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IMPROVING COLOUR QUALITY OF CORAL TROUT GROUPER *Plectropomus leopardus (Lacepede)* OF ASTAXANTHIN AND REBON MEAL IN FEED

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Abstract

The coral trout grouper (*P. leopardus*) found in nature often has a red hue, while others cultured in captivity lack this coloring. This disparity has been reported to negatively affect consumer interest in fish products. To address this issue, there is a need to add an additive that can improve the appearance of the red coloration. Therefore, this study aims to determine the effectiveness of adding astaxanthin and rebon meal to feed to improve the red color quality of coral trout grouper (*P. leopardus*) during the enlargement phase. The treatments tested included (feed A) 1% astaxanthin; (feed B) 15% rebon meal; (feed C) 0.5% astaxanthin and 7.5% rebon meal; and feed D as control. The fish were given dry pellet feed twice a day in the morning and evening (2-3% biomass per day). The samples consisted of coral trout groupers with a weight of ± 185 g and a density of 25 fish/net, which were cultured in floating net cages measuring 2x2x2 m. The parameters observed in this study were growth, survival rate, feed consumption rate, color quality, as well as levels of carotenoids in feed and fish. The results showed that the addition of astaxanthin and its combination with rebon meal improved the red color quality. Furthermore, the addition of only astaxanthin caused an increase in pigment concentrations, as indicated by changes in the red color indicator value, but the combination treatment had no significant effect.

Keywords: coral trout grouper, astaxanthin, rebon meal, growth, color

Introduction

Coral trout grouper (*Plectropomus leopardus*) is a highly valued export commodity in the Asian market, but overfishing has limited its availability in nature (Agustina *et al*. 2019; Bawole *et al*. 2018; Campbell and Northrop 2020; Ernaningsih *et al*. 2019; Sajriawati *et al*. 2019). Therefore, aquaculture activities are needed for species diversification to protect the fish population and meet market demand (Amaya and Nickell 2015; Parichy 2021; Susatyo *et al*. 2016). Several studies on Coral trout grouper have used various methods, including domestication, gonad maturation, brood spawning, and breeding (Indarjo *et al.* 2020; Lutviana, Widodo, and Armando 2020; Susatyo *et al*. 2016). These methods have led to controlled spawning and breeding, high hatchery ratios, and the production of juveniles since 2005 (Kusumawati, Asih, and Seti 2019; Setiawati and Melianawati 2020; Sudewi, Asih, and Nasukha 2020).

Several reports have shown that coral trout grouper cultured in floating net cages often have quality problems, such as paler and less red coloration, leading to low customer interest (Indarjo *et al*. 2020; Karyanto, Wisnawa, and Rusmana 2020; Sudewi *et al*. 2020). This indicates that efforts to improve the color quality of cultivated species are necessary. The addition of additives in the feed can improve the coloration performance of several types of fish, including astaxanthin (Gómez-Estaca *et al*. 2017; Rahman *et al*. 2016) and rebon (Ravidhia *et al*. 2019; Sholichin, Haetam, and Suherman 2012). Furthermore, astaxanthin has been proven to produce better color performance in clownfish *Amphiprion ocellaris* (Zulfikar, Erlangga, and Fitri 2018) and rainbow kurumoi fish *Melanotaenia parva* (Meilisza *et al*. 2018). Similar results have also been obtained in betta fish (*Betta splendens*, Regan, 1910) (Prasetyo *et al*. 2020; Syaifudin, Sulmartiwi, and Andriyono 2016) and mickey mouse platy after the use of rebon (*Xiphophorus maculatus*) (Amin, Rahimi, and Mellisa 2019).

Fish color is widely associated with healthy and high-quality products (Pulcini *et al*. 2020; Wishkerman *et al*. 2016), and is often considered the most important quality parameter for salmon besides freshness (Ytrestøyl *et al*. 2021). It is also an essential parameter for several types of red, orange, and yellow species, including bream (*Pagrus pagrus*) (Kim *et al*. 2023; Quigley, Fisheries, and Authority 2021; Seong *et al*. 2020) and Australian grouper (*Pagrus auratus*) (Kishimoto *et al*. 2018; Militelli *et al*. 2017; Yang *et al*. 2022). Furthermore, the coloration and pigmentation of fish are determined by the absorption and accumulation of pigmented carotenoids in the body (Hasidah, Mukarlina, and Rousdy 2017; Labola and Puspita 2018; Saini *et al*. 2022). Carotenoids are natural pigments that are responsible for a wide range of colors and functions (Maleta *et al*. 2018; Šovljanski *et al*. 2022). Several studies have shown that there are approximately 800 fat-soluble pigments that occur naturally in plants, algae, fungi, animals, photosynthetic bacteria, and some non-photosynthetic bacteria (Nisar *et al.* 2015; Saini *et al*. 2022; Yao, Goh, and Kim 2021). Although plants, bacteria, fungi, and algae can synthesize carotenoids, they cannot be produced by animals and are often derived from diet (Maleta *et al*. 2018; Nisar *et al*. 2015; Schmalzer, Thompson and Simpson 2008; Yao *et al*. 2021). Certain fish

species can only convert one form into another, which highlights the importance of adding pigments to their feed.

Carotenoids are important micronutrients found in fish that serve as a precursor of vitamin A (Leclaire *et al*. 2019; Park *et a*l. 2014; Sharma *et al*. 2019; Sun *et al*. 2022). They also providea wide range of benefits, including enhancing reproductive performance (Kotíková *et al*. 2007; Rodionova *et al*. 2020; Wahyuni, Shalihah, and Nurtiana 2020), acting as antioxidants (Adam *et al.* 2021, 2022; Darvin *et al*. 2022; Havaux 2014), improving enhance the immune system (Lee *et al*. 2021; Zhao *et al*. 2023), and affecting the structure of the liver (Saini *et al.* 2022; Yao *et al.* 2021). Species with high levels have been shown to be more resistant to bacterial and fungal diseases (Kleppel and Lessard 1992; Rodionova *et al*. 2020; Schmalzer *et al*. 2008; Šovljanski *et al*. 2022). Various types of carotenoids are present in fish, such as tunaxanthin (yellow), lutein (greenish yellow), doradexanthin (yellow), zeaxanthin (yellow-orange), canthaxanthin (orange- red), carotene (orange), astaxanthin (red), eichinenone (red), and taraxanthin (yellow) (Kleppel and Lessard 1992; von Lintig 2020; Su 2022; Wahyuni *et al.* 2020; Yuan *et al*. 2015). Astaxanthin, a red pigment, is commonly found in species consuming crustaceans (Amaya and Nickell 2015; Gómez-Estaca *et al*. 2017). Meanwhile, tunaxanthin is responsible for the yellow color of the fins and skin of marine fish and can be metabolized from astaxanthin via zeaxanthin (Matsuno 2001). Salmonids, which include salmon, contain more polar carotenoids, specifically astaxanthin, followed by canthaxanthin, zeaxanthin, and carotenes (Misawa 2021; Pradel *et al*. 2021; Ranjan 2016). Several studies have shown that approximately 50% of the astaxanthin absorbed is metabolized (Ytrestøyl *et al.* 2021).

Aquaculture commonly uses synthetic carotenoids, such as synthetic astaxanthin and cantaxanthin (Cheng *et a*l. 2019; Daniel *et al*. 2017) to enhance the growth and survival of aquatic animals (Das 2016; Joy, Joseph, and Anandan 2021; Maoka 2011).This indicates that the consumption of a diet rich in these pigments is an efficient way to improve the pigmentation process in coral trout grouper. Based on previous findings, there are no studies on the addition of astaxanthin and rebon meal to the feed of this species. Therefore, this study aims to determine the effectiveness of adding astaxanthin and rebon meal to feed to improve the red color quality of coral trout grouper (*P. leopardus*) during the enlargement phase.

Material and Methods

Animal, Facilities, and Study Design

This study was carried out in BBRBLPP floating marine cages (KJA) located in Pegametan Village, Gerokgak District, Buleleng, Bali, Indonesia. Furthermore, the test animals used were large coral trout grouper hatcheries in BBRBLPP, which were spread into 12 nets measuring 2x2x2 m.

A completely randomized design (CRD) was used with 4 treatments and 3 replications. Each replication was carried out using 25 fish. *Plectropomus leopardus* grouper fish measuring ± 185 g. Treatments were adding astaxanthin and rebon meal in feed namely 1% astaxanthin (feed A), 15% rebon meal (feed B), combination of 0.5% astaxanthin, 7.5%

rebon meal (feed C) and without astaxanthin and rebon meal (feed D) as control (Table 1). The astaxanthin used was the synthetic variant, which had a purity level of 10% (Carophyl Pink - DSM). Feeding was carried out twice a day in the morning and evening for 56 days with 2-3%biomass/day.

Table 1. Composition (g/100 g diet), proximate analysis of tested feeds

Ingredient	Tested feed			
	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
Rebon 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 tested 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

The parameters tested were: (1) growth, including initial body weight (IBW), final body weight (FBW), weight gain (WG), initial body length (IBL), final body length (FBL), specific growth rate (SGR) and feed consumption (FC); (2) fish color quality, including lightness (L*), redness (a*), yellowness (b*), chroma (C*) and hue (H*) parameters; and (3) Astaxanthin concentration. Color quality and astaxanthin concentration measurements were performed on the dorsal anterior (DA), dorsal posterior (DP), ventral anterior (VA), ventral posterior (VP), and caudal fin (CF).

Growth Performance

Growth parameters, including IBW, FBW, IBL, and FBL were monitored and measured directly once a week. Meanwhile, other parameters, such as WG, SGR, and FC were obtained using the equations below:

WG (g) = FBW (g) – IBW (g)

$$\text{SGR } (\%) = \frac{\text{Ln (FBW)} - \text{Ln (IBW)}}{\text{TrialPeriod (day)}} x\ 100\%$$

$$\text{FC} = \left(\frac{\text{Total Feed offered (g)}}{\text{Sum of fish (head)}}\right) : \text{Trial Periods (days)}$$

Chemical Analysis

Feed proximate composition (moisture, crude protein, crude fat, ash) as well as water quality (ammonia, nitrite, nitrate) in the maintenance media were analyzed based on the AOAC method (2005). Furthermore, analysis of astaxanthin concentrations in feed and fish tissue was carried out based on the method proposed by Tolasa et al (2005) with few modifications. All the procedures were performed under yellow light. A total of 10 g of each sample was extracted three times with 40 ml of 0.05% acetonic BHT solution with ultra turax for 1 min. The samples were cooled during the examination to prevent warming. After each extraction, they were centrifuged at 4000 U/ min for 5 min. Acetone extracts obtained from the process were then collected in a 250 ml separatory funnel. A total of 40 ml n-hexane, 100 ml water, and 0.5 g salt (NaCl) were added to separate the water-soluble compounds. After the shaking process, approximately 20–30 min later, the entire white phase was taken. The upper layer was then poured into a measuring cup up to 50 ml (raw extract).

Photometric valor was determined at a maximum wavelength of 470–474 nm using the 350–600 nm spectrum. Furthermore, the calculation of the total carotenoid concentration was carried out based on the standard curve of astaxanthin. Analysis of the samples was performed in triplicate, as the curve was established with approximately 3 mg of standard.

Astaxanthin Call−E−Astaxanthin (Fa. Acros, 97–103%) and 100 mg of butyl-hydroxytoluene (BHT) were stored in a 10 ml volumetric flask and dissolved in dichloromethane (without acid) using an ultrasonic bath (stock solution). A total of 1 ml of the stock solution was then diluted to a volume of 10 ml using n-hexane. Maximum absorbance was determined immediately after the production of the solution at the 350–600 nm spectrum interval. Subsequently, the concentration was measured using the maximum absorbance of approximately 472 nm).

The formula below was used for astaxanthin calculation:

$Call = E - Astaxanthin$ (µg/ml or g/L or ppm) = Absorbances x $\frac{(10000)}{(2100)}$

The value of E (1%, 1 cm) was determined to be 2100 for Call-E-Astaxanthin solution with a concentration of 1% (w/v) in hexane at 470 nm using a spectrophotometer. The scale factor was calculated to be 10,000.

A total of 0.1, 0.5, 1.0, 1.5, and 2.0 ml of each diluted astaxanthin stock solution were placed in a 10 ml flask using a pipette to prepare the standard curve. The mixture was made to the marked level using a ratio of n-Hexane: acetone: water of 40:120:100. Photometric tenacity was determined immediately after the production of the solution. A mixture of hexane, acetone, and water was also treated as a blank.

An illustration of the standard curve for astaxanthin concentration is presented in Figure 1.

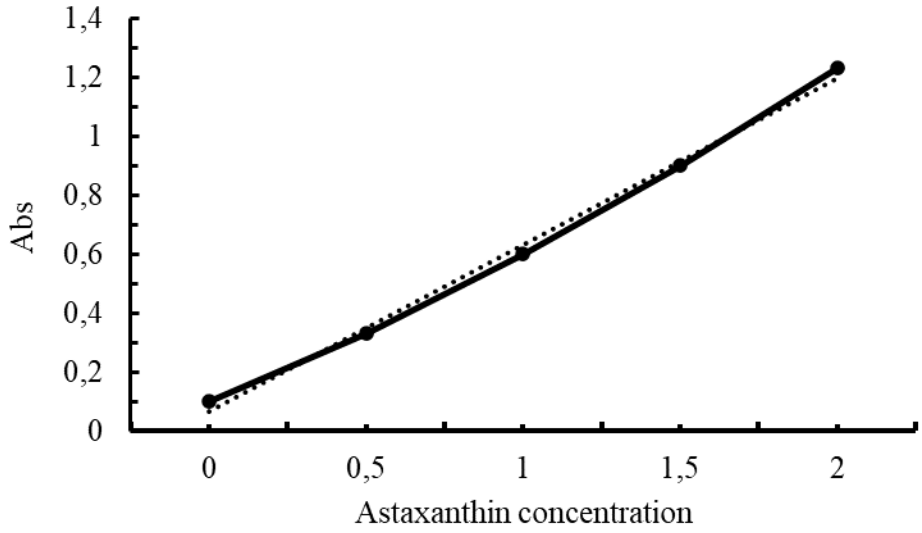


Figure 1. Standard Curve of Astaxanthin

Color Quality Measurement

The skin color quality of fish was measured using a portable colorimeter (Minolta Chroma CR-400) calibrated to a white plate standard. The original adjusted value of the white standard was L*=97.40 ± 0.01; a*=−0.10 ± 0.01; b*=1.92 ± 0.01; C* =1.92; H* =93.8. During the measurement, the fish were anesthetized with 2-phenoxyethanol (4 ml l−1), with dorsal anterior (DA), dorsal posterior (DP), ventral anterior (VA), ventral posterior (VP) skin, and caudal fin (CF) as the major targets.

The parameters employed during the evaluation of coloration included L for lightness (−100 black, +100 white), a* for redness or greenness (−100 green, +100 red), and b* for yellowness or blueness (−100 blue, +100 yellow), C* for chromaticity value (%) and H* for hue value or purity of the color (^{0}) (CIE 1976; Sukarman *et al.* 2023; Yilmaz, Ergun, and Soytas 2013).

Data Analysis

The data obtained in this study were presented as mean ± standard deviation (SD). Furthermore, statistical analysis was performed using Minitab 17.0 statistical software with a two-way ANOVA for multiple comparisons. If the data had significant differences, the turkey test method was used to analyze the level of difference among the treatments at $P<0.05$.

Results and Discussion

Growth Performance

The results showed that the addition of astaxanthin, rebon meal, and their combination affected the feed composition and the growth of coral trout grouper. Furthermore, the growth response of coral trout grouper reared in floating net cages (KJA) for 56 days are presented in Table 2.

Table 2. Growth performance of coral trout grouper treated for 56 days

Parameters	Feed Treatment			
	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/day)	2.31±0.22	2.42±0.02	2.24±0.05	2.23±0.03

The initial body weight did not differ among the treatments, but after being fed for 56 days, the final body weight of samples given feed A and feed C was higher ($P<0.05$) compared to those with A and D. Furthermore, the body weight gain and specific growth rate differed among the various groups, and the highest values were obtained in fish given treatment feed B, followed by feed C, and feed A. These values were significantly ($P<0.05$) higher than those in the control, with an average increase of 0.6% in the SGR parameter. The highest growth performance was obtained from the addition of bamboo shoots, followed by the ataxanthin- rebon meal combination, astaxanthin, and the control.

The body weight gain of groupers (WG) fed with an additional 1% astaxanthin increased from 18.69 g in the control treatment to 22.87 g within 56 days. The treatment also caused an increase of 0.06% from 0.31% to 0.37% in the SGR parameter. These results are consistent with previous studies that the presence of astaxanthin in feed can increase the growth of *Pagrus pagrus, Melanotaenia parva, Takifugu obscurus, Micropterus salmoides, Lates calcarifer, Carracius auratus,* and blood parrot fish (Cheng *et al*. 2018; Feng *et al*. 2018; Kalinowski,

Robaina, and Izquierdo 2011; Lim *et al.* 2019; Meilisza *et al*. 2017; Wu and Xu 2021; Xie *et al.* 2020). Furthermore, its ability to support fish growth performance was due to its role as an antioxidant that can reduce stress, increase disease resistance, enhance immunity, and increase the efficiency of nutrient use (Han *et al*. 2018; Heng *et al.* 2021; Lim *et al*. 2018, 2019, 2021; Song *et al*. 2017; Zhu *et al*. 2022).

The WG and SGR values of grouper increased after the intake of combination treatment feed C due to an increase in feed protein content of 1.5%, as shown in Table 2. The protein levels of treatment B, which used 15% rebon meal, also increased by 4% compared to the control. This increment was due to the proteinous nature of Acetes indicus rebon meal with a content range of 43 to 72% (Hertrampf and Piedad-Pascual 2000; Komilus and Mufit 2021; Nunes *et al*. 2019; Parmar *et al.* 2016; Pinandoyo *et al*. 2019) and digestibility level of 74-76% in grouper *Epinephelus sp* (Eusebio, Coloso, and Mamauag 2004). The results also showed that the commercial basal feed only contained approximately 49.85% protein. Based on these findings, the 25.52g and 0.43% increase in WG and SGR among samples fed with treatment C was suspected to be more influenced by differences in feed protein compared to astaxanthin. The best growth performance was obtained from the addition of 15% rebon meal with a content of 53.83%, resulting in WG and SGR of 34.75g and 0.54%, respectively. These findings are consistent with previous reports that the best performance of *Plectropomus leopardus* grouper was obtained after being fed a diet with protein levels of 53.14% and low ammonia secretion (Xia *et al*. 2015). However, Xia *et al*. (2019) still recommended feeds with 50% content. This was due to the beneficial effects of amino acids, particularly lysine with an optimal level of 2.84% (Giri *et al*. 2009). These results showed that the addition of 15% rebon meal as a mixture for coral trout grouper feed gave optimal effects.

Grouper Color Quality

The addition of astaxanthin and its combination with rebon meal in feed was shown to increase the redness value (a*) compared to the control ($P<0.05$), but the addition of rebon meal alone produced the same a* value as the control. Increases in a*, and b* as well as decreases in L* in salmon meat were reported to be closely related to the concentration of carotenoids and the duration of administration (Rahman *et al*. 2016; Shekarabi *et al.* 2020). Boonyapadee (2015), Song (2017), and Nogueira *et al*. 2021) also stated that astaxanthin can increase the redness of the skin of koi *Cyprinus carpio, discus Symphisodon* spp and red porgy *Pagrus pagrus*. The results of this study showed that the addition of 1% astaxanthin as well as a combination of 0.5% astaxanthin and 7.5% rebon meal in feed increased the red color of the skin and tail of coral trout grouper. However, an increase in the a* value was not followed by an increment in b* (yellowness). This was assumed to be related to the type and structure of the carotenoid compound given.

Astaxanthin was one of the most widely used carotenoids in the aquaculture industry (Amaya *et al*. 2014; Amaya and Nickell 2015; Bjerkeng 2008; da Costa, D.P.; Miranda- Filho 2019; García-Chavarría and Lara-Flores 2013; Lim *et al*. 2018; Stachowiak and Szulc 2021; Zhao *et al*. 2023), while *Acetes indicus* rebon is a crustacean that is commonly utilized

used as a feed ingredient and contains its natural form (Hertrampf and Piedad-Pascual 2000; Kalinowski *et al*. 2007; Šimat *et al.* 2022; Weeratunge and Perera 2016). The results of this studyshowed that the addition of 1% synthetic astaxanthin (feed A), 15% rebon meal (feed B), as well as a combination of 0.5% synthetic astaxanthin and 7.5% rebon meal (C), affected the color quality parameters, including a*, C *, and H* in the DA, DP, VA, VP, and C. However, the treatments did not affect L* and b*, as shown in Table 3 and Figure 2.

The effect of treatment feed on the color quality of coral trout grouper is presented in Table 3.

Table 3. Effect of Treatment on Color Quality Parameters of Coral Trout Grouper Fish

	Treatment			
	Astaxanthin (A)	**Rebon (B)**	**Astaxanthin + Rebon (C)**	**Control (D)**
Effect on DA Skin's Colour				
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* (^{o})	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 Colour				
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* (^{o})	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 Colour				
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* (^{o})	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 Colur				
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±0.62^{b}	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* (^{o})	37.21±7.61^{a}	65.18±13.41^{b}	43.42±1.40^{ab}	73.05±14.86^{b}
Effect on C Zone's Colour (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* (^{o})	29.57±6.90^{a}	73.3 ± 8.44^{b}	33.50±11.21^{ab}	47.90±15.26^{b}

Note: The value of pigmen indicator in coral trout grouper were observed for DA (Dorsal Anterior), DP (Dorsal Posterior), VA (Ventral Anterior), VP (Ventral Posterior) and C (Caudal) in each treatment during the experiment; - super script letters that differ in 1 line show significantly different results at the 95% level of confidence ($P<0.05$).

The difference in the quality of the color of the fish was assumed to be caused by the unequal concentration of the active substance astaxanthin in treatments A, B, C, and D with a content of 1000, 72, 566, and 45 ppm, respectively. According to Choubert (2001) reported that pigmentation in fish was influenced by several factors, including carotenoid sources, structure, stability, and quantity, withan optimal dose recommendation of 100 ppm.

Astaxanthin derived from synthetic materials or rebon meal is an oxy-carotenoid with a red color (Amaya and Nickell 2015; Joy *et al*. 2021; Tacon, Hasan, and Metian 2011), and was often stored in its form after consumption by aquatic animals (Choubert 2001; Latscha 1990). Consequently, the color of the grouper remained red and the b* was unaffected. Several studies have shown that the b* value can be increased through the administration of lutein-type carotenoids, which are abundant in phytoplankton and zooplankton (Kleppel and Lessard 1992; Pradel *et al.* 2021; Šovljanski *et al*. 2022). These organisms were theoretically reported to be carnivorous fish that can only convert a small amount of lutein to astaxanthin, and the remaining was often deposited in the form of yellow lutein (Meyer and Latscha 1997). Sukarman *et al*. (2023) stated that the administration of lutein caused a yellower skin color in *Schlerogapes formosus arowana* compared to other treatments. However, this effect must be further studied in coral trout grouper, due to the complexity and specificity of carotenoid metabolism in fish (Maoka 2011). There were also limited studies related to the effect of carotenoids on coral trout grouper.

The ANOVA analysis results showed that the addition of astaxanthin, rebon meal, and astaxanthin-rebon meal combination in the feed for 56 days did not affect the L* and b* parameters, but had a significant effect on a*, C*, and H* of the DA, DP, VA, VP, and C sections. Furthermore, the best results on parameters a* and C* were obtained from the samples fed with feed A, followed by C, B, and D. The addition of astaxanthin produced grouper with the reddest color compared to other treatments, and this was indicated by the lower H* values. These findings are in line with the visual appearance presented in Figure 2.

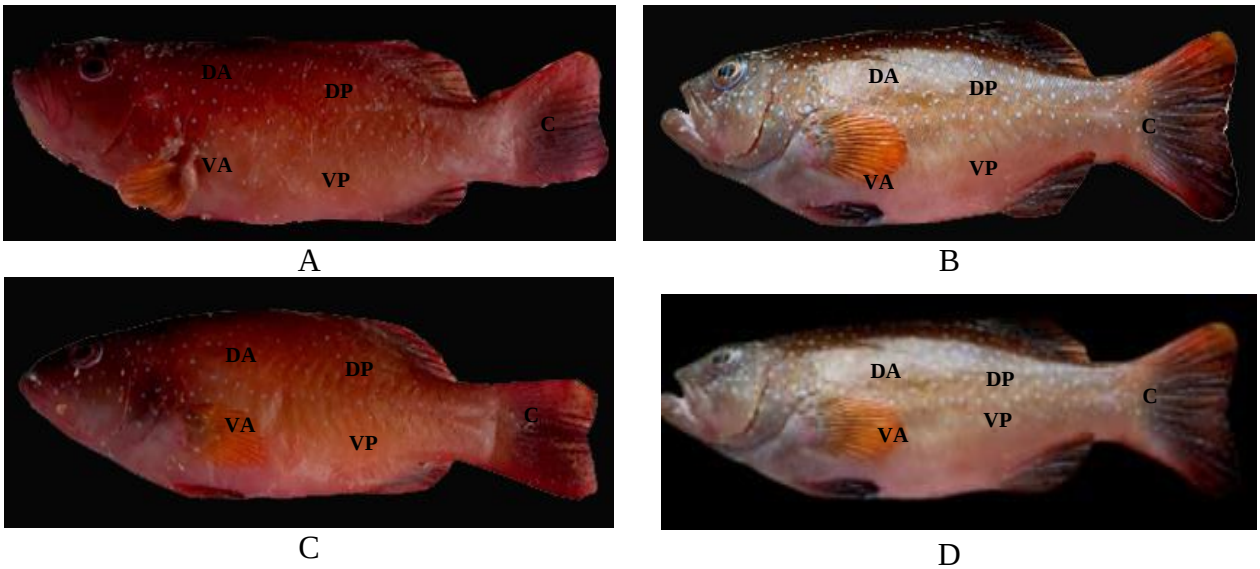


Figure 2. The appearance of color quality for treatments A, B, C, and D in the DA, DP, VA, VP, and C sections.

Changes in a* and/or b* can affect C* (Chroma) or saturation values, as C* represents the concentration of carotenoids in fish tissue (Choubert 2001; Sukarman *et al*. 2023; Sukarman and Hirnawati 2014; Teimouri, Amirkolaie, and Sakineh Yeganeh 2013) Carotenoids have been reported to possess both yellow and red color properties. The results showed that the addition of astaxanthin and its combination with rebon meal gave the same chroma value, which was higher compared to other treatments ($P<0.05$). Furthermore, the highest C* was obtained from the VP section, followed by VA, C, DP, and DA with values of 8.64, 7.00, 6.29, 4.41, and 4.17, respectively. This indicated that pigmentation in grouper occurred from fastest to slowest in the aforementioned sequence. These results are consistent with previous studies that the addition of astaxanthin increased the chroma value in salmon, Red *Oreochromis mossambicus*, and red porgy (Choubert 2010; Choubert, Cravedi, and Laurentie 2009; Choubert, Mendes-Pinto, and Morais 2006; Nickell and Bromage 1998; Nogueira *et al*. 2021; Yilmaz *et al.* 2013). Several studies can also be developed in the future because only a few combined Lab with LCH color space.

One of the unique parameters in evaluating fish color was hue (H*), which can be correlated with the dominance of carotenoids in the tissue tested. The results showed that the hue values of grouper given treatments A and C ranged from 29-43o, and were significantly lower than those obtained from B and D, namely 47-75o). These findings indicated that the color of the fish became redder. Furthermore, the results are consistent with previous studies that the administration of astaxanthin reduced the skin hue value of salmon, anemonefish, discus fish, king crabs, and *Pagrus pagrus* (Christiansen *et al.* 1995; Daly, Swingle, and Eckert 2013; Haque *et al.* 2021; Ho, Orlando Bertran, and Lin 2013; Nogueira *et al*. 2021; Smith, Hardy, and Torrissen 1992). Maoka *et al.* (2017) reported that the type of carotenoids in grouper *Plactropomus leopardus* was dominated by free-astaxanthin (3.1%), astaxanthin monoester (28.5%) and astaxanthin diester (52.71%) with red color (Higuera-Ciapara, Félix-Valenzuela, and Goycoolea 2006; Stachowiak and Szulc 2021).

The results showed that the addition of astaxanthin, rebon meal, and astaxanthin-rabon combination did not affect the L* value ($P>0.05$). However, the values obtained in the DA, DP, and C sections were lower compared to VA and VP. This indicated that the body and tail of coral trout grouper tended to be darker compared to the lower body due to interference with melanin pigment. These findings are consistent with several recent studies that astaxanthin does not affect the skin color of Bighead catfish, salmon, and tilapia (Booth *et al*. 2004; Harith *et al*. 2022; Hien *et al*. 2022). However, conflicting results were obtained by Micah (2022) and Choubert, Mendes-Pinto, and Morais (2006) that the carotenoid can reduce L* in blood parrot fish and salmon. Choubert (2001) stated that the lightness value (L*) in salmon was related to the physical status of the meat (crushed or not). The light or dark color was more closely related to melanin pigment in both salmon and grouper (Tserevelakis *et al* 2022; Zhu *et al*. 2021) compared to carotenoid

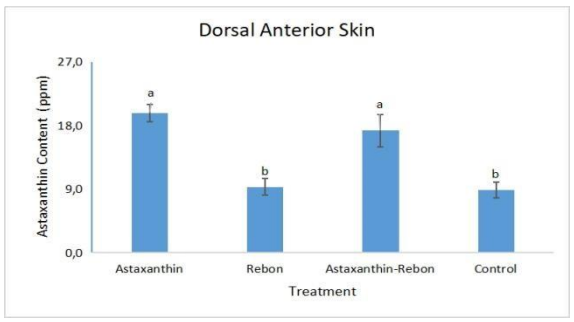
intake. This indicated that the addition of astaxanthin, and its combination with rebon meal does not affect the L* value in all parts of the grouper's skin and tail.

Based on the results, treatments A and D can improve the color quality of coral trout grouper to be redder. These findings are consistent with previous reports that the administration of astaxanthin can improve the color quality of fish skin (Gómez-Estaca *et al*. 2017; Rahman *et al.* 2016; Susatyo *et al.* 2016), and this treatment was the most effective for carnivorous fish,such as salmon (Das 2016; Joy *et al*. 2021; Ytrestøyl *et al.* 2021). The human eye does not evaluate changes in coloration solely based on one parameter but combines H*, C*, and L* values to become a unique color designation (Hariyadi *et al.* 2018; Moreau 2011; Syaifudin *et al*. 2016; Tacon *et al*. 2011). However, changes in quality visually can be seen in Figure 2.

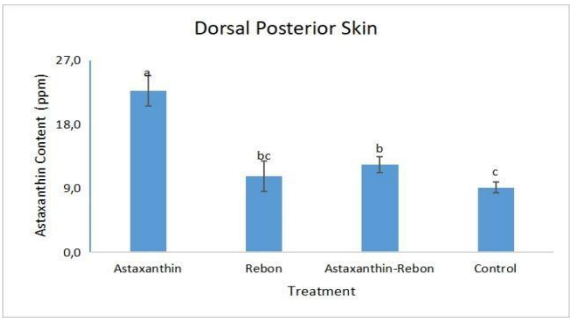
The results also showed that the intake of rebon meal did not significantly affect color quality, although it was recommended in several studies for other species (Gobel, Naiu, and Yusuf 2016; Ravidhia *et al*. 2019; Sholichin *et al*. 2012). This was because the treatment only contained low astaxanthin (Hertrampf and Piedad-Pascual 2000; Lim *et al*. 2021; Rahman *et al.* 2016), and cannot meet the needs of coral trout grouper.

Astaxanthin Content in Grouper Body Tissue

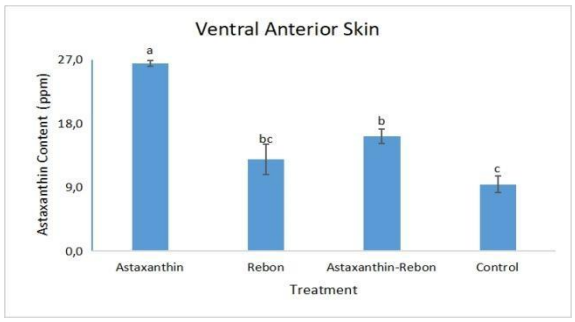
The results of this study showed that the addition of astaxanthin, rebon meal, and astaxanthin-rebon meal combination to feed increased the astaxanthin content in fish skin tissue of the DA, DP, VA, VP, and CF sections, as shown in Figure 3. The samples fed with feed A had the highest levels of astaxanthin ($P<0.05$) compared to the other treatments, with a value range of 19.76 - 26.47 ppm, followed by C (11.06 -17.98 ppm), B (8.95-12.92 ppm), and D (8.02-9.41 ppm). The results also showed that the content in DA and DP was relatively higher compared to the VA and VP sections, and this occurred in all treatments. In the control treatment, the astaxanthin levels in all sections were relatively the same.



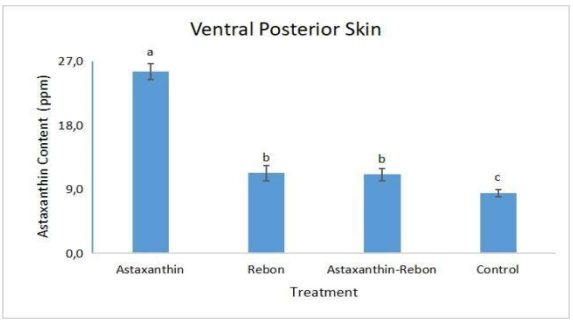
DA



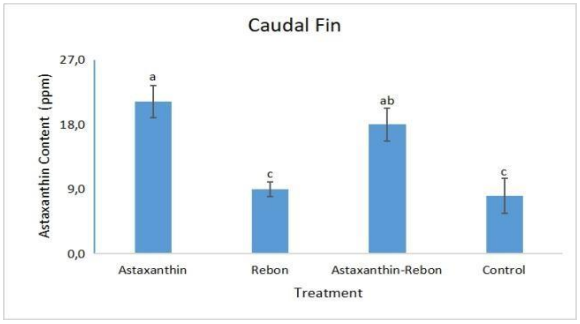
DP



VA



VP



C

Figure 3. Astaxanthin content in the body tissues of groupers located in the Dorsal Anterior, Dorsal Posterior, Ventral Anterior, Ventral Posterior, and Caudal after being fed for 56 days.

The concentration of carotenoids, including astaxanthin, in fish diet was reflected in the pigment levels in the tissues, which was assessed with chemical analysis. The results of the study showed that there was an increase in the astaxanthin content in fish that were given treatments A and C, specifically in the skin of the DA, VA, and C sections, as shown in Figure 3. Furthermore, treatments A, C, B, and D had levels of 19.76-26.47 ppm, 11.05-17.98 ppm, 8.94-12.92 ppm, and 8.01-9.41 ppm, respectively. These results are consistent with previous studies, where the levels in salmon meat treated with astaxanthin and Spirulina plants increase from a range of 0.63-3.95 ppm (Teimouri, Amirkolaie, and Sakineh Yeganeh 2013) to 8-12 ppm (Choubert 2001). Several studies revealed that the concentration varied in skin tissue of various species with values of 5-18 ppm, 3-171 ppm, 37–138 ppm, 4.33–27.7 ppm,33.75 ppm, and 171 ppm in olive flounder (Pham *et al*. 2014), goldfish (Sukarman and Hirnawati 2014), yellow croaker (Yi

et al. 2014), red porgy (Chatzifotis *et al.* 2005), *Melanotaenia parva* (Meilisza *et al*. 2017), and discus fish (Song *et al*. 2017). The levels of astaxanthin also depended on the nature of the fish tissue, species, genetics, development stage, metabolism, sexual maturity, target tissue, the amount of carotenoids in the feed, the structure and stability of the carotenoids, bioavailability, administration period, feed quality, environmental conditions, and disease (Choubert 2001; Meyer and Latscha 1997). Based on previous findings, there are no studies on the administration of astaxanthin to coral trout grouper.

Color Regression Analysis with Astaxanthin Content

The regression analysis results showed that the relationship between astaxanthin content in grouper tissue and lightness (R2 = 0.03), as well as yellowness, was very low, as shown in Table 4. Meanwhile, the relationship with redness and chroma was positive, indicating that the higher the astaxanthin in the tissue, the higher the value of these two parameters. The results also showed that there was a negative correlation with the Hue parameter (H*) (R2 = 0.47).

Table 4. Regression analysis between color quality parameters and astaxanthin concentration (Ast) in coral trout grouper tissue (n = 40 samples).

Model Regresi	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

Findings showed that there were only a few studies on the relationship between the administration of various types of carotenoids and color parameters in fish in the form of regression analysis. However, it was important to strengthen the theory that the color in fish tissue was caused by the presence of carotenoids, including astaxanthin) in the chromatophore cells (Kaleta 2009; Latscha 1990; Nilsson Sköld, Aspengren, and Wallin 2013; Sköld *et al.* 2016). The results of this study showed that the concentration of astaxanthin in the tissues of the coral trout grouper had a positive relationship with a* (R2 = 0.67) and C* (R2 = 0.51), but was negatively associated with H* value (R2 = 0.47). Although the content had a very low correlation with yellowness/b* (R2 = 0.22), it did not correlate with lightness (R2 = 0.03). These findings are consistent with previous studies that a*, C*, and b* had a positive correlation with carotenoid content in anemonefish, salmon, and goldfish, but H* negatively correlated (Ho, O'Shea, and Pomeroy 2013; Safari and Mehraban Sang Atash 2015; Sukarman, Astuti, and Utomo 2017; Teimouri, Amirkolaie, and Sekineh Yeganeh 2013). The difference in the regression analysis was in the yellowness (b*), which was linear with an R2 value, but the parameter was high in previous reports. This was because the type of carotenoid used in this

study was very specific, namely red astaxanthin red (Matsuno 2001) compared to previous reports, which used various sources.

Water Quality

The results of observations of water quality during the study are presented in Table 5. The results showed that the media's water quality was suitable for aquaculture activities. Furthermore, there were no limiting factors that can cause bad conditions in the coral trout grouper. The water quality in each treatment did not show a significant difference, hence, the parameter was considered an external factor with no effect on the results of this study.

Table 5. Water quality during coral trout grouper fish treatment

Parameter	Treatment			
	A	B	C	D
Ammonia (mg/L)	0.0870	0.0751	0.0767	0.0748
Nitrite (mg/L)	< 0.0087	< 0.0087	< 0.0087	< 0.0087
Phosphate (mg/L)	0.0214	0.0239	0.0255	0.0285

The quality of water in the rearing medium was not affected by the addition of astaxanthin, rebon meal, and astaxanthin-rebon meal combination in the feed. This was due to the automatic change of water from the high seas, which ensured that there was no environmental interference in improving the quality of fish color and the growth of grouper.

Conclusion

The results showed that the addition of astaxanthin and its mixture with rebon meal improved the red color quality of coral trout grouper. Furthermore, the presence of astaxanthin in the feed led to an increase in pigment concentration, as indicated by changes in the red color and pigment indicators, but the combination treatment had no significant effect.

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