Combination of beetroot and barley grass as a supplementary diet to enhance color brightness in koi fish seed (Cyprinus carpio L.)

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Abstract. Color is a key quality trait in koi (*Cyprinus carpio* L.) that can be enhanced through dietary carotenoids. This study evaluated the effects of beetroot and barley grass supplementation on color brightness of koi seed. Fish (5–7 cm) were reared for 28 days in 25-L tanks, five fish per tank, under three dietary treatments and one control group, each with three replicates. Diets containing beetroot and barley grass powders were fed at 5% of body weight per day. Coloration was assessed weekly using a visual scoring method to obtain relative color brightness, and growth performance was recorded. Data were analyzed using one-way ANOVA ($p < 0.05$). The combination of beetroot and barley grass significantly increased relative yellow–orange–red hues brightness ($p = 0.048$). The 10% beetroot + 10% barley grass diet resulted the highest improvement (7.02 ± 3.38%), although not significantly different from the 5% beetroot + 15% barley grass diet (2.81 ± 3.22%). Both treatments outperformed the 15% beetroot + 5% barley grass diet (2.46 ± 0.61%) and the control (0.35 ± 0.61%). No significant effects were found on growth performance. These results indicate that balanced beetroot and barley grass supplementation can enhance koi color brightness.

1 Introduction

The visual appeal of koi fish (*Cyprinus carpio* L.) is primarily determined by their body coloration, which strongly influences market value and hobbyist interest. The diverse colors and intricate patterns across different body regions form the aesthetic allure that makes koi one of the most prized ornamental freshwater fishes. However, dull or faded coloration can significantly reduce both consumer preference and commercial value. Therefore, enhancing koi body color is essential to maintain their attractiveness and market competitiveness.

The pigmentation of koi fish is governed by specialized cells known as chromatophores located in the dermal layer. These cells, such as melanophores (black), erythrophores (red), xanthophores (yellow), leucophores (white), and iridophores (reflective), contribute to color intensity and brightness depending on their number, distribution, and ability to reflect light [1]. Since fish are unable to synthesize pigments de novo, they rely on dietary intake from natural sources such as plants, phytoplankton, zooplankton, and crustaceans to maintain

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vibrant coloration [2, 3]. In contrast, koi cultured in tanks have limited access to natural pigment sources [4, 2]. Hence, supplementing pigment-rich ingredients diets is crucial to improve color brightness and pattern intensity.

Carotenoids are the main group of pigments responsible for yellow, orange, and red hues in fish. These lipophilic compounds, naturally found in plants, fruits, and vegetables, stimulate chromatophore activity and enhance coloration while also functioning as antioxidants [3]. Beta-carotene is the most common pigment in commercial koi diets, but combining multiple carotenoid sources can yield more vibrant and stable coloration. For instance, the inclusion of carrot powder at 5–10% improves koi color brightness [5]. This highlights the importance of using natural dietary supplements for optimal koi pigmentation.

Natural pigments are more sustainable and environmentally friendly alternatives to synthetic colorants. Beetroot (*Beta vulgaris*) contains various carotenoids, including α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, violaxanthin, neoxanthin, and lycopene [6]. Barley grass (*Hordeum vulgare*), rich in nutrients and fiber, is also a promising source of carotenoids, particularly β-carotene and lutein, comparable to those found in carrots [7]. Both beetroot and barley grass contain antioxidants and bioactive compounds that can enhance pigmentation without adverse effects on fish health or the environment [6, 8, 9].

Therefore, this study aimed to improve the coloration quality of koi fish seed through dietary supplementation using natural ingredients derived from a combination of beetroot and barley grass. The main purpose was to explore a natural approach and develop a sustainable and effective strategy to enhance the visual quality and market value of koi fish.

2 Materials and Methods

2.1 Experimental design

The experiment was conducted using a completely randomized design with three dietary treatments and one control group, each replicated three times. The treatments consisted of the following diet formulations: 5% beetroot combined with 15% barley grass (5 BR : 15 BG), 10% beetroot combined with 10% barley grass (10 BR : 10 BG), 15% beetroot combined with 5% barley grass (15 BR : 5 BG), and a control group receiving commercial diet without any supplementation. Koi were reared for 28 days under controlled conditions and fed three times daily. Sampling of body length, body weight, and relative color brightness was conducted weekly. The collected data were subsequently analyzed statistically.

2.2 Diet formulation and rearing setup

Commercial fish feed pellets containing 30% crude protein served as the basal diet. Commercial pure extract beetroot and barley grass powders were sprayed onto the pellets according to the treatment levels, and the pellets were then dried. Koi with an average size of 5–7 cm were cultured in 25-L circular black tanks equipped with aeration and biological filtration systems. The water level was maintained at approximately 30 cm, and the stocking density was five fish per tank. Prior to the experiment, koi were selected for uniformity in size and color domination to minimize variation.

2.3 Fish culture

Koi fish seed were acclimated for seven days prior to the feeding trial. During the 28 days experimental period, fish were fed at a rate of 5% of their body weight per day, divided into three feeding sessions (08:00, 12:00, and 16:00). Water quality was maintained by weekly water replacement, while temperature and pH were monitored daily. Fish health and behavior were observed to ensure normal activity and to confirm that changes in growth or coloration were attributable to the dietary treatments rather than environmental factors.

2.4 Data collection

Sampling was conducted once a week to record fish length, weight, and coloration. Color quality was assessed using a scoring method based on a modified Tocca Color Finder scale [10], adapted to the color variations typically observed in koi. Each fish was assigned an observed color score based on visual matching, with scores ranging from 1–19 for yellow–orange–red hues (1 = yellow, 19 = red). To allow standardized comparison across individuals and sampling periods, observed color scores were converted into a relative color brightness value, expressed as a percentage of the maximum score within each color range. Individual fish were monitored consistently for individual-based evaluation, as each koi possesses unique color patterns. Photographic documentation was carried out to support visual comparison of coloration changes over time.

2.5 Data calculation

Relative color brightness (*RCB*, %)

$$RCB = \frac{C}{C_{max}} \times 100 \quad (1)$$

where:
C = observed color score
C_{max} = maximum score in the scale
Change in relative color brightness (Δ*RCB*, %)

$$\Delta RCB = RCB_f - RCB_i \quad (2)$$

where:
RCB_i = initial RCB value
RCB_f = final RCB value
Weight gain (*WG*, g)

$$WG = W_f - W_i \quad (3)$$

where:
W_i = initial body weight
W_f = final body weight
Length gain (*LG*, cm)

$$LG = L_f - L_i \quad (4)$$

where:
L_i = initial body length
L_f = final body length
Specific growth rate (*SGR*, % day^{-1})

$$SGR = \frac{(\ln W_f - \ln W_i)}{t} \times 100 \quad (5)$$

where:
W_i = initial body weight
W_f = final body weight
t = duration of the experiment (days)
Feed efficiency (*FE*, %)

$$FE = \frac{(W_f + W_d - W_i)}{F_t} \times 100 \quad (6)$$

where:
W_i = initial body weight
W_f = final body weight
W_d = total weight of dead fish
F_t = total feed consumed
Survival rate (*SR*, %)

$$SR = \frac{N_f}{N_i} \times 100 \quad (7)$$

where:

N_i = initial number of fish
N_f = final number of fish

2.6 Statistical analysis

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software version 26.0 (IBM Corp., USA). One-way analysis of variance (ANOVA) was applied to determine differences among treatments, with a significance level set at 5% ($p < 0.05$). When significant differences were detected, Duncan's multiple range test was used to identify the best-performing treatment. The analyzed data were subsequently visualized in graphical form to support interpretation of results.

3 Results

3.1 Relative Color Brightness

Relative color brightness was evaluated weekly on an individual basis, as each koi exhibits unique color patterns, with representative samples from each treatment shown in **Table 1**. Yellow–orange–red hues were the dominant colors observed in the koi body patterns, while blue–black tones occasionally appeared and were evaluated as supplementary information.

Table 1. Representative koi (5–7 cm) from each dietary treatment containing beetroot (BR) and barley grass (BG). Images are for qualitative reference only, based on live visual scoring, color may vary due to photographic conditions. Scale bars are not applicable.

Treatments	Data retrieval (weeks)				
	0	1	2	3	4
Control					
5 BR : 15 BG					
10 BR : 10 BG					

15 BR : 5 BG

Weekly measurements of relative yellow–orange–red hues brightness ranged from 49–54% initially and increased to 55–58% by the end of the 28 days experiment (**Fig. 1**). Despite weekly fluctuations, the 10% beetroot + 10% barley grass diet showed a consistent upward trend from 49% to 58%. In the control group, relative color brightness rose from 55% to 59% in the first week, then declined to 54%. The 15% beetroot + 5% barley grass diet peaked at 60% in the second week but decreased to 56% by the end of the experiment. The 5% beetroot + 15% barley grass diet also showed a slight decline starting from the third week.

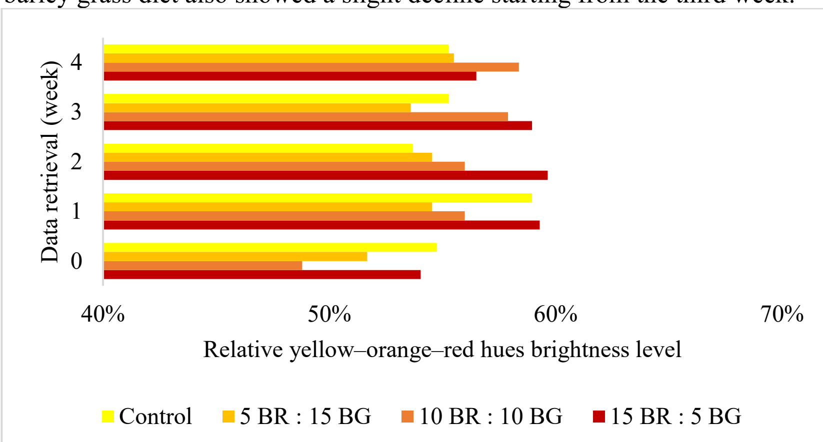

Fig. 1 Relative yellow–orange–red hues brightness of koi fed different combinations of beetroot (BR) and barley grass (BG) measured weekly.

3.2 Change in Relative Color Brightness

Statistical analysis revealed that dietary supplementation significantly enhanced the change in relative yellow–orange–red hues brightness ($p = 0.048$) (**Fig. 2**). Duncan's multiple range test indicated that the 10% beetroot + 10% barley grass diet yielded the highest average increase in relative color brightness (7.02 ± 3.38%). This increase was not significantly different from the 5% beetroot + 15% barley grass diet (2.81 ± 3.22%), due to high within-treatment variability. The 10% beetroot + 10% barley grass diet was significantly different from the 15% beetroot + 5% barley grass diet (2.46 ± 0.61%) and the control group (0.35 ± 0.61%), while the 5% beetroot + 15% barley grass diet did not differ significantly from either.

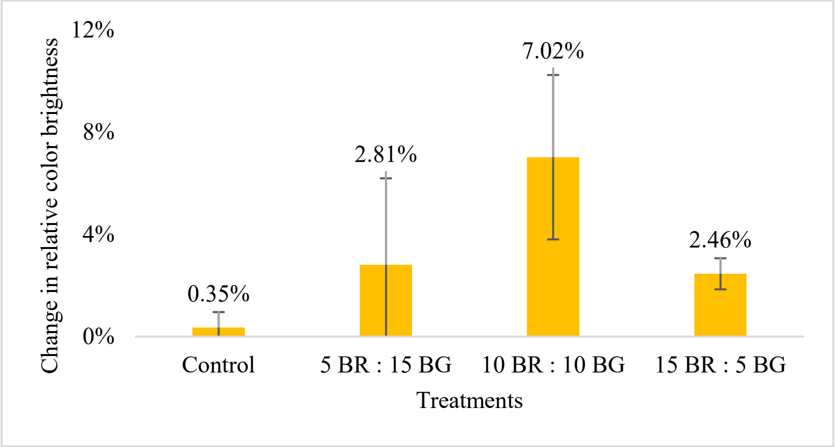

Fig. 2 Change in relative color brightness of yellow–orange–red hues in koi fed diets containing different combinations of beetroot (BR) and barley grass (BG).

3.3 Fish Growth and Water Quality

No significant differences were found in weight gain ($p = 0.160$) or length gain ($p = 0.521$) among treatments (**Table 2**). The control group showed the highest weight gain (2.27 ± 0.40 g) and length gain (0.70 ± 0.08 cm), followed by the 15% beetroot + 5% barley grass diet (1.80 ± 0.53 g; 0.54 ± 0.20 cm), the 5% beetroot + 15% barley grass diet (1.47 ± 0.61 g; 0.53 ± 0.17 cm), and the 10% beetroot + 10% barley grass diet (1.37 ± 0.25 g; 0.59 ± 0.10 cm). Specific growth rate (SGR) followed the same trend, highest in the control group (2.00 ± 0.22% day^{-1}) and lowest in the 5% beetroot + 15% barley grass diet (1.46 ± 0.68% day^{-1}).

Feed efficiency was highest in the control group (54.95 ± 9.80%), followed by the 15% beetroot + 5% barley grass diet (43.64 ± 12.83%), the 5% beetroot + 15% barley grass diet (35.56 ± 14.81%), and the 10% beetroot + 10% barley grass diet (33.13 ± 6.10%), reflecting their lower growth performance.

Survival rates ranged from 86% to 100%. The highest survival was observed in the 15% beetroot + 5% barley grass diet and 5% beetroot + 15% barley grass diet treatments (100% ± 0.00%), followed by the 10% beetroot + 10% barley grass diet (93.33 ± 11.55%), while the control group had the lowest survival (86.67 ± 11.55%).

Water quality remained stable across all treatments, with temperature ranging from 25°C to 27°C and pH consistently at 7.00. These conditions were optimal and uniform for all treatments, minimizing potential environmental effects on growth and coloration.

Table 2. Growth performance of koi and water quality parameters (mean ± SD)

Parameters	Treatments			
	Control	5 BR : 15 BG	10 BR : 10 BG	15 BR : 5 BG
Initial body weight (g)	2.73±0.12	2.73±0.46	2.67±0.23	2.87±0.61
Final body weight (g)	5.00±0.50	4.20±0.40	4.03±0.47	4.67±0.31
Weight gain (g)	2.27±0.40	1.47±0.61	1.37±0.25	1.80±0.53
Initial length(cm)	5.40±0.14	5.39±0.14	5.43±0.22	5.51±0.16
Final length (cm)	6.10±0.22	5.93±0.14	6.03±0.31	6.05±0.12
Length gain (cm)	0.70±0.08	0.53±0.17	0.59±0.10	0.54±0.20
SGR (% day^{-1})	2.00±0.22	1.46±0.68	1.51±1.37	1.67±0.68
Feed efficiency (%)	54.95±9.80	35.56±14.81	33.13±6.10	43.64±12.83
Survival rate (%)	86.67±11.55	100.00±0.00	93.33±11.55	100.00±0.00
Water temperature (°C)	25.34±1.69	26.48±1.88	26.18±2.07	26.36±1.59

pH	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00

4 Discussion

The supplementation of beetroot and barley grass in koi diets positively influenced the enhancement of yellow–orange–red hues in body coloration compared with the control group. Among all treatments, the 10% beetroot and 10% barley grass combination resulted the highest increase in relative yellow–orange–red hues brightness, reaching an average enhancement of 7%. Although it was not significantly different from the 5% beetroot and 15% barley grass combination, the balanced 10% + 10% formulation differed significantly from the 15% beetroot and 5% barley grass combination as well as the control group, whereas the 5% beetroot and 15% barley grass combination remained statistically similar.

The enhancement of yellow–orange–red hues coloration in koi is primarily associated with the accumulation of carotenoid pigments. This relative color brightness improvement was driven by the presence of supplementary diets combining beetroot and barley grass, both of which are natural sources of carotenoids that cannot be synthesized endogenously by fish. Dietary carotenoids bind within pigment cells, enhancing the deposition of yellow–orange–red hues on koi skin. Similar findings were reported that the combination of beetroot, carrot peel, and tomato peel powders effectively improved koi pigmentation [11].

In the control group, the final coloration of koi appeared yellowish (**Table 1**). This outcome reflects insufficient carotenoid intake for optimal yellow–orange–red hues deposition. A slight increase occurred during the first week, followed by a decline toward baseline levels in subsequent weeks (**Fig. 1**). The near-zero improvement in the control group suggests that commercial pellets containing 30% protein can maintain but not enhance coloration. Comparable outcomse have been documented, where commercial pellets alone failed to significantly improve koi relative color brightness despite containing carotenoids from fishmeal [9, 11]. This highlights the need for supplemental natural carotenoids to achieve noticeable color enhancement.

Yellow–orange–red hues coloration is predominantly regulated by erythrophores, which store dietary carotenoids such as those derived from beetroot and barley grass. Carotenoid accumulation in erythrophores is a gradual, resulting in a steady weekly increase in brightness [1, 2]. This trend was clearly observed in the 10% beetroot and 10% barley grass combination, which consistently improved relative color brightness across weeks. Other combinations showed a slight decline in the third week, likely due to limited carotenoid availability, pigment cell saturation, or competition for nutrients that arises when carotenoid intake is unbalanced or exceeds optimal levels.

The synergistic effect observed in the 10% beetroot and 10% barley grass combination is likely derived not only from the carotenoid diversity of both plant sources but also from their abundant antioxidants [6]. The density and absorption capacity of pigment cells determine carotenoid utilization efficiency [12], and excessive supplementation can reduce uptake and interfere with hormone-mediated pigment regulation. Therefore, proper dosage is essential.

The duration of supplementation also influences the extent of color enhancement. Carotenoid supplementation generally improves ornamental fish coloration after two weeks of feeding [1], although the response depends on pigment type, dosage, and intrinsic pigment cell capacity. The 10% beetroot and 10% barley grass combination likely reflect an optimal balance between carotenoid concentration and antioxidant protection, maximizing deposition in erythrophores. This balanced synergy facilitates effective carotenoid transport, stabilization, and storage within pigment cells.

The highest relative color brightness peak occurred in the second week in the 15% beetroot and 5% barley grass combination, indicating that higher beetroot levels, can initially elevate relative yellow–orange–red hue brightness. However, this coloration could not be sustained, likely due to insufficient nutritional support for continuous carotenoid deposition.

Although previous observations suggested that barley grass alone may enhance relative color brightness [9], in this study, beetroot appeared more effective for early coloration, whereas barley grass likely contributed to pigment stabilization over time through its broader nutrient profile. An unbalanced ratio with excessive beetroot and insufficient barley grass may therefore boost early brightness but cannot maintain it in the following weeks.

The relative yellow–orange–red hues brightness pattern across treatments and the control formed a triangular trend, indicating the presence of an optimal carotenoid–nutrient balance point. This reflects a biological threshold where pigment cell absorption approaches maximum efficiency; beyond this point, additional carotenoid input yields diminishing returns or reduced effectiveness due to cellular saturation.

Coloration enhancement in ornamental fish is a complex interaction among genetic, neurohormonal, nutritional, and environmental factors [2]. Nutritional deficiency or stress can cause pigment degradation. Neuroendocrine regulation also influences pigmentation through melanin-aggregating hormone (MAH) and melanin-dispersing hormone (MDH) produced by the pituitary gland [13]. In this experiment, blue–black tones, regulated by melanophores, did not exhibit notable changes among treatments. These tones were present occasionally but minimally. This indicates that beetroot and barley grass specifically enhanced yellow–orange–red hues coloration, consistent with carotenoid functions, but do not affect melanin-based pigmentation.

The present findings confirm that a balanced ratio of 10% beetroot and 10% barley grass diet achieved the greatest coloration improvement within 28 days, aligning with previous suggestions that supplementation exceeding 10% offers no additional benefit and may even decrease survival due to carotenoid precipitation [5]. In summary, the statistically significant enhancement in relative yellow–orange–red hues brightness confirms that the 10% + 10% formulation provides optimal nutritional and biochemical conditions for carotenoid deposition. The combination improves pigment cell performance through both carotenoid availability and antioxidant support, effects not achieved by higher or unbalanced doses.

Growth performance did not differ significantly among treatments and the control. Although the 10% beetroot and 10% barley grass combination achieved the best coloration, it showed the lowest feed efficiency (33.13 ± 6.10%). Feed efficiency reflects nutrient conversion into biomass. The control group achieved the highest specific growth rate (2.00 ± 0.22% per day), while the lowest (1.46 ± 0.68%) remained within acceptable limits comparable to reported values of 0.79% and 1.14% per day [14]. The lower feed efficiency observed in this treatment may reflect allocation of nutrients toward pigment deposition rather than growth, highlighting a trade-off between coloration and feed utilization.

In swordtail fish, 15% beetroot supplementation increased specific growth rate [15]. The trend in the present study also shows a slight tendency for higher beetroot levels to improve growth performance, although the effect was not statistically significant. Diets containing only barley grass, however, may enhance growth more effectively than beetroot alone, likely due to its broader nutrient profile [9]. These findings suggest that both beetroot and barley grass can support growth and coloration, but their relative contributions may vary depending on nutrient composition and experimental conditions. Variations among treatments are likely influenced by both intrinsic (metabolic efficiency, genetic potential) and extrinsic (diet formulation, nutrient interactions) factors.

Beetroot and barley grass provide diverse nutrients that enhance carotenoid absorption and bioavailability. Carotenoids not only act as pigment enhancers but also improve nutrient uptake and function as antioxidants that prevent oxidative stress [3]. These combined effects support fish health, maintain pigment stability, and increase survival. In this study, survival rates ranged between 86% and 100%, consistent with findings in goldfish fed natural pigment sources [4]. The control group, which did not receive any supplementation, showed the lowest survival, likely due to increased susceptible to oxidative stress and minor environmental challenges during the experiment. These results confirm that natural pigment supplementation is safe and may even improve survival compared with unsupplemented diets through its antioxidant role [8].

Overall, maintaining good water quality throughout the experiment supported both coloration and survival outcomes. Temperature and pH remained within optimal ranges for koi culture [1], ensuring that observed effects were primarily attributable to dietary treatments rather than environmental factors. Stable conditions likely minimized stress prevented pigment degradation, and supported normal feeding behavior and metabolism. These findings underscore the importance of integrating proper environmental management with dietary strategies to maximize ornamental fish coloration.

5 Conclusion

Dietary supplementation with beetroot and barley grass effectively enhanced the relative yellow–orange–red hues brightness of koi fish seed without negatively affecting growth performance. The balanced combination of 10% beetroot and 10% barley grass yielded the highest enhancement in relative color brightness, with an approximate increase of 7%. Future studies should evaluate the long-term effects of this supplementation on color stability, fish health, and growth under varying culture conditions.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements

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