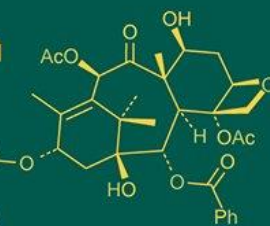
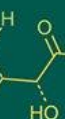
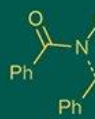


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Functional role of *Chlorella* sp. and *Porphyra* sp. as natural feed additives in ornamental goldfish culture

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Abstract

The present study evaluated the effects of dietary supplementation of *Chlorella* sp. and *Porphyra* sp. on growth performance, pigmentation and hematological response in goldfish (*Carassius auratus*). Seven experimental diets were formulated, including a control and graded levels of *Chlorella* (5%, 7.5%, and 10%) and *Porphyra* (5%, 10%, and 15%). Fish were reared for 75 days under controlled laboratory conditions. Results revealed significant improvements ($p < 0.05$) in final body weight, weight gain, specific growth rate, feed utilization, carotenoid deposition, hematological parameters, antioxidant enzyme activity, and resistance against *Aeromonas hydrophila* in algae-supplemented groups compared to the control. Among all treatments, 10% *Chlorella* supplementation (CT₃) exhibited the best overall performance, while 10% *Porphyra* (PT₂) showed optimal results within the macroalgal treatments. The findings suggest that algal supplements, particularly *Chlorella* sp., can serve as effective functional feed additives for sustainable ornamental fish culture.

Keywords: *Carassius auratus*, *Chlorella*, *Porphyra*, functional feed additive, pigmentation, hematology

1. Introduction

The ornamental fish industry represents one of the fastest-growing sectors of global aquaculture, with an estimated annual trade value of approximately USD 15 billion and more than two billion live fish traded worldwide. Asian countries contribute over 60% of global ornamental fish exports, while the major markets are concentrated in the United States, Europe, China, and Japan. In India, ornamental fish farming has emerged as an important enterprise supported by governmental initiatives such as the Pradhan Mantri Matsya Sampada Yojana (PMMSY). However, despite its rich aquatic biodiversity and favorable climatic conditions, India's contribution to the international ornamental fish trade remains comparatively low, highlighting the need for nutritional strategies that improve fish quality and production efficiency.

Among ornamental fish species, *Carassius auratus* (goldfish) is one of the most commercially important owing to its remarkable diversity of body forms and vibrant coloration. Consumer preference and market value are largely determined by body pigmentation, in addition to growth performance and overall health status. Since fish are unable to synthesize carotenoids *de novo*, these pigments must be supplied through the diet. Although synthetic pigments are commonly used to enhance coloration, their high cost and environmental concerns have increased interest in sustainable natural alternatives.

Marine and freshwater algae have attracted considerable attention as functional ingredients in aquafeeds because they are rich sources of highly digestible proteins, essential amino acids, vitamins, minerals, carotenoids, polyunsaturated fatty acids, antioxidants, and other bioactive compounds. These constituents not only improve nutrient utilization but also enhance antioxidant defense, immune competence, stress tolerance, and intestinal health, making dietary algal supplementation a promising strategy for sustainable aquaculture.

Among microalgae, *Chlorella* sp. is recognized as an excellent nutritional supplement because of its high protein content and abundance of chlorophyll, carotenoids, essential fatty acids, vitamins, and biologically active growth-promoting compounds (CGF). Previous studies have demonstrated that dietary *Chlorella* supplementation improves growth performance, feed utilization, pigmentation, antioxidant capacity, immune responses, and

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disease resistance in several cultured fish species. Similarly, *Porphyra* sp., a commercially important red macroalga, contains high-quality proteins, β -carotene, phycobiliproteins, taurine, polyunsaturated fatty acids, vitamins, minerals, and sulfated polysaccharides such as porphyran. These bioactive constituents exhibit antioxidant, immunomodulatory, antimicrobial, and pigmentation-enhancing properties and have shown positive effects on fish growth and physiological health.

Although numerous studies have independently evaluated the effects of microalgae and macroalgae in aquaculture diets, direct comparative information on the functional efficacy of *Chlorella* sp. and *Porphyra* sp. in ornamental fish remains limited. Therefore, the present study was undertaken to comparatively evaluate the effects of dietary supplementation with *Chlorella* sp. and *Porphyra* sp. on growth performance, feed utilization, pigmentation, hematological responses in goldfish (*Carassius auratus*), and to identify their optimum dietary inclusion levels for the development of sustainable functional feeds for ornamental aquaculture.

2 Materials and Methods

2.1 Experimental location

The feeding trial was conducted during 2024-2025 at the Wet Laboratory of the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India. All experimental procedures were performed under controlled laboratory conditions for a period of 75 days.

Treatment Group I	T ₀	CT ₁	CT ₂	CT ₃
	Control	<i>Chlorella</i> powder 5%	<i>Chlorella</i> powder 7.5%	<i>Chlorella</i> powder 10%
Treatment Group II	T ₀	PT ₁	PT ₂	PT ₃
	Control	<i>Porphyra</i> powder 5%	<i>Porphyra</i> powder 10%	<i>Porphyra</i> powder 15%

2.5 Diet preparation

Feed ingredients, including fish meal, groundnut oil cake, rice bran, wheat flour, tapioca flour, vegetable oil, fish oil, and a vitamin-mineral premix, were obtained from local commercial suppliers. Commercially available powders of *Chlorella* sp. and *Porphyra* sp. were used as dietary supplements. The proximate composition of individual feed ingredients was determined according to the standard methods of AOAC (2005) [2]. Moisture content was measured by oven drying, crude protein by the Kjeldahl method, crude lipid by Soxhlet extraction, and ash by incineration in a muffle furnace. Experimental diets were formulated using Pearson's square method to achieve a uniform crude protein level of 30%.

2.6 Feeding management

Fish were hand-fed twice daily throughout the experimental period. The daily ration was provided at 5% of the total biomass and was adjusted every 15 days following bulk weighing of fish to account for growth. Fish were carefully observed throughout the trial for feeding behavior, health status, and mortality.

2.7 Growth performance

Fish from each aquarium were weighed individually at 15-day intervals after 24 h of feed deprivation to minimize

2.2 Experimental fish and acclimatization

Healthy goldfish (*Carassius auratus* Linnaeus, 1758) fingerlings were procured from a local ornamental fish hatchery and transported to the laboratory under continuous aeration. Upon arrival, the fish were acclimatized for 30 days in 500-L fiberglass-reinforced plastic (FRP) tanks supplied with continuous aeration. During the acclimation period, fish were fed a commercial basal diet twice daily and monitored regularly to ensure good health. After acclimatization, healthy fish with a uniform initial body weight of 1.85 ± 0.003 g were randomly selected for the feeding experiment.

2.3 Experimental system

The experiment was conducted using twenty-eight rectangular plastic aquaria (60 × 30 × 30 cm; approximately 35 L capacity). Before stocking, all aquaria were thoroughly cleaned, disinfected, and filled with filtered freshwater. Continuous aeration was supplied throughout the experimental period to maintain adequate dissolved oxygen levels. To maintain water quality, uneaten feed and fecal matter were removed daily by siphoning, followed by the replacement of approximately 10% of the tank water with fresh disinfected water.

2.4 Experimental design

The experiment followed a completely randomized design (CRD) consisting of seven dietary treatments, each with four replications. The treatments were divided into two groups based on dietary inclusion:

variation due to gut contents. The following growth and feed utilization indices were calculated:

2.7.1 Weight gain (WG, g)

WG = Final body weight – Initial body weight

2.7.2 Weight gain percentage (WG %)

WG (%) = [(Final body weight – Initial body weight)/Initial body weight] × 100

2.7.3 Specific growth rate (SGR, % day⁻¹)

SGR = [(ln Final body weight – ln Initial body weight)/Experimental days] × 100

2.7.4 Feed conversion ratio (FCR)

FCR = Feed intake (g)/Weight gain (g)

2.7.5 Feed efficiency ratio (FER)

FER = Weight gain (g)/Feed intake (g)

2.7.6 Protein efficiency ratio (PER)

PER = Weight gain (g)/Protein intake (g)

2.7.7 Survival (%)

Survival (%) = (Final number of fish/Initial number of fish) × 100

2.8 Pigmentation analysis

Whole-body carotenoid concentration was determined following the method described by Olson (1989). Approximately 1 g of fish muscle tissue, excluding the head and viscera, was homogenized with anhydrous sodium sulfate and extracted using chloroform. The homogenate was incubated overnight at 0 °C to facilitate pigment extraction. After extraction, ethanol was added, and the absorbance of the extract was measured spectrophotometrically between 380 and 500 nm.

Total carotenoid concentration was calculated as:

$$\text{Total carotenoids } (\mu\text{g g}^{-1}) = (\text{Absorbance} \times \text{Dilution factor}) / (0.25 \times \text{Sample weight})$$

2.9 Hematological analysis

At the end of the feeding trial, fish were anesthetized using clove oil (150 mg L⁻¹), and blood samples were collected by cardiac puncture using sterile EDTA-coated syringes. Blood samples were immediately analyzed using an automated hematology analyzer to determine red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), and hematocrit (HCT). These parameters were used to evaluate the physiological and health status of experimental fish.

2.10 Statistical analysis

All experimental data were analyzed using the Statistical Package for the Social Sciences (SPSS). Before statistical analysis, the data were examined for normality and homogeneity of variance. Differences among treatment means were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) for multiple comparisons. Statistical significance was accepted at $p < 0.05$. Results are expressed as mean $\pm$ standard error (SE).

3 Results

3.1 Proximate composition of experimental diets

The proximate composition of the experimental diets is presented in Table 1. All diets were formulated to be isonitrogenous, containing approximately 30% crude protein on a dry weight basis. Moisture content ranged from 7.27 to 7.89%, while dry matter varied between 91.09 and 91.32%. Crude protein remained consistent among all dietary treatments (30.02-30.10%), indicating successful formulation of nutritionally comparable diets. Similarly, crude fat (7.78-8.09%) and ash (8.43-8.88%) exhibited only minor variations among treatments, confirming that the inclusion of *Chlorella* sp. and *Porphyra* sp. did not substantially alter the overall nutritional composition of the experimental diets.

3.2 Growth performance and feed utilization

The effects of dietary *Chlorella* and *Porphyra* supplementation on growth performance and feed utilization of *Carassius auratus* after 75 days are summarized in Table 2. No significant differences ($p > 0.05$) were observed in the initial body weight among treatments, confirming uniformity of fish at the commencement of the experiment. However, dietary supplementation significantly influenced ($p < 0.05$) all growth and feed utilization parameters at the end of the feeding trial.

Among all treatments, fish fed the 10% *Chlorella* diet (CT₃) exhibited the highest final body weight (5.87 ± 0.003 g), weight gain (4.13 ± 0.005 g), weight gain percentage ($223.00 \pm 0.772\%$), and specific growth rate ($1.56 \pm 0.003\%$ day⁻¹), followed by CT₂ and CT₁. In contrast, the control group recorded the lowest values for these parameters.

Similarly, the 10% *Porphyra* diet (PT₂) produced significantly higher ($p < 0.05$) final body weight (5.45 ± 0.008 g), weight gain (3.60 ± 0.008 g), weight gain percentage ($194.31 \pm 0.580\%$), and specific growth rate ($1.43 \pm 0.002\%$ day⁻¹) compared with PT₁, PT₃, and the control. However, the highest inclusion level of *Porphyra* (PT₃) did not improve growth performance and showed values comparable to the control. Feed utilization was also significantly affected by dietary treatments. Fish receiving CT₃ exhibited the lowest feed conversion ratio (1.36 ± 0.002) together with the highest feed efficiency ratio (0.73 ± 0.001) and protein efficiency ratio (2.08 ± 0.003). Within the *Porphyra* treatments, PT₂ recorded the lowest feed conversion ratio (1.71 ± 0.007) and the highest feed efficiency ratio (0.58 ± 0.003) and protein efficiency ratio (1.67 ± 0.007). The control and PT₃ groups showed comparatively lower feed utilization efficiency.

Overall, dietary supplementation with *Chlorella* produced greater improvements in growth and feed utilization than *Porphyra*, with 10% *Chlorella* representing the most effective dietary inclusion level.

3.3 Carotenoid deposition

Dietary supplementation with both algal species significantly affected ($p < 0.05$) carotenoid deposition in *C. auratus* (Table 3; Figure 1). The control group exhibited the lowest carotenoid concentration (3.25 ± 0.006 $\mu\text{g g}^{-1}$). Carotenoid accumulation increased progressively with increasing dietary *Chlorella* inclusion, reaching the highest value in CT₃ (5.94 ± 0.020 $\mu\text{g g}^{-1}$), followed by CT₂ (5.75 ± 0.017 $\mu\text{g g}^{-1}$) and CT₁ (4.86 ± 0.011 $\mu\text{g g}^{-1}$).

Among the *Porphyra*-supplemented groups, PT₂ recorded the highest carotenoid concentration (5.66 ± 0.011 $\mu\text{g g}^{-1}$), followed by PT₁ (5.26 ± 0.012 $\mu\text{g g}^{-1}$). In contrast, PT₃ showed carotenoid levels (3.23 ± 0.009 $\mu\text{g g}^{-1}$) comparable to those of the control group. Temporal changes in carotenoid accumulation throughout the feeding period are illustrated in Figure 1, demonstrating a gradual increase in pigmentation with increasing dietary supplementation, particularly in the CT₃ and PT₂ treatments.

3.4 Hematological responses

The effects of dietary algal supplementation on hematological parameters are presented in Table 3 and Figure 2. Significant differences ($p < 0.05$) were observed among treatments for red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), and hematocrit (Hct). Within the *Chlorella* treatments, RBC, Hb, and Hct increased significantly as dietary inclusion increased. Fish fed CT₃ exhibited the highest RBC ($1.31 \pm 0.037 \times 10^6$ μL^{-1}), Hb (8.51 ± 0.012 g dL⁻¹), and Hct ($25.52 \pm 0.027\%$), whereas the control group showed the lowest values. Conversely, WBC count declined progressively from $3.32 \pm 0.014 \times 10^3$ μL^{-1} in the control to $2.64 \pm 0.013 \times 10^3$ μL^{-1} in CT₃. Among the *Porphyra* treatments, PT₂ exhibited the highest RBC ($1.56 \pm 0.018 \times 10^6$ μL^{-1}), Hb (8.64 ± 0.013 g dL⁻¹), and Hct ($25.48 \pm 0.025\%$). The lowest WBC count ($2.15 \pm 0.585 \times 10^3$ μL^{-1}) was also observed in PT₂, whereas

PT₃ recorded the highest WBC value ($3.46 \pm 0.014 \times 10^3 \mu\text{L}^{-1}$) among all treatments.

The temporal variation in hematological parameters during the experimental period is presented in Figure 2, which

shows a progressive improvement in erythrocytic parameters with increasing dietary supplementation of *Chlorella* and moderate supplementation of *Porphyra*.

Table 1: Proximate composition (% dry weight basis) of control and *Chlorella* and *Porphyra*-supplemented diets across different treatments

Treatment	T ₀	CT ₁	CT ₂	CT ₃	PT ₁	PT ₂	PT ₃
Moisture	7.27	7.51	7.64	7.78	7.89	7.75	7.86
Dry matter	91.21	91.09	91.13	91.24	91.27	91.32	91.24
Protein	30.06	30.04	30.05	30.10	30.02	30.08	30.03
Fat	7.83	8.09	7.85	8.04	7.96	7.89	7.78
Ash	8.82	8.81	8.43	8.86	8.71	8.88	8.79

Note: T₀-control treatment, CT₁, CT₂ and CT₃-5%, 7.5% and 10% *Chlorella* supplemented diets respectively, PT₁, PT₂ and PT₃-5%, 10% and 15% *Porphyra* supplemented diets respectively.

Table 2: The comparative evaluation of growth performance and feed utilization parameters at the end of the experimental period

Parameter	T ₀ (Control)	CT ₁ (5% <i>Chlorella</i>)	CT ₂ (7.5% <i>Chlorella</i>)	CT ₃ (10% <i>Chlorella</i>)	PT ₁ (5% <i>Porphyra</i>)	PT ₂ (10% <i>Porphyra</i>)	PT ₃ (15% <i>Porphyra</i>)
IW(g)	1.85 ± 0.005 ^a	1.85 ± 0.005 ^a	1.85 ± 0.005 ^a	1.85 ± 0.005 ^a	1.85 ± 0.003 ^a	1.85 ± 0.002 ^a	1.85 ± 0.004 ^a
FW (g)	5.28 ± 0.005 ^c	5.55 ± 0.011 ^c	5.68 ± 0.027 ^b	5.87 ± 0.003 ^a	5.36 ± 0.005 ^b	5.45 ± 0.008 ^a	5.27 ± 0.010 ^c
WG (g)	3.43 ± 0.010 ^c	3.69 ± 0.009 ^c	3.83 ± 0.026 ^b	4.13 ± 0.005 ^a	3.50 ± 0.002 ^b	3.60 ± 0.008 ^a	3.42 ± 0.012 ^c
WG (%)	185.42 ± 1.073 ^c	198.83 ± 0.545 ^c	206.63 ± 1.263 ^b	223.00 ± 0.772 ^a	188.57 ± 0.338 ^b	194.31 ± 0.580 ^a	184.97 ± 0.999 ^c
SGR	1.39 ± 0.005 ^c	1.45 ± 0.002 ^c	1.49 ± 0.005 ^b	1.56 ± 0.003 ^a	1.41 ± 0.001 ^b	1.43 ± 0.002 ^a	1.39 ± 0.004 ^c
FCR	1.47 ± 0.004 ^a	1.43 ± 0.003 ^b	1.40 ± 0.010 ^c	1.36 ± 0.002 ^d	1.74 ± 0.004 ^a	1.71 ± 0.007 ^b	1.75 ± 0.006 ^a
FER	0.67 ± 0.001 ^d	0.69 ± 0.001 ^c	0.71 ± 0.005 ^b	0.73 ± 0.001 ^a	0.57 ± 0.002 ^b	0.58 ± 0.003 ^a	0.56 ± 0.002 ^b
PER	1.93 ± 0.004 ^d	1.99 ± 0.004 ^c	2.03 ± 0.014 ^b	2.08 ± 0.003 ^a	1.64 ± 0.005 ^b	1.67 ± 0.007 ^a	1.63 ± 0.006 ^b

Table 3: The comparative evaluation of carotenoid deposition, hematological indices, and antioxidant enzyme activities at the end of the feeding trial.

Treatment	Carotenoid	RBC	WBC	Hg	Hct
T ₀ (Control)	3.25 ± 0.006 ^c	1.09 ± 0.015 ^c	3.32 ± 0.014 ^a	8.00 ± 0.025 ^c	24.30 ± 0.026 ^c
CT ₁ (5% <i>Chlorella</i>)	4.86 ± 0.011 ^c	1.14 ± 0.024 ^b	3.23 ± 0.027 ^b	8.25 ± 0.025 ^c	24.70 ± 0.095 ^c
CT ₂ (7.5% <i>Chlorella</i>)	5.75 ± 0.017 ^b	1.27 ± 0.011 ^a	2.91 ± 0.027 ^c	8.37 ± 0.017 ^b	25.04 ± 0.122 ^b
CT ₃ (10% <i>Chlorella</i>)	5.94 ± 0.020 ^a	1.31 ± 0.037 ^a	2.64 ± 0.013 ^d	8.51 ± 0.012 ^a	25.52 ± 0.027 ^a
PT ₁ (5% <i>Porphyra</i>)	5.26 ± 0.012 ^b	1.35 ± 0.016 ^b	3.04 ± 0.014 ^{ab}	8.47 ± 0.009 ^b	24.78 ± 0.134 ^b
PT ₂ (10% <i>Porphyra</i>)	5.66 ± 0.011 ^a	1.56 ± 0.018 ^a	2.15 ± 0.585 ^b	8.64 ± 0.013 ^a	25.48 ± 0.025 ^a
PT ₃ (15% <i>Porphyra</i>)	3.23 ± 0.009 ^c	1.06 ± 0.013 ^c	3.46 ± 0.014 ^a	8.01 ± 0.023 ^c	24.30 ± 0.012 ^c

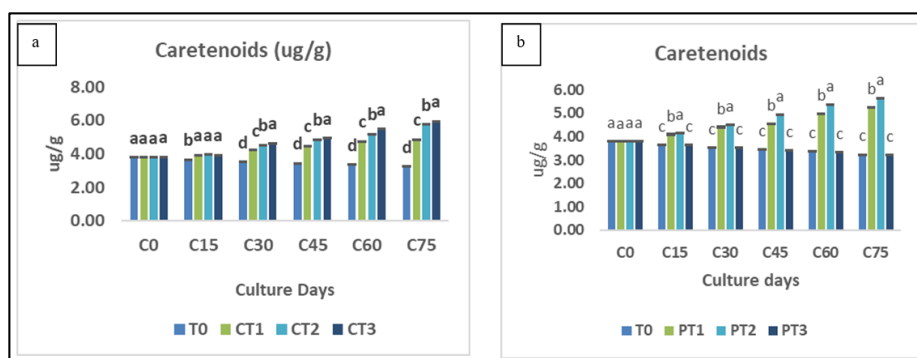


Fig 1: Effect of (a) chlorella (b) Porphyra supplementation on carotenoid (C) (ug/g) accumulation in *C. auratus* over the experimental period.

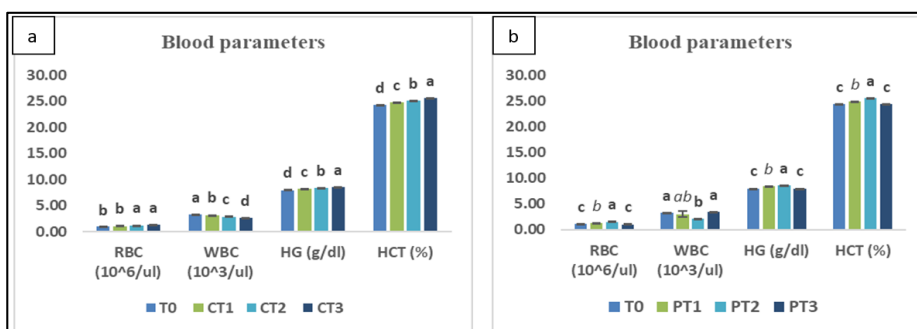


Fig 2: Effect of (a) chlorella (b) Porphyra supplementation on blood parameters of *C. auratus* over the experimental period.

4. Discussion

The present study demonstrated that dietary supplementation with *Chlorella* sp. and *Porphyra* sp. significantly enhanced growth performance, feed utilization, pigmentation, and hematological status of *Carassius auratus*. Among the tested dietary treatments, supplementation with 10% *Chlorella* consistently produced the greatest improvements in most evaluated parameters, whereas 10% *Porphyra* represented the optimum inclusion level among the macroalgal diets. These findings highlight the nutritional and functional potential of algal biomass as a sustainable feed additive for ornamental aquaculture.

4.1 Effect of algal supplementation on diet composition

The proximate composition of the experimental diets showed only negligible variations in moisture, crude protein, crude lipid, ash, and dry matter, confirming that all diets were successfully formulated as isonitrogenous. Consequently, the differences observed in fish performance can be attributed primarily to the physiological effects of algal supplementation rather than variations in nutrient composition. Maintaining comparable dietary protein and energy levels is essential in nutritional experiments to accurately evaluate the biological effects of functional ingredients. Similar observations have been reported in studies evaluating microalgae and macroalgae as feed additives, where dietary inclusion of algal biomass did not markedly alter proximate composition but significantly influenced fish growth and physiological responses through their bioactive constituents. *Chlorella* sp. is recognized as a rich source of highly digestible protein, essential amino acids, chlorophyll, carotenoids, vitamins, minerals, nucleotides, and polyunsaturated fatty acids, whereas *Porphyra* sp. contains abundant proteins, phycobiliproteins, taurine, carotenoids, sulfated polysaccharides, vitamins, and trace minerals (Holdt & Kraan, 2011) [14]. These bioactive compounds function not only as nutrients but also as physiological modulators capable of improving digestion, metabolism, antioxidant defense, and immune function. Therefore, the beneficial responses observed in the present study are likely associated with the synergistic effects of these biologically active compounds rather than simple nutrient supplementation.

4.2 Growth Performance and Feed Utilization

The present study demonstrated that dietary supplementation with *Chlorella* and *Porphyra* significantly improved growth performance and feed utilization of *Carassius auratus*. Among the dietary treatments, fish fed the 10% *Chlorella* diet exhibited the highest final body weight, weight gain, specific growth rate, feed efficiency ratio, and protein efficiency ratio, together with the lowest feed conversion ratio. Similarly, 10% *Porphyra* supplementation produced the best growth response among the macroalgal diets, whereas further increasing *Porphyra* inclusion to 15% resulted in reduced performance.

The superior growth performance observed in the *Chlorella*-supplemented groups may be attributed to its high-quality protein content and abundance of bioactive compounds, including essential amino acids, vitamins, carotenoids, polyunsaturated fatty acids, and nucleotides. These components are known to improve nutrient digestibility, stimulate digestive enzyme activities, and enhance protein synthesis and tissue accretion (Becker, 2013) [3]. Similar

improvements in growth and feed utilization following dietary *Chlorella* supplementation have been reported in gibel carp (*Carassius auratus gibelio*) by Xu *et al.* (2014), who observed enhanced specific growth rate and protein efficiency ratio in fish fed diets containing 0.8-1.2% *Chlorella*. Likewise, Abdel-Tawwab and Ahmad (2009) [11] reported significant improvements in growth and feed conversion efficiency in Nile tilapia fed *Chlorella vulgaris*-supplemented diets.

The beneficial effects of *Porphyra* supplementation may be associated with its highly digestible proteins, taurine, carotenoids, vitamins, and sulfated polysaccharides, particularly porphyran, which have been reported to improve nutrient metabolism and physiological performance (Holdt and Kraan, 2011) [14]. Similar findings have been reported by Kalla *et al.* (2008) [17], who observed improved growth and feed utilization in red sea bream fed diets supplemented with *Porphyra* spheroplasts. Furthermore, Cheung (2021) reported that inclusion of 5-10% *Porphyra amplissima* significantly improved wet weight and condition factor in Atlantic salmon.

However, growth performance declined at the highest inclusion level of *Porphyra* (15%), suggesting that excessive macroalgal incorporation may adversely affect nutrient utilization. Red macroalgae contain relatively high concentrations of structural polysaccharides and dietary fiber that can reduce nutrient digestibility and limit nutrient absorption when included at excessive levels (Holdt and Kraan, 2011) [14]. Similar reductions in growth performance at higher seaweed inclusion levels have been reported in rainbow trout and gilthead seabream (Soler-Vila *et al.*, 2009) [31].

Collectively, the present findings indicate that moderate dietary inclusion of algal biomass improves growth performance and feed utilization in goldfish, with 10% *Chlorella* providing the greatest biological response under the present experimental conditions.

4.3 Carotenoid Deposition and Pigmentation

Body coloration is one of the principal quality attributes determining the commercial value of ornamental fish. Since fish are unable to synthesize carotenoids *de novo*, dietary supplementation with natural pigment sources is essential for maintaining desirable body coloration. In the present study, both *Chlorella* and *Porphyra* significantly enhanced carotenoid deposition compared with the control diet, with the highest values observed in fish fed 10% *Chlorella*.

The enhanced pigmentation observed in the *Chlorella*-fed groups can be attributed to the presence of natural pigments such as lutein, β -carotene, zeaxanthin, and chlorophyll derivatives, which serve as precursors for chromatophore pigmentation (Becker, 2013) [3]. Moreover, the presence of essential fatty acids may facilitate intestinal absorption and transport of carotenoids, thereby improving pigment deposition in skin tissues. Similar findings were reported by Teimouri *et al.* (2013), who observed improved body coloration and carotenoid accumulation in goldfish fed diets supplemented with *Spirulina* and *Chlorella*. Likewise, Gouveia *et al.* (2003) [12] reported significant enhancement of pigmentation in ornamental fish following supplementation with carotenoid-rich microalgae.

Dietary *Porphyra* supplementation also significantly improved carotenoid deposition, particularly at the 10% inclusion level. Red macroalgae contain carotenoids,

phycobiliproteins, and antioxidant compounds that contribute to pigmentation enhancement and pigment stability (Holdt and Kraan, 2011) [14]. However, the reduced carotenoid deposition observed at 15% *Porphyra* inclusion suggests that excessive macroalgal supplementation may reduce carotenoid bioavailability, possibly because of impaired lipid digestion and nutrient absorption associated with higher fiber levels. Similar observations have been reported in marine fish and ornamental species receiving excessive seaweed supplementation (Wan *et al.*, 2019) [35]. The results indicate that moderate supplementation with *Chlorella* and *Porphyra* can serve as an effective and sustainable strategy for improving pigmentation and market quality of ornamental goldfish.

4.4 Hematological Responses

Hematological parameters are sensitive indicators of nutritional status and physiological health in fish. In the present study, dietary supplementation with both algal species significantly improved erythrocytic indices, including RBC count, hemoglobin concentration, and hematocrit values, with the highest responses observed in fish fed 10% *Chlorella* and 10% *Porphyra*.

The improvement in hematological parameters following *Chlorella* supplementation may be attributed to its rich supply of high-quality protein, iron, folic acid, vitamin B₁₂, and chlorophyll, which are essential for erythropoiesis and hemoglobin synthesis (Becker, 2013) [3]. Enhanced erythrocyte production increases oxygen transport to tissues, thereby supporting improved metabolism and growth. Similar improvements in hemoglobin concentration and erythrocyte count following dietary *Chlorella* supplementation have been reported in Nile tilapia (Abdel-Tawwab and Ahmad, 2009) [1] and gibel carp.

The positive hematological responses observed in the *Porphyra*-supplemented groups may be associated with the presence of bioavailable iron, taurine, essential amino acids, vitamins, and sulfated polysaccharides that support hematopoietic activity and cellular protection (Holdt and Kraan, 2011) [14]. However, the comparatively lower responses observed at 15% inclusion suggest that excessive incorporation of macroalgal biomass may reduce the availability of essential nutrients because of higher levels of indigestible polysaccharides and dietary fiber.

The improved erythrocytic parameters obtained in the present study indicate enhanced physiological condition and metabolic efficiency in algal-fed fish. Therefore, dietary supplementation with 10% *Chlorella* and 10% *Porphyra* appears to be effective for improving blood physiological status and promoting better overall health in *C. auratus*.

5. Conclusion

The present study demonstrated that dietary supplementation with *Chlorella* sp. and *Porphyra* sp. significantly improved the growth performance, feed utilization, carotenoid deposition, and hematological status of *Carassius auratus*. Among the evaluated dietary treatments, 10% *Chlorella* supplementation consistently produced the most pronounced improvements in final body weight, weight gain, specific growth rate, feed conversion ratio, feed efficiency ratio, protein efficiency ratio, carotenoid accumulation, and erythrocytic parameters. Similarly, 10% *Porphyra* supplementation enhanced growth performance, pigmentation, and hematological indices;

however, its overall effectiveness was comparatively lower than that of *Chlorella*. In contrast, increasing the dietary inclusion of *Porphyra* to 15% did not provide additional benefits and resulted in reduced performance, suggesting that excessive macroalgal supplementation may impair nutrient utilization because of its higher fiber and ash contents.

The improved performance observed in algal-supplemented fish can be attributed to the rich nutritional profile of both algae, including high-quality proteins, essential amino acids, carotenoids, vitamins, minerals, polyunsaturated fatty acids, chlorophyll, and other bioactive compounds that enhance nutrient utilization and physiological function. The increased carotenoid deposition further demonstrates the effectiveness of these natural algal resources as sustainable pigmentation enhancers for ornamental fish, offering an environmentally friendly alternative to synthetic colorants.

Overall, the findings indicate that *Chlorella* sp. and *Porphyra* sp. are promising functional feed additives for ornamental aquaculture, with 10% dietary *Chlorella* identified as the optimum inclusion level under the present experimental conditions. The incorporation of *Chlorella* at this level can improve growth, feed efficiency, pigmentation, and physiological health, thereby increasing the commercial quality and market value of goldfish. Future investigations should focus on the long-term effects of algal supplementation on reproductive performance, intestinal microbiota, molecular immune responses, and nutrigenomic regulation to further elucidate the mechanisms underlying their beneficial effects and to optimize their application in sustainable ornamental fish production.

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