JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(10), 116–125
https://doi.org/10.12911/22998993/205686
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Received: 2025.05.16
Accepted: 2025.07.15
Published: 2025.07.22

Cochineal powder as an eco-friendly carotenoid supplement to enhance coloration in *Betta splendens*

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ABSTRACT

The growing demand for natural and sustainable additives in aquaculture has driven interest in eco-friendly pigmentation enhancers for ornamental fish. This study evaluated the efficacy of cochineal powder (*Dactylopius coccus*), a natural insect-derived source of carminic acid and carotenoids, in enhancing red coloration in Betta fish (*Betta splendens*). A total of 60 Betta fish were randomly assigned to four dietary treatments containing 0%, 5%, 10%, and 15% cochineal powder for 30 days. Color intensity was quantified through visual analysis using Adobe Photoshop (hue and RGB values) and supported by histological analysis of chromatophore cell counts in dorsal and caudal skin tissues. The 10% cochineal inclusion yielded the most significant enhancement in red coloration, with the lowest hue values (7.3° dorsal, 6.0° caudal) and the highest red (R) values in RGB analysis. Histological examination confirmed a corresponding increase in chromatophore density, peaking at the 10% dose. This study highlights the novel application of cochineal powder in fish feed as a pro-ecological and sustainable alternative to synthetic pigments, supporting visual quality improvements in ornamental aquaculture. The research responds to the urgent need for environmentally responsible practices in the aquaculture sector by demonstrating that optimal inclusion of natural colorants can enhance product value without ecological compromise. These findings contribute to the advancement of green aquafeed technologies and provide a replicable model for sustainable pigment enhancement in the ornamental fish industry. For consumers and ornamental fish enthusiasts, incorporating feed containing 10% cochineal powder is recommended to naturally enhance red coloration in Betta fish without relying on synthetic additives. Regular use of such eco-friendly feed can improve fish appearance while supporting environmentally responsible aquaculture practices.

Keywords: aquatic nutrition, aquaculture, carminic, chromatophore, pigmentation, sustainability.

INTRODUCTION

In the ornamental fish industry, color vibrancy is a crucial determinant of both aesthetic appeal and market value. The demand for vividly colored fish has led enthusiasts and breeders to explore various methods for enhancing the natural pigmentation of these species. Research indicates that consumers often prefer fish with brighter, more vibrant colors, which can significantly influence purchasing decisions and market dynamics (Alam et al., 2024; Lee et al., 2010). The pigmentation of fish is predominantly influenced by carotenoids, a class of naturally occurring pigments that impart red, orange, and yellow hues to many aquatic species. Notably, fish are unable to synthesize carotenoids internally, making dietary supplementation essential for achieving optimal pigmentation. (Kiswara et al., 2022; Lee et al., 2010).

Traditionally, synthetic carotenoids have been employed extensively in aquaculture to enhance fish coloration. However, there is a growing trend towards natural and sustainable alternatives due to increasing consumer awareness and demand for environmentally friendly practices (Hien et al., 2022; Li et al., 2022). This shift has sparked interest in plant-based and insect-derived carotenoids. One notable source of natural carotenoids is Cochineal (*Dactylopius coccus*), an insect that produces carminic acid, a potent red dye used across various industries. The dried and powdered form of Cochineal is rich in carminic acid and other carotenoids, presenting a promising natural dietary supplement for improving fish coloration (Li et al., 2022; Tran et al., 2022).

This study aims to investigate the impact of Cochineal powder as a carotenoid supplement on the color performance of ornamental fish. By analyzing the effects of Cochineal supplementation on pigmentation, the research seeks to provide insights into sustainable practices for enhancing ornamental fish appearance. This aligns with the broader trend towards natural additives in aquaculture nutrition, which is increasingly recognized as essential for both fish health and marketability (Hien et al., 2022; Sallam et al., 2016).

However, while numerous studies have explored plant-based sources of carotenoids such as paprika, spirulina, or marigold for color enhancement in ornamental fish, limited research has investigated insect-derived carotenoids, particularly from *Dactylopius coccus* (cochineal), despite its established use as a natural red dye in other industries. Furthermore, prior studies often lack quantitative image-based analysis and histological validation to confirm pigment cell response, especially in species such as *Betta splendens* that exhibit complex pigmentation mechanisms. This presents a research gap in evaluating both visual and cellular effects of cochineal powder as a dietary pigment enhancer.

This study addresses this gap by evaluating the efficacy of cochineal powder as a natural carotenoid source through an integrated approach combining visual color analysis (RGB and hue values via Adobe Photoshop) with chromatophore cell quantification. The originality of this research lies in its focus on insect-derived pigments and the application of dual analytical methods to validate color enhancement. The objective of this study is to determine the optimal inclusion level of cochineal powder in ornamental fish feed to achieve enhanced red pigmentation in *Betta splendens* in an eco-friendly and sustainable manner.

MATERIAIS AND METHODS

Research preparation

The research utilized 60 *Betta splendens* with relatively uniform red-orange coloration. Fish were housed in individual glass aquaria (2 L capacity) equipped with aeration systems to ensure optimal water quality. Water parameters were maintained at 26–28 °C temperature, pH 6.8–7.2, and dissolved oxygen above 5 mg/L. Cochineal powder (*Dactylopius coccus*) was procured from a certified natural pigment supplier and incorporated into formulated diets at concentrations of 5%, 10%, and 15%. A commercial feed mill was used for pelletizing.

The formulated diets were prepared to contain 30% crude protein, 6.5% fat, and varied carotenoid content depending on cochineal inclusion (analyzed using spectrophotometry). Feed was stored in vacuum-sealed bags at 4 °C. Microscopic analysis of chromatophores was conducted using a compound microscope (Olympus CX23) at 400× magnification. Histological preparations were processed using hematoxylin-eosin staining, paraffin embedding, and sectioning with a microtome (5 μm thick).

Experimental treatments

Three different levels of Cochineal powder supplementation were administered: 5%, 10%, and 15% in the diet. The experiment was conducted at the Fish Breeding Laboratory, Division of Fish Reproduction, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, for fish maintenance. The feed content analysis was carried out at the Food Quality and Safety Testing Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya.

Chromatophore cell analysis was conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya. The research was conducted over a 30-day period from February to March 2024.

Feed formulation

The experimental diets were formulated with varying levels of Cochineal powder and a control group without supplementation (Table 1). The feed composition for all treatments included 30% protein, 6.5% fat, 4% crude fiber, 9% ash, and 10% moisture, while nitrogen-free extract accounted for 40.5%. Carotenoid levels varied as follows: 0% for the control, 1.57% for the 5% Cochineal addition, 3.14% for the 10% addition, and 4.71% for the 15% addition.

Feeding procedure

Feed management is a critical aspect of aquaculture, particularly in optimizing fish growth and minimizing environmental impacts. In this study, feed was provided at 5% of the fish biomass and distributed twice daily at 08:00 and 16:00. This feeding regimen is designed to ensure that fish consume all the feed, thereby minimizing leftover waste that could adversely affect water quality (Ballester-Moltó et al., 2016).

Chromatophore cell analysis

Chromatophore cell counts were conducted on dermal tissue samples taken from the fish. Chromatophore cells were observed under a microscope at 40 × 10 magnification, with counts performed in five fields of view per slide. This standardized method divides each field into five regions, with counts averaged across fields (Schroeder et al., 2020). Samples were prepared using hematoxylin-eosin staining. Tissue samples were sectioned, processed in an automatic staining machine, embedded in paraffin, and trimmed using a microtome to obtain histological slides. After deparaffinization and staining, slides were examined microscopically to determine any changes in chromatophore cell morphology and quantity.

Chromatophore cells were quantified by analyzing microscopic images using ImageJ software. The counting process involved several adjustment steps tailored to the specific characteristics of chromatophore cells. First, the microscopic images of chromatophores were imported into ImageJ. Brightness and contrast settings were adjusted to enhance the distinction between chromatophores and the background. The images were then converted to 8-bit grayscale format to facilitate the separation of chromatophores based on intensity.

Thresholding was subsequently applied using the threshold function to isolate chromatophore cells by exploiting differences in pixel intensity, as each chromatophore typically exhibits a single-color tone (Li et al., 2023). The particle size settings within the "Analyze Particles" function were adjusted to ensure that only objects within the known size range of chromatophores (0.01 to 1 mm) were detected (Hadjisolomou et al., 2021). Additionally, circularity parameters were configured to filter for round-shaped objects, corresponding to the morphological characteristics of chromatophore cells (Hadjisolomou et al., 2021).

Visual analysis

Color intensity analysis was performed using Adobe Photoshop's eyedropper tool to measure hue values from images of the fish. Photos of

Table 1. Feed formulation

Parameters	Feed control (%)	Cochineal powder addition (5%)	Cochineal powder addition (10%)	Cochineal powder addition (15%)
Protein	30%	30%	30%	30%
Fat	6.50%	6.50%	6.50%	6.50%
Crude fiber	4%	4%	4%	4%
Ash	9%	9%	9%	9%
Water	10%	10%	10%	10%
Nitrogen free extract	40.50%	40.50%	40.50%	40.50%
Carotenoid (from cochineal powder)	0%	1.57%	3.14%	4.71%

the fish were taken under standardized lighting conditions, using a dark background for contrast. The hue value was recorded from the body area of each fish, with lower hue values indicating more intense coloration (Salsabila et al., 2024). The images were processed, and hue values were extracted to quantify differences in pigmentation across treatments (Izzah et al., 2023).

RESUITS AND DISCUSSION

Chromatophore cell observation results

The results of chromatophore cell observations in Betta fish treated with Cochineal flour for 30 days are presented in Table 2. The number of chromatophore cells in Betta fish was observed under a microscope with a magnification of 100x, focusing on the dorsal and caudal (tail) regions. The increase in chromatophore cells is indicative of a more vibrant coloration due to the enhancement of pigment deposition.

The results in Table 2 and Figure 1 show that the treatment of Betta fish with varying doses of Cochineal flour significantly affects the number of chromatophore cells. Treatment K (0%) resulted in the lowest average number of chromatophores, with 24.0 cells per field of view. This shows that without the addition of Cochineal flour, Betta fish exhibit fewer pigment cells, leading to a less vibrant color.

Conversely, treatment B (10%) produced the highest number of chromatophore cells with an average of 46.0 cells per field of view. This indicates that the addition of 10% Cochineal flour significantly enhances the number of pigment cells, making the color of the fish more vibrant. The presence of more chromatophores allows for greater pigment deposition, which results in more intense coloration.

The decrease in the number of chromatophore cells in treatment C (15%), which averaged 39.0 cells per field of view, suggests that excessive Cochineal flour does not further enhance pigmentation. The observed reduction in chromatophores at higher doses may be due to saturation or a negative impact on pigment production mechanisms.

These findings are consistent with previous research by (Salsabila et al., 2023), which suggested that increasing the concentration of carotenoid sources, such as Cochineal flour, can enhance the pigmentation of ornamental fish. However, there is an optimal range of concentration beyond which no further improvement in coloration is observed. Published article also indicated that the maximum chromatophore activity typically occurs at moderate levels of carotenoid supplementation (Rahman et al., 2021). The optimal dose of Cochineal flour for enhancing chromatophore cell numbers in Betta fish appears to be 10%. At this concentration, the number of chromatophore cells in both the dorsal and caudal regions is maximized, leading to the most intense coloration. Doses higher than 10% do not provide additional benefits and may even reduce the effectiveness of pigment production.

Visual color intensity observation results

The analysis of chromatophore cells and the visual enhancement of Betta fish coloration was conducted using Adobe Photoshop, focusing on the color intensity in the dorsal and caudal regions (Figure 2).

The color intensity was quantified in RGB (Red, Green, Blue) values, with particular emphasis on the Red channel, which is critical due to its association with carotenoid pigments derived from dietary sources (Gunawan et al., 2022). Chromatophores are specialized pigment cells responsible for the coloration in various fish species, including Betta fish. There are several types of chromatophores, including erythrophores, which specifically contribute to red pigmentation (Indriani et al., 2023). The analysis revealed that as the dosage of dietary

Table 2. Chromatophore cells in Betta fish dorsal and caudal regions (cells/field of view)

Treatment	Dorsal region	Caudal region	Total	Average ± STDEV
K (0%)	25	23	48	24.0 ± 1.00
A (5%)	35	34	69	34.5 ± 0.70
B (10%)	45	47	92	46.0 ± 1.41
C (15%)	40	38	78	39.0 ± 1.41

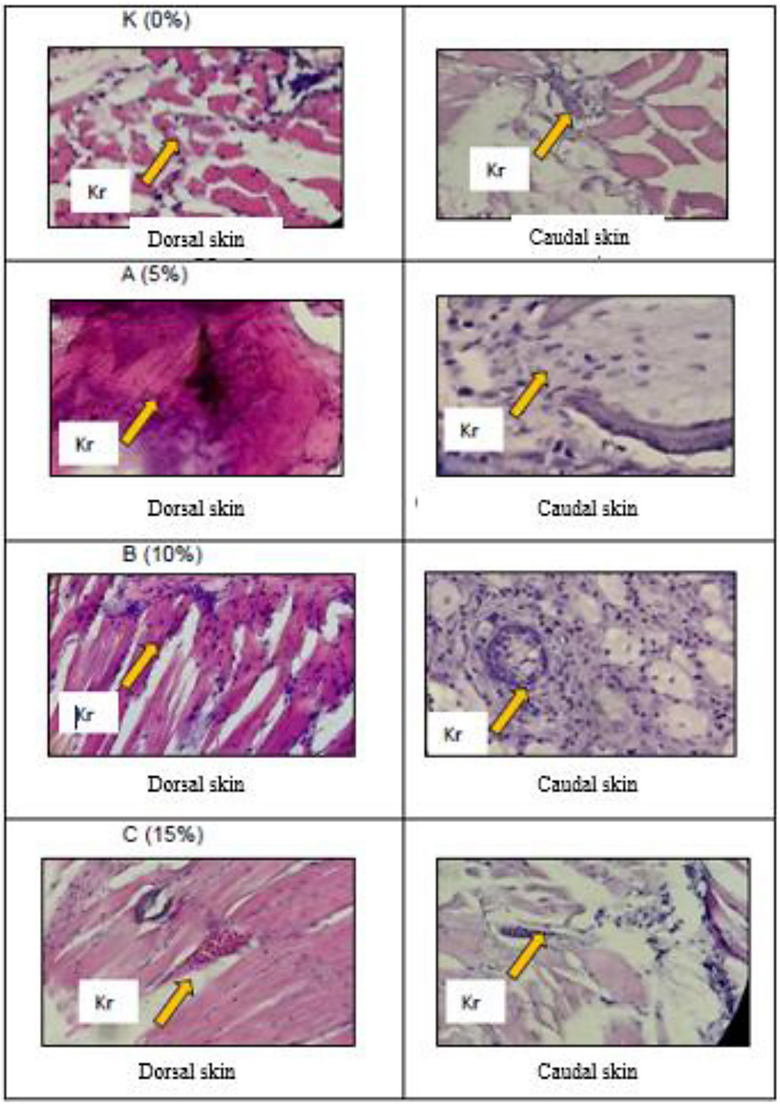

Figure 1. Chromatophore Cells (Kr) in Betta on the dorsal skin and caudal skin with 400x magnification (Photo documentation by S. Muarif)

carotenoids increased, the intensity of red pigmentation in the Betta fish also increased, which was reflected in the RGB values obtained from the image analysis. This correlation underscores the importance of carotenoids in enhancing the visual appearance of these ornamental fish (Wucherer and Michiels, 2012).

The use of Adobe Photoshop for color analysis allows for precise measurement of color intensity, which is essential for quantifying the effects of dietary supplements on fish coloration. Previous studies have demonstrated that chromatophores can respond dynamically to various stimuli, including dietary carotenoids, leading to observable changes in pigmentation (Dukovcic et al., 2010). This method of analysis not only provides a visual representation of color enhancement but also quantifies the degree of change in hue values, which is crucial for understanding the effectiveness of different concentrations of dietary supplements in improving the aesthetic qualities of Betta fish (Andrade et al., 2021). In summary, the chromatophore cell analysis and subsequent color intensity measurements using Adobe Photoshop highlight the significant role that dietary carotenoids play in enhancing the red coloration of Betta fish. The focus on the Red channel in RGB values aligns with the known effects of carotenoid pigments on fish pigmentation, providing a robust framework for future research in ornamental fish coloration (Kelsh et al., 2004).

The images of the Betta fish treated with Cochineal flour were processed and analyzed by converting them into RGB channels. The mean value for each channel was recorded to

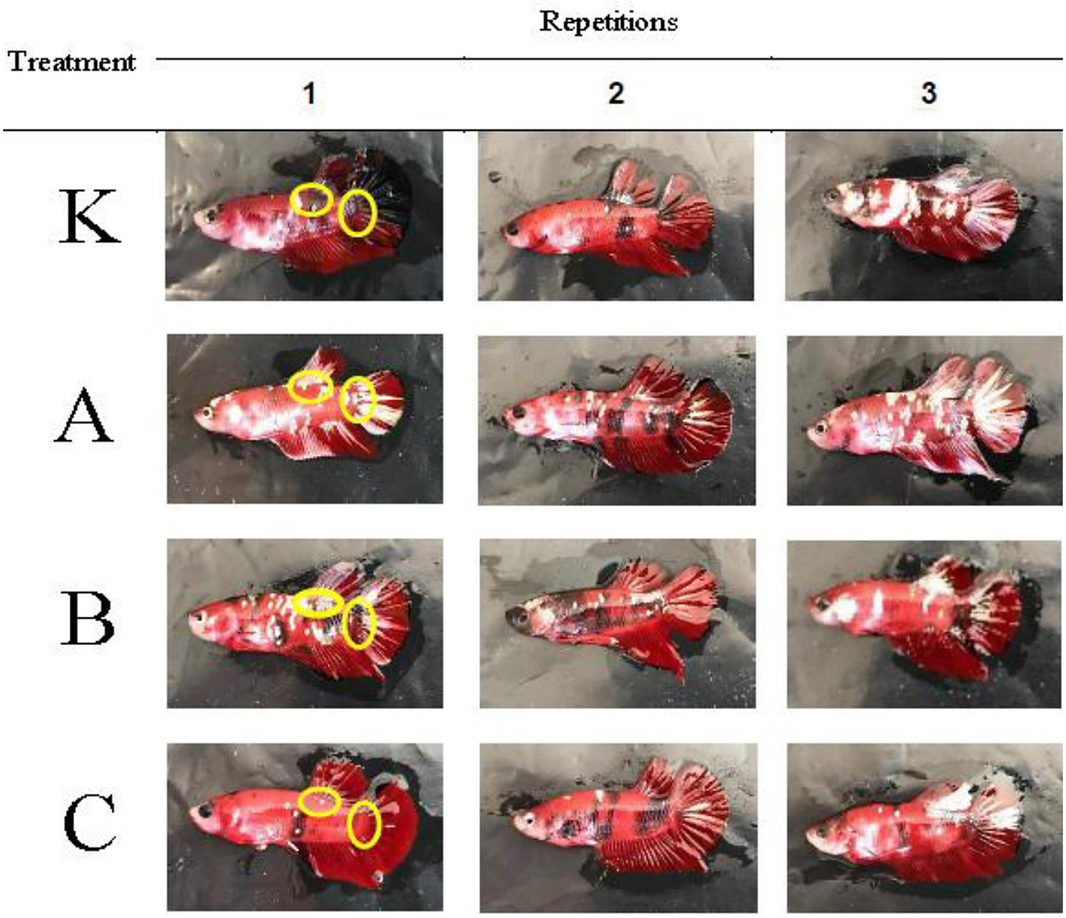

Figure 2. Color observation of *Betta splendens* on the dorsal and caudal skin regions (highlighted with yellow circles) across different treatments (Photo documentation by A.D.K. Putra)

evaluate the effect of different Cochineal flour concentrations on the visual appearance of the fish. The results are summarized in Table 3.

Table 3 presents the RGB color intensity values of Betta fish treated with varying concentrations of Cochineal flour. The analysis, utilizing Adobe Photoshop, indicates a significant enhancement in visual color intensity, particularly in the Red channel. This enhancement is associated with the pigmentation derived from carotenoids, which have a well-documented impact on the coloration of ornamental fish. Research indicates that diets rich in carotenoids, such as those containing Cochineal flour, can lead to substantial changes in coloration in various fish species, including Betta fish (Rauf et al., 2024). Specifically, treatments with Cochineal flour appear to have resulted in a notable increase in red pigmentation, supporting the notion that carotenoids are crucial for such coloration.

In examining the enhancement of coloration in ornamental fishes, prior investigations have highlighted the efficacy of specific pigment sources, particularly Cochineal flour and a variety of carotenoid supplements. Studies have confirmed that dietary carotenoid supplementation effectively enhances color intensity, especially in species like guppies and Koi (Fauziah et al., 2023; Soedibya et al., 2024). The control group, which received no treatment, exhibited the lowest average Red (R) value, suggesting a significantly duller appearance. This observation aligns with findings that coloration in fish can be heavily influenced by nutritional interventions.

Consequently, the differentiated RGB values reflecting the treatment conditions underscore the direct relationship between dietary carotenoid supplementation and modifications in color intensity in ornamental fish. The lack of treatment resulted in a more pronounced representation of the green and blue channels in the control group's color profile, which can be explained by the absence of red pigments that enhance visual appeal, as highlighted in the literature regarding carotenoid impact on coloration (Elagamy et al., 2023).

Table 3. RGB values of betta fish dorsal and caudal regions (Mean ± STDEV)

Treatment	Region	Red (R) value	Green (G) value	Blue (B) value	Total intensity (R+G+B)	Average intensity
K (0%)	Dorsal	110 ± 2.50	120 ± 1.75	130 ± 3.00	360	120 ± 2.42
	Caudal	115 ± 2.75	125 ± 2.10	135 ± 3.10	375	125 ± 2.65
A (5%)	Dorsal	145 ± 2.10	130 ± 1.85	120 ± 2.25	395	131.67 ± 2.07
	Caudal	150 ± 2.50	135 ± 2.00	125 ± 2.50	410	136.67 ± 2.33
B (10%)	Dorsal	180 ± 1.90	140 ± 2.10	130 ± 1.75	450	150 ± 1.92
	Caudal	185 ± 2.15	145 ± 2.25	135 ± 2.00	465	155 ± 2.13
C (15%)	Dorsal	170 ± 2.00	135 ± 1.90	125 ± 2.05	430	143.33 ± 1.98
	Caudal	175 ± 2.20	140 ± 2.30	130 ± 2.00	445	148.33 ± 2.17

When treated with 5% Cochineal flour (Treatment A), Betta fish showed a significant increase in red channel intensity, achieving an average R value of 147.5. This enhancement in red pigmentation underscores the positive impact of carotenoids derived from Cochineal flour on the vibrancy of fish (Lalita et al., 2022). In Treatment B, where the concentration was increased to 10%, the average R value reached 182.5, indicating that this concentration produced the most vibrant coloration. This finding suggests that 10% Cochineal flour is optimal for enhancing the Red channel significantly, resulting in a visually striking appearance (Lalita et al., 2022). However, in Treatment C, where the concentration was further increased to 15%, the average R value was recorded at 172.5.

Although this still represented an increase in red pigmentation compared to the control, it was lower than the values observed in Treatment B. This suggests that higher concentrations of Cochineal flour may not lead to a proportional increase in visual color intensity, potentially indicating diminishing returns or saturation effects (Lalita et al., 2022). The chromatophore analysis corroborates these findings, revealing that the optimal concentration for enhancing both pigment cell numbers and visual color intensity is around 10%. Higher concentrations, such as 15%, do not yield further improvements in color and may even result in slight reductions in intensity due to oversaturation (Khoo et al., 2014).

The relationship between the dosage of Cochineal flour and the hue values of Betta fish

The relationship between the dosage of Cochineal flour and the hue values of Betta fish, specifically in the dorsal and caudal (tail) sections (Figure 3; Figure 4), was analyzed using polynomial regression. The results indicate a significant correlation between the Cochineal dosage and the hue values, as illustrated in the corresponding graphs. For the dorsal section, the regression equation derived was ($y = -0.4333x + 13.333$) with a coefficient of determination (R^2) of 0.7578. This suggests that 75.78% of the variation in hue value can be attributed to the dosage of Cochineal flour. As the dosage increased, a downward trend in hue values was observed, with the 10% dose yielding the lowest hue value of 7.3°, indicating an enhancement in red coloration (Kwon et al., 2022; Wang et al., 2021; Zhang et al., 2021). Similarly, for the tail section, the regression equation was ($y = -0.46x + 12.367$), with an R^2 of 0.771, which shows that 77.1% of the hue variation was influenced by the Cochineal dosage. Again, the 10% dosage produced the lowest hue value (6.0°), aligning with the observation that lower hue values correspond to a more intense red color (Kwon et al., 2022; Zuo et al., 2021).

Both analyses demonstrate that increasing the Cochineal dosage leads to a reduction in hue values, with the optimal color intensity achieved at a 10% dosage. This indicates that the addition of Cochineal flour effectively enhances the red coloration of Betta fish, particularly at this concentration (Nogueira et al., 2021; Rees et al., 2022). The findings are consistent with previous research that has shown the impact of dietary carotenoids on fish pigmentation. Studies have indicated that carotenoids, including those derived from natural sources like Cochineal, can significantly enhance the coloration of various fish species, including Betta fish (Triantafyllou et al., 2020; Umaiyah et al., 2023). The results of this study contribute to the understanding of how specific dietary supplements can be utilized to improve the aesthetic qualities of ornamental

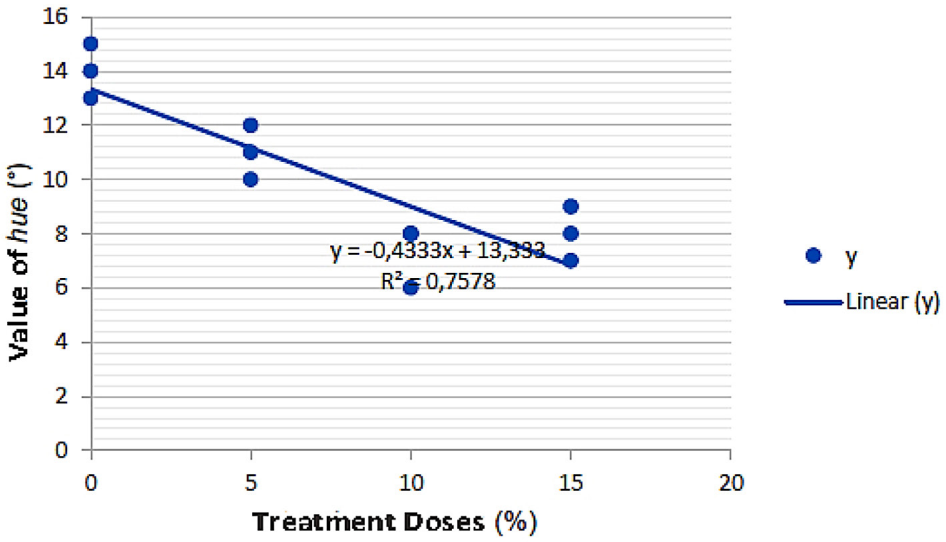

Figure 3. The relationship between the dosage of Cochineal flour and the hue values of Betta fish on the dorsal sections

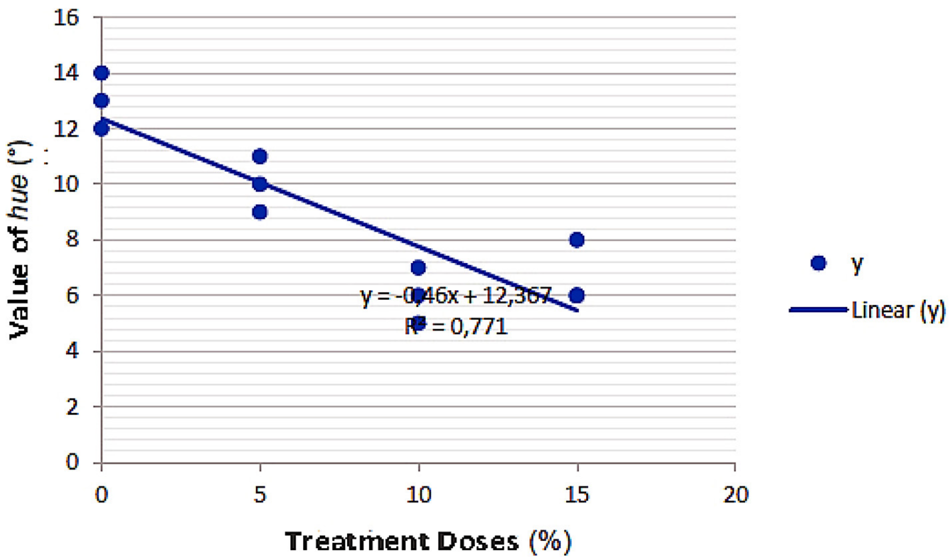

Figure 4. The relationship between the dosage of Cochineal flour and the hue values of Betta fish on the caudal (tail) sections

fish, thereby enhancing their market value (Batubara et al., 2022).

The visual color analysis conducted using Adobe Photoshop confirmed that Cochineal flour effectively enhances the red pigmentation in Betta fish. The optimal dosage for achieving the most vibrant and balanced coloration is indeed 10%, as indicated by the highest Red (R) values in the fish treated with this concentration. The addition of Cochineal flour thus provides a clear improvement in ornamental fish coloration, suggesting significant potential applications in commercial ornamental fish farming (Lalita et al., 2022).

CONCLUSIONS

The visual analysis using Adobe Photoshop confirms that the addition of Cochineal flour enhances the red coloration in Betta fish, with the optimal concentration being 10%. At this concentration, the fish displayed the highest red intensity (R value), indicating a more vibrant and visually appealing appearance. Higher concentrations, such as 15%, did not significantly improve coloration and may even lead to diminishing returns. Overall, Cochineal flour is effective in enhancing the visual color of ornamental fish, making it a valuable additive for improving the market appeal of Betta fish.

Acknowledgment

The authors also acknowledge the Integrated Research Laboratory (IRL) Brawijaya University, for all support and facilities. The authors also wish to thank ADK Putra, AN Ramadhani, and S Muarif for their assistance and contributions during the research process.

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