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Rose anthocyanin microencapsulation for improving the color of two strains of koi

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ABSTRACT

Koi fish coloration is genetically significant, but environmental factors and color quality can lead to degradation. Some individuals use synthetic dyes to enhance their appearance, which harms the environment. This study explored the effects of microencapsulated *Rose* sp. flower anthocyanin (MRFA) as a natural alternative dye to enhance the color of Sanke and Kohaku koi fish. Sanke exhibits orange-black hues, while Kohaku shows orange-white hues, allowing for comparative analysis. Before the experiment, MRFA was analyzed using FESEM and XRD to assess the particle size distribution and shape of the anthocyanin microcapsules. A Randomized Complete Design (CRD) with four treatments (T0, 150 mg (T1), 300 mg (T2), and 450 mg (T3)) and five replications were used. Data was analyzed with SPSS. Results showed that MRFA particles were distributed, though not entirely homogeneous (14 µm to 170 µm), with XRD spectra indicating interactions between maltodextrin and *Rose* sp. anthocyanin. Results showed that MRFA significantly improved certain color parameters in Sanke, particularly at moderate doses (T1 and T2), but the control group (T0) outperformed MRFA in some cases. In Kohaku, no significant improvement was observed compared to the control, suggesting that MRFA was less effective in enhancing white–orange pigmentation. Overall, our findings indicate that MRFA supported koi growth but showed limited, strain-specific effects on coloration. Further studies with longer trials and larger populations are needed to confirm its potential as a natural feed additive.

Introduction

Koi fish are characterized by patterns and colors that affect market sales (Hermawan *et al.*, 2024; Luo *et al.*, 2018). There is a direct correlation between the color quality and the koi fish price (Hakim *et al.*, 2025; Kalidupa *et al.*, 2018; Lili *et al.*, 2020). Koi fish are noted for their gorgeous colors, but environmental factors may influence them (Afnan *et al.*, 2019; Jalila *et al.*, 2021). Consequently, synthetic dyes are often added to their diets to improve ornamental fish skin color (Kurnia *et al.*, 2019). However, it generally has relatively high prices and negatively influences the ecosystem (García-Chavarría and Lara-Flores, 2013). Scientists are calling for natural pigment coloring compounds to replace synthetic dyes due to their harmful effects on the ecosystem and fish growth (Sathyaruban *et al.*, 2021; Zutshi and Madiyappa, 2020). In a case study on *Hyphessobrycon callistus*, dietary zeaxanthin and astaxanthin negatively correlated with antioxidant indices (Wang *et al.*, 2006). In addition, the North American yellow perch grows slowly, which could explain our finding. Yellow perch-fed canthaxanthin (CTX) enriched meals grew more slowly than those fed non-supplemented diets, possibly due to a preference for non-supplemented foods over carotenoids (Brown *et al.*, 2009).

Natural materials offer the potential to enhance object color while remaining cost-effective (Khoo *et al.*, 2017). For instance, a 15% dose of dragon fruit yielded optimal results by intensifying black and orange hues in koi carp (Kalidupa *et al.*,

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2018). Similarly, carrots have been employed to maintain the vibrancy of ornamental fish colors (Malide *et al*., 2020; Wagde *et al*., 2018). However, the widespread use of these ingredients as human food may lead to future competition. Consequently, *Rose* sp. petals could be a natural coloring agent for koi fish. *Rose* sp. crowns contain water, vitamins, carotene, anthocyanins, sugar, and essential oils (Saati *et al*., 2021; Wulandari *et al*., 2016). Anthocyanins, a type of flavonoid and significant plant secondary metabolite (Delgado-Vargas *et al*., 2000), could potentially enhance koi fish coloration when used as feed additives.

A high rate of anthocyanins can be extracted from *Rose* sp. petals, and their use as natural colorants has been demonstrated in various fields (Salman *et al*., 2023; Yan *et al*., 2023). These examples highlight the stability and versatility of anthocyanins, which support their potential application as natural feed additives to enhance ornamental fish coloration. Furthermore, Rose sp. is widely cultivated in Malang, East Java, Indonesia, making this resource locally abundant and accessible (Darliah *et al*., 2010; Prabavathy *et al*., 2018; Ula *et al*., 2019). Regarding its application to organisms, a study conducted by Vanegas-Espinoza *et al.* (2019) demonstrated that 450 mg of microencapsulated anthocyanin from *Hibiscus sabdariffa* significantly enhanced the noticeable distribution of chromatophores in the head, dorsal, and caudal fins of *Carassius auratus*. The discovery was also supported by Yong *et al.* (2022) found that the inherent pigment anthocyanin extracted from *Hibiscus sabdariffa* can enhance the coloration of the shrimp's body. Anthocyanins are stable and resistant to thermal and photodegradation when incorporated into clay mineral structures, making them suitable for use as colorants (Câmara *et al*., 2022). Overall, anthocyanins offer a promising alternative to synthetic dyes due to their stability, color fastness, and potential for various applications in textiles, food, cosmetics, and packaging.

In contrast, research has not used *Rose* sp. as the dye in koi fish. The objective of this study was to investigate the effects of microencapsulated rose anthocyanins on improving coloration and growth performance in two koi strains (Sanke and Kohaku).

Materials and Methods

The study took place between June and October 2024 at the Fishery Laboratory within the Aquaculture Department of the University of Muhammadiyah Malang, and at the Central Laboratory of Life Sciences at Brawijaya University in East Java, Indonesia.

Experimental design

The study utilized a completely randomized design (CRD) with four treatments and five repetitions. They consisted of the control group (T0), 150 mg (T1), 300 mg (T2), and 450 mg of microencapsulated *Rose* sp. flower anthocyanin (MRFA), which was added to the commercial feed to enhance the coloration of Kohaku and Sanke varieties. This approach aligns with the findings reported by Vanegas-Espinoza *et al.* (2019).

Fish and *Rose* sp. preparation

Kohaku and Sanke (10 to 15 cm) koi were provided by Koi farmers in Blitar, East Java, Indonesia, with as many as 240 fish each. These fish were of various colors, including orange and black (Sanke) and orange and white (Kohaku), which allowed for the evaluation of MRFA's efficacy for two different fish. Those fish were then acclimated for seven days without feeding in a tank (200 × 100 × 50 cm), complete with water aeration, filtration, and a heater. Afterward, 20 Kohaku and Sanke fish were divided into 12 aquariums (60 × 30 × 30 cm) and fed twice daily with a mixture of treated feed and commercial feed (protein ratio 32%), equal to 3% of their body weight, for four weeks. The tanks were completed with water aerations to maintain water quality during the study. Meanwhile, *Rose* sp. was purchased from a florist in Batu City, East Java, Indonesia, and weighed approximately 5 kg. The flowers were separated from their stem, washed under running water for a minute, and kept until use.

Extraction and quantification of anthocyanins from *Rose* sp. flower

The anthocyanin extraction method followed those provided by Saati *et al.* (2022) and Vanegas-Espinoza *et al.* (2019), with some light modifications. The *Rose* sp. flowers were crushed using a blender (LY-388-2L, King Chef) before being lyophilized with a Freeze Dryer Biobase BK-FD10S. Two hundred forty grams of *Rose* sp. flower powder were homogenized in 3 L of 96% ethanol and 30 mL of 0.01 N hydrochloric acid for 15 minutes using a magnetic stirrer and macerated for 24 hours. Afterward, the mixture was filtered using Whatman paper No. 42 before being powdered using a Freeze Dryer Biobase BK-FD10S. Moreover, the anthocyanin concentration of the extract was measured using a UV-VIS T80 spectrophotometer at wavelengths of 502 nm and 700 nm by adding solutions at pH levels of 1 and 4.5.

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Anthocyanin microencapsulation

The microencapsulation process, as described by Vanegas-Espinoza *et al.* (2019), involved several steps. First, 4 mg of *Rose* sp. flour was blended with 15% (w/v) commercial maize starch, maltodextrin, and 1% (w/v) sucrose using a homogenizer (Model HR-6B). The resulting mixture was spun at 4000 rpm for 20 minutes. Next, the suspension was introduced to a Freeze Dryer (Biobase BK-FD10S). The final product, microencapsulated *Rose* sp. flower anthocyanin (MRFA), was collected and stored at 4°C for further analysis.

Particle size distribution and shape of anthocyanin microcapsules

The measurements of MRFA were conducted using a Field Emission Scanning Electron Microscope (FE-SEM) (Quanta FEG 650) and an X-Ray Diffractometer (XRD) (PANalytical: X'Pert3 Powder), in collaboration with the Central Laboratory of Life Sciences at Brawijaya University, East Java, Indonesia. The sample (MRFA) was observed at an on-screen Polaroid display magnification of 100,000× and an In-lens detector at 10 kV. Meanwhile, the techniques of XRD rely on the diffraction of X-rays by periodic atomic planes and the detection of the diffracted signal at a precise angle or energy. The XRD diffractogram data were analyzed qualitatively for the phase type of the deposition layer sample using Match! Version 2 software.

Diet preparation

The diet was prepared by combining commercial feed and MRFA. One kilogram of the commercial feed was divided into three groups based on treatments, including T1 (150 mg of MRFA), T2 (300 mg of MRFA), and T3 (450 mg of MRFA). MRFA was incorporated into the commercial feed by mixing with Progol (5 mg) as a binder and 125 mL of water. The mixture was thoroughly blended with the pellets and then dried using a spray dryer. This process allowed MRFA to adhere firmly to the feed surface without full repelleting. The feed was kept in black plastic at room temperature until use.

Fish color analysis

Kohaku and Sanke's color quality was measured three times, including the 2nd week and 4th week, during the feeding trials, according to some resources (Fabanjo and Abdullah, 2021; Lalita *et al.*, 2022). At the same time, a permanence test was then conducted during a 2-week post-treatment period (weeks 5–6) after MRFA supplementation was stopped, to evaluate whether the effects persisted, according to Malini and Agustin (2018) studied. Five fish samples were isolated for each treatment, and their color qualities were carefully observed using the Color Reader CR-10 Plus. The tool provided the color values, including L/Lightness, A/Red, B/Yellow, C/Chrome, and H/Dull.

Growth performance and survival rate analysis

The growth performances of Sanke and Kohaku, as well as their survival rates, were also examined. These analyses included the growth rate (GR), the absolute length growth (AL), the feed conversion ratio (FCR), the total feed consumption (TFC), and the survival rate (SR) (Handajani *et al.*, 2021; Robbani *et al.*, 2022).

GR (g) = Wt – W0
AL (cm) = Lt – L0

$$\text{FCR} = \frac{\sum F}{(Wt - W0)}$$

TFC = F1 – F2

$$\text{SR} = \frac{S0}{St} \times 100\%$$

Note:
GR = Growth rate (g)
W0 = Initial weight (g)
Wt = Final weight (g)
AL = Absolute length (cm)
L0 = Initial length (cm)
Lt = Final length (cm).
FCR = Feed conversion rate
F = The number of feed (g)
TFC = Total feed consumption (g)
F1 = Uneaten feed
F2 = Eaten feed

Water quality analysis

According to Sanke and Kohaku cultures, water changes (50%) every four days, resulting in better growth performance and nutrient utilization, based on Ikpe *et al.* (2022). The water qualities, including temperature, pH, and ammonia, were measured twice weekly using proper tools based on a method from Ulfa (2018). The present study determined the temperature using a liquid thermometer (Alla France 5152T110-qp) and measured pH values with a pH meter (pH Meter Smart Sensor AS218). To assess ammonia levels in the water, this study followed a standard curve that included concentrations of 0 mg/mL, 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.5 mg/mL. In each Erlenmeyer flask, we combined 25 mL of the standard solution with 1 mL of phenol, 1 mL of sodium nitroprusside, and 2.5 mL of oxidizing solution (prepared by mixing 100 mL of alkaline citrate with 25 mL of sodium hypochlorite). After covering the flasks with paraffin film, we allowed them to stand for 1 hour to develop color. Finally, we analyzed the samples at 640 nm using a spectrophotometer (UV-1900, A124257).

Data analysis

ANOVA (One-way Analysis of variance) was utilized to examine the experimental results. Multiple comparisons (Duncan test) were conducted using IBM SPSS Statistics 26 to identify significant differences between treatments. The results were displayed as a mean ± SD. Differences between groups at each time point were deemed significant (*p<0.05*).

Results

Rose sp. anthocyanin levels

The results showed that *Rose* sp. flower methanolic extract proved that anthocyanins could be extracted, as seen in

Table **1**. Qualitative determination of *Rose* sp. anthocyanins was carried out by scanning at a wavelength of 520 nm to 750 nm on samples dissolved in the two buffer pH conditions (pH 1 and 4.5). The present study found that *Rose sp.* anthocyanins peaked at an acidic pH of 1.0, reaching 0.701 and 0.171 (520 nm and 700 nm, respectively). However, neither sample showed differences in MAP (Monomeric Anthocyanin Pigments) results (1.24 mg/L and 1.22 mg/L), as indicated by their numbers. Regarding those discoveries, *Rose* sp. anthocyanins extraction could employ methanol in the maceration process for 24 hours.

Table 1. Anthocyanin content of *Rose* sp. flower extract was measured at pH 1.0 and 4.5. Values represent duplicate measurements of the same sample.

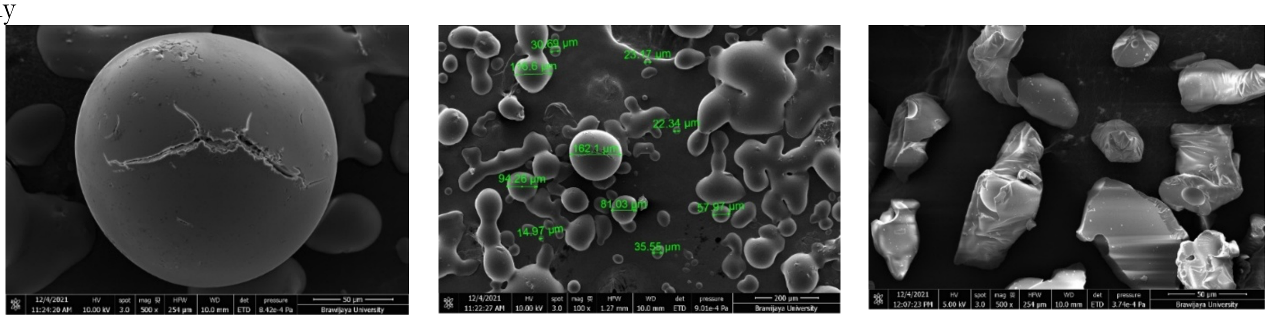
Technical replicates	pH 1		pH 4.5		A	MW	DF	ε	MAP ($mg{\cdot}L^{-1}$)
	520	700	520	700					
Sample 1	0.670	0.158	0.582	0.144	0.074	449.2	1	26900	1.24
Sample 2	0.701	0.171	0.614	0.157	0.073	449.2	1	26900	1.22

A : Solution absorbance
MW : Molecular weight
DF : Dilution factor
ε : Molecular absorptivity of cyanide-3-glucoside
MAP : Monomeric Anthocyanin Pigment

Field emission Scanning Electron Microscopy (FESEM) analysis of MRFA

The surface morphology analysis of MRFA particles, conducted using FESEM at a magnification of 500 times, revealed an irregular form among the particles. The morphology of maltodextrin looked like a large lump with a rough surface texture. The analysis of anthocyanin–maltodextrin microcapsules showed that the morphology of particles was still clearly distributed, even though it was not homogeneously distributed (14 µm to 170 µm) (Figure 1).

Furthermore, the present study conducted FESEM analysis to examine the surface morphology of MRFA nanoparticles (as shown in Figure 1). Notably, the barrier layer has a significant influence on particle wrinkling. This study employed maltodextrin to produce nearly spherical and smooth particles.



A B C

Figure 1. Surface morphology of particles using FESEM analysis, (A and B) MRFA, (C) maltodextrin.

X-ray diffraction (XRD) analysis of *Rose* sp. anthocyanin

The XRD test results (Figure 2) indicate that the silica ash obtained from well-burned rice husks during the initial treatment is not amorphous. This is evidenced by the sloping peak at 2θ = 22°, characteristic of amorphous silica. Previous research has shown that silica isolated from husked rice exhibits an amorphous structure.

Figure 2 displays the XRD diffractograms for the MRFA and maltodextrin layers, analyzed using Match! Version 2 software. Overall, both sample layers demonstrate an amorphous phase, as the diffraction peaks do not correspond to any crystalline phase. This qualitative analysis confirms the successful growth of MRFA and maltodextrin samples with an amorphous layer structure.

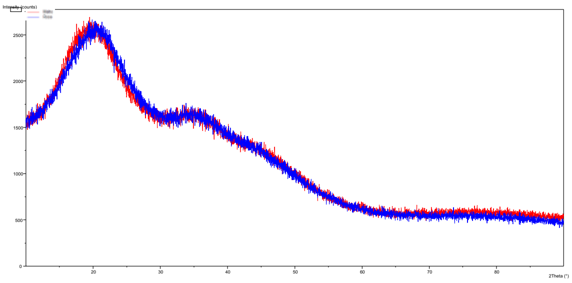


Figure 2. XRD analysis of MRFA with maltodextrin.

Effect of MRFA on Kohaku and Sanke Coloration

During week 2, MRFA supplementation produced significant differences only in chroma values, with T3 showing the highest level (42.33 ± 2.52). However, by week 4, although overall color values increased across treatments, no significant differences were observed among groups (*p>0.05*). This indicates that MRFA had only a short-term effect on Kohaku coloration (Figure 3).

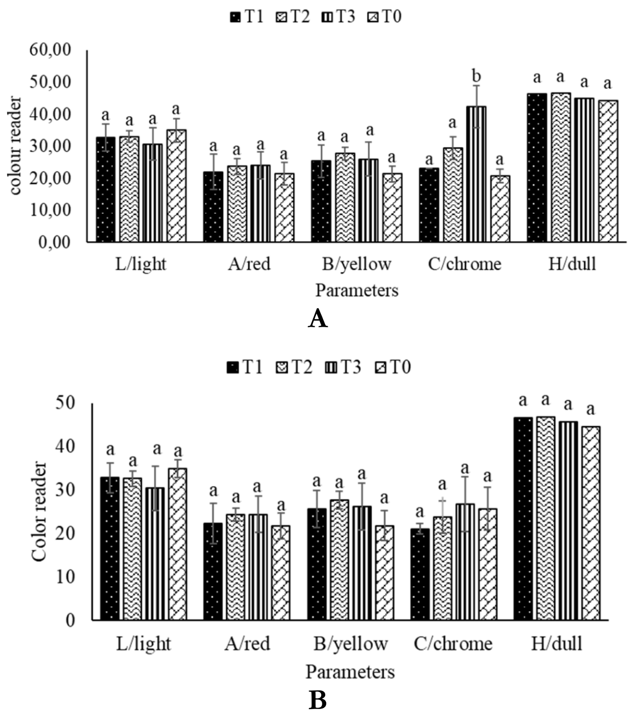


Figure 3. The Kohaku color level at (A) week 2 treatment and (B) week 4 treatment with MRFA.

In contrast, Sanke Koi responded more clearly to MRFA supplementation. At week 2, T1 and T2 significantly improved red, yellow, and chroma parameters compared to the control (*p<0.05*). However, higher supplementation (T3) was less effective. By week 4, some parameters decreased slightly, but Sanke maintained higher color intensity in T2 compared to the control, suggesting a more sustained effect of MRFA on black-based pigmentation (**Figure 4**).

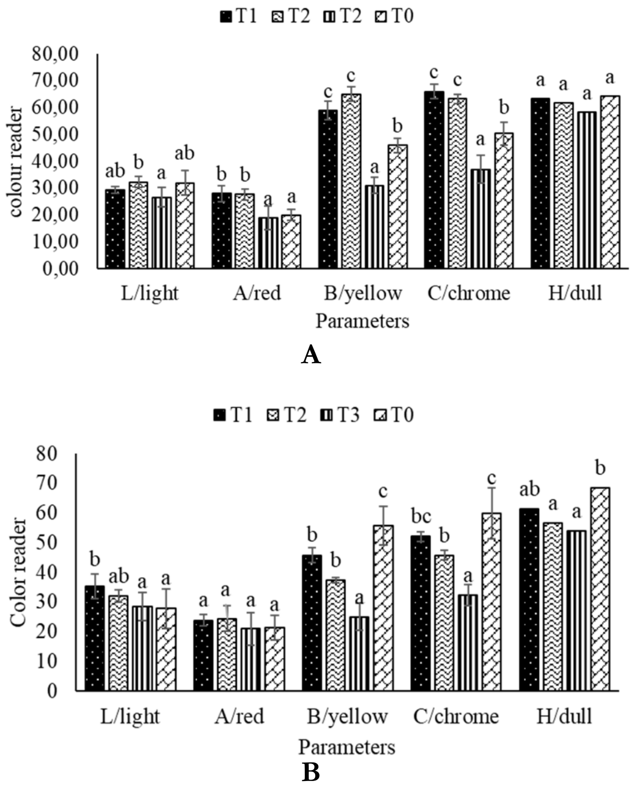
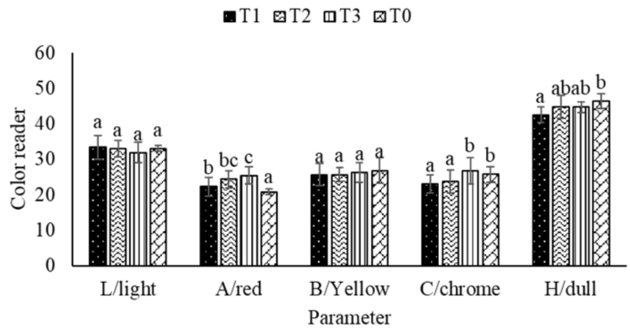


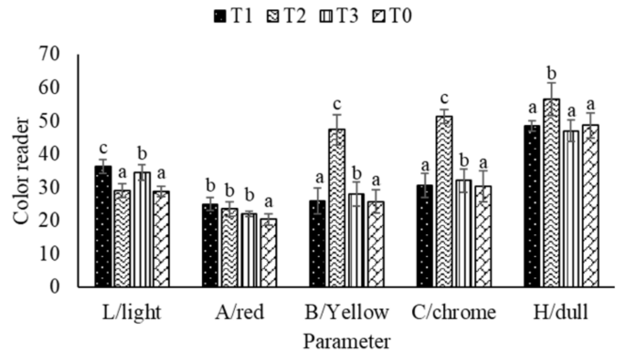
Figure 4. The Sanke color level, (A) week 2nd treatment, (B) week 4th treatment with MRFA.

Color permanence after MRFA feeding

The color permanence test aimed to determine whether the various dosages of MRFA in this study could remain and be absorbed by the koi fish body after all treatments were stopped. The data showed significant differences in all parameters (*p<0.05*) in Kohaku and Sanke at week 6 (two weeks after MRFA supplementation was stopped) (Figure 5). Overall, Kohaku and Sanke could absorb the MRFA very well, as proved by the numbers of several parameters compared to the control group.



A



B

Figure 5. Permanence of color parameters in koi species: (A) Kohaku, (B) Sanke, measured two weeks post-treatment with MRFA supplementation.

Based on the data, T3 and T2 were the most effective treatments for Kohaku (A) and Sanke (B), respectively, during a 2-week observation period. The T3 could promote Kohaku's red and chrome color higher than other treatments, reaching 26.4 ± 1.42 and 26.7 ± 3.77, respectively. Meanwhile, T2 was determined to be the best treatment for Sanke according to the numbers for red (23.46 ± 2.19), yellow (47.3 ± 4.55), and chrome (51.3 ± 2.11). Based on the analysis data, it can be concluded that MRFA could be absorbed and significantly increase the color of the koi fish body. Moreover, our study also discovered that MRFA was more effective in promoting Sanke's color than Kohaku's color.

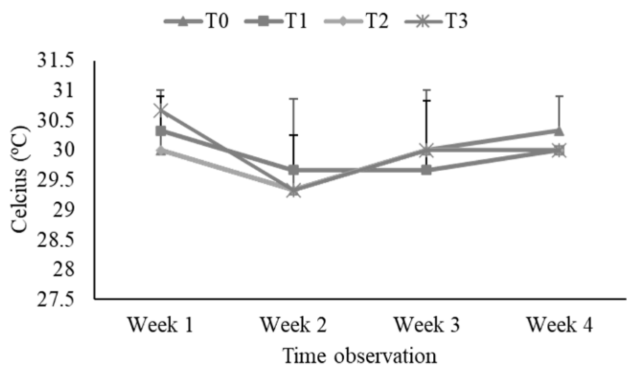
On the other hand, a significant figure in permanent color analysis showed that all treatments performed better than the control group (*p<0.05*) on the 14th day after the feeding trials were terminated. This means that the color of Sanke and Kohaku improved and became brighter permanently after the treatments were finished. Figure 5 indicates that Sanke and Kohaku completely absorbed the MRFA.

Growth performance and survival rate analysis

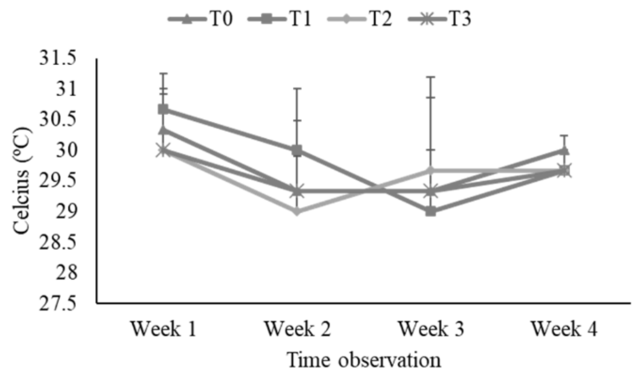
This study observed significant differences across all treatments for five measurements by comparing the growth performance and feed consumption of MRFA-supplemented commercial feed with that of Sanke and Kohaku strains over 30 days (as shown in Table 2 and Table 3). Overall, T3 exhibited the most favorable parameters, while T0 performed the weakest in promoting Sanke and Kohaku growth. T3 demonstrated superior Kohaku growth performance compared to Sanke, with an absolute weight difference of 1.02 g, while other parameters remained nearly consistent.

Water quality analysis during observations

Three water quality parameters were measured in this study: temperature, pH, and ammonia, which were recorded three times a week for one month. All water quality values remained suitable for Kohaku and Sanke cultures during the study. The water medium was consistent at 28.00 to 33.00°C (Figure 6), which was an optimum range for fish development.



A



B

Figure 6. The temperature level of Kohaku (A) and Sanke (B) rearing media water during the study.

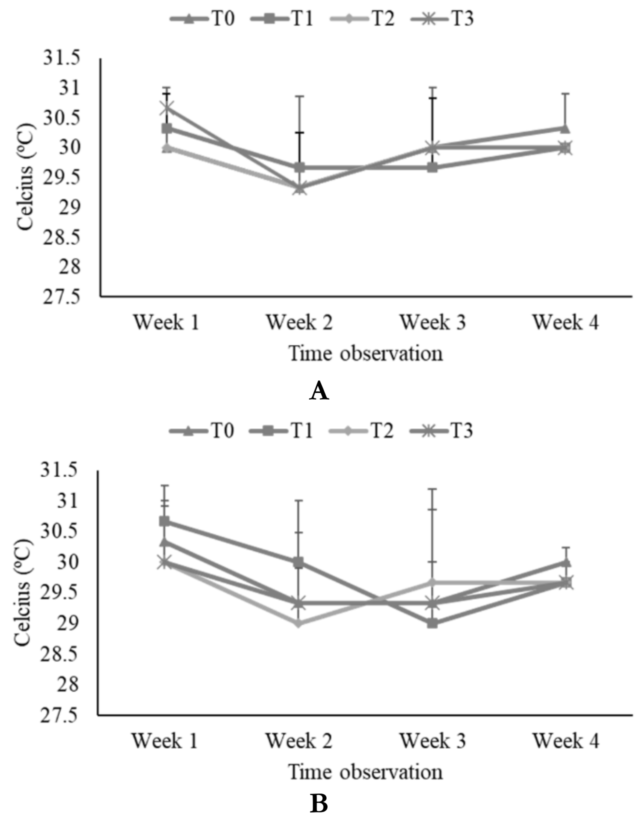


Figure 7. pH level of Kohaku (A) and Sanke (B) rearing media water during the study.

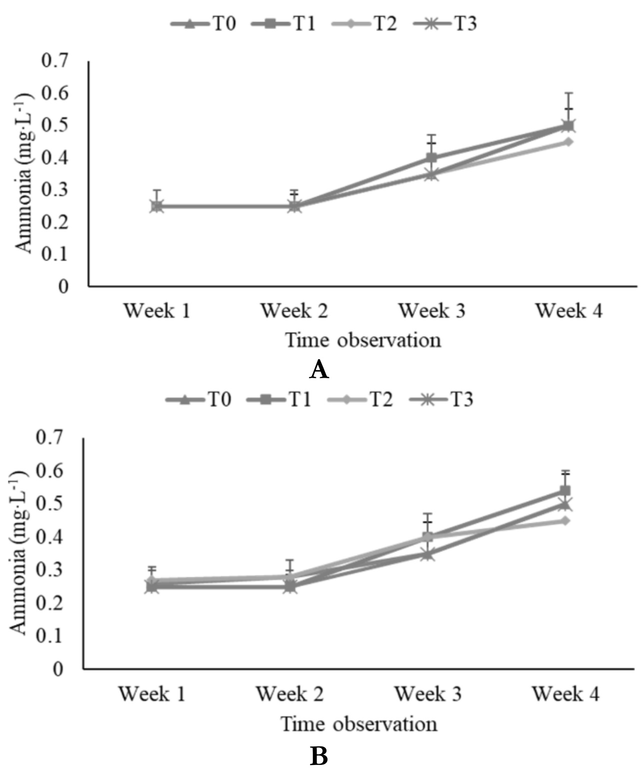


Figure 8. Ammonia level of Kohaku (A) and Sanke (B) rearing media water during the study.

At the same time, the pH level determined could support fish growth during the experiment, ranging from 7 to 8 (Figure 7). Moreover, throughout the experiment, ammonia concentrations remained within the acceptable range (0.25 mg·L^{-1} to 1 mg·L^{-1}) (Figure 8). Based on those discoveries, the MRFA supplemented in commercial feed did not negatively affect water quality during the study.

Discussion

Anthocyanins are pigments as a subgroup of water-soluble flavonoids found in most vascular plants, which are significant secondary plant metabolites (Alappat and Alappat, 2020). This study found that the *Rose* sp. flower contained anthocyanin (1.24 mg/L and 1.22 mg/L), observed using a UV-VIS T80 spectrophotometer at different wavelengths (Table 1). Some researchers also supported our study, which showed that the flower contains many anthocyanins (Barani *et al.*, 2022; Li *et al.*, 2023). FESEM evaluated Microencapsulated Rose sp. flower anthocyanin (MRFA) nanoparticle surface morphology (Figure 1). As shown, the barrier layer affects particle wrinkling. This study used maltodextrin to create almost seamless particles. Using maltodextrin DE24 as a wall material resulted in a softer surface than tapioca starch (Loksuwan, 2007). In addition, both MRFA and maltodextrin samples have been successfully cultivated using a structure consisting of an amorphous phase layer. This result was also supported by Saleh (2019) and Yang *et al.* (2019) demonstrated the amorphous phase of the a-C: H thin layer structure and the Crystallization of Ni Nanocrystals, respectively.

On the one hand, according to this study, the data were insignificant (*p>0.05*) in week 4, which contradicted some previous findings regarding Kohaku pigments. For instance, Lili *et al.* (2020) proved that adding marigold meal at a concentration of 1.5% increased the color intensity of Kohaku strain koi fish in the head and tail by 5.97 and 6.50%, respectively. Furthermore, one study found that four botanical additives, including flower petals of *Hibiscus rosasinensis*, *Rosa indica*, *Ixora coccinea*, and *Crossandra infundibuliformiss*, significantly increased the color pigments in an ornamental fish species (Singh *et al.*, 2021). As a result, based on our findings, all MRFA dosages were unable to promote Kohaku's pigment after the 4th week of observation.

On the other hand, Figure 4 illustrates variations in color parameters of Sanke koi across different treatments throughout the second and fourth weeks of identification. These findings are also consistent with various research studies. Other plant extracts, such as *Mucuna pruriens* seed methanol, *Tribulus terrestris* seed ethanol, and *Basella alba* leaves ethanol, have been shown to enhance the pigmentation of fish species like *Botia rostrata* (Mukherjee *et al.*, 2023).

Pumpkin extract, specifically at a concentration of 30ml·100gr, has also been found to increase the brightness of the color of ornamental fish (Sari *et al.*, 2022).

Although MRFA supplementation improved Kohaku coloration initially, the effect was not sustained by week 4, which contrasts with previous studies where marigold meal and other botanical additives enhanced Kohaku pigmentation (Lili *et al.*, 2020; Singh *et al.*, 2021). Several factors may explain these differences. First, genetic variation among Kohaku strains could influence their capacity to absorb and deposit pigments (Dani *et al.*, 2012; Shi *et al.*, 2024). Second, the type and dosage of pigment sources vary across studies, which may affect bioavailability and effectiveness (Ayvaz *et al.*, 2023). Finally, anthocyanins are more commonly associated with black pigmentation (Ahmad *et al.*, 2022; Bao *et al.*, 2022; Kim *et al.*, 2021), which may explain why Sanke responded more strongly than Kohaku. These factors highlight the need for further strain-specific and pigment-specific studies to clarify the mechanisms involved.

Furthermore, the present study found that the application of MRFA boosted Kohaku and Sanke's colors promptly after the second week of feeding trials (Figure 3 and Figure 4, respectively). The discovery might be assisted by other research that plant extract microencapsulation can improve the absorption of bioactive compounds by the body (Calderón-Santoyo *et al.*, 2022; Pertiwi *et al.*, 2023). The microcapsules formed have spherical shapes and small particle sizes, facilitating their absorption (Figure 1) (Lengyel *et al.*, 2019). It could be assumed that MRAF was absorbed quickly by the Koi fish through intestinal absorption during feeding trials. A similar study proved that 450 mg of *Hibiscus sabdariffa* anthocyanin stimulated *Carassius auratus* colors during eight weeks of feeding trials (Vanegas-Espinoza *et al.*, 2019).

Based on these findings, this suggests that microencapsulated rose anthocyanins (MRFA) can serve as a potential eco-friendly feed additive in aquaculture to support koi coloration and growth. However, MRFA did not significantly improve coloration in Kohaku compared to the control (T0), and in Sanke, the control group outperformed MRFA in some parameters, particularly yellow and chroma values at week 4. These results highlight that the effectiveness of MRFA may vary depending on koi strain and color traits, and further studies with longer durations (color permanence test, Figure 5) and larger populations are recommended to validate its broader application in ornamental fish culture.

Furthermore, MRFA demonstrated growth-enhancing effects in Koi compared to the control group (as shown in Table 2 and Table 3). These findings aligned with previous research that highlighted anthocyanins as fish growth promoters. For instance, dark rice bran anthocyanin extracts at concentrations of 4 to 8 g·kg^{-1} improved growth, feed utilization, and the expression of innate immune and antioxidant genes in Nile tilapia cultured within a biofloc system (Linh *et al.*, 2022).

Table 2. Sanke's growth rate, survival rate, and feed utilization.

Treatment	Absolute weight (g)	Absolute Length (cm)	SR (%)	TFC (g)	FCR
T0	5.00±0,56^{a}	1.56±0.23^{a}	100±00^{a}	195.50±4.02^{a}	1.99±0.23^{b}
T1	9.93±0.90^{bc}	1.63±0.45^{a}	100±00^{a}	199.75±3.05^{a}	1.32±0.21^{a}
T2	8.47±1.03^{b}	1.67±0.55^{a}	100±00^{a}	227.80±3.05^{b}	2.03 ±0.18^{b}
T3	10.60±1.25^{c}	1.90±0.70^{a}	100±00^{a}	195.50±3.29^{a}	1.45±0.14^{a}

Values with different superscript letters in the same column are significantly different (*p<0.05*).

Table 3. Kohaku's growth rate, survival rate, and feed utilization.

Treatment	Absolute weight (g)	Absolute Length (cm)	SR (%)	TFC (g)	FCR
T0	5.03±0,25a	1.62±0.22a	100±00a	196.00±3.12a	2.00±0.43b
T1	8.43±0.70bc	1.61±0.15a	100±00a	200.50±3.25a	1.62±0.14a
T2	9.12±1.33b	1.58±0.33a	100±00a	210.45±2.85b	1.53±0.18a
T3	11.62±1.27c	1.72±0.26a	100±00a	200.50±4.13a	1.42±0.14a

Values with different superscript letters in the same column are significantly different (*p<0.05*).

Similarly, a dietary supplement derived from *Hylocereus polyrhizus* fermentation waste, containing 0.57g/100g of anthocyanin, enhanced weight gain (WG) and specific growth rate (SGR) in *Platax pinnatus* during an 8-week feeding trial (Chu *et al.*, 2021). Anthocyanins may also influence gut microbiota colonization, impacting the development of intestinal bacteria (Tian *et al.*, 2019). Notably, they promote the growth of beneficial bacteria, such as *Bifidobacterium* spp. and *Lactobacillus-Enterococcus* spp., in vitro (Boto-Ordóñez *et al.*, 2014). Given the role of gut microbiota in processes like epithelial proliferation, nutrient metabolism, and innate immune responses (Yukgehnaish *et al.*, 2020). The microbiota residing in a fish's digestive tract can serve as a source of growth-promoting probiotics and contribute to vitamin production (Ramos *et al*., 2017). Based on these findings, the present study concludes that MRFA has growth-enhancing properties.

This study calculated temperature, pH, and ammonia three times a week for one month. All water quality values were adequate for the Kohaku and Sanke cultures during the investigation. Warm-water fish can survive in temperatures ranging from 22 to 32°C, as demonstrated by Nuwansi *et al.* (2021). The optimal pH range for fish in a fish culture system is between 6 and 8.5. Nutrient absorption was most efficient at slightly low pH levels (Heydarnejad, 2012; Tumwesigye *et al*., 2022). The ammonia level in water is still tolerable for fish when it does not exceed 1 mg·L^{-1} (Robbani *et al*., 2022).

On top of that, this study still has some limitations. The relatively short duration (four weeks of feeding plus two weeks post-treatment) may not reflect long-term effects. Genetic variation among koi strains could also have influenced responses, and the sample size, though adequate for analysis, limits generalization. Future studies with longer trials, larger populations, and multiple strains are needed to validate the broader application of rose anthocyanins in ornamental fish culture.

Conclusion

This study demonstrated that microencapsulated rose anthocyanins (MRFA) supported the growth performance of both Kohaku and Sanke koi but showed limited and strain-specific effects on coloration. At week 2, MRFA improved certain color parameters, particularly in Sanke, while Kohaku showed only temporary chroma enhancement. By week 4, MRFA did not significantly improve Kohaku coloration compared to the control (T0), and in Sanke, the control group was even superior in yellow and chroma values. The permanence test indicated that MRFA did not further increase coloration once supplementation was stopped, but helped maintain the coloration achieved during the feeding period.

Overall, these findings suggest that MRFA has potential as an eco-friendly, locally available feed additive in ornamental aquaculture, although its effects vary between strains and color traits. The relatively short experimental duration, modest sample size, and potential genetic variability among fish are limitations of the present study. Future research with longer feeding trials, larger populations, and multiple koi strains is recommended to validate the long-term applicability of rose anthocyanins in fish pigmentation.

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References

Afnan, M.A., G.A. Sutarjo, H. Hariyadi. 2019. Analysis of fresh water ornamental fish production (Goldfish, Koi and Betta Fish) in Tulungagung district, East Java, Indonesia. Indonesian Journal of Tropical Aquatic, 2(2): 80-87.

Ahmad, S., J. Chen, G. Chen, J. Huang, Y. Zhou, K. Zhao, S. Lan, Z. Liu, D. Peng. 2022. Why black flowers? An extreme environment and molecular perspective of black color accumulation in the ornamental and food crops. Frontiers in Plant Science, 13: 885176.

Alappat, B., J. Alappat. 2020. Anthocyanin pigments: beyond aesthetics. Molecules, 25(23): 1-17.

Ayvaz, H., T. Cabaroglu, A. Akyildiz, C.U. Pala, R. Temizkan, E. Ağçam, Z. Ayvaz, A. Durazzo, M. Lucarini, R. Direito, Z. Diaconeasa. (2023). Anthocyanins: Metabolic Digestion, Bioavailability, Therapeutic Effects, Current Pharmaceutical/Industrial Use, and Innovation Potential. *Antioxidants*, *12*(1), 48.

Bao, X., Y. Zong, N. Hu, S. Li, B. Liu, H. Wang. 2022. Functional R2R3-MYB transcription factor NsMYB1, regulating anthocyanin biosynthesis, was relative to the fruit color differentiation in Nitraria sibirica Pall. BMC Plant Biology, 22(1): 1-12.

Barani, Y.H., M. Zhang, A.S. Mujumdar, L. Chang. 2022. Preservation of color and nutrients in anthocyanin-rich edible flowers: Progress of new extraction and processing techniques. Journal of Food Processing and Preservation, 46(9): e16474.

Boto-Ordóñez, M., M. Urpi-Sarda, M.I. Queipo-Ortuño, S. Tulipani, F.J. Tinahones, C. Andres-Lacueva. 2014. High levels of Bifidobacteria are associated with increased levels of anthocyanin microbial metabolites: a randomized clinical trial. Food & Function, 5(8): 1932-1940.

Brown, T., B. Runciman, M. Bradford, S. Pollard. 2009. A biological synopsis of yellow perch (*Perca flavescens*). Canadian manuscript report of fisheries and aquatic sciences, 2883: 1-28.

Calderón-Santoyo, M., E.M. González-Cruz, M. Iñiguez-Moreno, O. Ramos-Martínez, A. Burgos-Hernández, J.A. Ragazzo-Sánchez. 2022. Microencapsulation of phenolic extract from sea grape (*Coccoloba uvifera* L.) with antimutagenic activity. Chemistry & Biodiversity, 19(11): e202200806.

Câmara, J.S., M. Locatelli, J.A. Pereira, H. Oliveira, M. Arlorio, I. Fernandes, R. Perestrelo, V. Freitas, M. Bordiga. 2022. Behind the scenes of anthocyanins—from the health benefits to potential applications in food, pharmaceutical and cosmetic fields. Nutrients, 14(23): 5133.

Chu, J.-H., K.-J. Liu, T.-M. Wu. 2021. Effects of pitaya (*Hylocereus polyrhizus*) fermentation waste dietary supplement on growth performance, and anti-oxidation of Pinnate batfish, *Platax pinnatus*. Aquaculture Research, 52(12): 6689-6698.

Dani, B., K. Sigal, S. Lior, D. Gad. 2012. Genetic diversity of color phenotypes in the Koi (*Cyprinus carpio* L.) as identified by molecular markers. Journal of Biophysical Chemistry, 3(3): 249-255.

Darliah, D., D. Kuriasih, W. Handayati. 2010. [Crossing and selection for varieties new superior red-colored cut roses]. Jurnal Hortikultura, 20(2): 103-110.

Delgado-Vargas, F., A.R. Jiménez, O. Paredes-López. 2000. Natural pigments: carotenoids, anthocyanins, and betalains characteristics, biosynthesis, processing, and stability. Critical Reviews in Food Science and Nutrition, 40(3): 173-289.

Fabanjo, M., N. Abdullah. 2021. Effect of addition of pro-enzymes and *Spirulina* flour on growth and color intensity of betta fish (*Betta* sp). IOP Conference Series: Earth and Environmental Science, 890(1): 012025-012033.

García-Chavarría, M., M. Lara-Flores. 2013. The use of carotenoid in aquaculture. Research Journal of fisheries and Hydrobiology, 8(2): 38-49.

Hakim, R.R., R.R. Hidayat, D. Hermawan, D. Prasetyo. 2025. Optimizing the addition of *Skeletonema Costatum* flour in feed for the growth and survival of koi fish (*Cyprinus carpio*). Acta Aquatica: Aquatic Sciences Journal, 1(1): 28-34.

Handajani, H., S. Andriawan, R. Gilang, W. Warkoyo. 2021. Enrichment of commercial feed with plant proteins for *Oreochromis niloticus* diet: digestibility and growth performance. AACL Bioflux, 14(5): 2894-2904.

Hermawan, D., G.A. Sutarjo, D. Prasetyo, L. Dahlia, D. Arisandy. 2024. Reproductive performance of crossbreeding between local female and imported male koi at CV. Indokoi Malang. BIO Web of Conferences, 143: 02005.

Heydarnejad, M.S. 2012. Survival and growth of common carp (*Cyprinus carpio* L.) exposed to different water pH levels. Turkish Journal of Veterinary and Animal Sciences, 36: 245-249.

Ikpe, J., Z. Bashir, A. Ajadi, A.B. Dauda. 2022. Frequency of water change on growth performance, nutrient utilization and liver histology of nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758). Asian Journal of Fisheries and Aquatic Research, 16: 42-50.

Jalila, R.S., A.R. Scabra, N. Cokrowati. 2021. [Influence of different color of wades on production performance of koi fish (*Cyprinus carpio*)]. Indonesian Journal Of Aquaculture Medium, 1(2): 83-97.

Kalidupa, N., A. Kurnia, I. Nur. 2018. [Study on the utilization of red dragon fruit peel flour (*Hylocereus polyrhizus*) in feed against the coloration of koi carp (*Cyprinus carpio* L.)]. Media Akuatika, 3(1): 590-597.

Khoo, H.E., A. Azlan, S.T. Tang, S.M. Lim. 2017. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food & Nutrition Research, 61(1): 1361779.

Kim, Y.B., R. Sathasivam, S.-U.K.S.U. Park. 2021. Differential expression profiles of anthocyanidin biosynthesis gene during black rice seed development. Journal of Phytology, 13: 36-40.

Kurnia, A., I. Nur, W.H. Muskita, M. Hamzah, W. Iba, R.S. Patadjai, A.M. Balubi, N. Kalidupa. 2019. Improving skin coloration of koi carp (*Cyprinus carpio*) fed with red dragon fruit peel meal. Aquaculture, Aquarium, Conservation & Legislation, 12(4): 1045-1053.

Lalita, A., W. Lili, F.M. Pratiwy. 2022. The effect of differences in the addition of astaxanthin and several sources of natural ß-carotene in increasing color intensity of koi fish (*Cyprinus carpio* L.). Asian Journal of Fisheries and Aquatic Research, 18(2): 38-47.

Lengyel, M., N. Kállai-Szabó, V. Antal, A.J. Laki, I. Antal. 2019. Microparticles, microspheres, and microcapsules for advanced drug delivery. Scientia Pharmaceutica, 87(3): 20.

Li, J., X. Guo, R. Wang, Z. Geng, J. Jia, S. Pang, Y. Du, S. Jia, J. Cui. 2023. Ultrasonic assisted extraction of anthocyanins from rose flower petal in DES system and enzymatic acylation. LWT, 180: 114693.

Lili, W., I. Iskandar, R.M. Rhamdhan, R. Grandiosa. 2020. The effect of addition marigold-meal to artificial feeds for increasing color intensity of koi fish (*Cyprinus carpio* Linnaeus, 1758) strain kohaku. World News of Natural Sciences, 32: 49-60.

Linh, N.V., D.V. Nguyen, N. Khongdee, S. Wannavijit, P. Outama, C. Le Xuan, S. Mahatheeranont, P. Sookwong, T.D. Le, S.H. Hoseinifar, Y.-H. Moon, H. Van Doan. 2022. Influence of black rice (*Oryza sativa* L.) bran derived anthocyanin-extract on growth rate, immunological response, and immune-antioxidant gene expression in Nile tilapia (*Oreochromis niloticus*) cultivated in a biofloc system. Fish & Shellfish Immunology, 128: 604-611.

Loksuwan, J. 2007. Characteristics of microencapsulated β-carotene formed by spray drying with modified tapioca starch, native tapioca starch and maltodextrin. Food Hydrocolloids, 21(5): 928-935.

Luo, M., L. Wang, W. Zhu, J. Fu, F. Song, M. Fang, J. Dong, Z. Dong. 2018. Identification and characterization of skin color microRNAs in koi carp (*Cyprinus carpio* L.) by Illumina sequencing. BMC Genomics, 19(1): 1-15.

Malide, S.M., A. Hendri, B. Budiman. 2020. [Addition of carrot and tubifex as a natural source of beta-carotene in artificial feed on the color quality of koi fish (*Cyprinus carpio* Linnaeus)]. Jurnal Akuakultura Universitas Teuku Umar, 2(2): 64-71.

Malini, D.M., R. Agustin. 2018. [The effect of adding *Spirulina fusiformis* flour to feed on the brightness level of koi fish (*Cyprinus carpio* L.)]. Jurnal Pro-Life, 5(2): 579-588.

Mukherjee, M., D.N. Das, S.B. Chakraborty. 2023. Dietary administration of three medicinal plant extracts enhance innate immunity and skin pigmentation of *Botia rostrata* (Günther, 1868). Journal of Applied Aquaculture, 35(4): 1069-1087.

Nuwansi, K.K.T., A.K. Verma, M.H. Chandrakant, G.P.W.A. Prabhath, R.M. Peter. 2021. Optimization of stocking density of koi carp (*Cyprinus carpio* var. koi) with gotukola (*Centella asiatica*) in an aquaponic system using phytoremediated aquaculture wastewater. Aquaculture, 532: 735993.

Pertiwi, A.K., C. Annisa, Z. Ningsih, A. Safitri. 2023. Microencapsulation of *Ruellia tuberosa* L. extracts using alginate: Preparation, biological activities, and release. Indonesian Journal of Chemistry, 23(2): 321-332.

Prabavathy, N., S. Shalini, R. Balasundaraprabhu, D. Velauthapillai, S. Prasanna, G. Balaji, N. Muthukumarasamy. 2018. Algal buffer layers for enhancing the efficiency of anthocyanins extracted from rose petals for natural dye-sensitized solar cell (DSSC). International Journal of Energy Research, 42(2): 790-801.

Ramos, M.A., S. Batista, M.A. Pires, A.P. Silva, L.F. Pereira, M.J. Saavedra, R.O.A. Ozório, P. Rema. 2017. Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. Animal, 11(8): 1259-1269.

Robbani, A.A., O. Ariyanti, D. Calvin, G.A. Sutarjo, H. Handajani, S. Andriawan. 2022. The combination of tofu dregs and *Rhodopsudomonas palustris* on *Oreochromis niloticus* growth performances. Aquaculture, Aquarium, Conservation & Legislation, 15(4): 2113-2124.

Saati, E.A., E.E. Haprinata, S. Winarsih, M. Wachid, V.A. Wahyudi. 2021. Physicochemical and sensory characteristics of Rose-spices drink with red ginger, lemongrass, and cinnamon extract as functional beverage. Food Science and Technology Journal (Foodscitech): 16-26.

Saati, E.A., V.A. Wahyudi, A. Dyah, S. Andriawan. 2022. Anthocyanin extract of *Rosa* sp. as a natural preservative in *Euthynnus affinis*. Aquaculture, Aquarium, Conservation & Legislation, 15(1): 136-146.

Saleh, M. 2019. [Optical properties and electrical properties of thin films of hydrogenated amorphous carbon (a-C:H) fabricated using the rf-pecvd technique]. Jurnal Sains dan Pendidikan Fisika, 15(3): 9-16.

Salman, M., S. Adeel, N. Habib, F. Batool, M. Usama, F. Iqbal, A. Fatima. 2023. Extraction of anthocyanin from Rose petals for coloration of biomordanted wool fabric. Coatings, 13(3): 623.

Sari, M.P., K. Khotimah, L. Ramonda. 2022. Growth response and color brightening enhancement of cupang fish (*Betta* sp.) fed yellow pumpkin extract (*Cucurbita moscheta* durch). Journal of Global Sustainable Agriculture, 3(1): 46-50.

Sathyaruban, S., D.I. Uluwaduge, S. Yohi, S. Kuganathan. 2021. Potential natural carotenoid sources for the colouration of ornamental fish: a review. Aquaculture International, 29(4): 1507-1528.

Shi, X., W. Zhu, J. Guo, K. Lin, J. Fu, L. Wang, Y. Dong, M. Luo, Z. Dong. 2024. Genome-wide association study reveals candidate genes critical for skin pigmentation in common carp (Cyprinus carpio) strains including koi. Aquaculture, 590: 741075.

Singh, M.K., P. Borgohain, K. Kaur, S. Gogoi. 2021. Emergence of plants in fish pigmentation. Agricultural Research, 10(4): 535-542.

Tian, L., Y. Tan, G. Chen, G. Wang, J. Sun, S. Ou, W. Chen, W. Bai. 2019. Metabolism of anthocyanins and consequent effects on the gut microbiota. Critical Reviews in Food Science and Nutrition, 59(6): 982-991.

Tumwesigye, Z., W. Tumwesigye, F. Opio, C. Kemigabo, B. Mujuni. 2022. The effect of water quality on aquaculture productivity in Ibanda District, Uganda. Aquaculture Journal, 2(1): 23-36.

Ula, D.Q., N. Azizah, A. Suryanto. 2019. [Reflowering on roses (*Rosa* sp.) as display garden through pruning and foliar fertilizer]. PLANTROPICA: Journal of Agricultural Science, 4(1): 1-10.

Ulfa, R.F. (2018). *[Analysis of cadmium levels in water and sediments of the Lesti River, Malang Regency using the Atomic Absorption Spectroscopy (AAS) method]* Universitas Islam Negeri Maulana Malik Ibrahim]. Malang.

Vanegas-Espinoza, P.E., V. Pérez-Escalante, G. Aguirre-Guzman, J.D. Hoyos-Leyva, A.A. Del Villar-Martínez. 2019. Microencapsulation of anthocyanins from roselle (*Hibiscus sabdariffa*) and its application on a pigment supplied diet to fantail goldfish (*Carassius auratus*). Aquaculture International, 27(6): 1801-1811.

Wagde, M.S., S.K. Sharma, B.K. Sharma, A.P. Shivani, N.R. Keer. 2018. Effect of natural β-carotene from-carrot (*Daucus carota*) and Spinach (*Spinacia oleracea*) on colouration of an ornamental fish-swordtail (*Xiphophorus hellerii*). Journal of Entomology and Zoology Studies, 6(6): 699-705.

Wang, Y.-J., Y.-H. Chien, C.-H. Pan. 2006. Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus*. Aquaculture, 261(2): 641-648.

Wulandari, R., M.A.K. Budiyanto, L. Waluyo. 2016. The influence of various concentration of red roses (*Rosa damascena* Mill) flower extract to anthocyanin color stability jelly as biology learning source. JPBI (Jurnal Pendidikan Biologi Indonesia), 2(1): 48-56.

Yan, Y., J. Zhao, S. Lin, M. Li, J. Liu, O. Raymond, P. Vergne, W. Kong, Q. Wu, X. Zhang. 2023. Light-mediated anthocyanin biosynthesis in rose petals involves a balanced regulatory module comprising transcription factors RhHY5, RhMYB114a, and RhMYB3b. Journal of Experimental Botany, 74(18): 5783-5804.

Yang, J., J. Koo, S. Kim, S. Jeon, B.K. Choi, S. Kwon, J. Kim, B.H. Kim, W.C. Lee, W.B. Lee, H. Lee, T. Hyeon, P. Ercius, J. Park. 2019. Amorphous-phase-mediated crystallization of Ni nanocrystals revealed by high-resolution liquid-phase electron microscopy. Journal of the American Chemical Society, 141(2): 763-768.

Yong, A.S.K., M.T. Mohamad Lal, N. Mohd @ Mohamad. 2022. Dietary inclusion of roselle improves body colouration and survival of the whiteleg shrimp (*Litopenaeus vannamei*) juveniles challenged against *Vibrio harveyi*. Borneo Journal of Marine Science and Aquaculture (BJoMSA), 6(1): 16-19.

Yukgehnaish, K., P. Kumar, P. Sivachandran, K. Marimuthu, A. Arshad, B.A. Paray, J. Arockiaraj. 2020. Gut microbiota metagenomics in aquaculture: factors influencing gut microbiome and its physiological role in fish. Reviews in Aquaculture, 12(3): 1903-1927.

Zutshi, B., R. Madiyappa. 2020. Impact of *Lantana camara*, a carotenoid source, on growth and pigmentation in Koi swordtail (*Xiphophorus helleri*). Aquaculture, Aquarium, Conservation & Legislation, 13(1): 286-295.

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