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One Percent Dietary Spirulina (*Arthrospira platensis*) Improves Color Brightness and Growth Performance in Zebrafish (*Danio rerio*)

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ABSTRACT

Spirulina (*Arthrospira platensis*) is widely used in ornamental fish diets because it contains carotenoids, phycocyanin, protein, vitamins, and other bioactive compounds that may enhance pigmentation and support growth. However, the optimal dietary inclusion level of spirulina for zebrafish (*Danio rerio*) remains unclear, particularly when colour performance and growth responses are evaluated simultaneously. This study examined the effects of dietary spirulina supplementation on colour performance, growth, and physiological responses in zebrafish. A 30-day feeding trial was conducted using a completely randomized design with four treatments: 0%, 1%, 3%, and 5% spirulina inclusion, each with three replicate aquaria. Data were analyzed using one-way ANOVA, with Welch ANOVA and Kruskal–Wallis tests used to assess result robustness. Dietary spirulina significantly affected RGB brightness, length gain, and weight gain. The most consistent improvement occurred at 1% inclusion. The highest RGB brightness (83.81 ± 2.13), length gain (0.18 ± 0.01 cm), and weight gain (0.15 ± 0.03 g) were recorded in the 1% treatment, which outperformed the control and higher inclusion levels. Total colour score and specific growth rate also tended to peak at 1%, although these responses showed lower statistical stability under non-parametric analysis. Condition factor, survival, and abnormality were not significantly affected by dietary treatment. Overall, 1% dietary spirulina supplementation provided the most effective balance between improved colour appearance and growth performance, while higher inclusion levels did not produce additional benefits under the tested conditions.

INTRODUCTION

Body coloration is a key quality trait in ornamental fish production because visual appearance strongly influences consumer preference and market value. Fish body color is shaped by genetic background, chromatophore distribution, environmental conditions, health status, and dietary pigment availability (Lau *et al.*, 2023; Luo *et al.*, 2021; Sathyaruban *et al.*, 2021). Because fish generally cannot synthesize carotenoids de novo at levels sufficient to support external pigmentation, dietary carotenoids and pigment-rich feed ingredients are central to color-enhancement strategies (Nakano & Wiegertjes, 2020; Sathyaruban *et al.*, 2021). This has encouraged the use of natural feed additives, including microalgae, as alternatives to synthetic pigments in ornamental aquaculture (Alagawany *et al.*, 2021; Lau *et al.*, 2023; Ma & Hu, 2024).

Zebrafish (*Danio rerio*) is widely used as a model organism in developmental biology, toxicology, behavior, nutrition, and aquaculture research. Its small body size, short life cycle, well-described husbandry requirements, and distinct pigmentation pattern make it suitable for controlled feeding trials and color-related assessments (Aleström *et al.*, 2020; Baier & Scott, 2024; Del Vecchio *et al.*, 2022; Zarantoniello *et al.*, 2020). Feed management is particularly important because zebrafish growth and physiological responses are sensitive to diet composition, feeding regime, water quality, photoperiod, temperature, pH, and dissolved oxygen (Abdollahpour *et al.*, 2020; Aguilar *et al.*, 2022; Hillman *et al.*, 2024; Licitra *et al.*, 2024). This sensitivity is consistent with recent local aquaculture studies showing that growth, survival, and feed-utilization endpoints in ornamental fish and shrimp can shift substantially under sublethal environmental stressors, reinforcing the need to interpret dietary trials together with husbandry and water-quality controls (Saptatriani *et al.*, 2026; Setyono, Hapriawan, *et al.*, 2026; Setyono, Negara, *et al.*, 2026; Utomo *et al.*, 2026).

Spirulina, commonly referred to in applied aquaculture literature as *Arthrospira platensis*, is a protein-rich cyanobacterium containing essential amino acids, vitamins, minerals, phycocyanin, chlorophyll, and carotenoid compounds. Its nutritional and bioactive profile has generated substantial interest in microalgae-based aquafeeds and functional feed additives (Al-Jabri *et al.*, 2021; Alagawany *et al.*, 2021; ElFar *et al.*, 2022; Khandual *et al.*, 2021; Siddik *et al.*, 2023). Recent zebrafish studies indicate that spirulina supplementation or fish-meal replacement with spirulina can modulate growth, metabolism, antioxidant status, and reproductive performance (Carneiro *et al.*, 2025; Coli *et al.*, 2024; Flagiello *et al.*, 2026). Comparable growth- and health-promoting effects have also been reported in other aquaculture species, including grass carp, Nile tilapia, Pacific whiteleg shrimp, and ornamental guppy (Ashour *et al.*, 2024; Bisht *et al.*, 2023; Faheem *et al.*, 2022; Youssef *et al.*, 2023). In a broader rearing-system context, plankton and microalgal resources can also function as natural food and ecological indicators, although their biological value depends on community balance and media management rather than abundance alone (Haeriah *et al.*, 2026).

Despite this evidence, few studies have specifically evaluated spirulina as a color-enhancing additive in zebrafish, and dose-response patterns remain uncertain. In ornamental fish, increasing the inclusion level of a pigment-rich additive does not necessarily produce a proportional improvement in color because pigment deposition, feed palatability, nutrient balance, digestive capacity, and environmental stress can constrain biological responses (Elbahnaswy & Elshopakey, 2023; Li *et al.*, 2022; Luo *et al.*, 2021; Rana *et al.*, 2023). Therefore, this study evaluated the effects of graded dietary spirulina powder levels on zebrafish color brightness using both visual TCF scoring and digital RGB analysis, while also assessing growth, survival, abnormality, condition factor, and water quality. The central hypothesis was that dietary spirulina would enhance zebrafish color brightness, but that the optimal response would occur at a low or moderate inclusion level rather than at the highest tested dose.

METHODS

Experimental Site, Fish, and Design

The feeding trial was conducted for 30 days, from May 10th to June 10th 2026, at the Fish Production and Reproduction Laboratory, Aquaculture Study Program, Faculty of Agriculture, University of Mataram, Indonesia. Healthy and active zebrafish of approximately two months of age and relatively uniform size were used. The experiment followed a completely randomized design with four dietary treatments and three replicate rearing units per treatment, yielding 12 experimental units. The experimental unit was the rearing unit, not the individual fish, to avoid pseudoreplication in statistical interpretation.

Diet Preparation and Feeding

A commercial floating pellet for juvenile fish was used as the basal diet. Based on the label information recorded in the source thesis, the commercial diet contained at least 35% crude protein, at least 5% crude lipid, a maximum of 3% crude fiber, a maximum of 13% ash, and a maximum of 12% moisture. Spirulina powder (*Arthrospira platensis*) was incorporated into the commercial feed at four inclusion levels: P0 = commercial diet without spirulina; P1 = 100 g commercial feed + 1 g spirulina powder; P2 = 100 g commercial feed + 3 g spirulina powder; and P3 = 100 g commercial feed + 5 g spirulina powder. Spirulina powder was first suspended in a small amount of clean water, gradually mixed into the feed, dried at room temperature in the shade, and stored in closed containers until use. Fish were fed three times daily, and uneaten feed and feces were removed periodically by siphoning.

The spirulina inclusion levels were selected to represent a graded dietary supplementation range for evaluating colour and growth responses in zebrafish. The 1% inclusion level was based on previous ornamental-fish studies showing that low dietary spirulina supplementation can improve colour-related traits without impairing growth. For example, spirulina levels of 1%, 1.5%, and 2% were tested in betta fish, with the strongest colour response reported at 1.5% inclusion (Saputra *et al*., 2023). Higher inclusion levels of 1%, 2%, and 3% have also been evaluated in polar blue cichlid, where 3% dietary spirulina improved colour quality, although growth responses were not significantly affected (Azril *et al*., 2024). Therefore, the present study used 1%, 3%, and 5% spirulina supplementation to cover a low dose, an intermediate dose supported by previous ornamental-fish research, and a higher exploratory dose. This design enabled assessment of whether increasing spirulina inclusion produced a linear improvement in zebrafish colouration and growth or whether an optimal inclusion level occurred within the tested range.

Table 1. Research Treatment

Treatment	Diet Composition	Spirulina Inclusion
P0	Commercial diet without spirulina	0%
P1	100 g commercial diet + 1 g spirulina powder	1%
P2	100 g commercial diet + 3 g spirulina powder	3%
P3	100 g commercial diet + 5 g spirulina powder	5%

Color and Growth Measurements

Color brightness was assessed using two complementary approaches. First, Toca Colour Finder (TCF) scoring was used as a semi-quantitative visual method by matching the body color of fish to the nearest TCF standard. Second, digital image analysis was used to calculate RGB brightness. Fish were photographed under the most consistent setup possible, and the same lateral body region was sampled using Adobe Photoshop. Red (R), green (G), and blue (B) values were averaged into a brightness index using $I = (R + G + B)/3$. Because digital color readings are affected by lighting, background, camera distance, exposure, and white balance, the RGB result is interpreted as an internally standardized brightness index rather than as an absolute colorimetric value.

Absolute length gain was calculated as the final mean length minus the initial mean length. Absolute weight gain was calculated as the final mean body weight minus the initial mean body weight. Specific growth rate (SGR) was calculated as $SGR = [(\ln Wt - \ln W0)/t] \times 100$, where Wt is the final body weight, W0 is the initial body weight, and t is the 30-day rearing period. Condition factor, survival, abnormality, and water-quality variables were recorded as supporting indicators.

Water Quality

Water-quality parameters were monitored throughout the 30-day feeding trial to confirm that culture conditions remained suitable and comparable among treatments. Temperature, pH, and dissolved oxygen were measured once every 7 days, resulting in five measurement occasions: day 0, day 7, day 14, day 21, and day 30. Ammonia, nitrite, and nitrate were measured twice, namely at the beginning of the experiment (day 0) and at the end of the experiment (day 30). Temperature was measured using a thermometer, pH using a pH meter, dissolved oxygen using a DO meter, and nitrogen compounds using commercial water-quality test kits.

Water-quality variables were treated as supporting data and interpreted descriptively because the experimental design evaluated dietary spirulina treatments rather than water-quality treatments. Nevertheless, the recorded values were considered during interpretation because temperature, pH, dissolved oxygen, ammonia, nitrite, and nitrate can influence fish physiology, feeding behavior, growth, and stress responses (Jannat *et al*., 2024; Kong *et al*., 2020; Lin *et al*., 2024; Mariz *et al*., 2023).

Statistical Analysis and Data Verification

Before manuscript preparation, the unit-level numerical data in the thesis appendices were independently reconstructed and verified. Descriptive statistics were recalculated for all response variables at the aquarium level (n = 3 replicate units per treatment). One-way ANOVA was used as the primary inferential model. Because replication was limited, Welch ANOVA was applied as a robustness check for unequal variances, and Kruskal-Wallis was used as a non-parametric sensitivity analysis. Tukey HSD was used to inspect pairwise differences under the ordinary ANOVA model. Shapiro-Wilk tests on ANOVA residuals and Levene-type variance checks were used as diagnostic audits rather than as rigid inclusion criteria. Statistical significance was considered at $p < 0.05$, but final interpretation prioritized convergence among tests, effect-size patterns, and biological plausibility.

RESULTS

Descriptive and Inferential Summary

Table 2. Results of Research Parameters

Variable	P0	P1	P2	P3	Statistical Interpretation
TCF score	3472 ± 286	4135 ± 467	3343 ± 52	3373 ± 50	ANOVA p = 0.024; Welch p = 0.218; supportive only
RGB brightness index	64.52 ± 5.83	83.81 ± 2.13	72.07 ± 1.29	72.19 ± 5.03	ANOVA p = 0.003; Welch p = 0.008; robust
Length gain (cm)	0.09 ± 0.01	0.18 ± 0.01	0.15 ± 0.01	0.14 ± 0.02	ANOVA p < 0.001; Welch p = 0.001; robust
Weight gain (g)	0.04 ± 0.01	0.15 ± 0.03	0.05 ± 0.03	0.05 ± 0.01	ANOVA p < 0.001; Welch p = 0.016; robust
SGR (% day^{-1})	0.59 ± 0.21	1.22 ± 0.02	0.50 ± 0.45	0.39 ± 0.26	ANOVA p = 0.027; Welch p = 0.020; Kruskal p = 0.091
Condition factor	2.02 ± 0.45	2.23 ± 0.12	1.95 ± 0.64	1.81 ± 0.05	No convincing treatment effect
Survival (%)	86.67 ± 5.77	86.67 ± 5.77	90.00 ± 0.00	80.00 ± 10.00	No convincing treatment effect
Abnormality (%)	16.67 ± 5.77	20.00 ± 10.00	13.33 ± 5.77	16.67 ± 5.77	No convincing treatment effect

Values are presented as mean ± SD (n = 3 experimental units per treatment). The final column of Table 2 summarizes the main inferential outcome using ordinary ANOVA and robustness checks. TCF = Toca Colour Finder; RGB = red-green-blue brightness index; SGR = specific growth rate.

Color Brightness

Dietary spirulina affected zebrafish color response, but the strength of evidence differed between color metrics. RGB brightness showed the clearest and most reproducible treatment effect (ANOVA F = 11.61, p = 0.003; Welch p = 0.008; eta-squared = 0.81). The highest RGB brightness was observed in P1 (83.81 ± 2.13), whereas P0, P2, and P3 remained lower. Tukey HSD confirmed that P1 was significantly higher than P0 and P2, while P2 and P3 did not differ. TCF score followed the same biological pattern, with P1 producing the highest mean value (4135.33 ± 466.97), followed by P0 (3472.33 ± 285.54), P3 (3373.00 ± 50.23), and P2 (3343.00 ± 51.96). However, the TCF result was less robust statistically: ordinary ANOVA was significant (F = 5.50, p = 0.024; eta-squared = 0.67), whereas Welch ANOVA (p = 0.218) and Kruskal-Wallis (p = 0.081) did not confirm the effect at alpha = 0.05. Accordingly, RGB analysis is treated as the primary evidence for color improvement, while TCF is interpreted as supportive but more tentative evidence.

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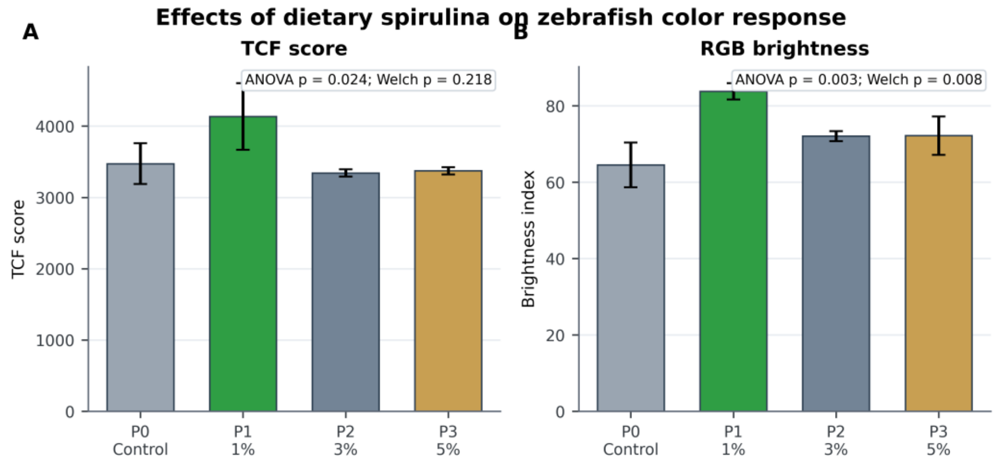

Figure 1. Effects of Dietary Spirulina on Zebrafish Color Response. Panel A Shows TCF Score and Panel B Shows RGB Brightness. Bars Represent Mean ± SD (n = 3 Replicate Aquaria per Treatment)

Growth Performance and Biological Condition

Length gain, weight gain, and SGR were all highest in the 1% spirulina treatment. Length gain differed strongly among treatments (ANOVA F = 39.08, p < 0.001; Welch p = 0.001; Kruskal-Wallis p = 0.017; eta-squared = 0.94), with mean values of 0.18 ± 0.01 cm in P1, 0.15 ± 0.01 cm in P2, 0.14 ± 0.02 cm in P3, and 0.09 ± 0.01 cm in P0. Weight gain also differed significantly (ANOVA F = 20.81, p < 0.001; Welch p = 0.016; eta-squared = 0.89), with P1 producing the highest gain (0.15 ± 0.03 g) compared with P0 (0.04 ± 0.01 g), P2 (0.05 ± 0.03 g), and P3 (0.05 ± 0.01 g). SGR was likewise highest in P1 (1.22 ± 0.02% day-1) and was significant in both ordinary and Welch ANOVA (ANOVA F = 5.27, p = 0.027; Welch p = 0.020; eta-squared = 0.66), although the Kruskal-Wallis test did not reach the 0.05 threshold (p = 0.091). By contrast, condition factor (p = 0.639), survival (p = 0.349), and abnormality (p = 0.728) showed no convincing treatment effects.

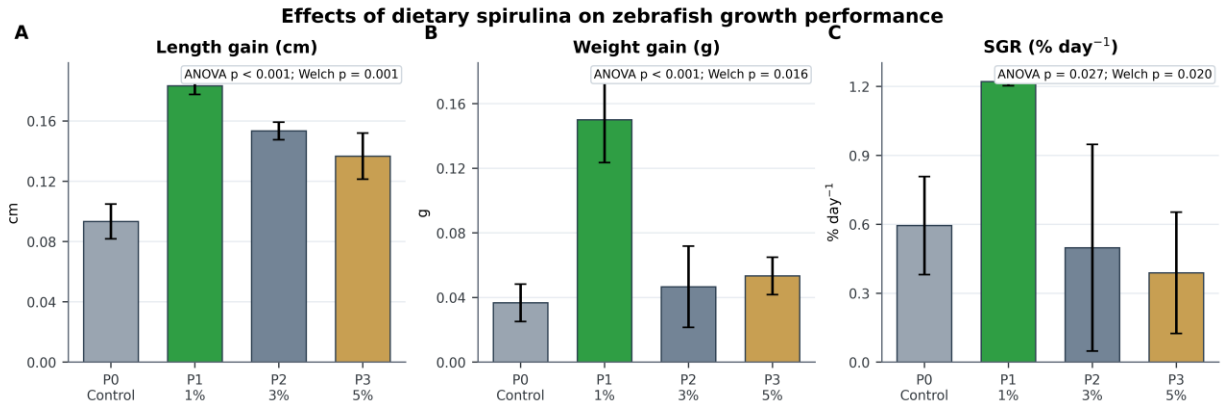

Figure 2. Effects of Dietary Spirulina on Zebrafish Growth Performance. Panel A Shows Length Gain, Panel B Shows Weight Gain, and Panel C Shows Specific Growth Rate (SGR). Bars Represent Mean ± SD (n = 3 Replicate Aquaria per Treatment)

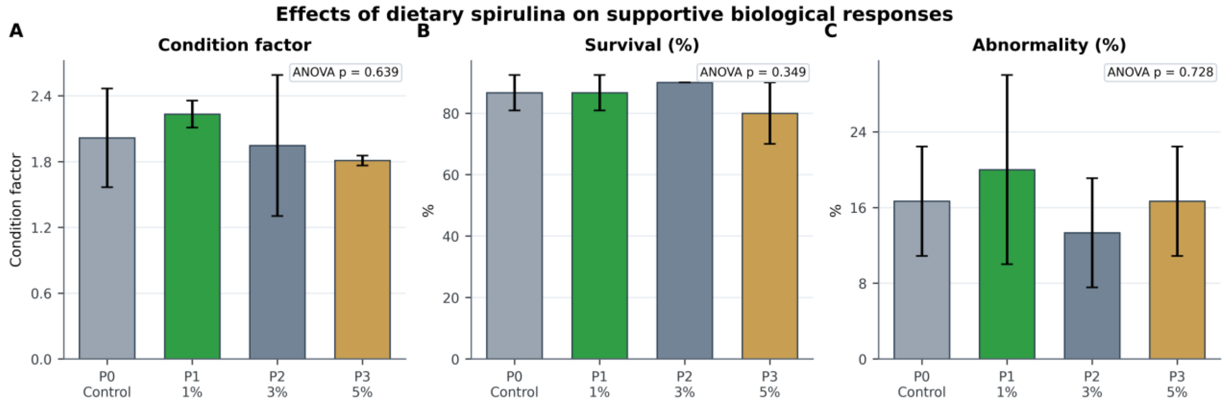

Figure 3. Effects of Dietary Spirulina on Supportive Biological Responses. Panel A Shows Condition Factor, Panel B Shows Survival, and Panel C Shows Abnormality. Bars Represent Mean ± SD (n = 3 Replicate Aquaria per Treatment)

Water Quality

Water quality was interpreted descriptively because it was not an experimental factor. Temperature remained narrow across treatments (26.7-27.6°C), and dissolved oxygen increased from 5.7-6.0 mg L^{-1} at the start to 6.6-6.9 mg L^{-1} at the end of the trial. Final pH ranged from 6.0 to 7.0, with the lowest value recorded in P3. Ammonia was <=0.25 mg L^{-1} at the beginning and below or near the test-kit threshold at the end. Nitrite decreased markedly from 2.0-4.0 mg L^{-1} initially to 0.1-0.5 mg L^{-1} at the final observation, while nitrate decreased from 25-100 mg L^{-1} initially to 5-25 mg L^{-1} at the end. These values suggest that the system remained biologically functional, although nitrogenous compounds and pH should be controlled more tightly in future trials to isolate dietary effects more clearly.

Table 3. Water Quality

Parameter	P0 Initial/Final	P1 Initial/Final	P2 Initial/Final	P3 Initial/Final	Interpretive Note
Temperature (°C)	27.4 / 26.9	27.2 / 27.1	27.5 / 26.7	27.6 / 27.0	Generally, within zebrafish range
DO (mg L^{-1})	5.9 / 6.6	6.0 / 6.8	5.8 / 6.9	5.7 / 6.8	Acceptable but should remain stable
pH	7.4 / 7.0	7.5 / 6.5	7.4 / 6.7	7.5 / 6.0	P3 final pH was low
Ammonia (mg L^{-1})	<0.15 / <0.15	<0.15 / <0.15	0.25 / <0.15	0.25 / <0.15	Initial P2-P3 ammonia requires caution
Nitrite (mg L^{-1})	2.0 / 0.1	4.0 / 0.5	2.0 / 0.5	4.0 / 0.5	Initial nitrite was high
Nitrate (mg L^{-1})	100 / 25	50 / 5	25 / 25	50 / 25	Initial nitrate was high in P0-P1-P3

DISCUSSION

The verified dataset indicates that the most dependable response to dietary spirulina occurred at the 1% inclusion level. Among the color-related variables, RGB brightness provided the strongest evidence because the effect remained significant in both ordinary and Welch ANOVA and clearly separated P1 from the control treatment. This finding supports the view that low-dose spirulina can enhance visible appearance in ornamental fish by supplying carotenoid-rich biomass and other bioactive compounds that contribute to pigment expression and physiological quality (Alagawany *et al.*, 2021; Lau *et al.*, 2023; Ma & Hu, 2024; Sathyaruban *et al.*, 2021).

The TCF result followed the same biological direction, but its inferential strength was weaker. The highest TCF score was also observed in P1, yet the result was sensitive to the statistical method and was not confirmed by the robustness checks. This distinction is important for manuscript interpretation. TCF is a practical field-oriented tool, but it is observer-dependent and semi-quantitative. In contrast, RGB analysis remains methodologically imperfect but is more reproducible when lighting, sampling region, and image processing are standardized. Therefore, this study frames RGB brightness as the primary color endpoint and TCF as corroborative evidence rather than as stand-alone proof of a dietary effect.

Growth responses reinforce the practical relevance of the 1% treatment. Length gain and weight gain increased consistently in P1, and both variables remained significant in the robustness analyses. SGR followed the same directional pattern and was also significant in the parametric models, although the non-parametric test was slightly above the conventional threshold. Collectively, these outcomes indicate that the color improvement observed in P1 was not achieved at the expense of growth. This is relevant for ornamental aquaculture because feed additives should improve visual quality without compromising biomass gain or general condition (Al-Jabri *et al.*, 2021; Siddik *et al.*, 2023).

The lack of additional benefit at 3% and 5% indicates a non-linear dose response. This pattern is biologically plausible. Moderate spirulina inclusion may optimize pigment availability and nutrient utilization, whereas higher inclusion may reduce palatability, alter nutrient balance, or exceed the capacity for efficient pigment absorption and deposition (Ashour *et al.*, 2024; Bisht *et al.*, 2023; Li *et al.*, 2022; Rana *et al.*, 2023; Youssef *et al.*, 2023). The present findings therefore support an empirical optimum near 1% spirulina under the tested husbandry conditions, rather than the assumption that higher spirulina inclusion necessarily produces a stronger response.

Condition factor, survival, and abnormality were not significantly altered by dietary treatment, suggesting that spirulina supplementation at the tested levels did not produce clear short-term adverse effects. Nevertheless, the trial lasted only 30 days and included three replicate aquaria per treatment. In addition, feed intake, feed conversion ratio, tissue carotenoid deposition, and molecular markers of pigmentation were not measured. These limitations constrain mechanistic interpretation and reduce the ability to detect subtler biological effects. They also justify a conservative reading of non-significant supporting variables, because related aquaculture experiments have shown that sublethal stressors may affect growth, feeding efficiency, behaviour, or survival before all general condition indicators respond in the same direction (Saptatriani *et al.*, 2026; Setyono, Negara, *et al.*, 2026; Utomo *et al.*, 2026).

Water quality and color-measurement methodology remain important caveats. Temperature and dissolved oxygen were generally acceptable for zebrafish, but pH reached 6.0 in P3 and nitrogenous compounds varied considerably between the initial and final observations. Such variation can influence fish performance and may confound diet effects when monitoring is infrequent. Recent regional studies by Setyono and collaborators strengthen this caution: waterborne PVC and polystyrene microplastics reduced survival, growth, feed utilization, or behavioural performance in guppy, betta fish, and whiteleg shrimp, while several experiments reported that the observed responses were more plausibly linked to the tested stressor than to overt water-quality deterioration (Saptatriani *et al.*, 2026; Setyono, Hapriawan, *et al.*, 2026; Setyono, Negara, *et al.*, 2026; Utomo *et al.*, 2026). Work on halophilic bacteria in Artemia culture further illustrates the importance of separating biological treatment effects from media-management effects when interpreting aquaculture responses (Al Humaero *et al.*, 2026). Future studies should use tighter control of nitrogenous waste, more frequent water-quality monitoring, larger replication, and standardized color-calibrated imaging or instrumental colorimetry, such as CIE L*a*b* measurements, to strengthen external validity and publication value.

CONCLUSION

In this 30-day zebrafish feeding trial, 1% dietary spirulina was the most effective inclusion level. The strongest evidence was observed for RGB brightness, length gain, and weight gain, all of which improved most clearly in P1 and remained significant in the verification analyses. TCF score and SGR also reached their highest mean values in P1, but the TCF result was sensitive to test choice and SGR should be interpreted with moderate caution because the non-parametric comparison was not significant. Condition factor, survival, and abnormality were not significantly affected. These findings support low-dose spirulina as a promising natural additive for ornamental zebrafish while indicating that future studies should incorporate larger replication, stricter color calibration, and tighter water-quality control.

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