



Effect of Dietary Supplementation with Green Spirulina on the Survival Rate and Color Intensity of Goldfish (*Carassius auratus*)

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Abstract: This study aimed to analyze the effect of adding natural carotenoid sources to feed on the enhancement of color intensity and survival rate of fish. The study employed a Completely Randomized Design (CRD) with four treatments and three replications, namely P1 (control), P2 (6% spirulina), P3 (8% spirulina), and P4 (10% spirulina). The experiment was conducted for 30 days using 60 experimental fish. The observed parameters included color intensity and survival rate. Data were analyzed using a one-way ANOVA. The results showed a significant difference among treatments on fish color intensity ($P < 0.05$). Treatment P3 (8% spirulina) produced the highest color intensity value (21.12 ± 0.27), followed by P4 (10%) (20.76 ± 0.19) and P2 (6%) (20.66 ± 0.07), while the lowest value was observed in P1 (control) (20.55 ± 0.14). However, no significant difference was found in the survival rate ($P > 0.05$), with the highest value in P1 (93%) and the other treatments showing similar values (87%). The addition of spirulina significantly improved fish color intensity, with the 8% dosage being the most effective treatment, but it did not significantly affect the survival rate.

Keywords: Goldfish (*Carassius auratus*); carotenoids; color intensity; pigmentation

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INTRODUCTION

Freshwater ornamental fish culture has developed rapidly as a commercial aquaculture sector in recent years, largely driven by increasing consumer demand for fish with superior aesthetic traits, particularly attractive body shape, brightness, and color intensity. Among the most valuable ornamental species, goldfish (*Carassius auratus*) are widely recognized for their diverse color patterns, distinctive body morphology, and high market preference. The demand for goldfish has continued to increase in line with the growing popularity of aquascaping and ornamental fish keeping in household and commercial settings (Rahman et al. 2021). In the ornamental fish industry, visual appearance is not merely an aesthetic attribute but also an important economic indicator, because fish with brighter and more stable coloration generally have higher market value (Gupta et al. 2007; Sathyaruban et al. 2021).

Fish body coloration is determined by complex interactions among genetic background, environmental conditions, physiological regulation, and nutrition. At the cellular level, fish coloration is produced by chromatophores, specialized pigment-containing cells located mainly in the dermal layer of the skin. These cells contain various pigment compounds, including carotenoids, which contribute to red, orange, and yellow coloration. Carotenoids are particularly important in ornamental fish because they directly influence visible color brightness and saturation. However, fish are generally unable to synthesize carotenoids *de novo*, making dietary intake the primary source of these pigments (Gupta et al. 2007; Sathyaruban et al. 2021). After

ingestion, carotenoids are absorbed in the digestive tract, transported through the bloodstream, and deposited in skin tissues through chromatophore activity, thereby influencing the intensity and stability of external coloration (Vissio et al. 2021).

Dietary supplementation with natural carotenoid sources is therefore considered an effective strategy to improve ornamental fish coloration. Spirulina, particularly *Spirulina platensis*, has received considerable attention because it contains natural pigments such as β -carotene, zeaxanthin, chlorophyll, and phycocyanin, along with protein, vitamins, and antioxidant compounds. These bioactive components may contribute not only to pigmentation but also to physiological condition and stress tolerance in fish (Batubara et al. 2023; Kargin & Dikbaş 2020). Compared with synthetic pigments, natural pigment sources are generally considered safer, more sustainable, and more environmentally acceptable for aquaculture feed development (Luciana et al. 2023; Sathyaruban et al. 2021). Previous studies have shown that spirulina and other carotenoid-rich ingredients can enhance color intensity in ornamental fish by increasing pigment deposition and chromatophore activity (Rouf et al. 2025; Yustiati et al. 2025).

Nevertheless, the effectiveness of spirulina supplementation varies among studies. These variations may be related to fish species, body size, physiological condition, feed formulation, supplementation dose, culture environment, and the method used to evaluate color. In goldfish, dietary *S. platensis* has been reported to improve skin pigmentation, although its effect on growth and other performance parameters is not always significant (Kargin & Dikbaş 2020). Similar findings have been reported in other ornamental fish, where spirulina supplementation improved color quality but produced variable effects on growth performance and survival rate (Azril et al. 2024). These findings suggest that the use of spirulina as a natural pigment source should not be evaluated solely based on color enhancement, but also in relation to biological responses that reflect fish health and culture performance.

Despite its potential, fish farmers still face challenges in maintaining stable and intense coloration in goldfish, particularly when commercial feeds contain insufficient carotenoid levels. Previous research has demonstrated that spirulina supplementation can improve color intensity; however, its effects on other parameters, such as survival rate, are often inconsistent (Rouf et al. 2025). Moreover, different supplementation doses have produced varying outcomes, and there is still no clear consensus regarding the optimal spirulina dose that is both effective for color enhancement and safe for goldfish culture (Andriansyah et al. 2020; Kargin & Dikbaş 2020). This indicates that although spirulina has strong potential as a natural dietary carotenoid source, further evaluation is required to determine an appropriate dose and to assess its broader biological effects.

Another important limitation in previous studies is the method used to assess fish coloration. Many studies still rely on visual scoring, which may introduce subjectivity due to differences in observer perception, lighting conditions, and scoring consistency. The Toca Color Finder (TCF) has been used in ornamental fish studies to score color brightness and saturation, including in koi and goldfish, and can provide a more standardized visual reference when combined with quantitative analysis (Lalita et al. 2022; Raming et al. 2024). However, the integration of TCF-based color evaluation with statistical analysis and survival assessment remains limited. Consequently, existing color evaluations may not fully represent the relationship between pigmentation response and the overall biological condition of the fish.

Based on these limitations, a specific research gap can be identified. Most previous studies have examined color enhancement as a single response variable,

without simultaneously evaluating the quantitative relationship between color intensity and survival rate. In addition, the combined use of TCF-based color assessment and statistical analysis remains relatively underdeveloped in studies on dietary spirulina supplementation for goldfish. Therefore, this study is positioned to address this gap by integrating quantitative evaluation of color intensity with simultaneous assessment of fish survival.

This study aims to analyze the effects of different dietary spirulina doses on color intensity enhancement and survival rate in goldfish (*Carassius auratus*) and to determine the most effective dose. The study contributes scientifically by applying an evaluation approach that combines Toca Color Finder (TCF)-based color assessment with statistical analysis to obtain more objective results. In addition, this study provides dose validation for dietary spirulina supplementation based on quantitative evidence, thereby supporting the development of more effective and environmentally friendly natural feed technology for ornamental fish culture.

METHOD

This study employed a completely randomized design (CRD) consisting of four treatments and three replicates, resulting in 12 experimental units. The treatments comprised P1 as the control without spirulina supplementation, P2 with 6% spirulina, P3 with 8% spirulina, and P4 with 10% spirulina, each incorporated into 1 kg of feed. The selected spirulina doses were based on previous studies indicating that supplementation within this range can enhance fish color intensity without negatively affecting survival. The aquarium served as the experimental unit, while the fish within each aquarium were treated collectively as a single analytical unit. The study was conducted over a 30-day rearing period in an aquaculture laboratory.

A total of 60 goldfish (*Carassius auratus*) measuring 3–5 cm in length and with an average body weight of 2.5 g were used as experimental fish. Healthy and uniform fish were selected and acclimatized for 7 days before treatment application. The fish were reared in aquaria measuring 40 × 30 × 30 cm, with a stocking density of five fish per aquarium, and each aquarium was equipped with an aeration system. Water quality was maintained within the optimal range, with temperature at 26–28°C, pH at 6.5–7.5, and dissolved oxygen above 5 mg/L. Water quality was monitored periodically throughout the rearing period, and partial water exchange was conducted to maintain suitable environmental conditions.

The experimental feed was prepared by mixing spirulina with commercial feed (PF1000 containing 39% protein) according to the designated treatment doses. The ingredients were mixed homogeneously, after which the feed was re-pelleted and dried before use. The prepared feed was stored under dry conditions to maintain its nutritional quality. Feeding was carried out two to three times daily *ad libitum*.

Color intensity was measured using the Toca Colour Finder (TCF) method through a visual scoring approach. Color assessment was performed by seven panelists by matching the body color of the fish with the TCF color standard, which has a score range of 20–34. A higher score indicated greater color intensity. Observations were conducted weekly on days 0, 7, 14, 21, and 28.

Data were collected by photographing the fish using a digital camera or smartphone against a white background under uniform lighting conditions, with the light source positioned approximately 30–40 cm from the object. The shooting distance was kept constant at approximately 20 cm for each observation to minimize visual bias. Image capture was standardized from the dorsal, lateral, and ventral sides of the fish. Each fish was observed at three measurement points, and the scores were averaged

to obtain a representative color value. Color intensity was calculated using the following equation:

$$\text{Color intensity} = \text{Final color score} - \text{Initial color score}$$

In addition to color intensity, survival rate and growth parameters were also measured as supporting variables. The survival rate (SR) was calculated using the following formula:

$$\text{SR (\%)} = (\text{Nt} / \text{N0}) \times 100$$

Note:

SR = survival rate (%)

Nt = number of fish at the end of the rearing period

N0 = number of fish at the beginning of the rearing period

Absolute weight gain was calculated using the following formula:

$$W = Wt - W0$$

Note:

W = absolute weight gain (g)

Wt = average fish weight at the end of the rearing period (g)

W0 = average fish weight at the beginning of the rearing period (g)

Absolute length gain was calculated using the following formula:

$$L = Lt - L0$$

Note:

L = absolute length gain (cm)

Lt = average fish length at the end of the rearing period (cm)

L0 = average fish length at the beginning of the rearing period (cm)

The primary parameter analyzed in this study was the color intensity of goldfish, while the supporting parameters included survival rate, absolute weight gain, and absolute length gain. The data obtained were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level or 5% significance level ($\alpha = 0.05$) to determine the effect of the treatments on the observed variables. Prior to ANOVA, the data were tested for statistical assumptions, including normality using the Shapiro–Wilk test and homogeneity of variance using Levene’s test, to ensure that the data met the requirements for parametric analysis. When ANOVA results indicated significant differences among treatments ($p < 0.05$), the analysis was followed by Duncan’s Multiple Range Test (DMRT) to identify significantly different treatment pairs and determine the best treatment within the tested dose range. All statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

This study aimed to analyze the effect of green spirulina supplementation at different dietary doses on the enhancement of color intensity in goldfish (*Carassius auratus*). The treatments consisted of P1 as the control, P2 with the addition of 6% spirulina per 1 kg of feed, P3 with the addition of 8% spirulina per 1 kg of feed, and P4 with the addition of 10% spirulina per 1 kg of feed. Data were analyzed using one-way ANOVA after the assumption of homogeneity of variance was met ($p > 0.05$).

Analysis of Fish Color Intensity

The ANOVA results showed a significant difference among treatments in the color intensity of goldfish ($F(3,80) = 3.685$; $p = 0.015$; $\eta^2 = 0.12$). The effect size indicated

that dietary treatment had a moderate effect on variation in fish color intensity. The color intensity values for each treatment are presented in Table 1.

Table 1. Increase in fish color intensity during the study

Treatment	Color Intensity (Mean \pm SD)
P1	20.55 \pm 0.14 ^a
P2	20.65 \pm 0.07 ^b
P3	21.11 \pm 0.27 ^b
P4	20.75 \pm 0.19 ^b

Note: Different superscript letters in the same column indicate significant differences based on Duncan's multiple range test at the 95% confidence level ($p < 0.05$).

Based on Table 1, the highest mean color intensity was observed in P3, which received 8% spirulina supplementation, followed by P4, P2, and the control treatment P1. Duncan's multiple range test showed that the control treatment differed significantly from all spirulina-supplemented treatments, whereas P2, P3, and P4 did not differ significantly from one another. This result indicates that the inclusion of spirulina in feed significantly improved goldfish color intensity compared with the control diet.

The improvement in color intensity is closely associated with the carotenoid content of spirulina. Ornamental fish generally depend on dietary carotenoids because they have limited ability to synthesize color-producing carotenoid pigments *de novo*. These pigments are absorbed through the digestive tract, transported to body tissues, and deposited in chromatophore cells, particularly xanthophores and erythrophores, which contribute to yellow, orange, and red coloration. Therefore, the higher color intensity observed in spirulina-supplemented treatments suggests that spirulina acted as an effective natural carotenoid source for enhancing pigmentation in goldfish.

The highest numerical value in P3 suggests that 8% spirulina may represent the most favorable dose within the tested range. However, the absence of a significant difference among P2, P3, and P4 indicates that increasing the dose above 6% did not produce a proportional improvement in color intensity. This pattern suggests a diminishing return response, in which additional carotenoid intake no longer results in a linear increase in pigment deposition. Such a response may occur when the physiological capacity for carotenoid absorption, transport, or deposition in chromatophores begins to reach saturation. Thus, although P3 produced the highest mean value, the practical effective range for spirulina supplementation in this study appears to be 6–8%.

These findings align with previous studies showing that spirulina and other natural carotenoid sources enhance pigmentation in ornamental fish. *Spirulina platensis* supplementation has been reported to improve skin pigmentation in goldfish (Kargin & Dikbaş, 2020), while spirulina- and/or canthaxanthin-enriched *Artemia* increased β -carotene content, pigmentation, and color intensity (Ahmed et al., 2023). Natural carotenoid sources, including microalgae, are therefore important feed additives for improving ornamental fish coloration (Sathyaruban et al., 2021). Thus, the present study further supports the use of spirulina as a natural pigment source to enhance the aesthetic quality of goldfish.

The present findings also align with Rouf et al. (2025), who reported that spirulina supplementation significantly enhanced fish color intensity without negatively affecting growth or survival. Similar responses were reported by Khairunnisa et al. (2020) and Rahman et al. (2021), who found that carotenoid supplementation increased color

intensity and chromatophore abundance. Differences in the magnitude of color improvement among studies may be attributed to variation in supplementation dose, rearing duration, fish size, carotenoid source, feed formulation, and color measurement method. Overall, the results indicate that the effect of spirulina on pigmentation is biologically relevant, although its response may not increase indefinitely with higher inclusion levels.

Survival Rate

In addition to color intensity, this study evaluated the survival rate of goldfish during the rearing period. The survival data are presented in Table 2.

Table 2. Survival rate of goldfish in each treatment

Treatment	Initial Number	Surviving Fish	Dead Fish	SR (%)
P1 (control)	15	13	2	87
P2 (6%)	15	13	2	87
P3 (8%)	15	14	1	93
P4 (10%)	15	13	2	87

Based on Table 2, the survival rate ranged from 87% to 93%. The highest survival rate was recorded in P3 at 93%, while P1, P2, and P4 each showed a survival rate of 87%. Statistical analysis using one-way ANOVA showed no significant difference among treatments in fish survival rate ($p > 0.05$).

The absence of significant differences in survival rate indicates that spirulina supplementation at 6–10% did not cause adverse effects on fish viability during the study. Biologically, this suggests that spirulina was well tolerated by goldfish and did not increase mortality risk under the experimental conditions. This finding is important because the use of feed additives in ornamental fish culture should not only improve color quality but also maintain fish health and survival.

These results are in line with Dewantari et al. (2024), who stated that spirulina supplementation plays a stronger role in improving color quality than in directly affecting survival or growth performance. Kargin & Dikbaş (2020) also reported that spirulina supplementation in goldfish feed contributed to pigmentation without producing negative effects on growth performance or survival. Similarly, Ahmed et al. (2023) found that spirulina-enriched feed treatments supported pigmentation and physiological performance in goldfish. Therefore, the survival rate observed in the present study supports the potential use of spirulina as a safe natural carotenoid supplement in goldfish feed.

Growth Performance

Fish growth during the study was evaluated based on increases in length and weight. The growth data are presented in Table 3.

Table 3. Growth performance of goldfish during the study

Treatment	Increase in Length (cm)	Increase in Weight (g)
P1 (control)	6.70 ± 0.87 ^a	2.79 ± 0.84 ^a
P2 (6%)	6.75 ± 1.26 ^a	2.84 ± 0.90 ^a
P3 (8%)	6.59 ± 1.23 ^a	2.86 ± 0.78 ^a
P4 (10%)	6.48 ± 0.78 ^a	3.07 ± 0.89 ^a

Note: Values followed by the same superscript letter in the same column are not significantly different ($p > 0.05$). \bar{x} = mean; SD = standard deviation.

The results showed that the mean increases in length and weight were relatively similar across all treatments. Statistical analysis indicated no significant differences among treatments in either length gain or weight gain ($p > 0.05$). This suggests that spirulina supplementation at the tested doses did not directly improve goldfish growth performance during the study period.

The lack of significant growth response may be explained by the functional role of spirulina in this study. Spirulina was mainly used as a natural carotenoid source to enhance pigmentation rather than as a primary protein or energy source. Although spirulina contains protein, essential amino acids, vitamins, minerals, and bioactive pigments, its inclusion as a supplement may not have been sufficient to substantially alter the overall nutritional balance of the diet. Fish growth is generally influenced more strongly by dietary protein quality, essential amino acid balance, energy availability, feeding rate, and environmental conditions. Therefore, when the basal feed already meets the nutritional requirements for growth, additional spirulina may have a more visible effect on pigmentation than on somatic growth.

This result supports the distinction between nutrients that primarily promote growth and pigments that primarily enhance coloration. The increase in color intensity observed in spirulina-supplemented treatments was not accompanied by a significant improvement in length or weight gain. Similar findings were reported by Andriani et al. (2018), who stated that spirulina supplementation was more effective in improving fish color quality than in increasing growth. Kargin & Dikbaşı (2020) also found that spirulina supplementation in goldfish feed contributed to skin pigmentation but did not significantly affect growth performance. However, other studies have reported growth improvement in different ornamental fish species, such as guppy and Oscar fish, when spirulina was used at specific inclusion levels, suggesting that the growth response to spirulina may depend on fish species, dose, feed formulation, and rearing duration.

Water Quality Conditions

Water quality was monitored to ensure that environmental conditions did not become a confounding factor influencing the study results. The observed parameters included temperature and pH, as presented in Table 4.

Table 4. Water quality parameters during the study

Treatment	Temperature (°C)	pH
P1	27	7.0
P2	26	6.8
P3	26	6.9
P4	26	6.9
Mean	26.25	6.9

Based on Table 4, water temperature during the study ranged from 26 to 27°C, while pH ranged from 6.8 to 7.0. These values indicate that the rearing environment remained relatively stable throughout the study. Stable water quality is important because environmental factors, particularly temperature and pH, can influence feeding activity, metabolism, physiological stress, survival, and pigment expression in fish.

The relatively stable water quality conditions were also reflected in the high survival rates observed across all treatments. Because temperature and pH were maintained within a narrow range, the differences in color intensity were unlikely to be caused primarily by environmental variation. Instead, the significant improvement in

color intensity was more strongly associated with the dietary supplementation of spirulina. This interpretation is consistent with previous studies showing that both diet and environmental conditions can influence pigmentation in ornamental fish, but controlled rearing conditions allow the dietary effect of carotenoid supplementation to be more clearly observed.

Overall, the results of this study demonstrate that spirulina supplementation significantly improved the color intensity of goldfish without negatively affecting survival, growth, or water quality conditions. The 8% dose produced the highest mean color intensity, although it was not statistically different from the 6% and 10% treatments. Therefore, spirulina supplementation at 6–8% may be considered an effective and efficient range for enhancing goldfish coloration under the conditions of this study.

CONCLUSION

This study demonstrated that spirulina supplementation as a natural carotenoid source in feed had a significant effect on enhancing the color intensity of goldfish (*Carassius auratus*). The best dose within the tested range was observed in the 8% treatment, which produced the highest color intensity compared with the other treatments. Increasing the dose to 10% did not result in an additional significant improvement in color intensity, indicating a tendency toward a diminishing return effect. Meanwhile, spirulina supplementation had no significant effect on fish growth or survival rate. Overall, the results indicate that spirulina is effective as a natural carotenoid source for improving fish color quality without compromising biological performance. These findings imply that the use of spirulina within an optimal dose range can serve as an efficient and safe strategy to enhance the aesthetic and economic value of goldfish in aquaculture.

RECOMMENDATION

Further studies are recommended to evaluate a wider range of spirulina powder doses to determine the optimum dose for improving color intensity in ornamental fish, particularly goldfish, with greater accuracy. Future research should also consider extending the rearing period and incorporating additional observation parameters, such as the physiological response of fish to carotenoid supplementation and pigment content in skin tissue.

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