Dietary astaxanthin and shrimp meal enhance skin pigmentation in *Plectropomus leopardus* (Lacepède, 1802) during the grow-out phase

[1]Muhammad Marzuqi, [1]Bejo Slamet, [2]Sukarman, [2]Regina Melianawati, [1]Moh. A. Adam, [1]Suko Ismi, [1]Siti Subaidah, [1]Joko Sumarwan, [1]Warih Hardanu, [3]Lisa Ruliaty, [1]Dwi H. Putro, [1]Ibnu Rusdi, [1]Supono, [1]Ketut M. Setiawati, [1]Ketut Mahardika, [4]Lia S. Nur'amaliyah, [5]Zafran

[1] Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), 83239 Mataram, Indonesia; [2] Research Center for Applied Zoology, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), 16911 Cibinong, Indonesia; [3] Research Center for Fresh Water Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), 16911 Cibinong, Indonesia; [4] Department of Animal Science, Faculty of Pharmacy, Health, and Science, University of Muhammadiyah Kuningan, 45515 Kuningan, Indonesia; [5] Research Center for Applied Microbiology, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), 16911 Cibinong, Indonesia. Corresponding author: Sukarman, sukarman.2@brin.go.id

Abstract. The Leopard coralgrouper, *Plectropomus leopardus* (Lacepède, 1802), is a high-value marine fish, mainly prized in the live reef food-fish trade for its bright red coloration, delicate flesh, and high consumer demand in Asian markets. However, individuals cultured in floating net cages often exhibit diminished pigmentation, reducing their market appeal. This study evaluated the effects of dietary supplementation with synthetic astaxanthin (1%), shrimp meal (15%), their combination (0.5% astaxanthin + 7.5% shrimp meal), and a control diet without astaxanthin or shrimp meal on growth performance, skin pigmentation, and astaxanthin deposition during a 56-day grow-out period. A total of 300 fish (average weight 185±12 g) were randomly assigned to four dietary treatments with three replicates each. Skin color parameters, including lightness (L*), redness (a*), yellowness (b*), chroma (C*), and hue angle (H*), as well as astaxanthin concentrations, were measured across five anatomical regions. Diets containing astaxanthin, alone or in combination with shrimp meal, significantly enhanced a* and C* compared with the control and shrimp meal-only groups ($p < 0.05$). The highest a* and C* values were observed in fish fed 1% astaxanthin, reaching 6.91±2.14 and 8.64±2.19, respectively, in the ventral posterior skin region. In contrast, the highest skin astaxanthin concentration (26.46 ppm) occurred in the ventral anterior region under the same treatment. Tissue astaxanthin content positively correlated with a* ($R^2 = 0.67$) and C* ($R^2 = 0.51$) but negatively with H* ($R^2 = 0.47$). Growth performance was highest in fish fed the shrimp-only diet, likely due to its high protein content. These findings demonstrate that combining synthetic astaxanthin with natural shrimp meal effectively enhances skin pigmentation in *P. leopardus* without compromising growth.

Key Words: *Plectropomus leopardus*, astaxanthin, shrimp meal, pigmentation, fish coloration.

Introduction. The Leopard coralgrouper, *Plectropomus leopardus* (Lacepède, 1802), is one of the most valuable marine fish species in the Asian market, with the majority still sourced from the wild. Skin coloration strongly influences market price, as normal to dark red individuals, particularly *P. leopardus*, command significantly higher auction prices than light brown or pale red fish (Shimose & Kanaiwa 2022). However, overexploitation of wild populations has led to declining stocks, necessitating sustainable aquaculture production to meet demand and preserve biodiversity (Agustina et al 2019; Campbell & Northrop, 2020). Significant progress has been made in the domestication, controlled spawning, and hatchery production of this species (Susatyo et al 2016; Indarjo et al 2020; Lutviana et al 2020), enabling large-scale grow-out in floating net cages.

Despite these advances, a major concern in captive *P. leopardus* culture is the loss of characteristic skin coloration, particularly the red pigmentation, which leads to reduced consumer preference and lower commercial value (Sudewi et al 2020; Karyanto et al 2020). Wild groupers typically display vivid red-brown hues, whereas farmed individuals often appear gray-brown, contributing to a lower market price (Wu et al 2023). Fish pigmentation is strongly influenced by dietary intake of carotenoids, naturally occurring pigments responsible for red, orange, and yellow hues (Maleta et al 2018; Saini et al 2022). Among them, astaxanthin, a red keto-carotenoid, has shown superior bioactivity in enhancing skin color in several fishes (Gómez-Estaca et al 2017; Lim et al 2019).

Since fish are unable to synthesize carotenoids de novo, their pigmentation depends on dietary inclusion of carotenoid-rich sources such as microalgae, krill, or crustacean meal (Maoka 2011; Yao et al 2021). One potential natural source is shrimp meal, derived from small marine crustaceans (*Acetes indicus*), which contain moderate levels of astaxanthin and have traditionally been used in Southeast Asian aquaculture feeds (Hertrampf & Piedad-Pascual 2000; Šimat et al 2022). While synthetic astaxanthin has higher pigment stability and bioavailability, shrimp meal may offer nutritional benefits such as additional protein and other micronutrients that support growth (Eusebio et al 2004; Nunes et al 2019).

Research on pigmentation in *P. leopardus* is still limited, particularly those exploring the combined use of synthetic astaxanthin and shrimp meal. Understanding the synergistic effects of these additives could provide practical strategies to improve both aesthetic and nutritional quality of cultured *P. leopardus*. Therefore, this study aimed to evaluate the impact of dietary supplementation with astaxanthin and shrimp meal, individually and in combination, on pigmentation, growth performance, and tissue astaxanthin deposition in *P. leopardus* during the grow-out phase.

Material and Method

Experimental site and fish. The research was conducted at the Research Institute for Brackishwater Aquaculture and Fisheries Extension (BBRBLPP), located in Pegametan Bay, Gerokgak District, Buleleng, Bali, Indonesia. Juveniles of *P. leopardus* with an average initial body weight of 185±12g were obtained from the hatchery. Fish were randomly distributed into 12 floating net cages (2 × 2 × 2 m) at a density of 25 fish per cage.

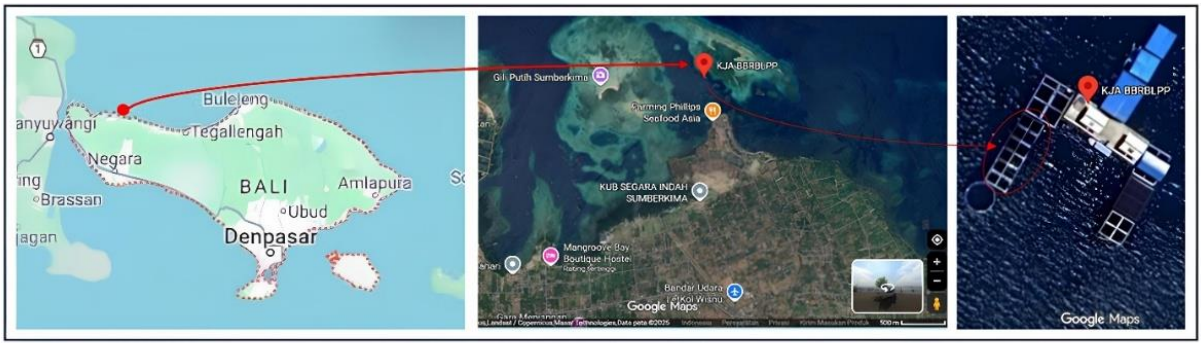

Figure 1. Map showing the location of the research site at Pegametan Bay, Gerokgak District, Buleleng, Bali, Indonesia.

Experimental design and diets. The experiment employed a completely randomized design with four dietary treatments and three replicates each:

1. Diet A: 1% synthetic astaxanthin.
2. Diet B: 15% shrimp meal.
3. Diet C: Combination of 0.5% astaxanthin + 7.5% shrimp meal.
4. Diet D: Control (basal feed without additives).

Fish were fed twice daily (morning and evening) at 2-3% of total biomass for 56 days.

Feed composition. All diets were formulated to be isonitrogenous and isolipidic, based on standard ingredients including fish meal, squid liver meal, soybean meal, flour meal, shrimp meal (derived from dried *Acetes indiscus* powder), fish oil, and vitamin-mineral premix. The composition and proximate analysis of the experimental diets are shown in Table 1.

Table 1

The composition and proximate analysis of the experimental diets

Ingredient	*Experimental diet*			
	A	*B*	*C*	*D*
Fish meal	56.5	48.7	50.9	56.4
Squid liver meal	14.0	10.0	14.0	14.0
Soybean meal	10.0	10.0	10.0	10.0
Flour meal	9.04	5.95	7.25	10.15
Vitamins mix	2.0	2.0	2.0	2.0
Minerals mix	2.5	2.5	2.5	2.5
Fish oil	2.46	3.35	2.85	2.45
Shrimp meal	0.0	15.0	7.5	0.0
Astaxanthin	1.00	0.0	0.5	0.0
Taurine	0.5	0.5	0.5	0.5
Lecithine	1.0	1.0	1.0	1.0
Carboxy methyl cellulose	1.0	1.0	1.0	1.0
Total	100	100	100	100
Proximate composition of feed				
Moisture (% DM)	3.21	3.14	3.35	3.15
Protein (% DM)	50.11	53.83	51.69	49.85
Lipid (% DM)	12.26	11.2	10.51	10.91
Ash (% DM)	13.24	14.15	13.57	13.39
Carbohydrate (% DM)	21.18	17.68	20.88	22.70
Astaxanthin (ppm)	1000	72	566	45

Notes: A = 1% synthetic astaxanthin, B = 15% shrimp meal, C = Combination of 0.5% astaxanthin + 7.5% shrimp meal, D = Control.

Growth performance evaluation. Growth performance was assessed weekly using the following parameters (Vesal et al 2016):

- Weight gain (WG, g) = Final body weight − Initial body weight
- Specific growth rate (SGR, % day^{-1}) = [(ln final weight − ln initial weight)/56 days] × 100
- Feed consumption (FC, g fish^{-1} day^{-1}) = Total feed offered ÷ (number of fish × trial duration)

Skin color measurement. Skin color was measured following the procedure of Zhang et al (2023) with minor modifications. Five anatomical regions were evaluated: dorsal anterior (DA), dorsal posterior (DP), ventral anterior (VA), ventral posterior (VP), and caudal fin (CF). For each anatomical region, three repeated readings were taken per fish and averaged to obtain representative values. A portable colorimeter (CR-400, Minolta, Osaka, Japan) equipped with a D65 illuminant was used to record color parameters in the CIELAB system (L*, a*, b*). The instrument was calibrated using the manufacturer's white standard plate before measurement. In the CIELAB color space, L* denotes lightness, a* represents redness, and b* corresponds to yellowness. Additional color parameters, including chroma (C*) and hue angle (H*), were calculated from the Lab* data to describe color saturation and hue tonality according to Pavlidis et al (2006).

Astaxanthin content analysis. Astaxanthin concentrations in feeds and fish tissues were determined according to the modified method of Tolasa et al (2005). Extraction was performed using 0.05% butylated hydroxytoluene (BHT) in acetone, followed by n-hexane partitioning and spectrophotometric reading at 470 nm. Quantification was based on a standard curve prepared using analytical-grade astaxanthin (Call-E-Astaxanthin, Fa. Acros, 97-103%) as shown in Figure 3.

Water quality monitoring. Water parameters, including ammonia, nitrite, and phosphate, were measured weekly using standard spectrophotometric methods (AOAC 2005). All cages were exposed to continuous water exchange from the open sea.

Statistical analysis. All data were analyzed using Minitab 17.0. Normality of the data was tested using the Shapiro-Wilk test before statistical analysis. Two-way analysis of variance (ANOVA) was then applied to determine significant differences ($p < 0.05$). When significance was detected, Tukey's post hoc test was used for pairwise comparisons.

Results

Growth performance. The growth performance of *P. leopardus* after 56 days of feeding with different experimental diets is summarized in Table 2. Fish fed with Diet B (shrimp meal) showed the highest final body weight (FBW), WG, and SGR, followed by those in Diet C (combination of astaxanthin and shrimp meal), Diet A (astaxanthin only), and the control (Diet D). FC did not significantly differ among treatments.

Table 2

Performance of *Plectropomus leopardus* (Lacepède, 1802) after 56 days fed by experimental diet (Mean ± SD)

Performance parameter	*Experimental diet*			
	A	*B*	*C*	*D*
FBW (g)	226.73±7.75^{b}	249.02±7.30^{a}	236.11±15.57^{ab}	219.52±8.27^{b}
IBL (cm)	23.69±2.88	23.48±3.13	23.34±3.20	23.50±3.08
FBL (cm)	24.69±2.81	24.76±3.12	24.38±3.06	24.57±2.98
WG (g)	22.87±0.92^{b}	34.75±1.39^{a}	25.52±0.60^{b}	18.69±1.48^{c}
SGR (%)	0.37±0.06^{c}	0.54±0.16^{a}	0.43±0.08^{b}	0.31±0.05^{d}
FC (g fish^{-1} day^{-1})	2.31±0.22	2.42±0.02	2.24±0.05	2.23±0.03

Notes: A = 1% synthetic astaxanthin, B = 15% shrimp meal, C = combination of 0.5% astaxanthin + 7.5% shrimp meal, D = control, FBW = final body weight, IBL = initial body length, FBL = final body length, WG = weight gain, SGR =specific growth rate, FC = feed consumption.

Skin color quality. Visual observation revealed apparent differences in skin pigmentation at the end of dietary treatments (Figure 2). Fish fed with Diets A and C displayed noticeably deeper red coloration compared to the control group (Diet D). The enhanced pigmentation was particularly prominent in the dorsal and caudal regions. However, coloration in fish from Diet C appeared unevenly distributed across body surfaces.

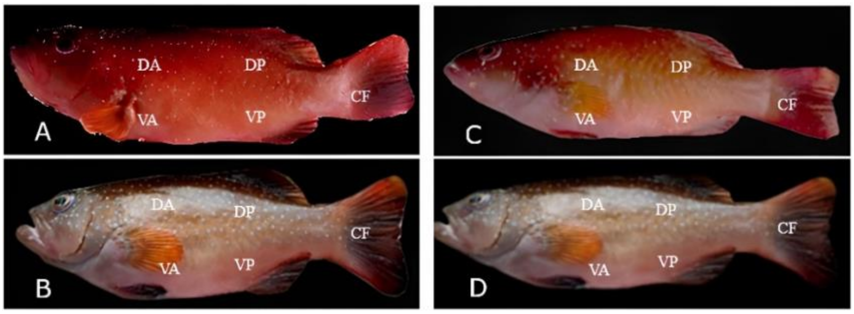

Figure 2. Visual observation revealed apparent differences in skin pigmentation of *Plectropomus leopardus* at the end of detary treatments. A = 1% synthetic astaxanthin, B = 15% shrimp meal, C = combination of 0.5% astaxanthin + 7.5% shrimp meal, D = control, DA = dorsal anterior, DP = dorsal posterior, VA = ventral anterior, VP = ventral posterior, and CF = caudal fin.

Quantitative color analysis confirmed these observations, showing significant differences ($p < 0.05$) in a* and C* values among treatments, especially in fish receiving Diets A and C. These groups consistently exhibited higher a* and C* values across five anatomical regions (dorsal anterior, dorsal posterior, ventral anterior, ventral posterior, and caudal fin). Conversely, H* values were significantly lower in Diet A and C groups, indicating a shift toward deeper red hues. No significant differences were observed in L* or b* values. The effects of dietary treatments on the quantitative color parameters of *P. leopardus* are presented in Table 3.

Table 3

Color quality parameters of *Plectropomus leopardus* (Lacepède, 1802) fed different diets

Parameters	*Experimental diets*			
	A	B	C	D
Effect on DA skin's color				
L* (%)	33.46±2.25	27.55±4.50	32.59±4.79	32.05±3.68
a*	3.60±1.48^{a}	1.38±0.15^{b}	2.68±1.08^{ab}	1.33±0.55^{b}
b*	2.40±1.81	1.65±0.29	1.53±0.77	1.52±0.39
C* (%)	4.41±2.09^{a}	2.15±0.31^{ab}	3.10±0.28^{ab}	2.03±0.64^{ab}
H* (°)	29.69±16.74^{b}	49.78±2.66^{a}	29.46±5.69^{b}	50.12±6.59^{a}
Effect on DP skin's color				
L* (%)	32.82±1.17	31.31±3.85	35.01±4.12	32.47±0.64
a*	3.45±1.77^{a}	1.21±0.28^{b}	3.06±1.01^{a}	1.22±0.15^{b}
b*	2.33±1.40	1.62±1.23	2.35±0.46	1.69±0.55
C* (%)	4.17±2.20^{a}	2.07±1.11^{ab}	3.87±1.00^{a}	2.10±0.53^{ab}
H* (°)	32.61±6.80^{b}	46.77±18.29^{a}	38.49±7.99^{ab}	53.03±6.57^{a}
Effect on VA skin's color				
L* (%)	44.56±2.88	44.77±4.55	42.98±2.93	41.53±4.97
a*	5.63±1.83^{a}	0.75±0.03^{b}	3.58±2.11^{ab}	1.05±0.35^{b}
b*	4.06±1.29	2.77±1.77	2.44±0.66	1.42±0.29
C* (%)	7.00±1.94^{a}	2.90±1.69^{b}	4.49±1.64^{ab}	1.79±0.30^{b}
H* (°)	36.49±9.60^{b}	70.26±12.38^{a}	38.18±17.83^{ab}	53.59±11.60^{ab}
Effect on VP skin's color				
L* (%)	43.68±3.78	42.89±2.74	45.83±4.13	43.23±3.14
a*	6.91±2.14^{a}	1.55±0.62b	5.99±2.38^{a}	0.77±0.49^{b}
b*	5.10±1.18	3.78±1.49	5.74±2.55	2.9±1.18
C* (%)	8.64±2.19^{a}	4.16±1.31^{b}	8.30±2.48^{a}	3.05±1.08^{b}
H* (°)	37.21±7.61^{a}	65.18±13.41^{b}	43.42±1.40^{ab}	73.05±14.86^{b}
Effect on CF zone's color (tail)				
L* (%)	30.77±3.27	33.16±3.26	32.80±3.64	31.91±2.86
a*	5.45±0.77^{a}	0.58±0.24^{b}	3.39±2.05^{ab}	0.62±0.56^{b}
b*	3.09±0.69	1.18±1.25	2.17±1.03	0.78±1.07
C* (%)	6.29±0.73^{a}	1.56±0.76^{b}	4.08±2.15^{ab}	1.14±1.00^{b}
H* (°)	29.57±6.90^{a}	73.3±8.44^{b}	33.50±11.21^{ab}	47.90±15.26^{b}

Note: Superscript letters that differ by 1 line show significantly different results at the 95% level of confidence ($p < 0.05$). A = 1% synthetic astaxanthin, B = 15% shrimp meal, C = combination of 0.5% astaxanthin + 7.5% shrimp meal, D = control, DA = dorsal anterior, DP = dorsal posterior, VA = ventral anterior, VP = ventral posterior, and CF = caudal fin.

Astaxanthin deposition in tissue. The standard curve of astaxanthin (Figure 3) showed a strong linear relationship between concentration and absorbance, confirming the reliability of the quantification method. Astaxanthin concentrations in skin tissue differed significantly among treatments (Figure 4). The highest astaxanthin levels were observed in fish fed Diet A, followed by Diet C, Diet B, and Diet D. Tissue accumulation was highest in dorsal regions, followed by ventral regions and CF.

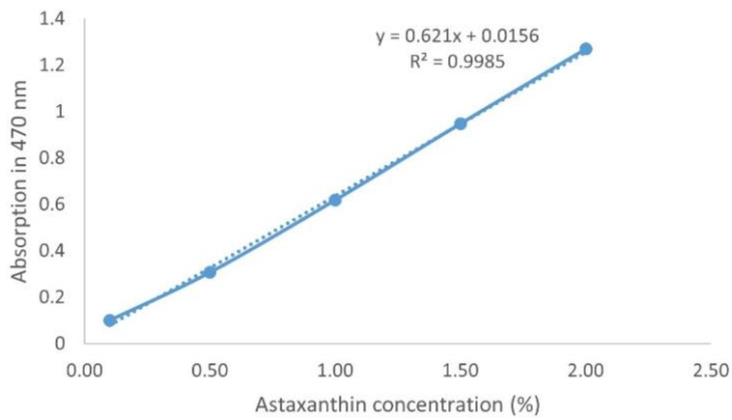

Figure 3. Standard curve of astaxanthin prepared from analytical-grade astaxanthin (call-E-Astaxanthin, Fa. Acros, 97-103%) and plotted using Microsoft Excel 2019

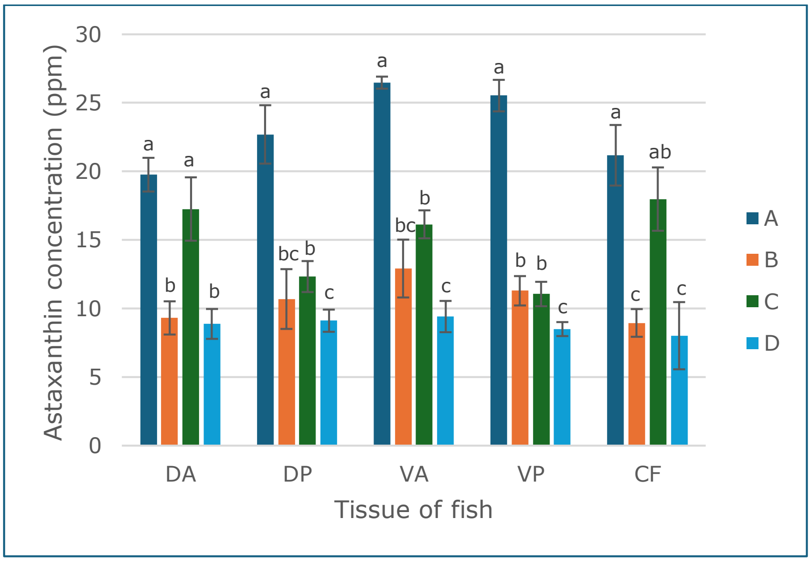

Figure 4. Astaxanthin concentration in the skin tissue of *Plectropomus leopardus* (Lacepède, 1802) fed by different feeds (A = 1% synthetic astaxanthin, B = 15% shrimp meal, C= combination of 0.5% astaxanthin + 7.5% shrimp meal, D = control, DA = dorsal anterior, DP = dorsal posterior, VA = ventral anterior, VP = ventral posterior, and CF = caudal fin).

Regression analysis between astaxanthin and color. Regression analysis showed a strong positive correlation between tissue astaxanthin concentration with redness (a*, $R^2 = 0.67$) and chroma (C*, $R^2 = 0.51$). Conversely, H* was negatively correlated with astaxanthin content ($R^2 = 0.47$), while L* and b* showed weak or no correlation (Table 4).

Table 4

Regression analysis between color quality parameters and astaxanthin concentration in *Plectropomus leopardus* (Lacepède, 1802) tissue

Regression model	*R^2*
L* = 0.1628 Ast + 8.3883	0.03
a* = 2.3073 Ast + 7.8896	0.67
b* = 2.1681 Ast + 8.8986	0.22
C* = 1.9589 Ast + 6.8042	0.51
H* = -0.2845 Ast + 27.626	0.47

n = 40.

Water quality. Water quality parameters, including ammonia, nitrite, and phosphate levels, remained within optimal ranges for marine fish culture throughout the experimental period (Table 5). No significant differences were found between treatments, indicating that feed additives did not affect water conditions.

Table 5

Water quality during *Plectropomus leopardus* (Lacepède, 1802) treatment

Parameters	*Treatments*			
	A	*B*	*C*	*D*
Ammonia (mg L^{-1})	0.0870	0.0751	0.0767	0.0748
Nitrite (mg L^{-1})	< 0.0087	< 0.0087	< 0.0087	< 0.0087
Phosphate (mg L^{-1})	0.0214	0.0239	0.0255	0.0285

Discussion. The current study demonstrated that dietary supplementation with synthetic astaxanthin and shrimp meal had distinct effects on pigmentation and growth performance in *P. leopardus*. The most intense red coloration was observed in fish fed diets containing astaxanthin (either alone or in combination), whereas the greatest growth performance was achieved in fish fed shrimp meal alone. These findings suggest that pigmentation and growth in *P. leopardus* may be enhanced through different dietary mechanisms.

Pigmentation response to dietary carotenoids. Fish coloration is primarily influenced by the deposition of dietary carotenoids into chromatophores in the skin. Astaxanthin, a potent red ketocarotenoid, has been widely documented to improve a* and C* values in various ornamental and food fish species (Bjerkeng 2008; Gómez-Estaca et al 2017; Lim et al 2018). In this study, the inclusion of 1% synthetic astaxanthin significantly increased a* and C* values in all body regions, consistent with previous reports in *Pagrus pagrus*, *Melanotaenia parva*, and *Symphysodon spp.* (Nogueira et al 2021; Meilisza et al 2017; Song et al 2017). Notably, *P. leopardus* has been reported to require approximately 200 ppm dietary astaxanthin (0.02% inclusion) to induce significant coloration enhancement in redness and chroma values (Zhang et al 2023).

Interestingly, the combination treatment (Diet C) also improved a* and C*, though to a lesser extent than astaxanthin alone, indicating a potential dilution effect when the synthetic pigment dosage is halved. In contrast, shrimp meal alone had minimal impact on pigmentation, likely due to its lower astaxanthin content (Hertrampf & Piedad-Pascual 2000). This supports the notion that while shrimp meal provides natural carotenoids, the concentration may be insufficient to drive substantial pigmentation changes in *P. leopardus* unless combined with synthetic sources.

The improvement of fish skin coloration through dietary astaxanthin occurs via several interconnected mechanisms. Firstly, astaxanthin regulates the expression of pigmentation-related genes, particularly those involved in carotenoid deposition and skin color development (Zhang et al 2023; Micah et al 2024; Zhao et al 2025). Secondly, astaxanthin increases the number of erythrophores, which are specialized red pigment cells that serve as sites for pigment accumulation. This physiological change contributes to the enhancement of a* and b*, as demonstrated in current studies. In addition, astaxanthin modulates key metabolic pathways related to antioxidant function and lipid metabolism, which are essential for red pigmentation (Liu et al 2025). Its strong antioxidant activity also plays a crucial role in minimizing oxidative stress, thereby preserving pigment stability and intensity (Ritu et al 2023). Although this study did not directly examine gene expression or metabolic responses, the observed pigmentation improvement in fish fed Diets A and B is consistent with these known mechanisms of astaxanthin action.

Distribution of coloration across body regions. Among the five anatomical regions assessed, the VP exhibited the highest C*, a*, and b* values, followed by the VA, CF, and dorsal regions (DA and DP). This pattern aligns with Zhang et al (2023), who reported that dietary astaxanthin enhanced ventral pigmentation more than dorsal regions.

Chemical verification of skin samples revealed astaxanthin concentrations ranging from 19-27 ppm in treatment A and 11-18 ppm in treatment C (Figure 4), confirming that ventral skin contained higher carotenoid levels. To our knowledge, this is the first report documenting region-specific carotenoid deposition in the skin of *P. leopardus*. The heterogeneous pattern likely reflects differences in skin thickness, vascularization, and chromatophore density (Sköld et al 2013; Sköld et al 2016).

Interestingly, the dorsal skin appeared visually darker (deep red) than the ventral surface (Figure 2), despite its lower astaxanthin content. This phenomenon was supported by lower L* values on the dorsal surface (Table 3). The darker appearance results from a higher proportion of melanophores in the basal dermis, which absorb light and mask the underlying carotenoids, whereas erythrophores in the superficial layer carry astaxanthin (Cal et al 2017; Ligon & McCartney 2016). This structural arrangement explains why dorsal skin visually appears deep red, even though ventral skin carries more pigment.

Correlation of astaxanthin content and color metrics. The regression analysis demonstrated that skin astaxanthin concentrations were strongly correlated with redness (a*, $R^2 = 0.67$) and chroma (C*, $R^2 = 0.51$) and negatively correlated with hue angle (H*, $R^2 = 0.47$). These relationships are consistent with the established principles of carotenoid-based coloration, where higher pigment deposition enhances saturation and redness while reducing hue angles (Choubert 2001). Similar correlations have been reported in *Amphiprion ocellaris* (Ho et al 2013a) and in ornamental koi fish fed carotenoid-rich diets from golden snail eggs (Sukarman et al 2025), confirming that carotenoid concentration is the primary determinant of a* and C* across ornamental fish species. The weak association between astaxanthin and L* or luminosity and b* suggests that these color components are less directly influenced by astaxanthin alone, likely involving other xanthophylls or structural pigmentation (Ho et al 2013b). Importantly, these regression relationships not only clarify the biological link between tissue carotenoid deposition and visual appearance but also provide a quantitative basis for non-destructive color assessment and for evaluating the effectiveness of dietary pigment sources in aquaculture applications.

Growth performance and protein contribution. Unlike pigmentation, the best growth performance was recorded in fish fed with shrimp meal alone. This may be attributed to its high protein content (43-72%) and good digestibility in grouper species (Eusebio et al 2004; Nunes et al 2019). Protein levels in Diet B (53.83%) were higher than the control (49.85%) and other treatments, suggesting that improved amino acid availability, possibly lysine, supported higher weight gain and specific growth rate (Giri et al 2009). The relatively lower growth in fish fed astaxanthin-only diets confirms that pigmentation enhancement does not necessarily translate to growth benefits unless coupled with nutritional improvements.

Practical implications. These findings provide practical insights for commercial *P. leopardus* aquaculture. The use of synthetic astaxanthin remains the most effective strategy to enhance skin redness, which is crucial for market acceptance. However, incorporating shrimp meal not only contributes protein for growth but may also serve as a sustainable carotenoid source when combined with synthetic pigments. Balancing pigmentation efficacy with feed cost and nutritional value should be a central consideration in diet formulation for high-value marine species.

Conclusions. This study demonstrated that dietary supplementation with synthetic astaxanthin significantly enhances skin pigmentation, particularly redness and chroma, in *P. leopardus* cultured in floating net cages. Although shrimp meal alone did not markedly improve pigmentation, it contributed to superior growth performance, likely due to its high protein content. The combination of astaxanthin and shrimp meal offered a balanced improvement in both pigmentation and growth, making it a promising strategy for high-value grouper production. Future research should explore optimization of dosage ratios and cost-benefit analysis for commercial application.

Acknowledgments. The authors thank the Research Institute for Brackishwater Aquaculture and Fisheries Extension (BBRBLPP), Gondol, Bali, for providing facilities and juvenile fish used in this study. This work was supported by the Indonesian Ministry of Marine Affairs and Fisheries under the APBN Research Grant Scheme.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Agustina S., Panggabean A. S., Natsir M., Retroningtyas H., Yulianto I., 2019 Yield-per-recruit modeling as biological reference points to provide fisheries management of Leopard coral grouper (*Plectropomus leopardus*) in Saleh Bay, West Nusa Tenggara. IOP Conferences Series: Earth and Environmental Sciences 278(1):012005.

Bjerkeng B., 2008 Carotenoids in aquaculture: fish and crustaceans. In Carotenoids: Volume 4: Natural Functions. Basel: Birkhäuser Basel, pp. 237-254.

Cal L., Suarez-Bregua P., Cerdá-Reverter J. M., Braasch I., Rotllant J., 2017 Fish pigmentation and the melanocortin system. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 211:26-33.

Campbell A. B., Northrop A. R., 2020 Stock assessment of common coral trout *(Plectromomus leopardus)* in Queensland. Technical Report. Department of Agriculture and Fisheries, Brisbane, Australia, 54 pp.

Choubert G., 2001 Carotenoids and pigmentation. In Nutrition and Feeding of Fish and Crustacean, edited by Guillaume J., Metailler R. Springer-Praxis Books, Chichester, United Kindom, 432 p.

Eusebio P. S., Coloso R. M., Mamauag R. E. P., 2004 Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coioides* (Hamilton). Aquaculture Research 35(13):1261-1269.

Giri I. N. A., Sentika A.S., Suwirya K., Marzuqi M., 2009 [Optimum lysine content in diet for growth of juvenile coral trout grouper (*Plectropomus leopardus*)]. Journal Riset Akuakultur 4(3):357-66. [in Indonesian]

Gómez-Estaca J., Calvo M. M., Álvarez-Acero I., Montero P., Gómez-Guillén M. C., 2017 Characterization and storage stability of astaxanthin esters, fatty acid profile and α-tocopherol of lipid extract from shrimp (*L. Vannamei*) waste with potential applications as food ingredient. Food Chemistry 216:37-44.

Hertrampf J. W., Piedad-Pascual L., 2000 Shrimp meal. In Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publishers, London, United Kingdom, pp. 364-371.

Ho A. L. F. C., Bertran N. M. O, Lin J., 2013b Dietary esterified astaxanthin concentration effect on dermal coloration and chromatophore physiology in spinecheek anemonefish, *Premnas biaculeatus.* Journal of the World Aquaculture Society 44(1):76-85.

Ho A. L. F. C., O'Shea S. K, Pomeroy H. F, 2013a Dietary esterified astaxanthin effects on color, carotenoid concentrations, and compositions of clown anemonefish, *Amphiprion ocellaris*, skin. Aquaculture International 21(2):361-374.

Indarjo A., Salim G., Amir F., Supriadi, Soejarwo P. A., Nugraeni C. D., Prakoso L. Y., Ambariyanto A., Firdaus M., Ransangan J., 2020 Growth characteristics layur fish *Lepturacanthus savala* in Juata waters, Tarakan, Indonesia. Indonesian Journal of Marine Sciences 25(3):127-134.

Karyanto K., Wisnawa G. S., Rusmana D., 2020 [Rearing of first-generation (F-1) and fourth-generation (F-4) grouper (*Plectropomus leopardus*) larvae]. Aquaculture Research Bulletin 18(2):93. [in Indonesian]

Ligon R. A., McCartney K. L.,2016 Biochemical regulation of pigment motility in vertebrate chromatophores: a review of physiological color change mechanisms. Current Zoology 62(3):237-257.

Lim K. C., Yusoff F. M., Shariff M., Kamarudin M. S., 2018 Astaxanthin as feed supplement in aquatic animals. Reviews in Aquaculture 10(3):738-733.

Lim K. C., Yusoff F. M., Shariff M., Kamarudin M. S., 2019 Dietary administration of astaxanthin improves feed utilization, growth performance and survival of Asian

seabass, *Lates calcarifer* (Bloch, 1790). Aquaculture Nutrition 25(6):1410-1421.
Liu Y., Wang L., Li Z., Li L., Ding T., Chen S., Duan P., Wang X., Qiu Y., Ding X., Tian Y., 2025 DNA methylation and transcriptome profiling reveal the role of the antioxidant pathway and lipid metabolism in *Plectropomus leopardus* skin color formation. Antioxidants 14(1):93.
Lutviana N. D., Widodo M. S., Armando E., 2020 Study on survival and growth rate of some variety of fish fry indigo (*Oreochromis* sp.) the different size of 3-5 centimeters. Journal of Aquaculture Development and Environment 3(1):143-47.
Maleta H. S., Indrawati R., Limantara L., Brotosudarmo T. H. P., 2018 [Various methods of carotenoid extraction from plant sources in the last decade (Literature Review)]. Journal of Chemical Engineering and Environment 13(1):40-50. [in Indonesian]
Maoka T., 2011 Carotenoids in marine animals. Marine Drugs 9(2):278-293.
Meilisza N., Jusadi D., Zairin M., Artika I. M., Utomo N. B. P., Kadarini T., Suprayudi M. A., 2017 Digestibility, growth and pigmentation of astaxanthin, canthaxanthin or lutein diets in Lake Kurumoi rainbowfish, *Melanotaenia parva* (Allen) cultured species. Aquaculture Research 48(11):5517-5525.
Micah A. D., Wen B., Yusuf A., Onimisi M. M., Adeyemi S. O., Gao J., Chen Z., 2024 Effects of dietary astaxanthin on chromatic, biochemical, and histological characteristics in juvenile blood parrotfish (*Vieja melanurus* ♀ × *Amphilophus citrinellus* ♂). Aquaculture International 32(5):5977-5996.
Nogueira N., Canada P., Caboz J., Andrade C., Cordeiro N., 2021 Effect of different levels of synthetic astaxanthin on growth, skin color and lipid metabolism of commercial sized red porgy (*Pagrus pagrus*). Animal Feed Science and Technology 276:114916.
Nunes A. J. P., Sabry-Neto H., Oliveira-Neto S., Burri L., 2019 Feed preference and growth response of juvenile *Litopenaeus Vannamei* to supplementation of marine chemoattractants in a fishmeal-challenged diet. Journal of the World Aquaculture Society 50(6):1048-1063.
Pavlidis M., Papandroulakis N., Divanach P., 2006 A method for the comparison of chromacity parameters in fish skin: preliminary results for coloration pettern of skin Sparidae. Aquaculture 258(1-4):211-219.
Ritu J. R., Ambati R. R., Ravishankar G. A., Shahjahan M., Khan S., 2023 Utilization of astaxanthin from microalgae and carotenoid rich algal biomass as a feed supplement in aquaculture and poultry industry: an overview. Journal of Applied Phycology 35(1):145-171.
Saini R. K., Prasad P., Lokesh V., Shang X., Shin J., Keum Y., Lee J., 2022 Carotenoids: dietary sources, extraction, encapsulation, bioavailability, and health benefits — A Review of recent advancements. Antioxidant 11(4):795.
Shimose T., Kanaiwa, M.,2022 Influence of the body color and size on the market value of wild captured colargroupers (Serranidae, *Plectropomus*): implication for fisheries management. Fisheries Research 248:106223.
Šimat V., Rathod N. B., Čagalj M., Hamed I., Mekinić I. G., 2022 Astaxanthin from crustaceans and their byproducts: a bioactive metabolite candidate for therapeutic application. Marine Drugs 20(3):206.
Sköld H. N., Aspengren S., Cheney K. L., Wallin M., 2016 Fish Chromatophores-From Molecular Motors to Animal Behavior. International Review of Cell and Molecular Biology 321:171-219.
Sköld H. N., Aspengren S., Wallin M., 2013 Rapid color change in fish and amphibians - function, regulation, and emerging applications. Pigment Cell and Melanoma Research 26(1):29-38.
Song X., Wang L., Li X., Chen Z., Liang G., Leng X., 2017 Dietary astaxanthin improved the body pigmentation and antioxidant function, but not the growth of discus fish (*Symphysodon* spp.). Aquaculture Research 48(4):1359-1367.
Sudewi, Asih Y. N., Nasukha A., 2020 [Study of the mouth opening of grouper larvae (*Plectropomus leopardus*) and the suitability of natural feed size]. Journal of Fisheries and Marine Research 4(1):87-93. [in Indonesian]
Sukarman, Sofyan A., Nur B., Cindelaras S.,Murniasih S., Rohmy S., Kadarini T., Nurhidayat, Zamroni M., Ginanjar R., 2025 Dietary effect of carotenoids from golden snail eggs on

colour enhancement in koi fish *Cyprinus carpio* Linnaeus 1758. Brazilian Journal of Biology 85:e292148.
Susatyo P., Sugiharto, Hana, Chasanah T., 2016 Effects of some feed suplements types to the growth of Javaen Barb/Brek Fish (*Puntius orphoides*) second filial as domestication product. Biosaintifika: Journal of Biology and Biology Education 8(3):277-284.
Tolasa S., Cakli S., Ostermeyer U., 2005 Determination of astaxanthin and canthaxanthin in salmonid." European Food Research and Technology 221(6):787-791.
Vesal S. E., Mooraki N., Vosooghi A. R., 2016 Effect of dietary fish oil on growth responses of severum (*Heros severus*). AACL Bioflux 9(1):81-90.
Wu H. Y., Chen,K. S., Huang Y. S., Hsieh, H. Y., Tsai, H. Y., 2023 Comparative transcriptome analysis of skin color-associated genes in leopard coral grouper (*Plectropomus leopardus*). BMC Genomics 24(1):5
Yao Y., Goh H. M., Kim J. E., 2021 The roles of carotenoid consumption and bioavailability in cardiovascular health. Antioxidants 10(12):1978.
Zhang J., Tian C., Zhu K., Liu Y., Zhao C., Jiang M., Zhu C., Li G., 2023 Effects of natural and synthetic astaxanthin on growth, body color, and transcriptome and metabolome profiles in the leopard coralgrouper (*Plectropomus leopardus*). Animals 13(7):1252.
Zhao N., Gao J., Fu S., Chen S. X., 2025 Effects of light intensity on erythrophore number and astaxanthin amount in the skin of coral trout *Plectropomus leopardus*. Aquaculture Reports 43:102883.
***AOAC (Association of Official Analytical Chemists), 2005 Official methods of analysis of AOAC International 18th ed. Available at: https://www.aoac.org/official-methods-of-analysis/. Accessed at: June 2021.

Received: 04 August 2025. Accepted: 19 September 2025. Published online: 15 December 2025.
Authors:
Muhammad Marzuqi, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No. 62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: muha298@brin.go.id
Bejo Slamet, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No.62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: bedjoselamet@yahoo.co.id
Sukarman, Research Center for Applied Zoology, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Bogor Raya Street Km 46, 16911 Cibinong, West Java, Indonesia, email: sukarman.2@brin.go.id
Regina Melianawati, Research Center for Applied Zoology, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), Bogor Raya Street Km 46, 16911 Cibinong, West Java, Indonesia, email: regina.melnawati@yahoo.com
Moh. Awaludin Adam, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No. 62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: moha044@brin.go.id
Suko Ismi, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No. 62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: sukoismi002@brin.go.id
Siti Subaidah, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No.62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: s.subaidah60@gmail.com
Joko Sumarwan, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No.62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: jokosumarwan90@gmail.com
Warih Hardanu, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No.62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: wari006@brin.go.id
Lisa Ruliaty, Research Center for Fresh Water Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Bogor Raya Street Raya Bogor Km 46, 16911 Cibinong, West Java, Indonesia, email: lisa005@brin.go.id
Dwi Handoko Putro, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Bogor Raya Street Km 46, 16911 Cibinong, West Java, Indonesia, email: dwih012@brin.go.id
Ibnu Rusdi, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No.62, 83239 Mataram, West Nusa Tenggara, Indonesia email: ibnu013@brin.go.id
Supono, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No.62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: supono@brin.go.id
Ketut Maha Setiawati, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No.62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: nike011@brin.go.id
Ketut Mahardika, Research Center for Marine Aquaculture, Research Organization for Agriculture and Food, National Research and Innovation (BRIN), Majapahit Street No.62, 83239 Mataram, West Nusa Tenggara, Indonesia, email: ketu006@brin.go.id
Lia Siti Nur'amaliyah, Department of Animal Science, Faculty of Pharmacy, Health, and Science, University of Muhammadiyah Kuningan, Pengeran Adipati Street No.D4, 45552 Kuningan, West Java, Indonesia, email: liasitinuramaliyah@umkuningan.ac.id
Zafran, Research Center for Applied Microbiology, Research Organization for Life Sciences and Environment, National Research and Innovation (BRIN), Bogor Raya Street Km 46, 16911 Cibinong, West Java, Indonesia, email: zafr001@brin.go.id

How to cite this article:
Marzuqi M., Slamet B., Sukarman, Melianawati R., Adam M. A., Ismi S., Subaidah S., Sumarwan J., Hardanu W., Ruliaty R., Putro D. H., Rusdi I., Supono, Setiawati K. M., Mahardika K., Nur'amaliyah L. S., Zafran, 2025 Dietary astaxanthin and shrimp meal enhance skin pigmentation in *Plectropomus leopardus* (Lacepède, 1802) during the grow-out phase. AACL Bioflux 18(6):2722-2733.