

Red dragon fruit peel as a natural pigment enhancer in goldfish (*Carassius auratus*)

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Abstract. Dahlia, Ardiansyah, Amrullah, Khatimah K, Dwinhoven I, Mega DAU. 2025. Red dragon fruit peel as a natural pigment enhancer in goldfish (*Carassius auratus*). *Asian J Agric* 9: 777-786. Color quality enhancement is an important factor in the ornamental fish industry, particularly for goldfish (*Carassius auratus*), as it directly affects market value and consumer preference. The use of carotenoid-based feed additives offers a sustainable alternative for improving the visual appearance of fish. Red dragon fruit (*Hylocereus polyrhizus*) peel, which is generally discarded as agro-industrial waste, is known to contain natural carotenoids, particularly betacyanin and beta-carotene, which have potential applications in aquaculture. This study aimed to evaluate the effectiveness of Red Dragon Fruit Peel Extract (RDFPE) in improving the color brightness of goldfish, along with related physiological parameters. A completely randomized design was used, consisting of five RDFPE dosage treatments (0, 50, 100, 150, and 200 mL/kg of feed), each with three replicates. The measured parameters included color brightness level, total carotenoid concentration, chromatophore cell count, growth, survival rate, and water quality. The results showed that RDFPE had a significant effect ($p < 0.05$) on color brightness level, with the optimum response observed at 150 mL/kg, followed by a decline at higher dosages. In contrast, RDFPE had no significant effect ($p > 0.05$) on growth or survival rate. Total carotenoid concentration and chromatophore cell count were reported descriptively (mean \pm SD) without statistical analysis, indicating that differences among treatments for these parameters require further confirmation. Water quality remained within the optimal range. These findings highlight the potential of RDFPE as a natural and eco-friendly feed additive for enhancing ornamental fish coloration while promoting the sustainable utilization of agro-industrial waste.

Keywords: *Carassius auratus*, carotenoids, chromatophore cells, color brightness, red dragon fruit peel extract

INTRODUCTION

Coloration plays a crucial role in determining the aesthetic and commercial value of ornamental fish, especially in goldfish (*Carassius auratus*), one of the most widely cultivated species in the global ornamental fish trade. Intense and stable pigmentation is not only a desirable visual trait but is also closely linked to consumer preference, market competitiveness, and economic return for producers. Pigment expression in fish is shaped by a combination of genetic, nutritional, physiological, and environmental factors, with dietary carotenoids being one of the most influential determinants of color quality (Saikia and Das 2023). Because fish are incapable of synthesizing carotenoids *de novo*, exogenous dietary sources become essential for developing red, orange, and yellow hues (Elbahnaswy and Elshopakey 2024). Ensuring consistent access to high-quality pigment sources is therefore critical for maintaining coloration across different production cycles and ensuring uniformity in commercial batches.

Carotenoids are absorbed through the intestinal epithelium, transported via lipoproteins, and deposited into chromatophores, particularly erythrophores and xanthophores, which regulate body coloration through pigment accumulation and dispersion (Duan et al. 2024). While synthetic carotenoids such as canthaxanthin and

astaxanthin have been widely utilized in aquaculture, concerns related to cost, long-term safety, and consumer acceptance have driven a shift toward natural pigment sources (Elbahnaswy and Elshopakey 2024). Natural pigments derived from plants, algae, and microbial biomass have gained attention due to their bioavailability, multifunctional antioxidant roles, and alignment with sustainable aquaculture practices (Aizpuru and González-Sánchez 2024). In addition to improving coloration, these natural compounds often provide complementary physiological benefits that support immune resilience and overall fish vitality, making them increasingly favorable options for producers seeking sustainable inputs.

In recent years, tropical fruit by-products have emerged as promising pigment sources because of their rich phytochemical compounds and year-round availability in Southeast Asia. Red dragon fruit (*Hylocereus polyrhizus*) peel is among the most abundant agro-industrial wastes, comprising up to 30-35% of total fruit mass (Corimayhua-Silva et al. 2024). Its peel contains carotenoids, betacyanins, polyphenols, and flavonoids that offer both pigmentation and antioxidant benefits. Betacyanins, in particular, provide red-purple coloration and possess higher stability than anthocyanins under moderate processing conditions, making them attractive for aquaculture feed applications (Nurhadi et al. 2024). Although dragon fruit

flesh has been widely studied for food and health uses, the utilization of its peel as a functional feed additive in ornamental fish remains limited, with most studies focusing on terrestrial animal models or food industry applications. This highlights the need to explore its potential role in aquatic species, especially those in the ornamental sector, where visual appearance is a primary determinant of value.

Valorizing agricultural waste through incorporation into aquaculture feed supports circular economy principles and enhances sustainability by reducing production costs and waste disposal issues. In ornamental aquaculture, which heavily relies on aesthetic traits and market-driven visual standards, innovative approaches that combine waste utilization and pigment enhancement offer significant ecological and economic benefits. Such strategies also help diversify feed ingredient sources, reducing dependence on imported or synthetic compounds, and potentially providing more stable long-term supply chains for small and medium-scale hatcheries.

However, despite its promising bioactive composition, scientific evidence on the dose–response relationship of Red Dragon Fruit Peel Extract (RDFPE) in fish pigmentation remains scarce. Important aspects such as carotenoid deposition efficiency, chromatophore response, physiological tolerance, and possible growth trade-offs require empirical verification. Furthermore, discrepancies between visual pigmentation and biochemical carotenoid concentration reported in previous studies suggest that pigment enhancement may involve not only carotenoid accumulation but also changes in chromatophore density, pigment redistribution, oxidative status, and cellular protection mechanisms (Lim et al. 2023). Understanding these dynamics is essential to determine whether the observed improvements in coloration result from direct pigment incorporation or from broader physiological adjustments within the integumentary system.

Thus, this study aims to evaluate the effect of different RDFPE dosages on multiple pigmentation-related parameters in goldfish, including color brightness, total carotenoid concentration, and chromatophore cell density. Complementary indicators such as growth, survival rate, and water quality are also assessed to determine the extract's safety and functional suitability as a natural pigment additive. The findings from this research are expected to provide a scientific basis for developing eco-friendly, cost-effective, and sustainable ornamental fish feeds using agro-industrial by-products. By integrating biological responses with practical considerations, the study contributes to advancing sustainable ornamental aquaculture practices.

MATERIALS AND METHODS

A collection of fish

Goldfish (*C. auratus*), aged 30 days, measuring 2 cm in length and weighing 0.4 g, were collected from the ornamental fish breeding business at Jl. Kancil 3, Mamajang Luar, Mamajang District, Makassar, South Sulawesi, Indonesia. The fish were acclimatized for 14

days in a 1,500-liter rearing container and were fed a commercial diet at a rate of 3% of their biomass weight per day, with feeding occurring three times daily. Water quality was maintained under optimal conditions, with a temperature range of 22–25°C and a pH of 6.5–7.5. During the acclimatization period, no diseases were observed, and there was no mortality, making the fish ready for the feeding trial. This acclimatization procedure followed the general guidelines for fish acclimatization in research (Gonzalez 2023).

Red dragon fruit peel extraction

The red dragon fruit (*H. polyrhizus*) used in this study was ripe, fresh, and large (approximately 500 g), sourced from a local fruit store in Makassar. The peel was separated from the flesh, thoroughly washed, and cleaned of the outer scales, then cut into small pieces. The extraction process followed the method of Nizori et al. (2020) with minor modifications to improve reproducibility.

A total of 50 grams of dragon fruit peel was mixed with distilled water acidified with 2% citric acid (v/v), using a material-to-solvent ratio of 1:4 (w/v). The mixture was blended for 30 seconds until homogeneous, then macerated in a dark bottle for 48 hours at room temperature ($\pm 27^\circ\text{C}$) to prevent pigment degradation due to light exposure. After maceration, the solution was filtered using gauze and then centrifuged for 5 minutes at 4,000 rpm to remove coarse sediment. The supernatant was further filtered using Whatman No. 41 filter paper, and the resulting filtrate was used as the Red Dragon Fruit Peel Extract (RDFPE).

To ensure extract consistency, the pH of each RDFPE batch was measured (with an average pH of 4.2–4.5) as a stability parameter. Although carotenoid content was not quantified using HPLC in this study, previous references (Arivalagan et al. 2021; Saenjum et al. 2021) indicate that red dragon fruit peel contains carotenoids, betacyanin, and polyphenols with bioactive properties relevant to functional feed applications. The use of spectrophotometric or HPLC methods is recommended for future research to enable more precise and comparative pigment quantification. The resulting filtrate was the Red Dragon Fruit Peel Extract (RDFPE), ready to be mixed with the experimental feed.

Experimental diets

The feed used in this study was a commercial pellet feed with a small pellet size, containing 30% protein, 3% fat, 4% fiber, 12% ash, as well as added vitamins and minerals. A protein content of 30% is consistent with the nutritional requirements of omnivorous ornamental fish such as goldfish (*C. auratus*), which recommends a protein level of around 30% (Sales and Janssens 2003). RDFPE supplementation was applied to the feed using a spraying method to ensure even distribution of the extract across the surface of the pellets. Each RDFPE dosage for the treatments was first diluted with boiled water to reach a total volume of 50 mL per kilogram of feed. This volume was chosen based on technical considerations to ensure uniform application without making the pellets overly moist or physically damaged.

The RDFPE solution was then transferred into a manual sprayer and evenly sprayed onto pre-weighed commercial pellet feed. The dosages for each treatment were as follows:

- Treatment A (Control): 0 mL RDFPE
- Treatment B: 10 mL RDFPE + 40 mL water/kg feed
- Treatment C: 20 mL RDFPE + 30 mL water/kg feed
- Treatment D: 30 mL RDFPE + 20 mL water/kg feed

After spraying, the feed was thoroughly mixed using a clean spatula to ensure the solution adhered evenly to the surface of the pellets. The processed feed was then air-dried for 24 hours in a shady place to reduce moisture and stabilize the active compounds, particularly carotenoids, which are sensitive to light and high temperatures. Once dried, the feed was stored in sealed containers at room temperature and used according to the feeding schedule.

Fish culturing conditioning

The experiment was conducted in two stages using the same maintenance procedures to avoid the effects of population decline in each experimental unit due to sampling. Stage 1 focused on measuring color brightness, chromatophore cell count in skin tissue, growth, and survival rate. Stage 2 focused on measuring the total carotenoid concentration in the fish body.

Each container was filled with 20 liters of water. A total of 240 fish were distributed into 12 containers at a stocking density of 1 fish per liter and maintained for 45 days. This rearing density refers to previous research showing that low to medium densities still support optimal growth and survival in both ornamental and food fish (Saraiva et al. 2022). During the rearing period, fish were fed according to the assigned treatments at a daily feeding rate of 3% of biomass, divided into three feeding times (morning, midday, and afternoon). This feeding procedure is consistent with common practices in intensive fish rearing in laboratories (Duan et al. 2025). To maintain water quality parameters, uneaten feed and feces were siphoned out, and water was partially replaced on a regular basis (once a week).

Color brightness

The goldfish's color brightness was measured at the beginning of the study (day 0) and the end of the study (day 45) using a color code scale on the indicator tool (Haser 2015). The color indicator was modified to resemble the Tocca Colour Finder (Figure 1).

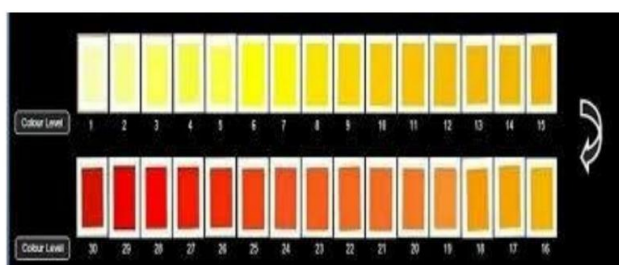


Figure 1. Tocca colour finder

Color brightness was measured visually by selecting five fish from each experimental unit and matching their coloration to the closest shade on a color indicator device, which had a scale ranging from 1 to 30. Scoring was performed to assess changes in fish coloration over time or in response to the treatments applied.

Total carotenoid concentration

The total carotenoid concentration in the bodies of goldfish was analyzed every week using spectrophotometry. Two fish from each experimental unit were caught with a scoop net and then anesthetized using cold temperatures.

To measure the total carotenoid concentration, a tissue sample of ± 40 -50 mg was weighed, put into a test tube, and then 5 mL of acetone was added. The mixture was homogenized until fully dissolved. More acetone was added to bring the volume to 10 mL, and the solution was stirred for several minutes to ensure uniformity. The homogenized solution was then filtered using Whatman paper. The solution was analyzed using a spectrophotometer at wavelengths of 380, 450, 475, and 500 nm. The highest result was used to calculate the total carotenoid value (Sukarman et al. 2014).

Number of chromatophore cells

The number of chromatophore cells in the skin tissue of goldfish was counted at the end of the experiment using histological preparations. Three fish were randomly selected from each treatment unit and anesthetized using a cold temperature method (ice-bath shock) until loss of motor response. A section of dorsal skin tissue (approximately 0.5×0.5 cm) was then taken from the upper posterior dorsal region and fixed in 10% buffered formalin for 24 hours.

The fixed tissues were dehydrated through a graded ethanol series (70%, 80%, 90%, 95%, and absolute), followed by clearing with xylene and embedding in paraffin. The paraffin blocks were sectioned using a microtome at a thickness of 5 μ m. Tissue sections were mounted on glass slides and stained using the Hematoxylin–Eosin (H&E) method following the guidelines of Ferdous et al. (2024). This technique was used to reveal the general structure of the skin tissue and to visually identify chromatophore cells, particularly erythrophores and xanthophores, based on their morphology and distribution within the epidermal and dermal layers.

Observations were performed under a light microscope at 400x magnification. For each fish sample, three non-overlapping random fields of view from the epidermal region were examined. Chromatophores were manually counted in each field, and the average from the three fields was recorded as the individual value. The individual values were then averaged across fish within each treatment unit for further statistical analysis.

Growth performance

The absolute growth of goldfish was measured at the end of the study (week 6) using the formula from Effendie (2002):

$$W_m = W_t - W_o$$

Where:

W_m: Absolute weight growth (grams)

W_t: Biomass weight at the end of the study (grams)

W_o: Biomass weight at the beginning of the study (grams)

Survival rate

The survival rate of goldfish (*C. auratus*) is calculated at the end of the study using the formula from Effendie (2002):

$$SR = N_t / N_0 \times 100$$

Where:

SR: Survival Rate (%)

N_t: Number of fish at the end of the study (individuals)

N₀: Number of fish at the start of the study (individuals)

Water quality parameters

Water quality parameters were measured twice a day (at 08:00 a.m. and 4:00 p.m.), including temperature using a thermometer, pH using a pH meter, and dissolved O₂ using a DO meter. The ammonia (NH₃) concentration was measured once a week using an ammonia test kit.

Data analysis

The data on color brightness, total carotenoid concentration, growth rate, and survival rate of goldfish were statistically analyzed using SPSS version 23.0. A one-way ANOVA was used to test the significance at the 95% confidence level, followed by Duncan's test if significant differences were found. The number of chromatophore cells in the skin tissue and the water quality data were analyzed descriptively.

RESULTS AND DISCUSSION

Color brightness level

Supplementation of Red Dragon Fruit Peel Extract (RDFPE) significantly affected the color brightness level of goldfish ($p < 0.05$). At the beginning of the study, the color brightness levels across all treatments were relatively uniform. However, at the end of the study, treatments B, C, and D showed higher color brightness levels compared to the control treatment (A) (Figure 2). The highest color brightness was achieved in treatment D (24.0), followed by treatment C (21.7), then treatment B (19.3), and the lowest in treatment A (15.7). Overall, there was a significant increase in color brightness of 83.2% in treatment D, 64.4% in treatment C, and 47.3% in treatment B. Treatment A did not show a significant increase, only 19.8%.

Total carotenoid concentration

The total carotenoid concentration in the bodies of goldfish showed an increasing trend from the first week to the sixth week in all treatments. The average carotenoid concentration values at week six reached 0.370 $\mu\text{mol/g}$ (treatment B), 0.375 $\mu\text{mol/g}$ (C), and 0.400 $\mu\text{mol/g}$ (D), compared to 0.350 $\mu\text{mol/g}$ in the control (A). Although not statistically significant ($p > 0.05$), the increase in carotenoids in treatments B, C, and D was 5.71%, 7.14%, and 6.67% higher, respectively, compared to the control (Figure 3).

Number of chromatophore cells

The number of chromatophore cells in the skin tissue of goldfish showed differences among treatments. There was a tendency for a higher number of chromatophore cells in fish supplemented with RDFPE (treatments B, C, and D) compared to the control treatment (A) (Figure 4).

Absolute growth

The absolute growth of goldfish during the study ranged from 3.75 cm to 3.77 cm (Figure 5). No significant differences were found in absolute growth among treatments ($p > 0.05$), indicating that the supplementation of RDFPE in the feed did not hinder the fish's growth.

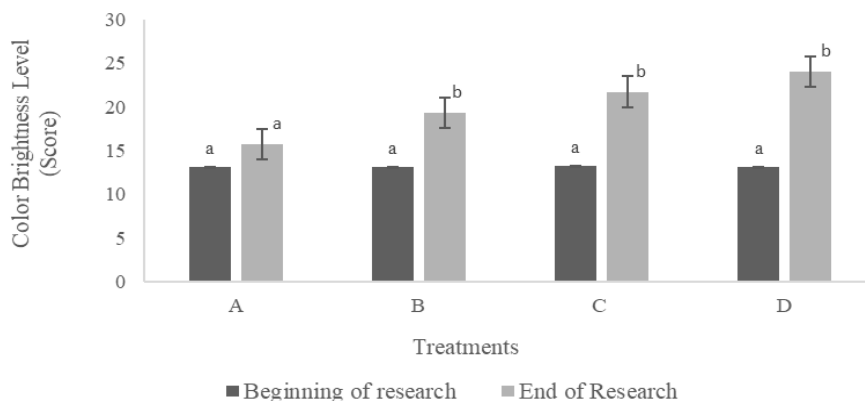


Figure 2. Color brightness score of goldfish (*C. auratus*) at various RDFPE doses at the beginning and end of the study. A: Control (0 mL RDFPE/kg feed), B: 10 mL/kg, C: 20 mL/kg, D: 30 mL/kg. Values are presented as mean \pm SD ($n=3$). Error bars represent Standard Deviation (SD). Different letters above the bars indicate significant differences among treatments ($p < 0.05$; Duncan's test)

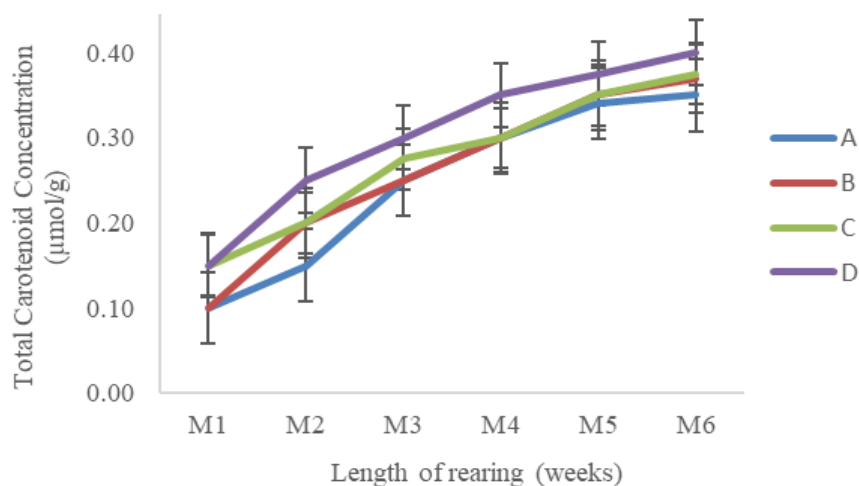


Figure 3. Profile of total carotenoid concentration ($\mu\text{mol/g}$) in goldfish (*C. auratus*) at various RDFPE doses during a 6-week rearing period (M1–M6). A: control (0 mL RDFPE/kg feed), B: 10 mL/kg, C: 20 mL/kg, and D: 30 mL/kg. Error bars represent standard deviation (SD). No significant differences were observed among treatments ($p>0.05$)

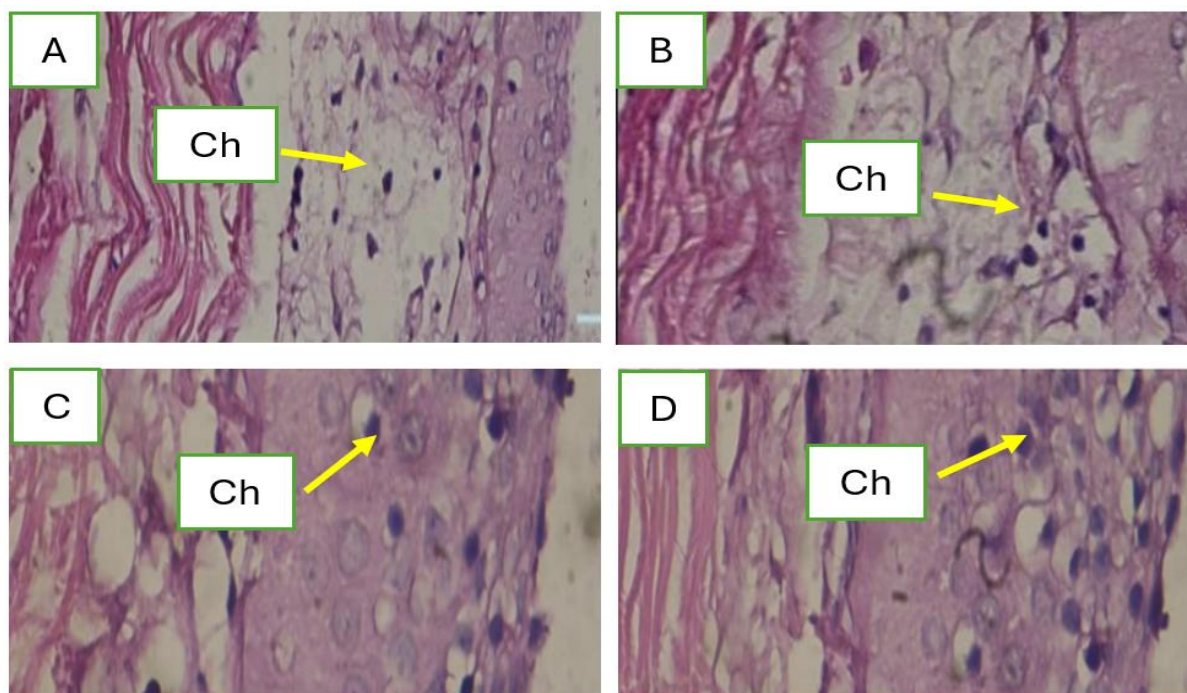


Figure 4. Histological micrographs of chromatophore cells (Ch, indicated by yellow arrows) in the skin tissue of goldfish (*C. auratus*) after dietary supplementation with Red Dragon Fruit Peel Extract (RDFPE). A. Control group 0 mL RDFPE/kg feed., B. 10 mL/kg, C. 20 mL/kg, and D. 30 mL/kg. Tissue sections were stained with Hematoxylin–Eosin and observed under light microscopy at 400 \times magnification. An increase in the density and visibility of chromatophores (black-pigmented cells) is observed in treatments C and D, indicating dose-dependent stimulation of pigment cell development

Survival rate

The survival rate of goldfish in all treatments was relatively high, ranging from 86% to 89% (Figure 6). There were no significant differences in survival between treatments ($p>0.05$), indicating that the supplementation of RDFPE did not negatively impact the fish's survival.

Water quality parameters

During the study, water quality parameters such as temperature, dissolved oxygen, pH, and ammonia concentration were within the optimal range for the rearing of goldfish (Table 1). These conditions supported the growth and survival of the test animals and minimized environmental stress that could affect the study's results.

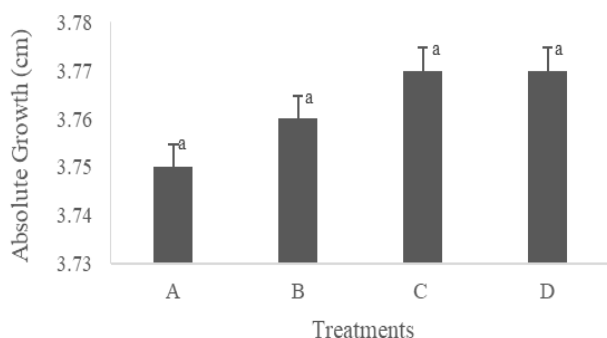


Figure 5. Absolute growth (cm) of goldfish (*C. auratus*) at various RDFPE doses during the rearing period. A: Control (0 mL RDFPE/kg feed), B: 10 mL/kg, C: 20 mL/kg, and D: 30 mL/kg. Error bars represent standard deviation (SD). No significant differences were observed among treatments ($p>0.05$), as indicated by the same letter (a) above all bars

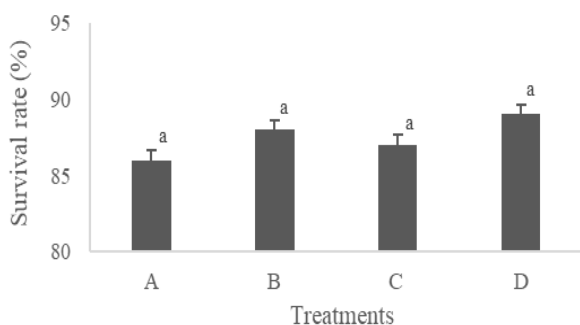


Figure 6. Survival rate (%) of goldfish (*C. auratus*) at various RDFPE doses during the rearing period. A: Control (0 mL RDFPE/kg feed), B: 10 mL/kg, C: 20 mL/kg, and D: 30 mL/kg. Error bars represent standard deviation (SD). No significant differences were observed among treatments ($p>0.05$), as indicated by the same letter (a) above all bars

Table 1. Range of water quality parameters for goldfish seed rearing during the study

Parameters	Ranges	SNI: 2018
Temperature (°C)	25.0-26.5	22-26
Dissolved oxygen (ppm)	5.6-6.5	Min 5
pH	6.8-7.5	6-7
Ammonia (ppm)	0.01-0.03	Max 0.05

Discussion

Color brightness level

Figure 2 revealed that the color brightness of goldfish increased with higher doses of RDFPE in the feed. Treatment D (30 mL/kg feed) resulted in the highest color brightness score, significantly greater than the other treatments ($p<0.05$), followed by Treatments C (20 mL/kg) and B (10 mL/kg). At the same time, Treatment A (control) showed no significant change between the beginning and end of the study. This increase indicates that higher RDFPE doses lead to greater carotenoid accumulation in the fish's skin tissue, contributing to more intense coloration. These findings support the important role of RDFPE as an

effective natural carotenoid source in enhancing the visual quality of ornamental fish through pigment deposition in chromatophores.

This increase in color brightness is consistent with the concept of dose-dependent carotenoid deposition, as reported in a review stating that astaxanthin supplementation enhances color saturation (redness, a^* and chroma) in ornamental fish (Elbahnaswy and Elshopakey 2024). Similarly, the use of red paprika (*Capsicum annum*) as a source of astaxanthin has also shown a positive relationship between pigment dosage and body color intensity in fish (Elshafey et al. 2023). The color brightness score of RDFPE approached that of astaxanthin despite its lower carotenoid content, indicating the contribution of betalains, water-soluble phenolic pigments with high antioxidant activity, which have been shown to maintain visual stability by protecting pigments from oxidative degradation (Wu et al. 2022).

The significant increase in body color brightness scores in goldfish (*C. auratus*) with RDFPE supplementation indicates a strong relationship between dietary carotenoid intake and pigment accumulation in integumentary tissue. RDFPE contains key bioactive compounds such as β -carotene, lycopene, and betacyanin, which contribute orange, red, and reddish-purple hues, respectively (Chen et al. 2024). These compounds are lipophilic pigments, and since fish cannot synthesize carotenoids endogenously (Nakano and Wiegertjes 2020), they are completely dependent on exogenous sources from feed.

Biologically, carotenoids are absorbed in the fish's gastrointestinal tract through passive diffusion, which is enhanced by the presence of lipids in the diet. Once inside enterocytes, these pigments are packaged into lipoproteins and transported via the circulatory system to target tissues such as the skin and eyes (Liang et al 2025). In the epidermal and dermal layers, carotenoids are stored in specialized chromatophores, erythrophores (red pigment cells) and xanthophores (yellow pigment cells), which are the primary determinants of coloration in ornamental fish (Cruz et al. 2021). This study demonstrated that the 30 mL/kg RDFPE dose resulted in the highest color brightness score, followed by the 20 mL/kg and 10 mL/kg doses, indicating a dose-dependent response in pigment accumulation.

However, the difference between Treatments C (20 mL/kg) and D (30 mL/kg) was not always statistically significant in individual cases. This may be due to a saturation mechanism in carotenoid absorption, where, beyond a physiological threshold, absorption efficiency decreases despite higher doses. Choubert and Heinrich (1993) reported this phenomenon, finding that high carotenoid concentrations in feed do not always result in linear accumulation in fish tissues due to limitations in intestinal membrane transport.

Compared to other commonly used carotenoid sources in aquaculture, such as spirulina (rich in zeaxanthin and β -carotene), red paprika (capsanthin), and marigold (lutein), RDFPE offers advantages in terms of raw material abundance, cost-efficiency, and its contribution to the circular economy. While spirulina is highly effective for

pigmentation, it is relatively expensive and less accessible in some regions (Gouveia et al. 2003). Paprika and marigold have shown similar outcomes but are more commonly used in large-scale, industrial aquaculture. Meanwhile, the utilization of dragon fruit peel waste presents a more affordable alternative that supports zero-waste principles in ornamental fish farming.

In addition to β -carotene, RDFPE contains betacyanin, an antioxidant, water-soluble phenolic compound with a characteristic red-purple color (Lin et al. 2022). While its direct role in fish integument pigmentation remains unclear, studies in other vertebrates suggest this antioxidant may enhance cell membrane stability and prolong chromatophore lifespan (Fang et al. 2022). This suggests that RDFPE's visual effects may not only stem from direct pigmentation but also from its synergistic function in structurally protecting pigment cells.

These findings reinforce the safety and sustainability of RDFPE's potential as a natural functional feed additive for the ornamental aquaculture industry. Its effect on improving color brightness can enhance the aesthetic and commercial value of ornamental fish without relying on synthetic colorants, which are often questioned for their safety and sustainability. The use of RDFPE also supports the valorization of agricultural waste as a nutraceutical source in sustainable aquaculture systems, aligning with global trends towards more environmentally friendly fish production practices (Onomu and Okuthe 2024).

Therefore, RDFPE serves not only as an effective alternative carotenoid source but also as an innovative approach to agricultural waste management, offering dual benefits in fish aesthetics and environmental sustainability in aquaculture.

Total carotenoid concentration

Trend data showed a progressive increase in total carotenoid concentration from the first to the sixth week across all treatments. This indicates that during the rearing period, natural carotenoids were accumulated from both the basal feed and the feed supplemented with Red Dragon Fruit Peel Extract (RDFPE).

Although the RDFPE supplementation significantly enhanced the body color brightness of goldfish, quantitative analysis of total carotenoid concentration did not show significant differences among treatments. This finding suggests that visual is not always directly proportional to total carotenoid accumulation in the tissue. This phenomenon has also been reported in African cichlid fish, where improvements in visual coloration do not always correspond proportionally to the measured carotenoid concentration (Ahi et al. 2020). One possible explanation is the degradation and redistribution of carotenoids within pigment cells, mediated by carotenoid-cleaving enzymes (Ahi et al. 2020). Moreover, the stability and retention of pigment in chromatophores may be enhanced by antioxidant protection, contributing to prolonged pigment life and sustained coloration (Liu et al. 2024).

Additionally, the synergistic effect of carotenoids and betalains from dragon fruit peel might contribute to visual

color enhancement without increasing the total carotenoid levels. Andriani et al. (2025) reported similar findings, observing that the addition of dragon fruit peel in feed significantly improved color vibrancy in comet fish, even though they did not report proportional increases in carotenoid concentration. This suggests that the visual effect of RDFPE may also come from efficient pigment distribution or stabilization in the integument.

Technical factors such as extraction methods, instrument sensitivity, and carotenoid distribution among body organs (e.g., skin vs. muscle) may also influence total carotenoid measurement outcomes. Therefore, advanced analytical methods like High-Performance Liquid Chromatography (HPLC) are recommended to detect specific carotenoid species accurately.

Figure 3 shows the changes in total carotenoid concentration in goldfish over a 6-week feeding period. Treatment D (30 mL RDFPE/kg feed) resulted in the highest final carotenoid concentration, followed by Treatments C, B, and A. The consistent increase observed across treatments indicates a dose-dependent response to red dragon fruit peel extract, suggesting that higher levels of RDFPE supplementation effectively enhance carotenoid accumulation in the fish. These findings are in line with the study by Kaur and Shah (2017), who reported that ornamental fish are unable to synthesize carotenoids endogenously, making dietary supplementation with natural carotenoid sources essential for enhancing color intensity. Thus, RDFPE can be considered an effective natural carotenoid source for improving pigmentation in ornamental fish.

Overall, these findings support the hypothesis that the enhancement of body color brightness in fish is not solely dependent on total carotenoid accumulation but is also influenced by the pigment distribution mechanisms within the skin tissue and other physiological factors such as hormonal activity, stress conditions, and metabolic function. While carotenoids are indeed the primary pigments responsible for red, orange, and yellow hues, their effectiveness in producing vivid coloration is highly dependent on the presence and density of chromatophores, particularly erythrophores and xanthophores, where these pigments are stored.

In addition, the absorption and transport of carotenoids within the fish body involve complex biological mechanisms that can vary between individuals and among treatments (Fang et al. 2022). Therefore, an increase in carotenoid content alone does not always directly correlate with enhanced body color brightness unless it is accompanied by optimal pigment cell distribution.

Chromatophore cell count

Observations of chromatophore cell counts in the skin tissue of goldfish showed a positive correlation with color brightness levels, though not directly correlated with the measured total carotenoid concentration. This indicates that the intensity of body coloration in fish is more influenced by the number and distribution of chromatophore cells than by the total carotenoid content in the tissue.

Chromatophores are pigment cells responsible for skin coloration through the accumulation and redistribution of pigments such as carotenoids and melanin. Their numbers can be affected by nutrition, environment, and genetic factors (Hien et al. 2022). Differences in chromatophore cell counts between treatments support the assumption that RDFPE supplementation supports the development of pigment structures in fish.

Figure 4 illustrates the increase in both the number and distribution of chromatophore (Ch) cells in the skin tissue of goldfish (*C. auratus*) fed diets containing varying doses of Red Dragon Fruit Peel Extract (RDFPE). In Treatment A (0 mL RDFPE), the number of chromatophores appeared to be the lowest, with a sporadic distribution. Treatment B (10 mL RDFPE) showed an increase in chromatophore numbers, indicated by the darker pigmentation more widely distributed across the epidermal tissue. Treatment C (20 mL RDFPE) presented a higher number of chromatophores with a more uniform spread than Treatment B. Meanwhile, Treatment D (30 mL RDFPE) exhibited the most prominent chromatophore count and size, indicating that RDFPE at this dose significantly promotes pigment accumulation.

Red dragon fruit peel extract is known to be rich in natural carotenoids such as β -carotene and lycopene, which serve as key precursors in the biosynthesis of pigmentation in fish. These compounds accumulate in chromatophore cells, particularly erythrophores and xanthophores, contributing to the red-orange coloration of ornamental fish integument. Beyond their role as pigments, carotenoids also possess strong antioxidant properties, potentially aiding in cell membrane stability and supporting the metabolic functions of chromatophores (Zhang et al. 2024).

Several studies on model species have suggested that carotenoid exposure can influence the expression of pigmentation-related genes, such as microphthalmia-associated transcription factor (MITF) and scavenger receptor class B type 1 (SCARB1), which are involved in pigment cell differentiation and pigment absorption (Liang et al. 2025). Although this study did not directly evaluate gene expression, these mechanisms may provide a biological hypothesis explaining the enhanced coloration resulting from RDFPE supplementation.

The combined nutritional, antioxidant, and bioactive effects of RDFPE may enhance the viability of chromatophore cells, increase the capacity for pigment uptake, and help maintain uniform color distribution in the skin tissue. Therefore, the higher the RDFPE dose in the feed, the greater the potential for increasing the number and functionality of pigment-producing cells, which is reflected significantly in the increase in the brightness of the fish's body color. Therefore, RDFPE supplementation in feed may serve not only as a pigment source but also as a trigger for chromatophore proliferation.

Chromatophores develop from neural crest cells during early fish development and can increase in number in response to environmental factors and nutritional intake. Carotenoids can stimulate the expression of genes that regulate chromatophore proliferation and differentiation, such as *mitf* (microphthalmia-associated transcription

factor), which mediates the formation of melanophores, xanthophores, and erythrophores (Ahi et al. 2020).

The discrepancy between carotenoid concentration and chromatophore cell count may also be explained by the fish's adaptive responses to its environment and aesthetic needs, where increasing the number of pigment cells becomes the primary mechanism to optimize visual appearance.

The integration of data on total carotenoid concentration, chromatophore cell count, and color brightness indicates that the improvement in the aesthetic coloration of goldfish is more strongly driven by skin tissue morphology (the number and distribution of chromatophores) rather than solely by pigment accumulation. This reinforces the importance of a holistic approach in understanding coloration mechanisms in ornamental fish.

Absolute growth analysis

The analysis of absolute growth in goldfish during the maintenance period showed no significant differences among treatments. All treatment groups, including the control and those supplemented with Red Dragon Fruit Peel Extract (RDFPE), exhibited relatively uniform growth.

This lack of difference indicates that RDFPE supplementation does not negatively or positively impact the fish's linear growth. In other words, the extract is safe to use as a feed additive to enhance coloration aesthetics without compromising growth performance. This finding aligns with Saikia and Das (2023), who reported that natural carotenoid supplementation from plant sources in ornamental fish feed tends to affect aesthetic parameters such as body color rather than physiological parameters like growth.

Carotenoids are neither energy sources nor primary proteins, so their contribution to biometric growth is generally minimal. Therefore, the use of red dragon fruit peel extract in goldfish feed can be considered a nutritional intervention that selectively enhances aesthetic quality without sacrificing growth. Under these conditions, growth proceeds optimally because the basal feed already meets the protein and energy requirements, while carotenoids act as a non-growth-related additive supplement (Biswas et al. 2023; Zhang et al. 2024).

Survival rate

Goldfish survival data throughout the study showed no significant differences among treatments, including between the control group (without RDFPE) and the groups supplemented with RDFPE. The consistently high survival rate across all groups (>85%) indicates that the aquaculture environment and husbandry practices applied during the experiment were optimal and did not exert notable stress on the fish.

The absence of significant differences in survival rates between the control and RDFPE-treated groups suggests that supplementation with red dragon fruit peel extract is safe, non-toxic, and does not disrupt the physiological balance of goldfish. The fish's metabolic system well tolerated RDFPE and did not induce stress or

immunological disturbances, functioning more as a color-enhancing agent rather than a factor influencing survival. These findings are consistent with Park et al. (2024), who reported that plant-based natural carotenoid sources are generally safe for use in ornamental fish feed formulations.

Moreover, the consistently high survival rate (>85%) across all treatments also indicates that environmental conditions during the study were well-maintained, thereby allowing the aesthetic outcomes to be interpreted validly without confounding effects from environmental stress.

Water quality

Throughout the maintenance period, the stability of water quality parameters, including temperature, dissolved oxygen, pH, and ammonia concentration, remained within optimal ranges played a crucial role in supporting the growth and survival of goldfish. This stability is a testament to the reliability of our research, ensuring that the observed outcomes, particularly in terms of color brightness, total carotenoid concentration, and chromatophore cell count, were solely attributable to the feed treatments rather than environmental stress.

Stable water quality provides a sound basis for interpreting all observed biological responses as effects of dietary treatment, not environmental stressors. Parameters such as temperature, pH, DO, and ammonia remained within optimal ranges for goldfish metabolism, feed absorption, and digestive enzyme efficiency (Menon et al. 2023). A stable environment also minimizes activation of stress pathways such as the HPI-axis, which, if triggered, can reduce pigmentation efficiency and growth (Yuan et al. 2024).

Specifically, the temperature was maintained between 25-26.5°C, dissolved oxygen above 5.6-6.5 ppm, pH between 6.8-7.5, and ammonia levels below 0.03 ppm, all within the optimal standards for goldfish culture (Elshafey et al. 2023). Under these consistently controlled conditions, it can be concluded that the positive responses to dietary treatment, including increased color brightness, were indeed the result of red dragon fruit peel extract supplementation. The optimal water quality throughout the study ensures the accuracy and reliability of the observational data for key parameters.

In conclusion, the supplementation of Red Dragon Fruit Peel Extract (RDFPE) in feed has been proven to significantly enhance the body color brightness of goldfish (*C. auratus*), making it a promising natural pigment source for ornamental aquaculture. Although the increase in total carotenoid levels was not statistically significant, the results suggest that other mechanisms, such as absorption efficiency or chromatophore stability, may play a role in color enhancement. RDFPE did not cause any negative effects on growth, survival, or water quality, indicating that this additive is safe, environmentally friendly, and suitable for application in aquaculture systems.

The use of RDFPE supports sustainability principles by utilizing agro-industrial waste as a feed additive ingredient. Its potential as a natural colorant presents an opportunity for developing functional feeds to improve the aesthetic value of ornamental fish while also reducing production

costs. Further studies are recommended to explore the molecular mechanisms of pigmentation and to analyze the economic feasibility of using RDFPE on an industrial scale.

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