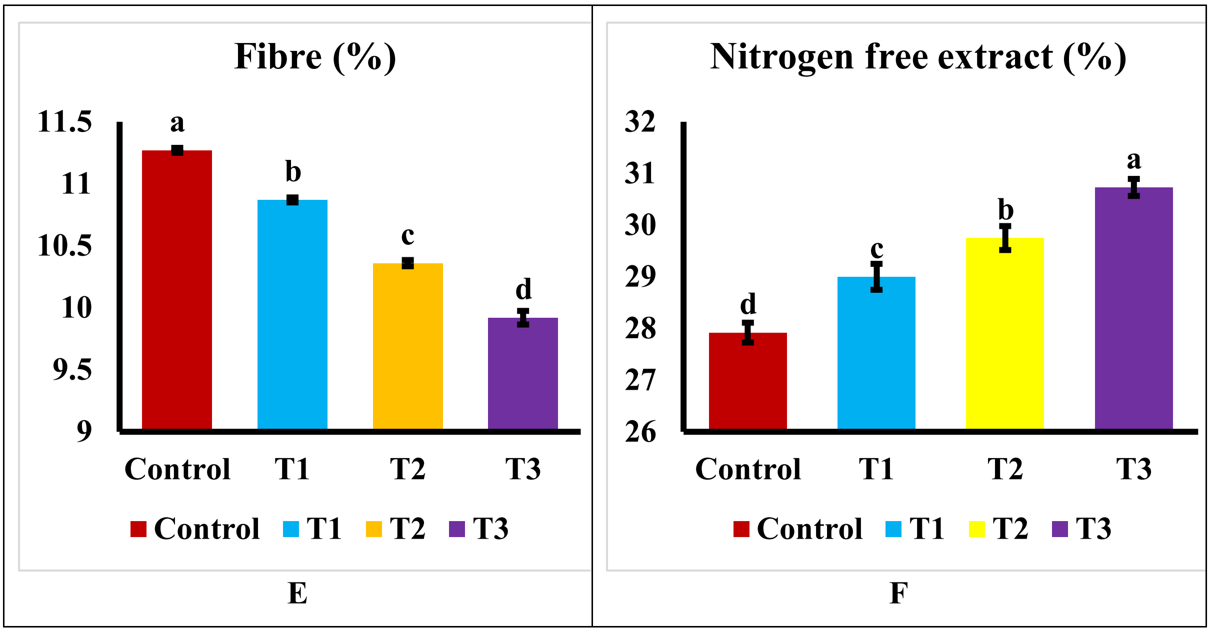


Fig: 1. Proximate composition of *L. rohita*; a. Protein; b. Lipds; c. Moisture; d. Ash; e. Fibre; f. Nitrogen Free Extract. *Data are presented as mean±SE. Different superscripts indicate statistically significant difference ($p<0.05$) among the experimental groups.

3.2 Survivability and mortality of *L. rohita*

The survivability and mortality of *L. rohita* are represented in the Fig. No. 2. The survivability of *Labeo rohita* during the experimental period varied among the different treatment groups. The highest survivability (70%) was recorded in the T3 group, indicating better health and adaptability of fish under this treatment. In contrast, the lowest survivability (56.66%) was observed in the control group, suggesting less favorable rearing conditions compared to the treatment groups. Similarly, mortality rates showed an inverse trend. The highest mortality (43.33%) occurred in the control group, while the lowest mortality (30%) was recorded in the T3 group. These results indicate that the T3 treatment provided more suitable conditions for fish survival, contributing to reduced mortality and improved overall performance during the experimental period.

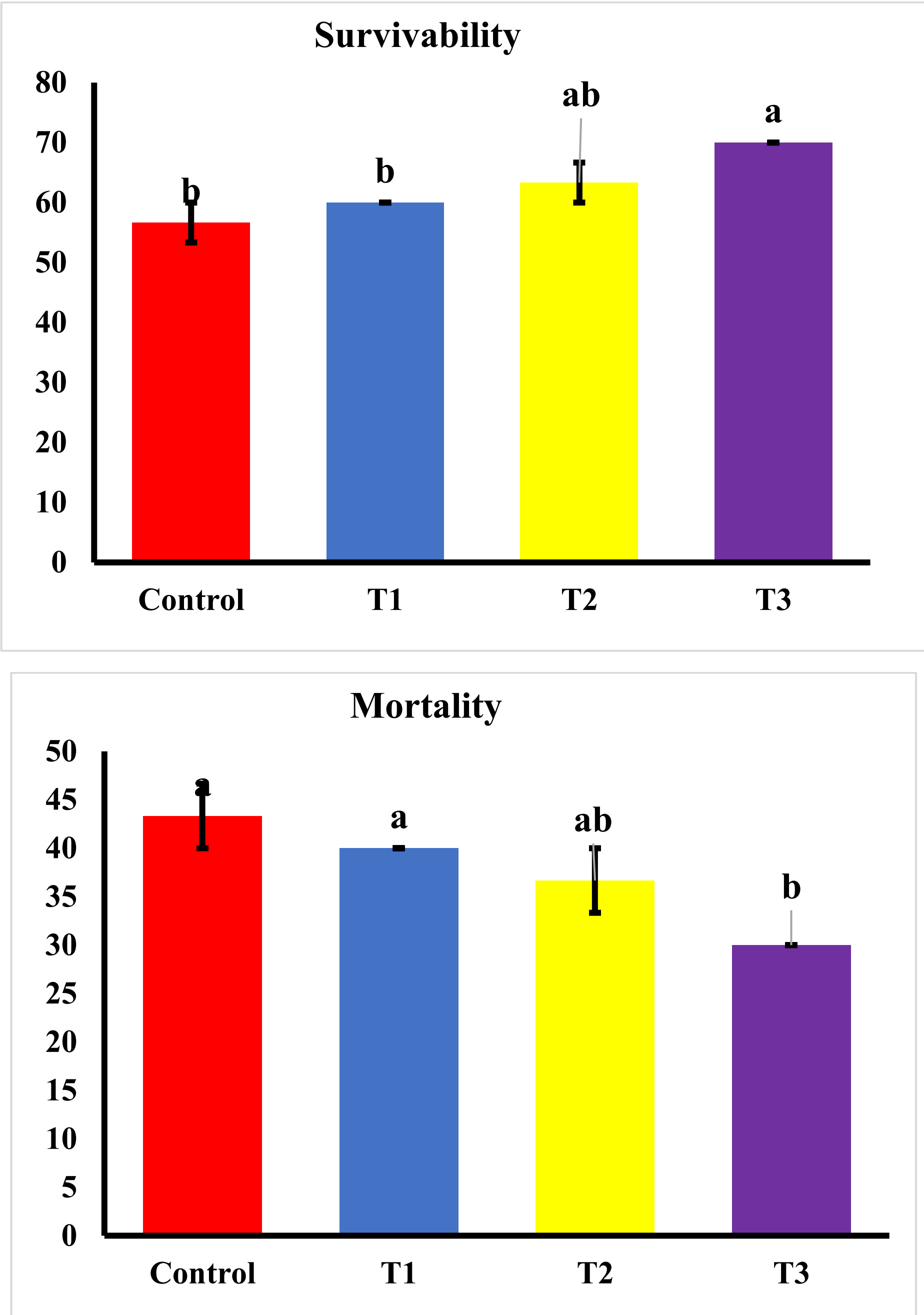


Fig. 2 Survivability and Mortality of rohu, *L. rohita* at end of the experiment. *Data are presented as mean ± SE. There is a statistically significantly difference ($p>0.05$) among the experimental groups.

3.3 The growth performance of *L. rohita*

The growth performance of *L. rohita* are repersented in the Table No. 2. The growth performance parameters of fish such as length gain, weight gain, percentage weight gain, specific growth rate, food conversion ratio, protein efficiency ratio, hepato-somatic index and intestinal-somatic index. The highest survivability

70% was observed in the T3 group compared to the other treatment groups. The highest mortality 40% was observed in the control group compared to the other treatment groups. There is a statistically significantly (p>0.05) difference in all among the treatment groups. The highest length gain (9.26±0.057^{a}) was observed in the T3 group while lowest (6.40±0.10^{d}) in the control group compared to the other treatment groups. The highest weight gain (12.80±0.10^{a}) was observed in the T3 group whreas the lowest (10.83±0.30^{c}) in control group compared to the other treatment groups. The highest percentage weight gain (304.76±2.38^{a}) was observed in the T3 group and lowest (246.62±16.07^{c}) in the control groups compared to the other treatment groups. The highest specific growth rate (0.67±0.002^{a}) was observed in the T3 group while the lowest (0.59±0.022^{c}) in the control groups compared to the other treatment groups. There is a statistically significantly (p>0.05) difference in all among the treatment groups.

The highest food conversion ratio (1.85±0.15^{a}) was observed in the control group while the lowest value (1.33±0.007^{c}) was oberved in T3 group compared to the other treatment groups. The highest protein efficiency ratio (2.43±0.014^{a}) was observed in the T3 while the lowest value (1.78±0.14^{c}) was observed in control groups compared to the other treatment groups. There is a statistically significantly (p>0.05) difference in length gain was found among the groups. The highest hepato-somatic index (0.040±0.003^{a}) was observed in the T2 group and lowest (0.036±0.004^{a}) in the control groups compared to the other treatment groups. The highest intestinal-somatic index (0.48±0.005^{a}) was observed in the T3 group and whereas the lowest (0.43±0.06^{a}) in the control groups compared to the other treatment groups. There is a statistically significantly (p>0.05) difference in length gain was found among the groups

Table: 2. The growth performance of *L. rohita* during the experimental periods.

Treatment	Initial length (cm)	Final length (cm)	Initial Weight (g)	Final weight (g)	Length gain (cm)	Weight gain (g)	% weight gain (%)	Specific growth rate (%)	Food conversion ratio (g)	Protein efficiency ratio (g)	Hepato Somatic Index (%)	Intestinal Somatic Index (%)
Control	5.30±0.10^{a}	11.70±0.152^{d}	4.40±0.173^{a}	15.23±0.152^{c}	6.40±0.10^{d}	10.83±0.30^{c}	246.62±16.07^{c}	0.59±0.022^{c}	1.85±0.15^{a}	1.78±0.14^{c}	0.036±0.004^{a}	0.43±0.06^{a}
T1	5.33±0.057^{a}	12.33±0.115^{c}	4.36±0.057^{ab}	16.50±0.100^{b}	7±0.17^{c}	12.13±0.15^{b}	277.92±7.12^{b}	0.64±0.009^{b}	1.61±0.026^{b}	2.03±0.03^{b}	0.037±0.001^{a}	0.45±0.014^{a}
T2	5.36±0.057^{a}	13.66±0.	4.26±0.057^{ab}	16.76±0.152^{a}	8.30±0.10^{b}	12.50±0.10^{a}	292.98±2.27^{ab}	0.66±0.002^{ab}	1.52±0.11^{b}	2.15±0.16^{b}	0.040±0.003^{a}	0.48±0.045^{a}

		057^{b}										
T3	5.30±0.0E0^{a}	14.56±0.100^{a}	4.20±0.0E0^{b}	17±0.100^{a}	9.26±0.057^{a}	12.80±0.10^{a}	304.76±2.38^{a}	0.67±0.002^{a}	1.33±0.007^{c}	2.43±0.014^{a}	0.039±0.001^{a}	0.48±0.005^{a}
F-Value	0.733	401.244	2.757	111.783	374.062	65.886	23.725	20.898	15.071	17.871	1.212	1.318

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

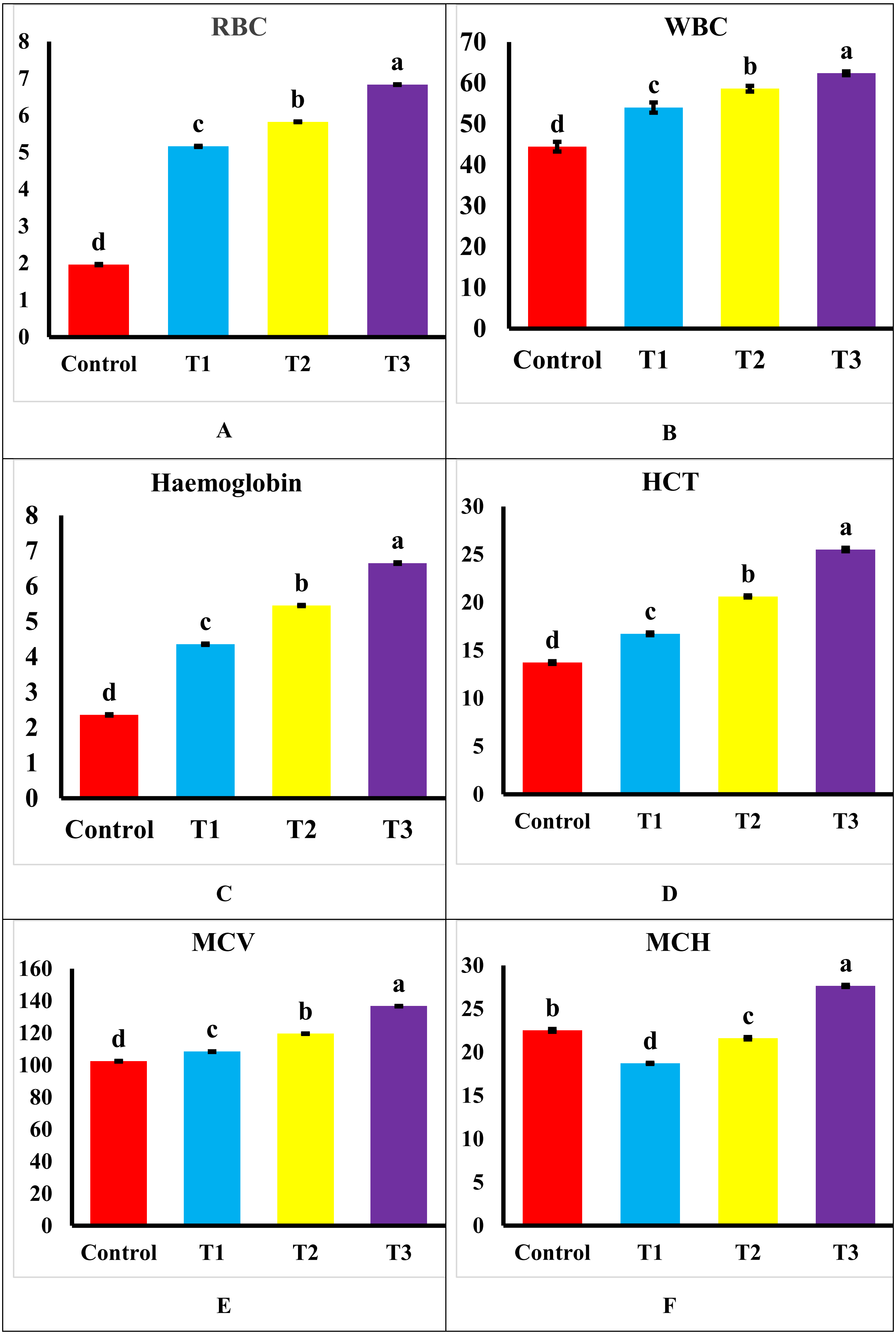
3.4 Haematological parameters of fish

The haematological parameters of fish was observed at the end of the experiment are represented in table No. 3 and Figure No. 3 The haematological parameters of fish such as Red Blood Cells, White Blood Cells, Packed Cell Volume (PCV), haemoglobin, hematocrit (HCT), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC). There is a statistically significantly (p>0.05) difference in length gain was found among the groups. The highest RBCs (6.83 ± 0.013^{a}) was observed in the T3 group whereas the lowest value (1.96 ± 0.028^{d}) in the control groups. The highest WBCs (62.36 ± 0.80^{a}) was observed in the T3 group whereas the lowest value (44.44 ± 2.054^{d}) in the control groups. The highest HCT (25.52 ± 0.31^{a}) was observed in the T3 group whereas the lowest value (13.73 ± 0.24^{d}) in the control groups. The highest haemoglobin (6.65 ± 0.036^{a}) was observed in the T3 group while the lowest (2.35 ± 0.031^{d}) in the control groups compared to the other treatment groups. The highest MCV (136.66 ± 0.20^{a}) was observed in the T3 group whereas the lowest value (102.40 ± 0.35^{d}) in the control groups. The highest MCH (27.64 ± 0.21^{a}) was observed in the T3 group whereas the lowest value (18.71 ± 0.11^{d}) in the T1 groups. The highest MCHC (23.55 ± 0.25^{a}) was observed in the T3 group and whereas the lowest (1.97 ± 0.019^{d}) in the control groups. There is a statistically significantly (p>0.05) difference in between all among the treatment groups.

Table: 3. Haematological parameters of *L. rohita* at the end of 90^{th} days of experiment.

Treatment	RBCs ($x10^6/\mu l$)	WBCs ($x10^3/\mu l$)	HCT	Hb (g dL^{-1})	MCV (fi)	MCH	MCHC (%)
Control	1.96 ± 0.028^{d}	44.44 ± 2.054^{d}	13.73 ± 0.24^{d}	2.35 ± 0.031^{d}	102.40 ± 0.35^{d}	22.51 ± 0.27^{b}	1.97 ± 0.019^{d}
T1	5.16 ± 0.025^{c}	53.99 ± 2.16^{c}	16.73 ± 0.24^{c}	4.35 ± 0.031^{c}	108.36 ± 0.30^{c}	18.71 ± 0.11^{d}	7.52 ± 0.24^{c}
T2	5.82 ± 0.015^{b}	58.60 ± 1.21^{b}	20.62 ± 0.18^{b}	5.45 ± 0.028^{b}	119.53 ± 0.20^{b}	21.60 ± 0.23^{c}	15.50 ± 0.34^{b}
T3	6.83 ± 0.013^{a}	62.36 ± 0.80^{a}	25.52 ± 0.31^{a}	6.65 ± 0.036^{a}	136.66 ± 0.20^{a}	27.64 ± 0.21^{a}	23.55 ± 0.25^{a}
F-Value	28440.28	65.35	9655.23	1240.48	8949.12	885.11	4418.67

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



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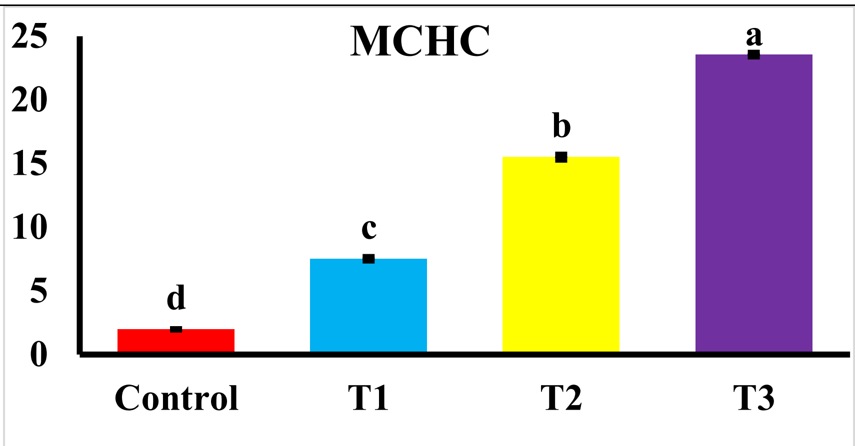

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Fig: 3. Haematological parameters of *L. rohita* at the end of the experiment; a. Total erythrocyte count; b. Total leucocyte count; c. Total haemoglobin; d. Hematocrit; e. Mean corpuscular volume; f. Mean corpuscular haemoglobin, g. Mean corpuscular haemoglobin concentration. *Data are presented as mean±SE. Different superscripts indicate statistically significant difference ($p<0.05$) among the experimental groups.

3.5 The water quality parameters

The water quality parameters of experimental fish was observed during the experimental period are repersented in Table No. 4 and Fig. No. 4. The acceptable range of water quality parameters was observed such as temperature, dissoved oxygen, pH, hardness and alkalinity. The highest water temperature range from (24.85-27.9) was observed in the control group. The highest dissolved oxygen range form (5.31-5.9) was observed in T1 group compared to the control groups. The highest pH range form (7.45-7.8) was observed in T2 group compared to the control groups. The highest water hadness (63.4-64.31) was observed in control group compared to the other treatment groups. The highest water alkalinity range form (77.5-78.7) was observed in T2 group while the lowest (76.2-77.7) in the control group.

Table: 4. 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.

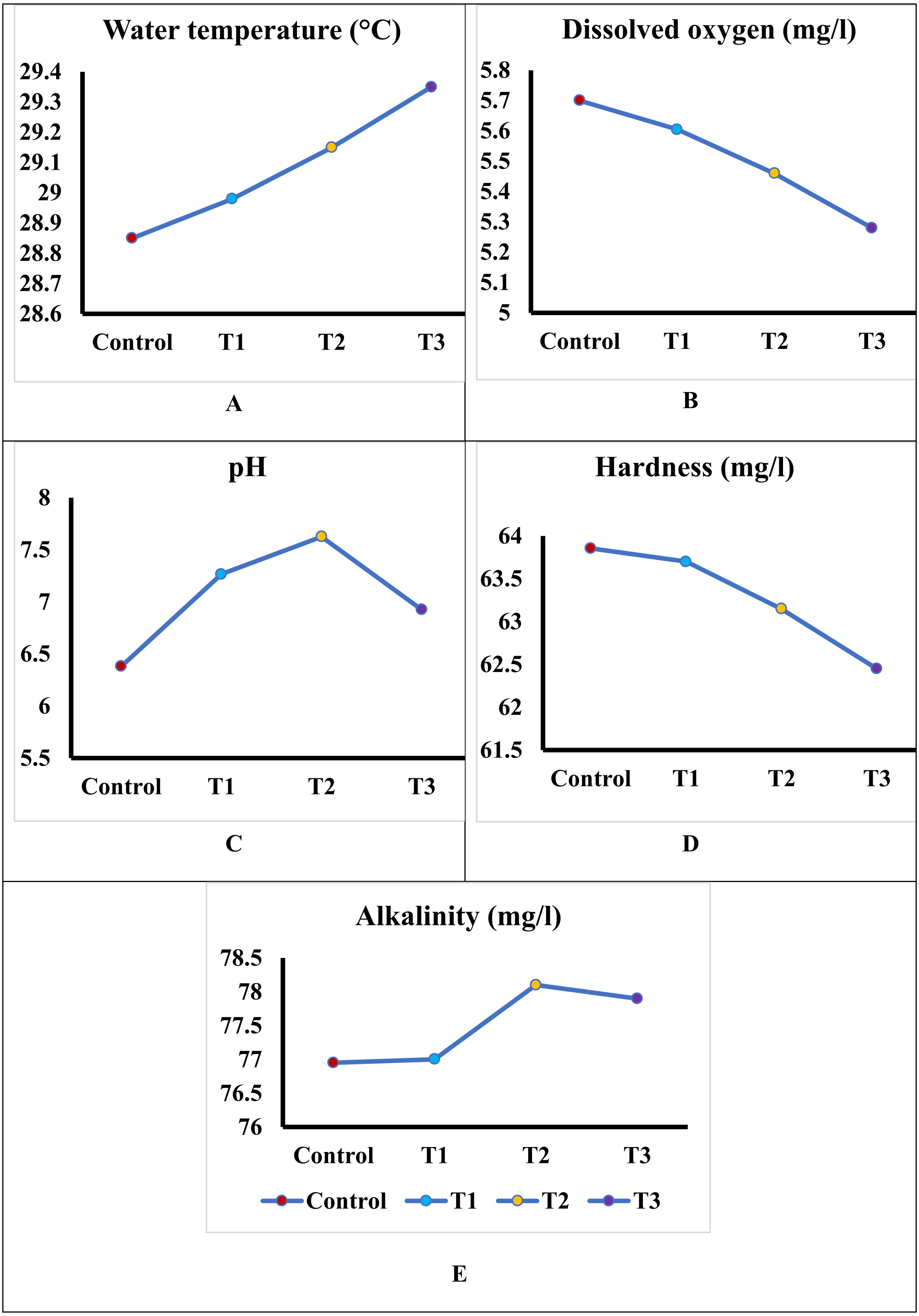

Fig: 4. The water quality parameters (dissolved oxygen) of *L. rohita* during the experimental period. *Data are presented as mean±SE.

4. Discussion

4.1 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).

In the fish diets with 30% spirulina inclusion show significantly higher protein content (around 55%) in fish body composition compared to lower inclusion levels and improve fatty acid profiles with increased omega-3 levels like EPA and DHA. Protein content in spirulina-based fish feed pellets can vary from 35% to nearly 39%, depending on the formulation, with injectable spirulina protein effectively replacing fishmeal protein without loss of growth performance. Thus, spirulina-containing fish feed is rich in protein, beneficial fats (omega-3s), vitamins, and minerals, making it a nutritious and sustainable alternative or supplement to conventional fishmeal-based feeds (Soma *et al.*, 2024). Spirulina has high digestibility of protein and amino acids in certain marine fish species such as snubnose pompano and sobaity seabream, with sobaity seabream showing better protein and amino acid digestibility. Spirulina inclusion did not reduce feed intake and showed potential as a sustainable feed ingredient substituting conventional proteins (Siddik *et al.*, 2025). Spirulina can replace fishmeal protein by up to 30% in feeds for juvenile Nile tilapia without negative impact on growth performance, feed intake, or mortality. Spirulina also preserves beneficial fatty acids such as n-3 LC-PUFA (EPA and DHA), which are important for fish and consumer nutrition (Soma *et al*., 2024). Feeding Spirulina enhances fish health by improving immune response, reducing disease mortality, and potentially lowering the need for medications and antibiotics. Spirulina stimulates beneficial gut bacteria and enzymatic activities that enhance nutrient utilization and growth (Al Mamun *et al*., 2023). Meta-analysis of multiple studies shows that dietary Spirulina meal (SPM) supplementation significantly improves fish growth (final body weight, specific growth rate), feed efficiency (feed conversion ratio), and protein utilization (protein efficiency ratio), without negative effects on fish condition or hepatosomatic index. Optimal inclusion rates are around 1.5-2.3% as a supplement or up to 22-25% replacement of fishmeal (Li *et al*., 2022). The nutraceutical properties of Spirulina, including antioxidants and immune stimulators, contribute to stress resistance and overall fish welfare, supporting sustainable aquaculture practices (Ujjwal *et al*., 2025).

4.2 Growth performance of *L. rohita*

The growth and feed utilization parameters in *L. rohita* in the current study indicated an upward trend up to 20% level of fish meal replaced with *S. platensis*, which may be attributable to increased feed intake and nutrient digestibility as well as essential vitamins, minerals, and amino acids. These results are in line with those of Teimouri *et al*. (2013), who noticed that rainbow trout fed 5% *S. platensis* had significantly worse growth performance than those fed 7.5% and 10% *S. platensis*, and in particular that the diet containing 2.5–10% of *S. platensis* increased weight gain percentage from 113.1 ± 4.8% to 131.4 ± 7.7%. Whereas, Akter *et al*., 2023) observed that dietary replacement of fishmeal by *S. platensis* in the diet of (*Ompok pabda* resulted in the best growth performance at 15% level compared to the control. *S. platensis* contains a high-quality protein as well as bioactive compounds that play an essential role in growth enhancement (Da Silva *et al*., 2021). Similar results were found in previous findings where fish grew better on algae-enriched diets (Riano *et al*., 2012). Abdel-Tawwab and Ahmed (2009) found a lower FCR value (1.22 ± 0.02) in the 5% level replacement of *S. platensis* and a higher FCR value observed in the control diet, they also observed a maximum PER value (2.91 ± 0.08) in 5% level of S. platensis incorporation while minimum PER value was found in the control diet. In the case of survival rate, similar results were found in the findings of Roohani *et al*. (2018). According to James *et al*. (2006), S. platensis also enhanced the intestinal flora in fish, breaking down indigestible feed components to extract more nutrients from the feed and promoting the synthesis of enzymes that transport lipids for metabolism rather than storage. The higher feed utilization pattern in the present study is also justified by the statement. Prior research suggests that the high vitamin, mineral, essential amino acid, linoleic acid, and linolenic acid content of *S. platensis* in the diet improve growth performance and feed utilization (Cao *et al*., 2018; Roohani *et al*., 2018).

The use of spirulina as a feed supplement positively influences fish growth performance. Studies show that including spirulina in fish feed improves survival rate, weight gain, and length increase in various fish species. For example, in *Pangasius sutchi*, a feed with 5% spirulina resulted in higher survival (94%), average weight gain (60.4 g), and length (13.07 cm) compared to control feeds without spirulina. Spirulina improves feed intake, nutrient digestibility, and supplies essential vitamins and minerals aiding growth promotion (Jana *et al*., 2014). Fish fed diets containing Spirulina (5 g/kg) had significantly better growth and feed utilization as compared to fish fed with the control diet. The present study proved that dietary supplementation of Spirulina enhanced fish growth. These results may possibly be due to the improved feed intake and nutrient digestibility. Moreover, Spirulina contains several nutrients especially vitamins and minerals that may help in fish growth promotion. These results agree with those found by several researchers (Belay *et al*., 1993; Hayashi *et al*., 1998; Hirahashi *et al*., 2002) who reported that feeding Spirulina to fish improved survival and growth rates. In this regard, (Watanabe *et al*., 1990) mentioned that feed supplemented with Spirulina powder improved the feed conversion ratio and growth rates for striped jack, *Pseudocaranx Dentex*. Lowest length gain was observed in case of control (20 cm) and highest was observed in case of fishes fed with 5% Spirulina (28.3 cm). Lowest weight gain was observed in case of control (20 gm) and highest was observed in case of fishes fed with 5% Spirulina. Survival was almost 100% in case of control and highest was observed in case of fishes fed with 5% Spirulina. Nandeesha *et al*. (2001) studied the influence of *Spirulina platensis* meal on the growth of two Indian major carps, catla (*Catla catla*) and rohu (*L. rohita*) for a 90-day culture trial. The specific growth rate and protein efficiency ratio recorded in rohu improved with higher levels of Spirulina inclusion, while in catla they did not differ significantly from the control treatment. But in our study, it was observed that length, weight gain, and survival of *Pangasius sutchi* was significantly best with the addition in Spirulina content in the feed.

A comprehensive meta-analysis confirms that dietary spirulina meal supplementation significantly improves fish final body weight, specific growth rate, protein efficiency ratio, and decreases feed conversion ratio, which means better feed utilization. The optimal spirulina supplementation levels for fish diets range from about 1.5% to 2.3%. As a fishmeal substitute, spirulina can be used up to around 22%-25% in fish diets without negative effects on growth (Li *et al*., 2022). Additional studies report growth improvements with spirulina inclusion up to 7.5-10% in some fish species, with enhanced immunity and feed utilization. Overall, spirulina is a promising sustainable additive for improving fish growth performance in aquaculture (Al Mamun *et al*., 2023).

4.3 Haematological parameters of fish

The hemoglobin and red blood cells count were significantly higher in the Spirulina-supplemented groups than the control group. The same results were reported by El Gammal *et al*. (2010) and El-Sheekh *et al*. (2014). Increased RBCs count may be due to Spirulina has 14% phycocyanin which stimulates the erythropoietin hormone production for hematopoiesis (Abdalla *et al*., 2014). In contrast, RBCs of carp fed 3.0–5.0 g/kg Spirulina were not affected by the dietary treatments (Abdulrahman *et al*., 2019). The PCV concentration was also increased by feeding of Spirulina. This result is supported by that of El Gammal *et al*. (2010) and Hegazi *et al*. (2014) who revealed that the PCV of tilapia fed 10.0–15.0% Spirulina was significantly higher than the control group. Feeding of Spirulina increased also the WBCs count. The same results were found by Hegazi *et al*. (2014) and Sayed and Fawzy (2014). This increase in WBCs count could be due to the presence of C-phycocyanin in the Spirulina algae, which can help in building the immune capacity (Vonshak, 1997). These results indicate an improvement of fish health when fed Spirulina-supplemented diets, because Spirulina contains carotenoids which increase the ability to fight off infections through the reduction of stress levels. In addition, the major functions of WBCs are to fight infection and protect the body against foreign organisms (Sayed and Fawzy, 2014).

Zahan *et al.* (2024) A prominent study on Nile tilapia (*Oreochromis niloticus*) demonstrated that dietary supplementation with *S. platensis* at levels of 2.5%, 5%, and 10% for eight weeks significantly increased hemoglobin concentration, packed cell volume (PCV), red blood cell (RBC) counts, and white blood cell (WBC) counts compared to controls. The increase in RBCs is attributed to the phycocyanin content in spirulina, which stimulates erythropoietin production, enhancing hematopoiesis. PCV and WBC enhancements were also observed, with the latter linked to C-phycocyanin's immunomodulatory properties. Importantly, lymphocytes and eosinophils increased, indicating stronger immune responses. Additionally, lysozyme activity, immunoglobulin M (IgM) levels, and phagocytic activity were elevated, showing enhanced innate immunity. Spirulina supplementation also lowered serum creatinine and urea, suggesting protective effects on kidney function. Intestinal histomorphometry showed increased villi height and goblet cell counts, supporting better nutrient absorption and local immunity (Hematological, biochemical, and immunological analysis in tilapia) (Zahan *et al*., 2024). Similar findings were reported in other fish species, including carp and catfish, with spirulina diets improving RBC, WBC, hemoglobin, and immunological parameters. For example, in studies on Heteropneustes fossilis, dietary *S. platensis* improved growth, haematological parameters, and immune responses. These findings reinforce Spirulina's role as a beneficial functional ingredient in aquafeeds that promotes fish health and disease resistance (Rahman *et al*., 2023). In Nile tilapia (*O. niloticus*), feeding diets with 2.5%, 5%, and 10% *S. platensis* for 8 weeks significantly increased hemoglobin, packed cell volume (PCV), red blood cell (RBC) count, and white blood cell (WBC) count. The study also found increases in lymphocytes, eosinophils, IgM levels, lysozyme activity, and phagocytic activity, indicating enhanced immune status. Serum creatinine and urea levels decreased, suggesting improved kidney function, while intestinal villi height and goblet cell counts were elevated, supporting better gut health (Youssef *et al*., 2023). Spirulina supplementation in fish has been linked to phycocyanin content stimulating erythropoietin production, which promotes RBC formation and improves oxygen transport capacity (Abdalla *et al*., 2014; Zahan *et al*., 2024).

Another study on *O. mossambicus* showed that Spirulina combined with commercial feed significantly improved growth and blood parameters (WBC, RBC, hemoglobin, MCV, MCH), further showing Spirulina's effectiveness in enhancing haematology (El-daim *et al*., 2021). Studies on *Heteropneustes fossilis* demonstrated improved growth, haematology, and immune response with dietary Spirulina, confirming benefits across different fish species (Rahman *et al*., 2023). Youssef *et al*. (2023) demonstrated that feeding spirulina to fish improved their hematological and biochemical parameters, including significantly increased red blood cell count (erythrocyte count), hemoglobin, and hematocrit (PCV), alongside decreased cholesterol and improved overall lipid profiles, indicating systemic health benefits for the fish. Supplementing fish diets with spirulina also enhanced growth performance and immune responses, showing it can serve as a beneficial natural feed additive in aquaculture. A feeding trial by Ringø, (2025) demonstrated that incorporating 7 g of Spirulina per kilogram of diet significantly improved weight gain and specific growth rates in fish, in addition to enhancing hematological parameters. This finding supports the use of Spirulina as a beneficial, functional feed additive in aquaculture. In rainbow trout, Spirulina in diets at 2.5% to 10% inclusion improved hematological and serum biochemical parameters, enhancing overall health and stress resistance in the fish (Yeganeh *et al*., 2015).

4.4 Water quality parameters of fish

The water temperature during the experimental period ranged from 24°C to 30°C. Other water quality parameters, pH (7.29-7.46) and dissolved oxygen (7.73-8.06ppm) were also within the favourable limits for carp growth (Jhingran, 1991). The growth and survival rates of aquatic organisms are closely linked to the optimal range of water quality parameters and the overall health of the aquatic environment they inhabit (Kembenya and Ondiba, 2021). Water quality parameters are fundamental to the culture and production of Labeo rohita, as they directly affect the fish's growth, health, and survival. Temperature, pH, dissolved oxygen (DO), total alkalinity, ammonia content, and hardness are among the critical parameters to monitor for successful aquaculture management. Labeo rohita generally thrives in water temperatures ranging from 25°C to 36°C, with the ideal being around 28–30°C. This temperature range facilitates optimum metabolic and enzymatic activities essential for growth and development. Deviations from this range can lead to metabolic stress and reduced feeding efficiency, ultimately negatively impacting growth performance. The pH of the rearing water should be maintained near neutral or slightly alkaline, typically between 7.0 and 8.5, which ensures the stability of physiological processes and enzymatic actions in the fish tissues (Mahamood *et al*., 2021).

Dissolved oxygen is a vital parameter in *L. rohita* culture, with optimum values around 6 to 7 mg/L or higher being necessary for adequate respiration and metabolic activity. Reduced dissolved oxygen levels create hypoxic conditions, which lead to stress, anemia, and increased susceptibility to diseases in the fish. Frequent aeration and effective water circulation are crucial to maintain these dissolved oxygen levels within the optimal range. Free carbon dioxide levels also need to be controlled since excessive CO_2 reduces pH and causes respiratory difficulties. Water chemistry elements such as total alkalinity and hardness contribute to buffering capacity and ionic balance in the water, with alkalinity values between 20 to 300 mg/L and hardness around 150 mg/L being favorable for maintaining water stability and fish health (Biswal *et al*., 2020).

Among chemical pollutants, ammonia, particularly in its un-ionized form (NH_3), is highly toxic to *L. rohita* even at low concentrations. Ammonia originates from fish excrement and the breakdown of uneaten feed and organic matter and affects gill function, oxygen uptake, and immune defense mechanisms. Maintaining ammonia concentrations below 0.02 mg/L as un-ionized ammonia is crucial to minimize physiological stress. Proper feeding management, regular water exchange, and/or application of biofloc or probiotic systems help mitigate ammonia accumulation and improve water quality. The presence of heavy metals such as cadmium, chromium, and nickel beyond permissible limits in culture water can bioaccumulate in organs of *L. rohit*a, disrupting biochemical pathways and causing toxic effects evident through altered hematological and enzymatic parameters (Tabrez *et al*., 2022).

Water quality also influences the biochemical composition and physiological health of *L. rohita*. Variations in environmental parameters like temperature and dissolved oxygen affect protein, lipid, and carbohydrate metabolisms in different body regions (head, trunk, tail) of the fish. Stress caused by poor water quality triggers oxidative damage and compromises tissue repair, reflected in changes to antioxidant enzyme activities and biochemical markers. Seasonal and spatial fluctuations in water quality correlate with shifts in biochemical content, underscoring the importance of continuous water monitoring to optimize feeding and growth strategies and reduce mortality (Kaur, 2020). The relationship between water quality and fish health extends to hematological parameters, which can serve as biomarkers for environmental stress in *L. rohita*. Exposure to suboptimal water conditions such as wastewater or sewage contamination leads to reductions in red and white blood cells and hemoglobin levels, indicating anemia, immunosuppression, and decreased oxygen transport capacity. The health status of fish can therefore be indirectly assessed by regular examination of these blood parameters alongside physicochemical water tests, providing a comprehensive overview of the culture environment and guiding timely interventions (Rout *et al.*, 2017). Efficient management of water quality parameters requires integrated practices such as periodic water testing, adequate oxygenation, balanced feeding regimes, and waste removal. Culture systems with appropriate water depth, flow rates, and natural or artificial aeration maintain stable temperature and oxygen levels. Use of probiotics and biofloc technologies further improve water quality by reducing toxic nitrogenous compounds and enhancing fish gut health. Collectively, these practices promote better feed conversion ratios, faster growth, reduced mortality, and sustainable production yields in *L. rohita* aquaculture (Nesara and Sheethal, 2020).

5. Conclusions

The present 60-day experimental study demonstrated that dietary supplementation of *Spirulina platensis* significantly improved the growth performance, feed utilization, and haematological parameters of *Labeo rohita*. Among all treatments, the T3 group (20 g spirulina powder per kg diet) showed the best results in terms of growth indicators such as length gain, weight gain, percentage weight gain, specific growth rate, and protein efficiency ratio, while recording the lowest feed conversion ratio, indicating enhanced feed efficiency. The haematological parameters including red and white blood cell counts, haemoglobin, haematocrit, and related indices were highest in the T3 group and remained within the normal physiological range, reflecting improved health status and immune competence of the fish. Water quality parameters also remained within optimal limits throughout the study, ensuring no environmental stress influence. Overall, the results indicate that dietary inclusion of 20 g spirulina powder per kg feed (T3) effectively enhances growth, nutrient utilization, and blood health in *Labeo rohita*, suggesting its potential as a natural growth promoter and health enhancer in aquaculture feed formulation.

Declaration of competing interests

The authors declare that they have no financial or other conflicts of interest pertaining to the study.

Data Availability

The corresponding author can provide the data from this study upon request.

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