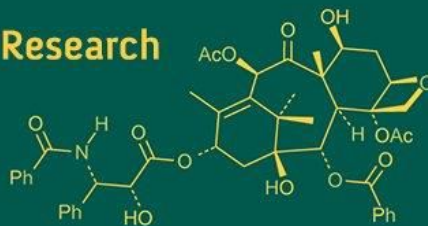
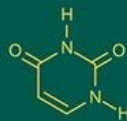


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Effect of partial replacement of fishmeal with *Chlorella* spp. and *Haematococcus* spp. in the diet of *Labeo rohita* (Hamilton, 1822)

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Abstract

This experiment evaluated the effect of partial replacement of fish meal with *Chlorella* spp. and *Haematococcus* spp. in the diet of *Labeo rohita* fry for 60 days. Four treatment diets were prepared with adding *Chlorella* spp. and *Haematococcus* spp. at 5% (T₀), 10% (T₁), 15% (T₂) and 20% (T₄) inclusion level and control diet was prepared without any microalgae. Fish were stocked at a density of 10 nos./aquarium in all 20 aquaria and were fed two times per day at 10% body weight. At the end of the experiment fish fed with 10% of both microalgae showed significantly higher growth rate, protein efficiency ratio (PER) and lower feed conversion ratio (FCR) ($p < 0.05$) compare to all other treatments ($p > 0.05$). While all the treatments showed 100% survival rate ($p > 0.05$). Proximate composition analysis of fish showed the highest protein (14.71%) in T₂, fat (3.65%) in T₃, ash (4.88%) in T₄, and moisture (76.85%) in T₀. Partial replacement of fish meal with *Chlorella* spp. and *Haematococcus* spp. improved growth rate, feed efficiency and survival.

Keywords: Aquaculture, fish meal, Indian major carp, nutrition, survival

Introduction

Fish is considered as a cheap source of protein (Muruganandam *et al.*, 2019) [22]. Aquatic food systems are diverse, providing environmental, economic, and social benefits. Globally, they are valued for their nutritional importance and role in supporting healthy diets and aquatic biodiversity. In 2022, the world fisheries and aquaculture production was 223.2 million tonnes. About 51% from total production was contributed by aquaculture sector 94.4 million tonnes. Carps dominate freshwater aquaculture in India, with Catla (*Catla catla*), Rohu (*Labeo rohita*), and Mrigal (*Cirrhinus mrigala*) as key species due to their fast growth and consumer preference (Rutaisire *et al.*, 2017; Bais, 2018) [30, 6]. *L. rohita*, a highly demanded food fish in India, is preferred for its taste (Rajikkannu *et al.*, 2015) [28]. Improved growth was observed with algae-supplemented feed compared to the control diet (Mukherjee *et al.*, 2011) [21].

The fish meal act as a main source of protein due to its high protein content, presence of essential amino acids, easy nutrient digestibility and minimum anti-nutritional factors. Due to decline in capture fisheries and less availability of fish meal is a major problem facing by feed manufacturers. To counter such problem there is a need to find alternative protein sources which can replace fish meal without any adverse effect (Daniel, 2018) [8]. Several protein sources are available which have the potential of replacing fishmeal in aquaculture feeds without affecting the growth performance of fish (Tacon, 2004) [35]. Microalgae are microscopic unicellular organisms which have many advantages (rich in a variety of important bioactive substance-protein, amino acids, polysaccharides, unsaturated fatty acids and cytochrome etc.), which make it become a new source of feed material (source of protein, fatty acids, vitamins, minerals, pigments etc.) (Li *et al.*, 2015) [17].

Chlorella is freshwater, unicellular, green algae which have huge nutritional value as it is enriched with proteins, minerals, lipids, etc. Some research has proved that *Chlorella* has a range of health benefits (Rani *et al.*, 2018) [29]. It consists of 55-60% protein, 1-4% chlorophyll, 9-18% dietary fibre, vitamins and minerals (Shim *et al.*, 2008) [33]. In the diet of fish dried biomass of *Chlorella* or their extract are added to improve the quality of cultured fish; for instance, it has been reported that enhancement of resistibility to diseases

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(Nakagawa *et al.*, 1981) ^[23]. The high nutritional value of *Chlorella* has used as dietary protein sources for marine and freshwater fish to improve weight gain and carcass quality (Kim *et al.*, 2002; Badwy *et al.*, 2008; Xu *et al.*, 2014) ^[16, 5, 36].

Haematococcus pluvialis, which is also an important microalgae having good amount of natural astaxanthin also considered as “super anti-oxidant”. Astaxanthin has important applications in the nutraceuticals, cosmetics, food, and aquaculture industries (Shah *et al.*, 2016) ^[32]. *H. pluvialis* is a freshwater species of Chlorophyta (Lorenz and Cysewski, 2000) ^[20]. Generally, it contains 1.5-6.0% natural astaxanthin. The natural astaxanthin of *H. pluvialis* is trans, ester form and stable (Zhao *et al.*, 2011) ^[37], which can be well utilized by aquatic animals (Gong *et al.*, 2014) ^[11]. *H. pluvialis* in dried powder form has been used in feed with specific functions such as for colouration and immune enhancer, because of such functions it is considered to be a potential feed additive for aquaculture feeds (Parisenti *et al.*, 2011) ^[25]. Many kinds of research have been carried out which shows that astaxanthin in *H. pluvialis* can protect from free radicals (Ambati *et al.*, 2014) ^[3]. *H. pluvialis* has gained acceptance in aquaculture and other markets as a concentrated form of natural astaxanthin (Lorenz and Cysewski, 2000) ^[20].

Considering the above factors, the present study was conducted to evaluate the effect of partially replacing fish meal with microalgae *Chlorella* spp. and *Haematococcus* spp. in the diet of *Labeo rohita* (Hamilton, 1822) ^[12] fry.

Materials and Methods

Experimental Setup

The experiment was conducted at Aquaculture Laboratory, College of Fisheries Science, Kamdhenu University, Veraval, for 60 days.

Collection of Feed Ingredients

The required ingredients for the preparation of the experimental diet such as fish meal (FM) and groundnut oil cake (GNOC) as a protein source, wheat flour (WF), fish oil, sunflower oil, tapioca powder (TP) and vitamin & mineral (V&M) for feed formulation were brought from the local market. *Chlorella* powder (CP) and *Haematococcus* powder (HP) was brought from Oceanic Seaweed Private Limited, Rajkot.

Proximate analysis of Ingredients

Proximate composition of ingredients (Table 1) was determined by the standard methods (AOAC, 2012). Moisture content was obtained by heating the samples to 105 °C for 30 minutes and then drying at 65 °C till a constant weight was obtained (Boyd, 2019) ^[7]. Crude protein values were obtained by the semi-automatic Micro-Kjeldahl digestion and distillation apparatus (Gerhardt, Germany). Crude fat was estimated with the Soxhlet apparatus. The total ash content was determined with the help of a muffle furnace.

Formulation and preparation of feed

The experimental diet was formulated with 30% protein level using locally available ingredients. In five experimental diets, the microalgae *Chlorella* spp. and *Haematococcus* spp. was added at the rate of 5%, 10%, 15% and 20%, while control diet was prepared with 0% of microalgae. The required quantity of ingredients was

weighed accurately as per feed formula as shown in (Table 2). The dough was prepared by mixing all the ingredients and then it was thermally processed at 121 °C and 15 lbs pressure for 10-15 minutes. After steam cooking of feed mixture, vitamin and mineral mixture was mixed. The dough was pelletized by using hand pelletizer. The pellets were spread on a plastic sheet, exposed to sunlight for sun drying. The dried feed was packed in air-tight plastic jars.

Treatments details

The treatments are selected as follows:

T₀ = Diet prepared with 0% level of *Chlorella* spp. and *Haematococcus* spp. (Control)

T₁ = Diet prepared with 5% level of (2.5% *Chlorella* spp. + 2.5% *Haematococcus* spp.)

T₂ = Diet prepared with 10% level of (5% *Chlorella* spp. + 5% *Haematococcus* spp.)

T₃ = Diet prepared with 15% level of (7.5% *Chlorella* spp. + 7.5% *Haematococcus* spp.)

T₄ = Diet prepared with 20% level of (10% *Chlorella* spp. + 10% *Haematococcus* spp.)

Experimental Setup

Labeo rohita fry was procured from a commercial hatchery, were transported to the Aquaculture Laboratory in Veraval. After 10 days of acclimatization in a 500 L plastic tank with continuous aeration, fry weighing approximately 1.065±0.013 g were selected for the experiment. The experiment utilized 20 disinfected rectangular plastic aquaria (size 2x1x1 feet) filled with 30 liters of chlorine-free filtered freshwater. Each tank was stocked with 10 fishes with continuous aeration. Fry were fed twice daily at 10% of their body weight, and tanks were cleaned daily to remove excess feed pellets and faecal matter. Sampling occurred fortnightly to assess juvenile fish body weight and adjust feed quantities. Physiochemical parameters (temperature, pH, alkalinity and dissolved oxygen) were monitored weekly throughout the experimental period.

Evolution Methods

The study involved assessing the bodyweight of fishes at 15-day intervals using an electronic weighing balance. Various growth and efficiency parameters, such as mean weight gain (WG), weight gain percentage (%), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), and Survival Rate (%), were calculated using specific formulas. Water quality measurements were recorded using a multi-parameter test kit for pH and temperature, total alkalinity was measured using HiMedia alkalinity testing kit, while dissolved oxygen was determined using Winkler's method.

The results of partial replacement of fish meal with *Chlorella* spp. and *Haematococcus* spp. on the growth performance and survival parameters were statistically analysed using completely randomized design (CRD) and one-way analysis (ANOVA) using IBM® SPSS® version 26.00 software and significant differences ($p < 0.05$) among treatment means were compared using duncan multiple range test (DMRT) (Snedecor and Cochran, 2014) ^[34].

Results

Physico-chemical Parameters of Water

The water quality parameters recorded during the experimental period shown in (Table 3). remained within the suitable range for the growth and survival of the

experimental fish. The water temperature varied slightly across treatments, with values ranging from 28.85 ± 0.19 to 29.15 ± 0.09 °C. Dissolved oxygen levels were relatively stable, ranging between 5.97 ± 0.13 and 6.14 ± 0.08 ppm, indicating adequate oxygen availability for fish metabolism. The pH values remained within the optimal range, varying from 7.94 ± 0.05 to 8.04 ± 0.09 , ensuring a suitable aquatic environment. Total alkalinity, an important parameter influencing buffering capacity, ranged from 157.50 ± 4.43 to 164.38 ± 5.86 ppm, reflecting stable water conditions throughout the study. These findings suggest that the water quality parameters were well-maintained and did not impose any adverse effects on fish growth and health during the experimental period.

Growth Performance

The study assessed the impact of partially replacing fish meal with *Chlorella* sp. and *Haematococcus* sp. in the diet of *Labeo rohita* fry over a 60-day period. Growth parameters, including weight gain, specific growth rate (SGR) and survival rate shown in (Table 4) were statistically analyzed to determine the effects of microalgal supplementation. The initial weight of *L. rohita* fry across treatments (T_0 , T_1 , T_2 , T_3 , and T_4) remained similar, ranging from 1.065 ± 0.006 gm to 1.073 ± 0.005 gm, with no significant difference ($p > 0.05$), indicating uniform stocking conditions. However, at the end of the experimental period (60 days), significant differences ($p < 0.05$) were observed in final weight and total weight gain among treatments. The highest final body weight was recorded in T_2 (3.11 ± 0.063 gm), which was significantly higher ($p < 0.05$) than the other treatments, followed by T_3 (2.80 ± 0.022 gm), T_1 (2.63 ± 0.107 gm), and T_4 (2.66 ± 0.044 gm), while T_0 (2.36 ± 0.056 gm) had the lowest final body weight. While treatments (T_1 , T_3 , and T_4) showed statistically similar growth but were still higher than control treatment. Similarly, the mean weight gain varied among treatments, with T_2 showing the highest value of 2.05 ± 0.068 gm, which was significantly different ($p < 0.05$) from the control (T_0) and other treatments. Treatments T_1 (1.55 ± 0.111 gm), T_3 (1.73 ± 0.023 gm), and T_4 (1.59 ± 0.039 gm) exhibited intermediate growth, whereas T_0 recorded the lowest mean weight gain (1.30 ± 0.053 gm). The weight gain percentage followed a similar trend, with the highest percentage observed in T_2 ($292.37 \pm 7.41\%$), significantly differing ($p < 0.05$) from the other treatments. T_3 ($261.70 \pm 2.43\%$), T_1 ($244.88 \pm 10.89\%$), and T_4 ($248.56 \pm 2.89\%$) showed moderate weight gain percentages, whereas the lowest was in T_0 ($221.57 \pm 4.67\%$). The specific growth rate (SGR) was also influenced by dietary treatments, with the highest SGR recorded in T_2 ($1.79 \pm 0.042\%$), which was significantly higher ($p < 0.05$) than the other groups. Treatments T_1 ($1.49 \pm 0.075\%$), T_3 ($1.60 \pm 0.016\%$), and T_4 ($1.52 \pm 0.019\%$) exhibited intermediate values, while the lowest SGR was observed in T_0 ($1.32 \pm 0.035\%$). These findings indicate that partial replacement of fish meal with *Chlorella* spp. and *Haematococcus* spp. had a positive impact on the growth performance of *L. rohita* fry, with the best results observed in T_2 , suggesting an optimal level of replacement in this treatment.

Feed efficiency

The results of feed conversion ratio (FCR) of *Labeo rohita* fry at the end of the 60-day experimental period are

presented in Table 4. The lowest FCR was recorded in Treatment T_2 (1.78 ± 0.048), indicating better feed efficiency, followed by T_3 (2.04 ± 0.049), T_4 (2.16 ± 0.034) and T_1 (2.22 ± 0.052), and the highest in T_0 (2.57 ± 0.111). The statistical analysis showed significant differences ($p < 0.05$) between all treatments. The protein efficiency ratio (PER) values for different treatments are presented in Table 4. The highest PER was recorded in Treatment T_2 (1.88 ± 0.050), followed by T_4 (1.55 ± 0.025), T_3 (1.63 ± 0.039), T_1 (1.52 ± 0.092), and the lowest in T_0 (1.30 ± 0.052). Statistical analysis indicated significant differences ($p < 0.05$) among all the treatments.

Survival rate

The survival rate of *Labeo rohita* fry across different treatments is presented in (Table 4). The survival rate was recorded at 100% in all experimental groups (T_0 , T_1 , T_2 , T_3 , and T_4) throughout the culture period. The data indicate that there was no mortality in any of the treatment groups, with survival rates remaining consistently at 100%. This finding suggests that all dietary treatments supported optimal health conditions for the fry. Statistical analysis showed there was no significant differences ($p > 0.05$) found between all the treatments.

Proximate composition of fish carcass

At the end of the feeding period, five randomly selected fish samples from each tank underwent proximate analysis, results are shown in (Table 5). The protein content was significantly highest in T_2 ($14.71 \pm 0.041\%$) and lowest in T_0 ($11.85 \pm 0.040\%$). The fat content was significantly highest in T_3 ($3.65 \pm 0.026\%$) and lowest in T_2 ($3.11 \pm 0.018\%$). The ash content was significantly highest in T_4 ($4.88 \pm 0.021\%$) and lowest in T_0 ($2.50 \pm 0.029\%$). The moisture content was highest in T_0 ($82.20 \pm 0.087\%$) and lowest in T_4 ($77.80 \pm 0.058\%$).

Discussion

The present study evaluated the growth performance of *Labeo rohita* fry fed with different dietary inclusions of *Chlorella* spp. and *Haematococcus* spp. over a 60-day experimental period. The results indicated that the final weight gain, percentage weight gain, and specific growth rate (SGR) were significantly higher ($p < 0.05$) in the treatment group T_2 (10% *Chlorella* spp. and *Haematococcus* spp.) compared to all other treatments. These findings are consistent with previous studies by Khani *et al.*, (2017) [15], who reported that *Cyprinus carpio* fed with a 5% *Chlorella* diet exhibited optimal weight gain. Similarly, Raji *et al.*, (2019) [27] observed enhanced growth parameters in *Clarias gariepinus* fingerlings when fish meal was partially replaced with *Chlorella vulgaris* powder. Abdulrahman *et al.*, (2019) [1] also demonstrated that *C. carpio* exhibited increased growth rate, improved FCR and PER when fed with *Chlorella pyrenoidosa*-incorporated diets. Furthermore, Galal *et al.*, (2018) [10] reported improved growth in *Oreochromis niloticus* fed with *C. vulgaris*, while Enyidi (2017) [9] found that *C. gariepinus* exhibited significantly better weight gain and SGR with *C. vulgaris* inclusion. Additionally, Ju *et al.*, (2012) [14] reported that dietary incorporation of *Haematococcus pluvialis* enhanced growth in large yellow croaker (*Pseudosciaena crocea*), and Li *et al.*, (2014) [18] observed similar trends in *Litopenaeus vannamei* when fed defatted *H. pluvialis* meal. The diet

containing *Chlorella* meal can significantly enhance the digestive enzymes activity of *Carassius auratus gibelio*, which in results showed better feed efficacy and growth rate (Xu *et al.*, 2014) [36]. Also according to Lordan *et al.*, (2011) essential amino acids, low molecular weight and rich protein of *Chlorella* makes *Chlorella* easily digestible. Pham *et al.*, (2014) observed better feed and protein efficiency in juvenile olive flounder (*Paralichthys olivaceus*) with *Hematococcus pluvialis* inclusion in their diet. The carotenoids content of *Hematococcus* shows beneficial role in metabolic process of aquatic animals (Segner *et al.*, 1989) due to which the utilization of nutrient increases which helps easy digestion and in results shows better growth (Amar *et al.*, 2001). The declining trend in growth at higher inclusion levels (T₃ and T₄) suggests that excessive microalgal incorporation may reduce digestibility, alter feed palatability, or introduce anti-nutritional factors. Previous studies indicate that high levels of microalgae can increase fiber content and reduce protein digestibility, leading to suboptimal growth. These results indicate that microalgal supplementation can positively influence growth performance in *L. rohita*. The observed variations among different studies could be attributed to species-specific responses, diet formulations, and experimental conditions. Due to such quality of this microalgae they have positively enhanced the growth performance and feed efficacy in *L. rohita* in this present study.

Throughout the experimental period, a 100% survival rate was recorded across all treatment groups, with no observed mortality. This suggests that the environmental conditions were optimal for *L. rohita* and that the formulated diets did not induce any nutritional deficiencies or incompatibilities. These findings align with Raji *et al.*, (2019) [27], who reported 100% survival in *C. gariepinus* fed with diets containing *Spirulina platensis* and *C. vulgaris*. Similarly, Pakravan *et al.*, (2018) [24] found that *L. vannamei* exhibited 100% survival when fed with *C. vulgaris*-supplemented diets. Jiang *et al.*, (2019) [13] also reported 100% survival in

juvenile yellow perch (*Perca flavescens*) when fed defatted *H. pluvialis* meal. However, it cannot be conclusively determined that dietary inclusion levels were solely responsible for the observed survival rates. The high survival rates suggest that the experimental diets provided adequate nutrition and did not induce any toxic effects.

The proximate analysis results of the experiment indicate that the partial replacement of fish meal with *Chlorella* spp. and *Haematococcus* spp. revealed significant differences ($p < 0.05$) among treatments. The significantly higher protein content in T₂ suggests that the inclusion of *Chlorella* spp. and *Haematococcus* spp. in the diet improved protein deposition, likely due to its high-quality protein content and bioavailable nutrients. This finding is consistent with Abdulrahman *et al.*, (2019) [1], who reported increased protein and ash content in *Cyprinus carpio* fed with *Chlorella pyrenoidosa*. Conversely, Pakravan *et al.*, (2018) [24] observed a reduction in protein content when *Chlorella vulgaris* meal was incorporated into the fish diet, possibly due to differences in species-specific protein utilization or diet formulation. The highest fat content in T₃ suggests that *Haematococcus* spp. contributed to lipid accumulation, likely due to its rich carotenoid and astaxanthin content, which may have influenced lipid metabolism. Similar findings were reported by Li *et al.*, (2014) [18] in large yellow croaker (*Pseudosciaena crocea*), where diets containing *H. pluvialis* led to increased lipid deposition. The significantly higher ash content in T₄ indicates increased mineral deposition, which may be attributed to the bioavailable minerals present in microalgae. The lowest moisture content in T₄ suggests a higher deposition of dry matter in the fish body, correlating with higher protein and ash content. However, Jiang *et al.*, (2019) [13] reported no significant changes in whole-body moisture, crude protein, or lipid content in juvenile yellow perch (*Perca flavescens*) when fed with defatted microalgae meal, highlighting potential variations due to species, diet formulation, and inclusion levels of microalgae.

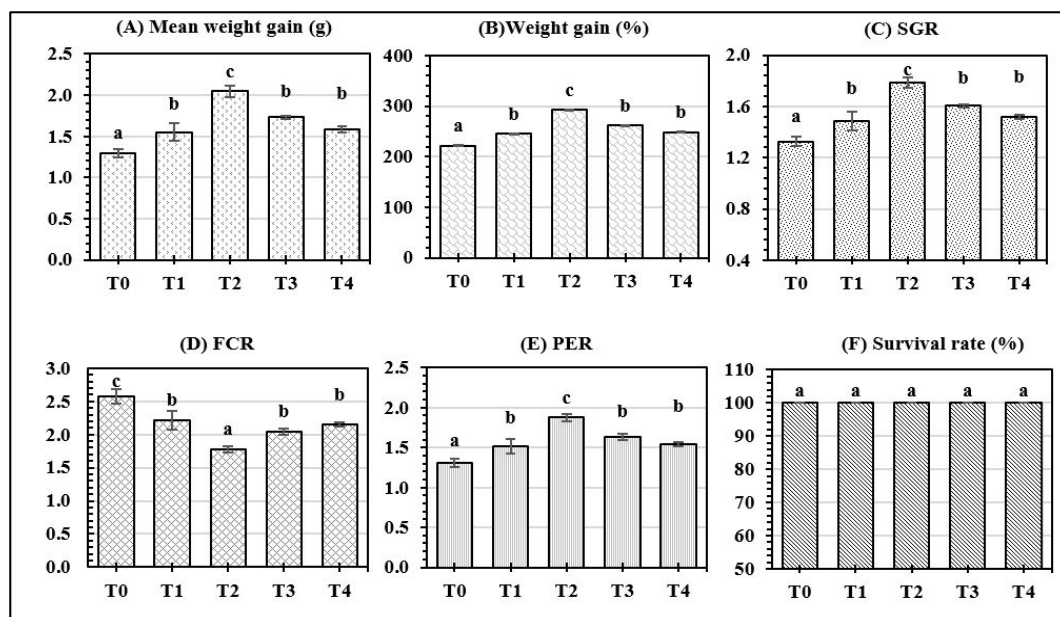


Fig 1 A-F: (A) Mean weight gain (gm), (B) Weight gain (%), (C) Specific growth rate (SGR), (D) Feed conversion ratio (FCR) (E) Protein efficiency ratio (PER) and (F) Survival rate (%) of *Labeo rohita* recorded in different treatments during the culture period.

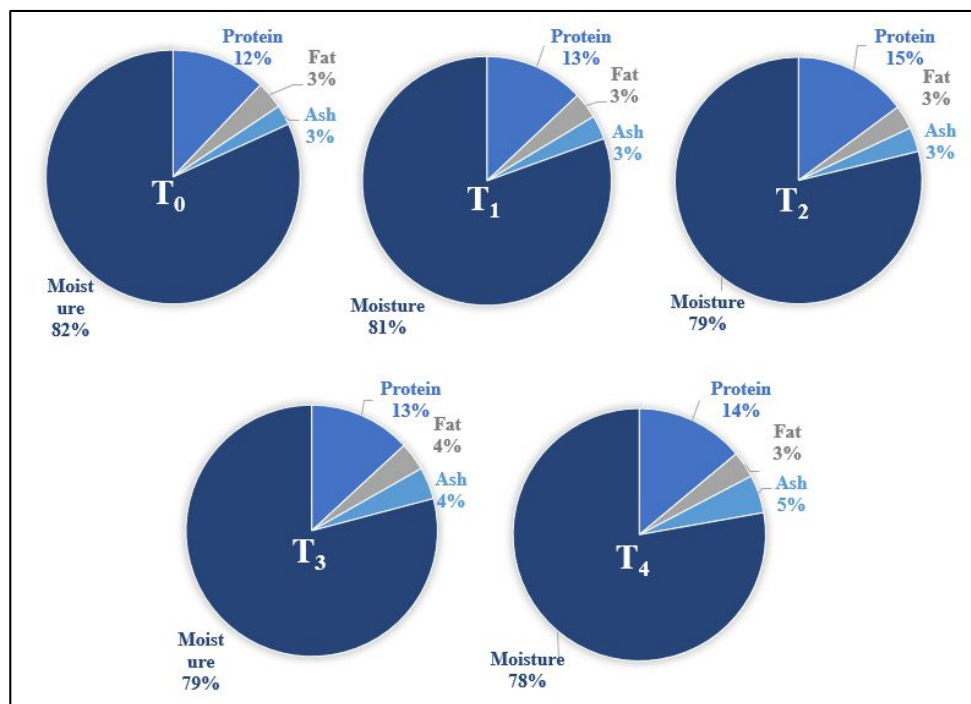


Fig 2: Proximate composition of fish body carcass of *Labeo rohita* recorded in different treatments at the end of experiment.

Table 1: Proximate composition of feed ingredients (%).

Ingredients	Crude Protein (%)	Crude Fat (%)	Ash (%)	Moisture (%)
Fishmeal	51±0.058	5.14±0.012	22.2±0.058	6.5±0.058
Groundnut oil cake	38.15±0.029	8.11±0.012	8.42±0.015	5.3±0.058
Wheat flour	8.4±0.058	2.14±0.006	1.5±0.012	11.32±0.043
Tapioca flour	0.22±0.006	0.8±0.006	0.05±0.006	9.45±0.029
<i>Chlorella</i> powder	48.5±0.058	12.18±0.012	9.5±0.029	5.85±0.029
<i>Haematococcus</i> powder	21.25±0.029	8.35±0.012	6.65±0.017	8.2±0.058

Note: Data expressed as Mean ± SE (n=3)

Table 2: Feed formulation and its proximate analysis.

Ingredient (%)	Treatments				
	T ₀	T ₁	T ₂	T ₃	T ₄
Fishmeal	42	40	38	36	34
Groundnut oil cake	37	35	33	32	30
<i>Haematococcus</i> powder	0	2.5	5	7.5	10
<i>Chlorella</i> powder	0	2.5	5	7.5	10
Wheat flour	7.5	7	6.5	5.5	5
Tapioca flour	7.5	7	6.5	5.5	5
Fish oil	4	4	4	4	4
Vitamins & Minerals	2	2	2	2	2
Total	100	100	100	100	100
Proximate analysis of experimental diets (%)					
Nutrients	T ₀	T ₁	T ₂	T ₃	T ₄
Crude Protein	29.25±0.055	29.9±0.118	30.06±0.193	30.1±0.081	30.05±0.095
Crude Fat	6.12±0.092	7.1±0.081	7.2±0.053	7.45±0.128	7.35±0.057
Moisture	8.12±0.048	9.15±0.032	8.35±0.050	8.45±0.114	8.25±0.054
Ash	10.15±0.077	12.15±0.119	13.15±0.066	15.65±0.082	12.45±0.155

Note: Data for proximate analysis is expressed as Mean ± SE (n=3)

Table 3: Water quality parameters during experimental period.

Experimental Fish	Water Temperature (°C)	Dissolved oxygen (ppm)	pH	Total alkalinity (ppm)
T ₀	28.88±0.23	5.97±0.13	8.00±0.03	161.25±3.50
T ₁	29.15±0.09	6.03±0.06	7.94±0.05	164.38±5.86
T ₂	29.10±0.18	6.09±0.07	8.04±0.09	162.50±4.01
T ₃	28.89±0.18	6.14±0.08	7.98±0.09	158.75±4.51
T ₄	28.85±0.19	6.01±0.10	8.01±0.05	157.50±4.43

Note: Data expressed as Mean ± SE (n=8)

Table 4: Performance for *Labeo rohita* fry fed with different experimental diets during culture period.

Diets	T ₀	T ₁	T ₂	T ₃	T ₄
Initial body weight (g)	1.065±0.006 ^a	1.073±0.005 ^a	1.065±0.006 ^a	1.07±0.004 ^a	1.07±0.006 ^a
Final body weight (g)	2.36±0.056 ^a	2.63±0.107 ^b	3.11±0.063 ^c	2.80±0.022 ^b	2.66±0.044 ^b
Mean weight gain (g)	1.30±0.053 ^a	1.55±0.111 ^b	2.05±0.068 ^c	1.73±0.023 ^b	1.59±0.039 ^b
Weight gain (%)	221.57±4.67 ^a	244.88±10.89 ^b	292.37±7.41 ^c	261.70±2.43 ^b	248.56±2.89 ^b
Specific growth rate (%)	1.32±0.035 ^a	1.49±0.075 ^b	1.79±0.042 ^c	1.60±0.016 ^b	1.52±0.019 ^b
Feed conversion ratio	2.57±0.111 ^c	2.22±0.139 ^b	1.78±0.048 ^a	2.04±0.049 ^a	2.16±0.034 ^b
Protein efficiency ratio	1.30±0.052 ^a	1.52±0.092 ^b	1.88±0.050 ^c	1.63±0.039 ^b	1.55±0.025 ^b
Survival rate (%)	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a

Note: Data expressed as Mean ± SE (n=4); Mean values with different superscripts within a column are significantly different ($p < 0.05$).

Table 5: Proximate composition of fish body carcass.

Experimental Fish	Protein%	Fat%	Ash%	Moisture%
T ₀	11.85±0.040	3.45±0.037	2.50±0.029	80.20±0.087
T ₁	12.95±0.050	3.41±0.018	3.12±0.012	80.52±0.044
T ₂	14.71±0.041	3.11±0.018	3.18±0.012	78.00±0.126
T ₃	13.11±0.038	3.65±0.026	4.11±0.023	79.08±0.076
T ₄	13.92±0.015	3.40±0.050	4.88±0.021	77.80±0.058

Note: Data expressed as Mean ± SE (n=3)

Common abbreviations

1. FM (fish meal)
2. GNOC (groundnut oil cake)
3. WF (wheat flour)
4. TP (tapioca powder)
5. FO (fish oil)
6. V&M (vitamin and mineral)
7. CP (Chlorella powder)
8. HP (*Haematococcus* powder)
9. MWG (mean weight gain)
10. WGP (weight gain percentage)
11. SGR (specific growth rate)
12. FCR (food conversion ratio)
13. PER (protein efficiency ratio)
14. CRD (completely randomized design)

Conclusion

The present study demonstrated that the partial replacement of fish meal with *Chlorella* spp. and *Haematococcus* spp. in the diet of *Labeo rohita* fry positively influenced growth performance, feed efficiency, and carcass composition. Among the different dietary treatments, T₂ (10% inclusion) showed the highest final weight, weight gain, specific growth rate (SGR), and protein efficiency ratio (PER), along with the best feed conversion ratio (FCR), suggesting that this level provides the most beneficial balance of nutrients and digestibility. The 100% survival rate across all treatments indicates that microalgal supplementation did not induce any adverse effects on fish health, confirming its suitability for aquafeed formulations. Proximate composition analysis revealed higher protein content in T₂, while T₃ exhibited increased lipid deposition, highlighting the potential of *Haematococcus* spp. as a source of beneficial carotenoids. However, the declining growth performance at higher inclusion levels (T₃ and T₄) suggests that excessive microalgal incorporation may reduce feed palatability or nutrient digestibility. Overall, these findings indicate that *Chlorella* spp. and *Haematococcus* spp. can serve as effective alternative protein sources in aquafeeds, reducing dependency on fish meal while maintaining optimal growth and health in *L. rohita* fry. Future research should focus on optimizing inclusion levels for different life

stages and evaluating the long-term effects of microalgal supplementation on fish physiology and immunity.

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