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Chlorella sp. and *Porphyra* sp. enhance final body weight, antioxidant defense, and resistance to *Aeromonas hydrophila* in goldfish (*Carassius auratus*)

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Abstract

Present study evaluated the effects of *Chlorella* sp. and *Porphyra* sp. supplementation on final body weight, antioxidant responses, resistance against *Aeromonas hydrophila*, and water quality in goldfish (*Carassius auratus*). Fish were fed isonitrogenous diets supplemented with graded levels of *Chlorella* sp. (5, 7.5, and 10%) or *Porphyra* sp. (5, 10, and 15%) for 75 days under controlled culture conditions. Water quality parameters remained within the optimum range throughout the experimental period and were not significantly influenced by algal supplementation ($P > 0.05$). Both algal supplements significantly improved the final body weight and antioxidant status of goldfish compared with the control ($P < 0.05$). Fish fed diets containing 10% *Chlorella* sp. exhibited the highest final body weight, enhanced superoxide dismutase (SOD) and catalase (CAT) activities, reduced malondialdehyde (MDA) levels, and the lowest cumulative mortality following experimental challenge with *Aeromonas hydrophila*. Similarly, dietary supplementation with 10% *Porphyra* sp. improved final body weight, antioxidant enzyme activities, and bacterial disease resistance, whereas no additional benefits were observed at the highest inclusion level. Overall, the findings demonstrate that both *Chlorella* sp. and *Porphyra* sp. serve as effective natural feed additives for enhancing growth, antioxidant defense, and resistance to bacterial infection without adversely affecting water quality. These results support the incorporation of algal supplements as sustainable functional ingredients for improving the health and production performance of ornamental goldfish.

Keywords: Macroalgae, microalgae, antioxidant enzymes, *Aeromonas hydrophila*

1. Introduction

The ornamental fish industry represents one of the fastest-growing sectors of global aquaculture, with goldfish (*Carassius auratus*) being one of the most popular cultured ornamental species because of its attractive appearance, adaptability, and high commercial value. However, intensive culture practices often expose fish to environmental stress and opportunistic pathogens, resulting in oxidative damage, reduced immunity, and increased disease outbreaks. Consequently, sustainable nutritional strategies that improve fish health while minimizing the use of antibiotics have become an important research focus in modern aquaculture (FAO, 2024; Reverter *et al.*, 2020) [8, 19].

Natural feed additives, particularly algae, have attracted considerable attention owing to their high nutritional value and abundance of bioactive compounds. Microalgae and macroalgae are rich sources of proteins, essential amino acids, vitamins, minerals, carotenoids, polysaccharides, and phenolic compounds that enhance physiological performance, antioxidant capacity, and immune function in fish (Hoseinifar *et al.*, 2019; Abdel-Tawwab *et al.*, 2023). Among microalgae, *Chlorella* sp. has been widely recognized as a promising functional feed ingredient because of its balanced nutrient composition and antioxidant properties. Similarly, the red macroalga *Porphyra* sp. contains bioactive compounds, including sulfated polysaccharides and phycobiliproteins, which have been associated with improved antioxidant activity, immune modulation, and disease resistance in aquatic animals (Holdt & Kraan, 2011; Dawood *et al.*, 2020) [11, 7].

Oxidative stress is a major physiological challenge in intensive aquaculture and is characterized by excessive production of reactive oxygen species that damage cellular lipids, proteins, and nucleic acids.

Endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) play a critical role in neutralizing reactive oxygen species, whereas malondialdehyde (MDA) serves as an indicator of lipid peroxidation and oxidative damage. Nutritional supplementation with antioxidant-rich algal ingredients has therefore been proposed as an effective strategy to enhance the antioxidant defense system and improve fish health (Lushchak, 2011; Birben *et al.*, 2012) [16, 5].

Among bacterial pathogens, *Aeromonas hydrophila* is one of the most important causative agents of disease in freshwater fish, leading to hemorrhagic septicemia and substantial economic losses in aquaculture. The increasing concern over antimicrobial resistance has encouraged the development of natural dietary immunostimulants capable of improving disease resistance without adverse environmental impacts (Austin & Austin, 2016; Reverter *et al.*, 2020) [4, 19]. Furthermore, any functional feed additive intended for aquaculture should maintain suitable water quality to ensure a healthy culture environment and sustainable production.

Although the beneficial effects of *Chlorella* sp. and *Porphyra* sp. have been reported individually in different fish species, information comparing their influence on final body weight, antioxidant status, resistance to *Aeromonas hydrophila*, and water quality in ornamental goldfish remains limited. Therefore, the present study evaluated the effects of dietary supplementation with *Chlorella* sp. and *Porphyra* sp. on final body weight, antioxidant enzyme activities, bacterial disease resistance, and water quality in *Carassius auratus*. The findings are expected to provide scientific evidence supporting the application of algal supplements as sustainable functional feed additives for ornamental aquaculture.

2. Materials and Methods

2.1 Experimental fish and rearing conditions

The feeding experiment was conducted at the Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India. Healthy juvenile goldfish (*Carassius auratus*) with an average initial body weight of 1.85 ± 0.01 g were procured from a local ornamental fish farm and acclimatized for 30 days prior to the experiment. During acclimatization, fish were maintained in fiberglass tanks and fed a commercial pellet diet twice daily.

A total of 280 fish were randomly distributed into 28 glass aquaria (35 L capacity) at a stocking density of 10 fish per aquarium. Continuous aeration was provided throughout the experimental period, and approximately one-third of the water was exchanged daily to maintain optimum water quality. The feeding trial was conducted for 75 days under natural photoperiod conditions.

2.2 Experimental diets and feeding regime

Two independent feeding experiments were conducted to evaluate the effects of *Chlorella* sp. and *Porphyra* sp. supplementation. In the first experiment, four isonitrogenous diets were formulated containing 0% (control), 5%, 7.5%, and 10% *Chlorella* sp. In the second experiment, four similar diets were prepared with 0% (control), 5%, 10%, and 15% *Porphyra* sp. supplementation. All diets were formulated to contain approximately 30% crude protein using locally available feed ingredients.

Fish were hand-fed twice daily at 5% of their body weight, and the feeding ration was adjusted periodically according to biomass.

2.3 Final body weight

At the end of the 75-day feeding trial, fish from each experimental unit were individually weighed using a digital analytical balance after a 24-h fasting period. Final body weight was recorded and expressed as mean $\pm$ Standard error for each treatment.

2.4 Antioxidant enzyme analysis

At the end of the feeding trial, liver tissues were collected aseptically from randomly selected fish for antioxidant analysis. Tissue homogenates were prepared in ice-cold phosphate buffer and centrifuged to obtain the supernatant for biochemical assays.

Superoxide dismutase (SOD) activity was determined according to the method of Marklund and Marklund (1974). Catalase (CAT) activity was estimated following the method described by Sinha (1972), whereas lipid peroxidation was quantified as malondialdehyde (MDA) concentration using the thiobarbituric acid reactive substances (TBARS) method described by Lykkesfeldt (2001).

2.5 Bacterial challenge test

Following completion of the feeding trial, fish from each treatment were subjected to an experimental challenge with *Aeromonas hydrophila*. A virulent bacterial culture (1×10^8 CFU mL⁻¹) was prepared, and each fish was intraperitoneally injected with 0.1 mL of the bacterial suspension. Challenged fish were maintained under identical culture conditions for 10 days and observed daily for mortality. Cumulative mortality was recorded and used to evaluate disease resistance among dietary treatments.

2.6 Water quality analysis

Water quality parameters, including temperature (°C), pH, dissolved oxygen (mg L⁻¹), total alkalinity (mg L⁻¹), total hardness (mg L⁻¹), ammonia-nitrogen (mg L⁻¹), and nitrite-nitrogen (mg L⁻¹), were monitored regularly throughout the experimental period following standard methods described by the American Public Health Association (APHA, 2017). All measured parameters remained within the acceptable range for goldfish culture.

2.7 Statistical analysis

Data were expressed as mean $\pm$ Standard error (SE). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test to determine significant differences among treatment means. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1 Final body weight

Dietary supplementation with *Chlorella* sp. and *Porphyra* sp. significantly influenced the final body weight of *Carassius auratus* after the 75-day feeding trial ($P < 0.05$). In the *Chlorella* sp. feeding trial, the initial body weight of fish did not differ significantly among treatments (1.85 ± 0.00 g; $P > 0.05$). However, significant differences were observed in the final body weight following dietary supplementation. Fish fed the diet containing 10% *Chlorella* sp. (CT₃)

attained the highest final body weight (5.87 ± 0.01 g), followed by the 7.5% *Chlorella* sp. group (CT₂; 5.68 ± 0.01 g) and the 5% *Chlorella* sp. group (CT₁; 5.55 ± 0.01 g). The control group recorded the lowest final body weight (5.28 ± 0.01 g) (Table 1). The progressive increase in final body weight with increasing levels of *Chlorella* sp. supplementation indicated a significant positive effect on fish growth.

Similarly, dietary inclusion of *Porphyra* sp. significantly affected the final body weight of goldfish ($P < 0.05$). Fish receiving the 10% *Porphyra* sp. diet (PT₂) exhibited the highest final body weight (5.45 ± 0.01 g), followed by PT₁ (5% *Porphyra* sp.; 5.36 ± 0.01 g). No significant improvement was observed at the highest inclusion level (15%; PT₃), where the final body weight (5.27 ± 0.01 g) was comparable to that of the control group (5.28 ± 0.01 g). These findings indicate that moderate dietary inclusion of *Porphyra* sp. was more effective than the highest supplementation level in improving the final body weight of *C. auratus*.

3.2 Antioxidant responses

Dietary supplementation with *Chlorella* sp. and *Porphyra* sp. significantly influenced the antioxidant status of *Carassius auratus* after the 75-day feeding trial ($P < 0.05$) (Table 1 & Figure 1). In the *Chlorella* sp. feeding trial, superoxide dismutase (SOD) activity increased significantly with increasing dietary inclusion, rising from 141.82 ± 0.09 U mg⁻¹ protein in the control group to 148.04 ± 0.58 , 153.61 ± 0.14 , and 158.51 ± 0.10 U mg⁻¹ protein in the 5%, 7.5%, and 10% *Chlorella* sp. treatments, respectively. Similarly, catalase (CAT) activity showed a significant improvement, reaching the highest value (102.63 ± 1.24 U mg⁻¹ protein) in fish fed the 10% *Chlorella* sp. diet compared with 95.09 ± 0.23 U mg⁻¹ protein in the control. In contrast, malondialdehyde (MDA) concentration decreased progressively from 9.42 ± 0.09 U mg⁻¹ protein in the control to 8.79 ± 0.06 , 7.47 ± 0.08 , and 6.37 ± 0.08 U mg⁻¹ protein in CT₁, CT₂, and CT₃, respectively, with the lowest value recorded in the 10% supplementation group.

A similar trend was observed in fish fed *Porphyra* sp.-supplemented diets. SOD activity increased significantly from 141.82 ± 0.09 U mg⁻¹ protein in the control to 146.04 ± 0.56 and 149.12 ± 0.68 U mg⁻¹ protein in the 5% and 10% *Porphyra* sp. treatments, respectively, whereas the 15% inclusion level (141.83 ± 0.94 U mg⁻¹ protein) did not differ significantly from the control. CAT activity followed the same pattern, with the highest activity observed in the 10% *Porphyra* sp. group (98.55 ± 0.39 U mg⁻¹ protein), while the 15% supplementation group exhibited values comparable to the control. Conversely, MDA concentration was significantly reduced in fish receiving 5% (7.70 ± 0.31 U

mg⁻¹ protein) and 10% (6.52 ± 0.04 U mg⁻¹ protein) *Porphyra* sp., whereas the 15% inclusion level (9.19 ± 0.21 U mg⁻¹ protein) was statistically similar to the control (9.42 ± 0.09 U mg⁻¹ protein).

3.3 Disease resistance against *Aeromonas hydrophila*

Dietary supplementation with *Chlorella* sp. and *Porphyra* sp. significantly affected the resistance of *Carassius auratus* to *Aeromonas hydrophila* infection following the experimental challenge ($P < 0.05$) (Figure 2).

In the *Chlorella* sp. feeding trial, cumulative mortality decreased significantly with increasing dietary inclusion level. Fish fed the control diet exhibited the highest cumulative mortality ($66.67 \pm 3.33\%$), whereas mortality declined to $53.33 \pm 3.33\%$, $36.67 \pm 3.33\%$, and $20.00 \pm 5.77\%$ in the 5% (CT₁), 7.5% (CT₂), and 10% (CT₃) *Chlorella* sp. treatments, respectively. The lowest cumulative mortality observed in CT₃ indicated a marked improvement in disease resistance following bacterial challenge.

Similarly, dietary supplementation with *Porphyra* sp. significantly enhanced resistance to *A. hydrophila*. The highest cumulative mortality was recorded in the control group ($66.67 \pm 3.33\%$), while fish receiving 5% (PT₁) and 10% (PT₂) *Porphyra* sp. diets showed significantly lower cumulative mortality of $46.67 \pm 3.33\%$ and $26.67 \pm 3.33\%$, respectively. However, increasing the supplementation level to 15% (PT₃) resulted in cumulative mortality of $53.33 \pm 3.33\%$, which was significantly higher than PT₂ but lower than the control.

3.4 Water quality parameters

The physicochemical characteristics of the culture water remained within the acceptable range for the successful rearing of *Carassius auratus* throughout the 75-day experimental period (Table 2). Water temperature gradually increased from 25.0–25.5°C at the beginning of the experiment to 28.4–28.9°C on day 75. The pH remained stable between 7.1 and 7.7, while dissolved oxygen concentrations varied from 5.58 to 6.68 mg L⁻¹ during the study. Total alkalinity ranged from 127 to 138 mg L⁻¹ as CaCO₃, indicating adequate buffering capacity throughout the experimental period.

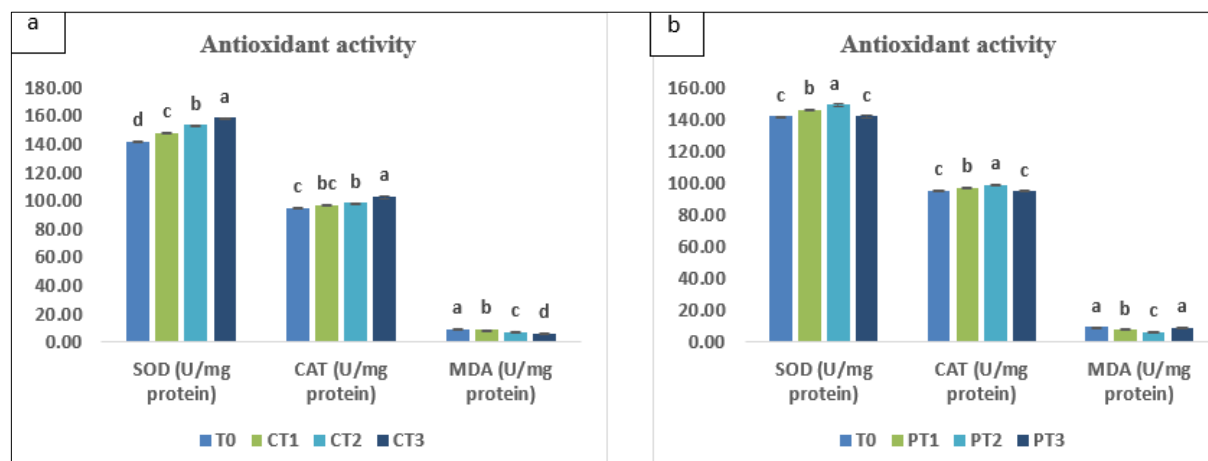
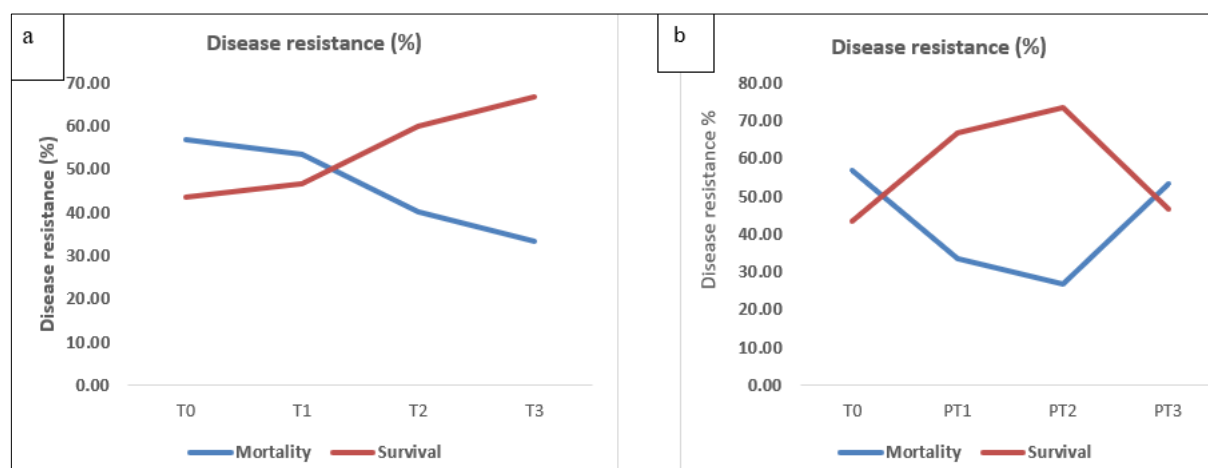
No abnormal fluctuations in any of the measured water quality parameters were observed during the feeding trial. All recorded values remained within the recommended range for ornamental fish culture, providing a stable culture environment throughout the experiment. Consequently, the improvements observed in final body weight, antioxidant responses, and disease resistance in fish fed *Chlorella* sp.- and *Porphyra* sp.-supplemented diets were unlikely to be influenced by environmental variation and were primarily associated with the dietary treatments.

Table 1: Effects of dietary *Chlorella* and *Porphyra* supplementation on growth performance and antioxidant status of goldfish (*Carassius auratus*) after a 75-day feeding trial

Parameter	IW(g)	FW (g)	SOD	CAT	MDA
T ₀ (Control)	1.85 ± 0.005^a	5.28 ± 0.005^c	141.82 ± 0.090^c	95.09 ± 0.234^c	9.42 ± 0.085^a
CT ₁ (5% <i>Chlorella</i>)	1.85 ± 0.005^a	5.55 ± 0.011^c	148.04 ± 0.584^c	96.99 ± 0.177^{bc}	8.79 ± 0.063^b
CT ₂ (7.5% <i>Chlorella</i>)	1.85 ± 0.005^a	5.68 ± 0.027^b	153.61 ± 0.136^b	98.32 ± 0.180^b	7.47 ± 0.079^c
CT ₃ (10% <i>Chlorella</i>)	1.85 ± 0.005^a	5.87 ± 0.003^a	158.51 ± 0.099^a	102.63 ± 1.235^a	6.37 ± 0.075^d
PT ₁ (5% <i>Porphyra</i>)	1.85 ± 0.003^a	5.36 ± 0.005^b	146.04 ± 0.564^b	97.16 ± 0.237^b	7.70 ± 0.308^b
PT ₂ (10% <i>Porphyra</i>)	1.85 ± 0.002^a	5.45 ± 0.008^a	149.12 ± 0.679^a	98.55 ± 0.390^a	6.52 ± 0.036^c
PT ₃ (15% <i>Porphyra</i>)	1.85 ± 0.004^a	5.27 ± 0.010^c	141.83 ± 0.943^c	94.75 ± 0.383^c	9.19 ± 0.208^a

Table 2: Physico-chemical water parameters during the experiment (Mean $\pm$ SE)

Day	Temperature ($^{\circ}$ C)	pH	Dissolved Oxygen (mg/L)	Alkalinity (mg/L as CaCO ₃)
Day 0	25.0 - 25.5	7.1 - 7.2	5.78-6.12	127 - 129
Day 15	25.7 - 26.1	7.2 - 7.4	5.68-6.68	128 - 133
Day 30	26.4 - 26.0	7.3 - 7.5	5.63-6.58	129 - 135
Day 45	27.1 - 27.7	7.4 - 7.6	5.58-6.61	131 - 137
Day 60	27.90 - 28.3	7.5 - 7.7	5.64-6.52	133 - 138
Day 75	28.4 - 28.90	7.4 - 7.6	5.67-6.75	131 - 136

**Fig 1:** Effect of (a) chlorella (b) Porphyra supplementation on antioxidant activity of *C. auratus* over the experimental period.**Fig 2:** Cumulative mortality (%) and survival of gold fish after a 10-day *A. hydrophila* challenge. Effect of (a) chlorella (b) Porphyra supplementation.

4. Discussion

4.1 Final body weight

The present study demonstrated that dietary supplementation with *Chlorella* sp. and *Porphyra* sp. significantly improved the final body weight of *Carassius auratus*, with the highest response observed at 10% inclusion in both feeding trials. The improvement in growth may be attributed to the rich nutritional composition of both algal ingredients, including high-quality proteins, essential amino acids, vitamins, minerals, and other bioactive compounds that enhance nutrient utilization and support tissue growth. In addition, these bioactive constituents are known to improve digestive efficiency and metabolic activity, thereby promoting better conversion of nutrients into body biomass (Dawood *et al.*, 2020; Abdel-Tawwab *et al.*, 2022) [7, 1].

The superior performance of *Chlorella* sp. compared with *Porphyra* sp. may be associated with its greater nutrient digestibility and higher bioavailability of essential nutrients.

Furthermore, the enhanced antioxidant status observed in the present study may have reduced oxidative stress, allowing greater metabolic energy to be utilized for growth rather than cellular maintenance. Similar growth-promoting effects of *Chlorella* sp. have been reported in several cultured fish species (Abdel-Tawwab *et al.*, 2022; Pradhan & Das, 2024) [1, 17].

Although *Porphyra* sp. also enhanced final body weight, increasing its inclusion level from 10% to 15% did not provide additional benefits. This reduction in growth response may be attributed to the higher content of structural polysaccharides and mineral ash in macroalgae, which can reduce nutrient digestibility and limit nutrient absorption when included at excessive levels. Freshwater fish have a limited capacity to digest complex algal carbohydrates, thereby decreasing the efficiency of nutrient utilization at higher dietary inclusion levels (Holdt & Kraan, 2011; Wan *et al.*, 2019) [11, 23]. These findings indicate that optimum dietary inclusion is more important than maximum

inclusion for achieving superior growth performance, which is consistent with previous reports on algal supplementation in aquaculture (Siddik *et al.*, 2024) ^[21].

4.2 Antioxidant responses

The present study demonstrated that dietary supplementation with *Chlorella* sp. and *Porphyra* sp. markedly improved the antioxidant status of *Carassius auratus*, as evidenced by enhanced SOD and CAT activities and reduced MDA levels. The improvement was most pronounced in fish fed 10% *Chlorella* sp. and 10% *Porphyra* sp., indicating that both algal supplements effectively strengthened the endogenous antioxidant defense system.

The increased activities of SOD and CAT suggest an enhanced capacity to neutralize reactive oxygen species (ROS) generated during normal metabolism and environmental stress. Bioactive compounds present in *Chlorella* sp. and *Porphyra* sp., including carotenoids, chlorophylls, polyphenols, vitamins, and sulfated polysaccharides, possess strong antioxidant properties that can stimulate the synthesis and activity of endogenous antioxidant enzymes. Consequently, efficient scavenging of ROS minimizes oxidative damage and helps maintain cellular homeostasis (Dawood *et al.*, 2020; Abdel-Tawwab *et al.*, 2022) ^[7, 1].

Although both algal supplements enhanced antioxidant responses, no further improvement was observed at the highest inclusion level of *Porphyra* sp. (15%). This finding suggests that excessive dietary supplementation may not proportionally increase the availability or utilization of antioxidant compounds. Reduced digestibility of macroalgal components or limited absorption of bioactive molecules at higher inclusion levels may have restricted their physiological effectiveness (Holdt & Kraan, 2011) ^[11]. Similar improvements in antioxidant enzyme activities following algal supplementation have been reported in several cultured fish species, demonstrating the ability of algal bioactive compounds to reinforce antioxidant defense mechanisms and reduce oxidative stress (Hoseinifar *et al.*, 2020; Siddik *et al.*, 2024) ^[2, 21].

4.3 Disease Resistance Strategy (with inside citations)

Dietary supplementation with *Chlorella* sp. and *Porphyra* sp. significantly improved the disease resistance capacity of *Carassius auratus* against *Aeromonas hydrophila* infection; however, the magnitude of protection differed among dietary treatments. Fish receiving 10% *Chlorella* supplementation (CT₃) showed the highest survival and reduced mortality following pathogen challenge, indicating enhanced immunocompetence and pathogen tolerance. The improved resistance observed in *Chlorella*-fed fish may be associated with its rich composition of bioactive molecules, including polysaccharides, carotenoids, essential fatty acids, vitamins, and antioxidant compounds, which stimulate innate immune responses and improve oxidative stress regulation in aquatic animals (Guzmán-Murillo *et al.*, 2019; Shah *et al.*, 2020) ^[10, 20]. Dietary microalgae supplementation has been reported to enhance immune-related parameters, including lysozyme activity, respiratory burst activity, antioxidant enzyme function, and resistance against bacterial infections through modulation of host defense mechanisms (Abdel-Tawwab *et al.*, 2020; Dawood *et al.*, 2020) ^[2, 7].

Similarly, *Porphyra*-supplemented groups exhibited improved resistance against *A. hydrophila* compared with the unsupplemented control, particularly at moderate inclusion levels (5-10%). The immunostimulatory effects of red macroalgae have been linked to sulfated polysaccharides, phenolic compounds, and antioxidant metabolites that enhance immune signaling and improve cellular defense mechanisms (Hoseinifar *et al.*, 2019; Rahman *et al.*, 2021) ^[13, 18]. However, the comparatively lower protective effect observed at 15% *Porphyra* inclusion suggests that excessive supplementation may not provide additional immunological benefits and could potentially influence nutrient utilization or immune homeostasis due to higher levels of structural polysaccharides and reduced digestibility (Wan *et al.*, 2019; Valente *et al.*, 2021) ^[23, 22]. Overall, the present findings demonstrate that optimal dietary inclusion is essential for maximizing disease resistance, with 10% *Chlorella* supplementation providing the most effective protection against bacterial challenge. The enhanced survival response in this treatment may be attributed to the combined effects of improved antioxidant status, nutritional enrichment, and immune modulation, supporting the application of *Chlorella* as a functional feed additive in ornamental fish production (Ahmadifar *et al.*, 2022; Hoseinifar *et al.*, 2023) ^[3, 14].

4.4 Water Quality Parameters

Throughout the experimental period, all water quality parameters remained within the suitable range for the culture of *Carassius auratus*, indicating that dietary supplementation with *Chlorella* sp. and *Porphyra* sp. did not negatively influence the rearing environment. The stable water quality conditions observed in all treatments suggest that the supplemented diets were efficiently utilized without causing excessive organic loading or deterioration of water conditions. Similar observations have been reported where dietary inclusion of algae-based feed additives maintained favorable water quality parameters due to improved feed utilization and reduced waste production (Shah *et al.*, 2020) ^[20].

The incorporation of microalgae and macroalgae in aquafeeds may also contribute to improved nitrogen utilization by enhancing nutrient digestibility, thereby reducing the accumulation of metabolic wastes in culture systems (Wan *et al.*, 2019) ^[23]. Maintenance of optimal dissolved oxygen, pH, temperature, and ammonia levels is essential for sustaining fish metabolism, immune function, and disease resistance, as fluctuations in these parameters can induce physiological stress and compromise health performance (Boyd & Tucker, 2012) ^[6]. In the present study, the absence of significant variations among treatments indicates that dietary *Chlorella* and *Porphyra* supplementation at the tested inclusion levels was environmentally safe and suitable for goldfish culture.

5. Conclusion

The present study demonstrated that dietary supplementation of *Chlorella* sp. and *Porphyra* sp. effectively improved the growth performance, physiological condition, antioxidant status, and disease resistance of *Carassius auratus*. Among the tested treatments, 10% *Chlorella* supplementation showed the most consistent positive effects, resulting in enhanced growth, feed utilization efficiency, immune protection, and survival

following *Aeromonas hydrophila* challenge. *Porphyra* supplementation also exhibited beneficial effects; however, the response was optimum at moderate inclusion levels (5-10%), while higher inclusion (15%) did not provide additional advantages.

Overall, *Chlorella* sp. showed greater potential as a functional feed additive for ornamental fish production due to its balanced nutritional composition and immunomodulatory properties. The findings suggest that incorporation of 10% *Chlorella* powder in goldfish diets can be recommended to improve performance, health status, and resistance against bacterial infections under culture conditions. Further studies involving molecular immune markers and long-term feeding trials are recommended to validate its application in sustainable aquaculture systems.

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