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RESEARCH

Effects of dietary astaxanthin on chromatic, biochemical, and histological characteristics in juvenile blood parrotfish (*Vieja melanurus* ♀ × *Amphilophus citrinellus* ♂)

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Abstract

This study investigated the effects of astaxanthin, a carotenoid supplement, on the physiology and coloration of juvenile blood parrotfish cultured in a recirculating aquaculture system. Fish were divided into three groups: a control group fed a basal diet for 84 days, a coloration (ASX) group fed an astaxanthin-enriched diet for 84 days, and a decoloration (ASX-) group initially fed an astaxanthin-enriched diet for 42 days and then switched to a basal diet for another 42 days. The results showed that astaxanthin increased the density of erythrophore cells in the skin of the fish, leading to increased ($P < 0.05$) redness (a^*), yellowness (b^*), chroma (C_{ab^*}), and hue (H°_{ab}) in the skin and muscle of the ASX group compared to the ASX- and control groups. It also led to increased ($P < 0.05$) villus height and muscular thickness in the anterior, mid, and posterior intestines, as well as increased production of Kupffer cells in the liver and red pulp in the spleen compared to those fed ASX- and control diets. Additionally, astaxanthin improved the concentration of high-density lipoprotein cholesterol (HDL-C) in the blood plasma and lowered low-density lipoprotein cholesterol (LDL-C) in the liver. The study concluded that astaxanthin significantly improved the concentration of pigment cells, chromatic parameters, villus height, and muscular thickness in blood parrotfish. The differences observed in the groups were attributed to short-term changes in the group that switched from an astaxanthin-enriched diet to a basal diet.

Keywords Astaxanthin · Blood parrotfish · Chromatic characteristics · Biochemical markers · Tissue morphology

Abbreviations

a^*	Redness
AI	Anterior intestine
ASX	Coloration diet (0.45 g/kg astaxanthin)
ASX-	Decoloration diet (0.45 g/kg + 0 g/kg)

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b^*	Yellowness
CA	Central arteriole
C_{ab^*}	Chroma
ELISA	Enzyme-linked immunosorbent assay
HDL-C	High-density lipoprotein cholesterol
H&E	Hematoxylin and eosin
HP	Hepatocytes
KCs	Kupffer cells
L^*	Lightness
LV	Liver
LDL-C	Low-density lipoprotein cholesterol
MI	Mid-intestine
MS-222	Tricaine methanesulfonate
MT	Muscular thickness
ns	Not significant
OD	Optical density
PI	Posterior intestine
RP	Red pulp
rpm	Revolution per minute
SD	Standard deviation
SN	Sinusoids
SPSS	Statistical Package for the Social Sciences
VH	Villus height
VW	Villus width
WP	White pulp

Introduction

Astaxanthin (3,3-dihydroxy-4,4-diketo-β,β-carotene) is an orange-reddish ketocarotenoid pigment used as feed supplement to improve the coloration and health condition of aquatic animals in the aquaculture and ornamental fish industries, thereby influencing its high market value (Lim et al. 2018; Elbahnaswy and Elshopakey 2023). It has been shown to have higher antioxidant activity and oxygen radical absorbance capacity compared with other carotenoids (Nakagawa et al. 2011; Merhan 2017). This biological pigment can generate the red/pink hue that attracts hobbyists in the ornamental trade (Lim et al. 2018). Astaxanthin can be obtained from natural or artificial sources where the former can be synthesized mainly from either plants, algae, phytoplankton, bacteria, or a few fungi (Grung and Metzger 1994; Qin et al. 2008; Katsumata et al. 2014; Novoveská et al. 2019; Yadavalli et al. 2021). Also, natural sources of astaxanthin can be acquired indirectly in our diet by eating crustaceans (such as krill, copepods, and shrimp) and Salmonidae (such as rainbow trout and salmon) species (Liu et al. 2016; Wang et al. 2018; Lu et al. 2021). On the other hand, artificial sources can be produced through chemical synthesis to meet up the global demand for this pigment in the feed market (Li et al. 2011). Depending on the fish species, time of culture, source, and concentration of astaxanthin are the major contributing components that can promote the skin pigmentation of fish (Ha et al. 1993). Aquatic animals especially fish cannot biosynthesize these pigments de novo. The pigmentation requires dietary supplementation (Kalinowski et al. 2007; Li et al. 2018a).

Previous studies have shown that dietary astaxanthin promoted growth performance and enhanced skin pigmentation in *Astronotus ocellatus* (oscar fish), *Cichlasoma synspilum* × *Cichlasoma citrinellum* (blood parrotfish), *Symphysodon* spp. (discus fish), and *Pagrus pagrus* (red porgy) by Alishahi et al. (2015), Li et al. (2018a), Song et al. (2017), and Nogueira et al. (2021), respectively. However, the information on the effects of astaxanthin on the integrity of chromatic assessment, some biochemical indices, and tissue morphology of these species is scarce. Also, astaxanthin digestion coefficients and carotenoid composition of the plasma, liver, muscle, and whole kidney in Atlantic salmon and Atlantic halibut show the ability to metabolically change ingested carotenoids among fish species and flesh coloration (Bjerkeng and Berge 2000). Hence, the large differences between salmonid fish species to absorb and deposit carotenoids in the muscle are a result of low uptake from the intestinal tract and low retention of the absorbed astaxanthin in the muscle (Storebakken and No 1992; March and MacMillan 1996). Total carotenoid concentration in the muscle of rainbow trout (*Oncorhynchus mykiss*) fed an astaxanthin (50 mg/kg) diet increased its redness deposition (Rahman et al. 2016). The study conducted by Song et al. (2017) demonstrated that astaxanthin accumulation reduced and later rapidly increased as time progresses which is like the results obtained on rainbow trout (Zhang et al. 2012). Australian snapper (*Pagrus auratus*) fed 39 mg/kg dietary astaxanthin improved skin astaxanthin concentration compared with fish fed 13 to 26 mg/kg dietary astaxanthin after a 63-day feeding trial (Ben et al. 2009). Inayah and Qin (2010) reported that false clownfish (*Amphiprion ocellaris*) fed enriched dietary astaxanthin enhanced the skin zeaxanthin content after 5 weeks of feeding trial.

Johnson and Bergmann (1984) suggested the use of histomorphology in the analysis of the effects of dietary supplements. The liver is considered the primary organ responsible for storing and metabolizing carotenoids in fish (Segner et al. 1989). Carotenoids are transported via lipoproteins to their target organs or tissues including muscle, skin and scale, testes, ovaries, and intestine (Bjerkeng 2008; Parker 1996). Segner et al. (1989) reported that dietary astaxanthin of red tilapia (*Oreochromis niloticus*) and sunset thick-lipped gourami (*Coliosa labiosa*) fed astaxanthin at 71–132 mg/kg and 32 mg/kg, respectively, enhanced liver histomorphology, thereby improving hepatic parenchyma arrangement consisting of hepatocytes. Furthermore, Page et al. (2005) established that the supplementation of dietary carotenoid pigment improves positively the structure of liver cells. However, astaxanthin has been reported to improve the gastrointestinal tract of golden pompano (*Trachinotus ovatus*) by increasing the length and width of the gut villus (Niu et al. 2020). Their findings revealed that the supplementation of dietary astaxanthin impacted positively the integrity of gut morphology. Nevertheless, higher supplementation impacted negatively on the gut morphology. Thus, the required quantity of dietary astaxanthin supplements necessary for gut morphology integrity needs to be managed.

Blood parrotfish, a hybrid between *Vieja melanurus*♀× *Amphilophus citrinellus* ♂, is a well-known ornamental freshwater fish globally. Because of their colorful red appearance and plump body, they are widely accepted in China and Japan (Sui et al. 2016). The red hue plays a significant role in the quality and market price of this ornamental fish. Some studies have used varying sources of dietary astaxanthin in blood parrotfish to promote body coloration. For instance, Li et al. (2016a, b) and Song et al. (2016) demonstrated that synthetic astaxanthin and alfalfa saponins with the addition of astaxanthin promoted body coloration. Also, blood parrotfish fed with natural astaxanthin from green microalgae *Haematococcus pluvialis* enhanced skin coloration (Li et al. 2018a, b). However, information on the effects of astaxanthin on the integrity of tissues morphology and chromatic and biochemical assessment on blood parrotfish has not been fully documented. Consequently,

this study was aimed at investigating the integrity of astaxanthin on the skin and muscle (chromatic assessment); liver, intestine, muscle, and blood (biochemical indices); and liver, spleen, anterior intestine, mid-intestine, and posterior intestine (histomorphology characteristics) of juvenile blood parrotfish.

Materials and methods

Animal care

All experimental protocols involving animals were approved by the animal care and welfare committee of the College of Fisheries and Life Science of Shanghai Ocean University, Shanghai, China (SHOU-DW-2021-027).

Experimental feed preparation and proximate composition

Two test diets with the same basal composition (Table 1) were prepared. The treatment diet was supplemented with 0.45 g/kg of synthetic astaxanthin (Carophyll® pink 10% CWS, DSM Nutritional Products Ltd). Feed ingredients were finely ground, sieved, and thoroughly mixed. Distilled water was added to form a dough and the dough was pelleted into 1-mm pellet size, air-dried, sieved, and sorted into uniform sizes. The formulated diets were later stored at −20 °C to prevent astaxanthin degradation.

The proximate composition analysis of basal and treatment diets was measured via standard methods of the Association of Official Analytical Chemists (AOAC 1995). Moisture was determined by oven-drying at 105 °C to a constant weight and dry matter content by subtracting moisture value from 100. Crude protein (N × 6.25) contents of diets were determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (2300-Autoanalyzer Foss Tecator, Sweden). Crude lipid was determined by a chloroform-methanol extraction method according to Cejas et al. (2004). Astaxanthin concentration in the basal and treatment diets was analyzed using a fish astaxanthin ELISA kit purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. (mlbio) Shanghai, China, following the manufacturer's procedures.

Experimental fish

A total of 360 juvenile blood parrotfish with an average weight of 10.16 ± 0.38 g were acquired from a commercial fish farm in Hainan, China. All fish were acclimated to laboratory conditions and fed the control diet for 14 days before the start of the trial. The fish were assigned to 18 glass aquaria measuring 48 cm × 45 cm × 30 cm with 20 fish per aquarium, and divided into three groups with sextuplicate each. The dissolved oxygen, water temperature, pH, and ammonia level were maintained at 7.2 ± 0.1 mgL^{-1}, 26 ± 1 °C, 7.1 ± 0.2, and 0.02 ± 0.01 mgL^{-1}, respectively. This was done during acclimatization and experimentation condition. Hanna equipment (model H198194) was used to measure the dissolved oxygen, water temperature, and pH by inserting the probe inside the water while ammonia was measured using HI-700. A total of 30% of the water was changed everyday from the bottom of the tanks as the fish were cultured in a recirculating aquaculture system. The fish were hand-fed twice until apparent satiation at 10:00 am and 4:00 pm daily. The fish were subjected to a natural photoperiod during the 84-day experimental period. The control group were fed a diet

Table 1 Dietary ingredients and proximate analysis of the basal and treatment diets

Feed ingredients	Basal diet (g/kg)	Treatment diet (g/kg)
[a]Fish meal (imported)	400	400
[a]Soybean meal	150	150
[a]Cotton seed meal	110	110
[a]Fish oil	25	25
[a]Soybean oil	20	20
[a]Wheat flour	160	160
[a]Wheat middling	100	100
[b]Mineral premix	15	15
[c]Vitamin premix	10	10
[a]Cellulose (binder)	10	9.55
[d]Astaxanthin	0	0.45
Total	1000	1000
Proximate analysis		
Moisture content (%)	7.69	7.65
Dry matter content (%)	92.31	92.35
Ash content (%)	2.08	2.34
Crude lipid (%)	12.06	12.04
Crude protein (%)	52.51	51.55
Astaxanthin (g/kg)	0.025	0.471

[a]Yuehai Feed Mill, Zhejiang, China. [b]Per kg mineral premix contains: 0.8 g Co; 0.02 g Se; 3 g Cu; 10 g Zn; 3.8 g Mn; 1 g Fe; 12 g Mg; 90 g K; 10.5 g Ca. [c]Per kg vitamin premix contains: 8 million IU of vitamin A; 5 g thiamine-HCl; 15 g riboflavin; 2 million IU of cholecalciferol; 50 g DL-α-tocopherol; 8 g pyridoxine-HCl; 10 g menadione; 0.02 g cyanocobalamin; 40 g nicotinamide; 25 g Ca-pantothenate; 2.5 g folic acid; 0.08 g biotin; 100 g inositol. [d]Carophyll® pink 10% CWS, DSM Nutritional Products Ltd

containing no supplemental astaxanthin for 84 days. The coloration (ASX) group were fed a diet containing 0.45 g/kg synthetic astaxanthin for 84 days. The decoloration (ASX-) group were initially fed the same diet as the ASX group for 42 days then fed the same diet as the control group for another 42 days (Fig. S1).

Sample collection

At the end of the trial, approximately 24 h after the last feeding, three fish from each aquarium were randomly taken then euthanized with tricaine methanesulfonate (MS-222, 50 mg/L) (Shanghai Reagent Corporation, China). After blood collection, the same fish were dissected. Skin, liver, spleen, and intestine were removed and stored at −80 °C for further analysis.

Color analysis

Five individual fish from each aquarium were randomly taken and euthanized with MS-222 for skin and muscle colorimeter analysis after the feed trial using a Minolta CM-600

Chroma Meter (Minolta Camera Co. Ltd, Asaka, Japan). The Chroma Meter was set to take absolute measurements in the L^*, a^*, and b^* measuring mode CIE (1976) using D65 illuminant. Color measurements were taken in the center (Fig. S2) of the lateral body. L^* is the lightness variable where $L^* = 100$ is white and $L^* = 0$ is black; a^* is for the red chromaticity coordinates where $+a^*$ stands for red and $-a^*$ stands for green, and b^* is for the yellow chromaticity coordinates where $+b^*$ stands for yellow and $-b^*$ stands for blue. Hue (H°_{ab}) and chroma (C_{ab^*}) values were calculated from the a^* and b^* values. Hue (e.g., red and yellow, which are the observable colors) is an angular measurement where 0° indicates a red hue and 90° denotes a yellow hue, and is calculated by the equation, $H^\circ_{ab} = \tan^{-1}(b^*/\, a^*)$ (Stokes and Brill 1992). Chroma is an expression of saturation or intensity of the color attained and is calculated by the equation $Cab\ *= \sqrt{(a^{*2} + b^{*2})}$ (Wyszecki and Stiles 1967).

Histomorphology and chromatophore analysis

At the end of the experiment, tissue samples of the liver, spleen, mid-intestine, anterior intestine, and posterior intestine (Fig. S3) were removed and immersed immediately in Bouin's solution after dissection. This was done on three fish randomly taken from each aquarium (the same as in the sample collection). Samples were then dehydrated in graded ethanol solution after 24 h, embedded in paraffin, and transversely sectioned into 4-µm cuts. The sectioned tissue was mounted on glass slides and stained with hematoxylin and eosin. Slides were examined under a NIKON Eclipse Ci microscope (Nikon Corporation, Japan) and photomicrographs of sectioned liver, spleen, mid-intestine, anterior intestine, and posterior intestine tissues were taken (×200 magnification). The muscular thickness, villus width, and villus height were determined by electronic images using the technique described by Poolsawat et al. (2021).

At the end of the experiment, skin samples of two randomly taken fish (control, ASX, and ASX-) were removed with forceps from the side area of each fish. The skin and scale of each were then carefully placed upon a 76×26 mm glass micro slide (Kimble micro slides, Owens-Illinois Co., USA) and covered with a translucent coverslip to immobilize it and then transferred to a compound fluorescent microscope (Nikon Eclipse 80i with digital sight DS-Ri1, Nikon Corporation, Japan). Both ×20 and ×10 amplitude objectives (Plan Achromatic series, Nikon Corporation, Japan) were used.

Biochemical analysis

High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined using assay kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and were used according to the manufacturer's instruction. The above parameters were measured spectrophotometrically (UV-2365, Unico Instruments Co. Ltd., Shanghai, China) at 546-nm wavelength and measurements were performed on the blood plasma, liver, intestine, and skin samples at day 84. A heparinized tube was used to collect the blood then centrifuged for 2500 rpm for 10 min and plasma was retrieved. The liver, intestine, and skin samples were homogenized with 0.86% saline in the ratio of 1:9 using a tissue homogenizer and then centrifuged in a low temperature (4°C) at 2500 rpm for 10 min to retrieve the supernatant. The optical densities (OD) of the blank and standard were used to generate the exact concentrations of the biochemical

parameters. This assay was done on three randomly taken fish (control, ASX, and ASX-) per replicate. The activities were expressed as units of millimole per gram of protein.

Determination of astaxanthin concentration in intestine, liver, scale, and skin

Astaxanthin concentrations in the intestine, liver, scale, and skin on three randomly taken fish (control, ASX, and ASX-) per replicate were analyzed using a fish astaxanthin ELISA kit purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. (mlbio) Shanghai, China, which was used following the manufacturer's instruction. The intestine, liver, scale, and skin samples were homogenized with 0.86% saline in the ratio of 1:9 using a tissue homogenizer and then centrifuged in a low temperature (4°C) at 2500 rpm for 10 min to retrieve the supernatant. Optical density at 450-nm wavelength was read using a microplate reader with 96 wells and actual activities of enzymes were calculated with reference to OD of different concentrations of a standard solution.

Data analysis

The data obtained from this experiment were subjected to a test of homogeneity to test if they were normally distributed. The differences in these parameters between treatments were compared using one-way analysis of variance in SPSS V24.0 (SPSS Inc., Chicago, IL, USA) for Windows. The differences between means were tested by Tukey's multiple range test. All data are presented as means ± SD. Two-way ANOVA was used to establish the effects of the interaction between treatment and section on intestinal morphometrics analysis. P values < 0.05 were considered to indicate statistical significance.

Results

Chromatic and chromatophore analysis

Color parameters such as redness (a^*), yellowness (b^*), chroma (C_{ab^*}), and hue (H°_{ab}) for the ASX group showed a significant effect ($^*P < 0.05$, $^{**}P < 0.01$) for skin and muscle color assessment of blood parrotfish compared to the ASX- and control groups (Fig. 1a, b). However, lightness (L^*) shows no significant difference ($P > 0.05$) in muscle among treatments.

The fish fed the astaxanthin-enriched diet (ASX group) showed significantly concentrated red-colored erythrophore cells (pigment cells) compared with the ASX- with dispersed red-colored erythrophore cells, whereas erythrophore cells were absent in the control diet (Fig. 2a–c) after 84 days of feed trial.

Astaxanthin concentration in the intestine, liver, scale, and skin of blood parrotfish

Astaxanthin impacts positively on the intestine, scale, and skin of the ASX group ($^{**}P < 0.01$) compared to the ASX- and control groups (Fig. 3a–c), whereas astaxanthin was significantly higher in the liver of the ASX- group ($^*P < 0.05$) in contrast to the ASX and control groups (Fig. 3d).

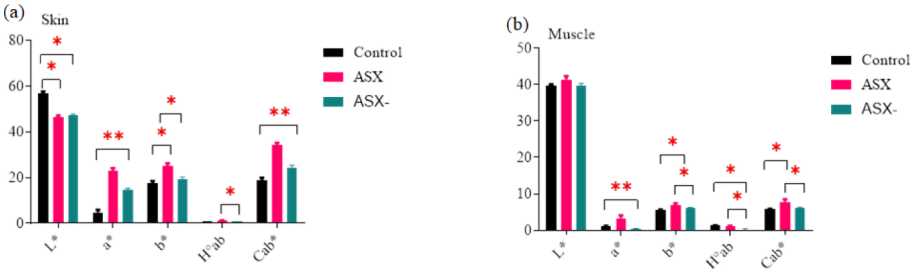

Fig. 1 L^*, a^*, b^*, C_{ab^*}, and H°_{ab} of blood parrotfish showing the **a** skin and **b** muscle. Data are expressed as mean ± SD ($n = 90$). The asterisk indicates significant difference ($^*P < 0.05$, $^{**}P < 0.01$)

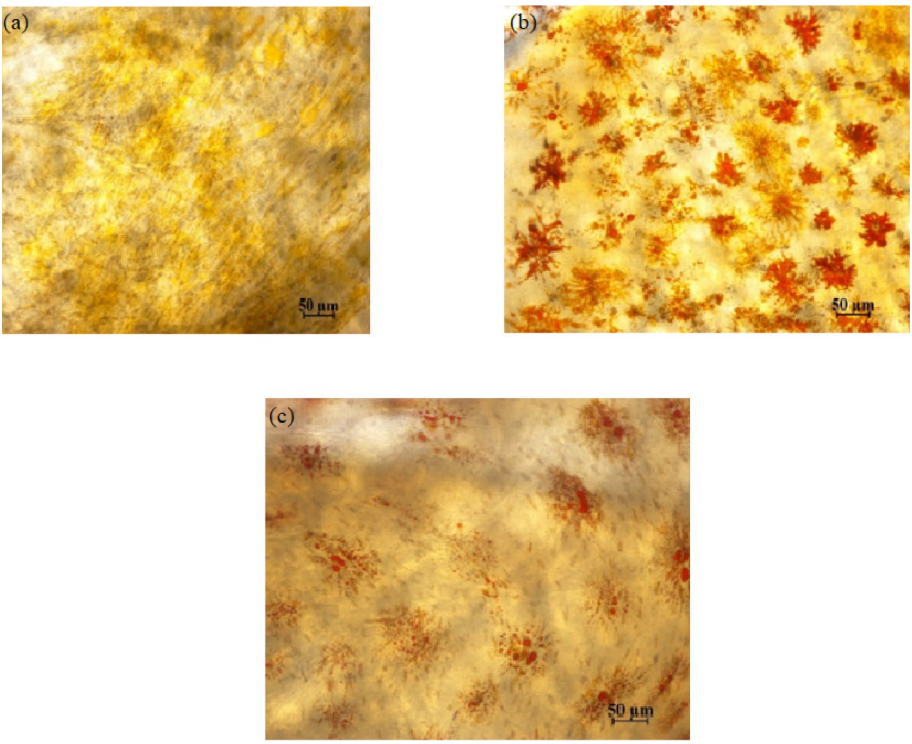

Fig. 2 The skin chromatophores of blood parrotfish fed control, ASX, and ASX- diets, respectively. **a** Absence of erythrophores in control, **b** concentrated red-colored erythrophores in ASX, and **c** dispersed structure of red-colored erythrophores in the ASX- group (magnification ×200)

Blood plasma lipoproteins assay

In Fig. 4a and b, blood plasma HDL-C and LDL-C differed significantly; fish fed the astaxanthin-enriched diet (ASX group) showed increased ($^{**}P < 0.01$) HDL-C and LDL-C compared with the ASX- and control groups. The HDL-C and LDL-C of blood plasma in the ASX and ASX- groups were significantly higher than in the control group.

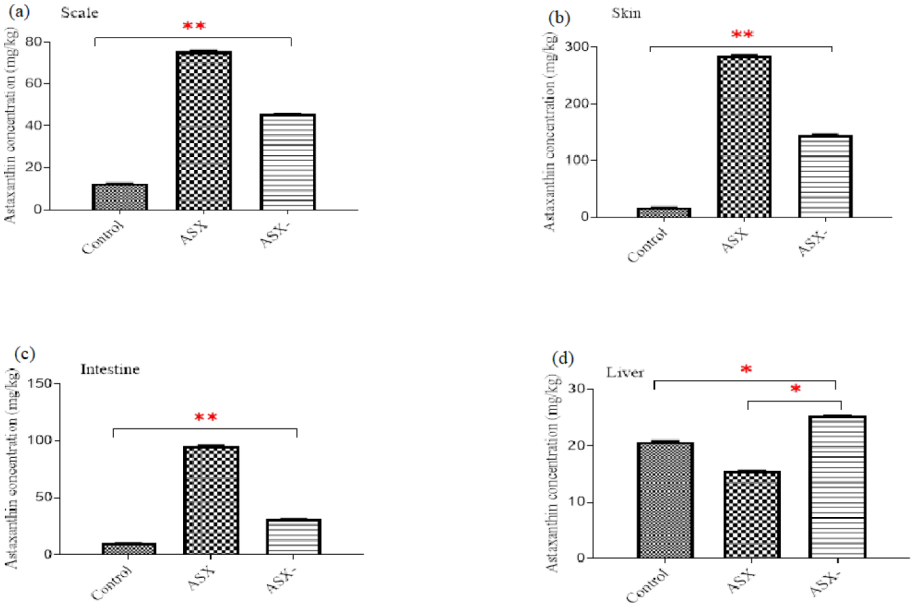

Fig. 3 Concentration of astaxanthin in **a** scale, **b** skin, **c** intestine, and **d** liver of blood parrotfish fed control, ASX, and ASX- diets, respectively. Data are expressed as mean ± SD ($n = 54$). The asterisk indicates significant difference ($^*P < 0.05$, $^{**}P < 0.01$) between treatments

Skin, liver, and intestine HDL-C assay

The results for skin, liver, and intestine HDL-C are shown in Fig. 5a–c. Dietary ASX showed significantly higher ($^*P < 0.05$, $^{**}P < 0.01$) HDL-C in contrast to the ASX- group. The lowest HDL-C was observed in the control group.

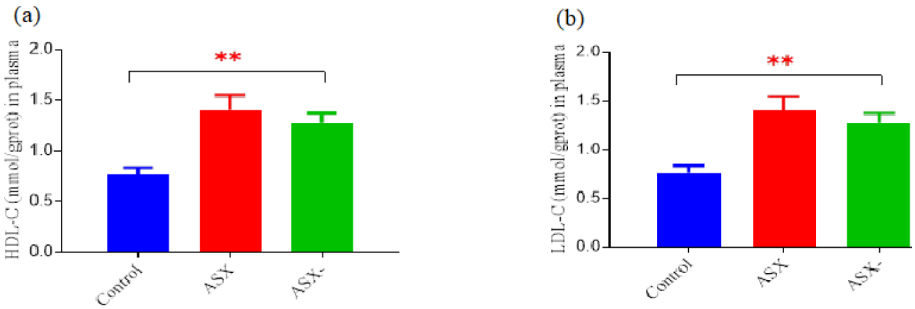

Fig. 4 The lipoprotein-cholesterol of blood parrotfish fed control, ASX, and ASX- diets, respectively. **a** HDL-C in plasma and **b** LDL-C in plasma. Data are expressed as mean ± SD ($n = 18$). The asterisk represents a highly significant difference ($^{**}P < 0.01$) between treatments

Skin, liver, and intestine LDL-C assay

Dietary astaxanthin (ASX) positively enhanced LDL-C (*$P < 0.05$, **$P < 0.01$) in the skin and intestine tissue than the ASX- and control groups, though the skin LDL-C of ASX- fish was higher compared to that of control fish and no significant difference was observed between the ASX- and control groups of intestine LDL-C. However, the LDL-C concentration in the liver of the group fed the control diet was observed to be higher (**$P < 0.01$) than in the ASX and ASX- groups (Fig. 6a–c).

Intestinal histomorphology and liver and spleen morphology of blood parrotfish

This is the first study that provides experimental evidence on the effects of astaxanthin on the integrity of the intestine, liver, and spleen of blood parrotfish. As shown in Table 2, the ASX group improved (*$P < 0.05$) muscular thickness (MT) of the posterior intestine (PI), mid-intestine (MI), and anterior intestine (AI) and villus height (VH) (**$P < 0.01$) of AI, followed by PI and MI of blood parrotfish than the control and ASX- groups. Nevertheless, villus width (VW) shows no significant ($P > 0.05$) effect on the PI, AI, and MI among the ASX, ASX-, and control groups (Table 2). Moreover, it caused a significant increment in the PI, followed by AI and MI of the ASX group compared to the ASX- and control groups. The histomorphology of AI, MI, and PI, stained with hematoxylin and eosin (H&E) displaying the MT, VW, and VH morphometrics, is shown in Fig. 7.

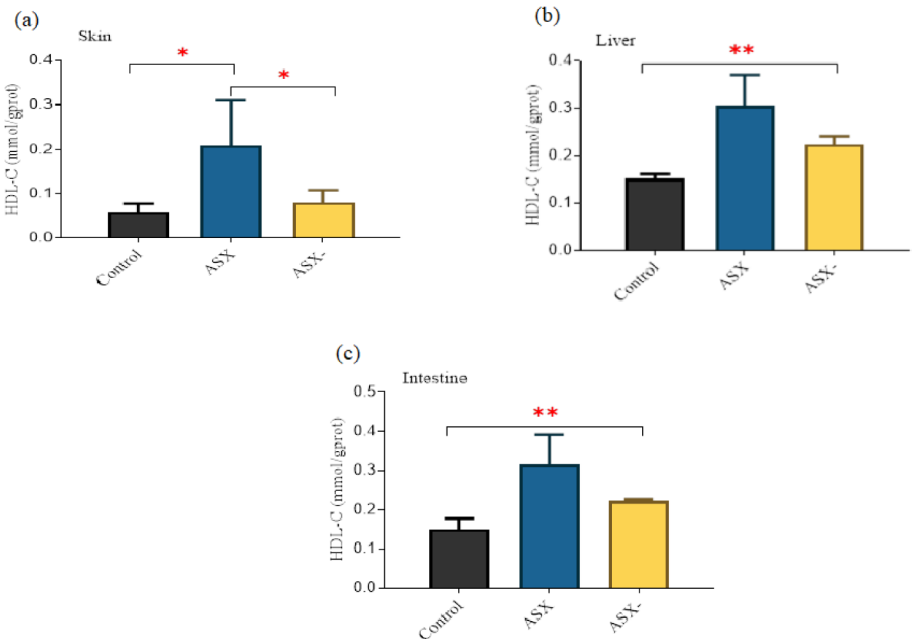

Fig. 5 The high-density lipoprotein-cholesterol of blood parrotfish fed control, ASX, and ASX- diets, respectively. **a** Skin, **b** liver, and **c** intestine. Data are expressed as mean ± SD ($n = 54$). The asterisk indicates significant difference (*$P < 0.05$, **$P < 0.01$) between treatments

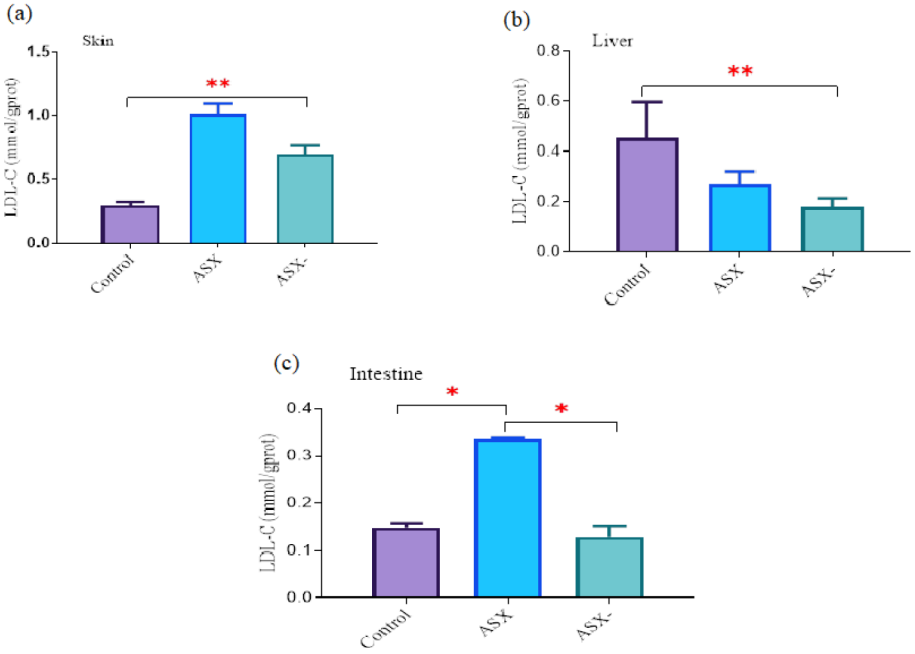

Fig. 6 The low-density lipoprotein-cholesterol of blood parrotfish fed control, ASX, and ASX- diets, respectively. **a** Skin, **b** liver, and **c** intestine. Data are expressed as mean ± SD ($n = 54$). The asterisk indicates significant difference ($^*P < 0.05$, $^{**}P < 0.01$) between treatments

Table 2 Intestinal morphometrics of anterior intestine, mid-intestine, and posterior intestine of blood parrotfish fed control, ASX (0.45 g/kg), and ASX- (0.45 g/kg + 0 g/kg) diets

Treatment	Section	MT (μm)	VH (μm)	VW (μm)
Control	AI	14.42 ± 4.59	299.87 ± 4.88	120.92 ± 2.44
	MI	33.56 ± 2.49	285.39 ± 3.89	115.18 ± 7.85
	PI	31.99 ± 11.72	232.21 ± 7.58	127.79 ± 5.05
ASX	AI	41.95 ± 0.84	680.44 ± 2.91	171.36 ± 2.46
	MI	54.61 ± 7.10	405.36 ± 2.53	149.26 ± 5.15
	PI	55.19 ± 4.07	475.56 ± 4.25	173.66 ± 9.23
ASX-	AI	26.31 ± 16.60	241.46 ± 5.77	130.70 ± 4.08
	MI	27.10 ± 1.95	337.58 ± 8.96	128.02 ± 7.93
	PI	51.98 ± 1.35	190.24 ± 7.99	122.70 ± 7.51
ANOVA	TRT	*	**	ns
	Section	ns	ns	ns
	TRT*Section (combined)	ns	ns	ns

Data are shown as mean ± SD of three replicates (3 fish/aquarium) in each treatment. The asterisk indicates significant difference ($^*P < 0.05$, $^{**}P < 0.01$), and *ns* indicates no significant difference. *ASX* coloration fish, *ASX-* decoloration fish, *AI* anterior intestine, *MI* mid-intestine, *PI* posterior intestine, *TRT* treatment, *TRT*Section* interaction between TRT and Section, *MT* muscular thickness, *VH* villus height, *VW* villus width

The hepatocytes (HP), sinusoids (SN), and Kupffer cells (KCs) of hepatocytes show a better parenchyma arrangement in the ASX group than in the ASX- and control groups (Fig. 8a–c). Nevertheless, the control group showed a better hepatocyte, sinusoids, and Kupffer cells parenchyma arrangement than the ASX- group. The red pulp (RP) was highly promoted in the ASX group compared to the ASX- and control groups (Fig. 9a–c). Furthermore, the white pulp (WP) was significantly higher in the ASX group in contrast to the ASX- and control groups. The central arteriole (CA) is responsible for the supply of oxygenated blood to the spleen of all the groups.

Discussion

Astaxanthin is a carotenoid of the xanthophyll family of hydroxy-carotenoids usually supplemented in cultured fish and crustaceans' diets to boost color pigmentation in the ornamental and aquaculture industry (Schuchardt and Izquierdo 2005; Kop 2008; Flores and Chien 2011; Li et al. 2016a, b; Abd El-Gawad et al. 2019). Previous studies have shown that astaxanthin impacts positively on fish blood serum, flesh, liver, and intestine lipoprotein-cholesterol (Torrissen et al. 1989; Torrissen 1995). Also, astaxanthin enhanced the liver histology of rainbow trout, Nile tilapia, and thick-lipped gourami (Segner et al. 1989; Page et al. 2005) and the gastrointestinal tract of golden pompano (Niu et al. 2020).

In the present study, the dietary astaxanthin in the astaxanthin-fortified diet influenced redness, yellowness, chroma, and hue of the skin compared to the muscle in blood parrotfish. Body color is attributed to the health status, quality, and market price of fish and crustaceans in the aquaculture and ornamental industry (Sathyaruban et al. 2021). The

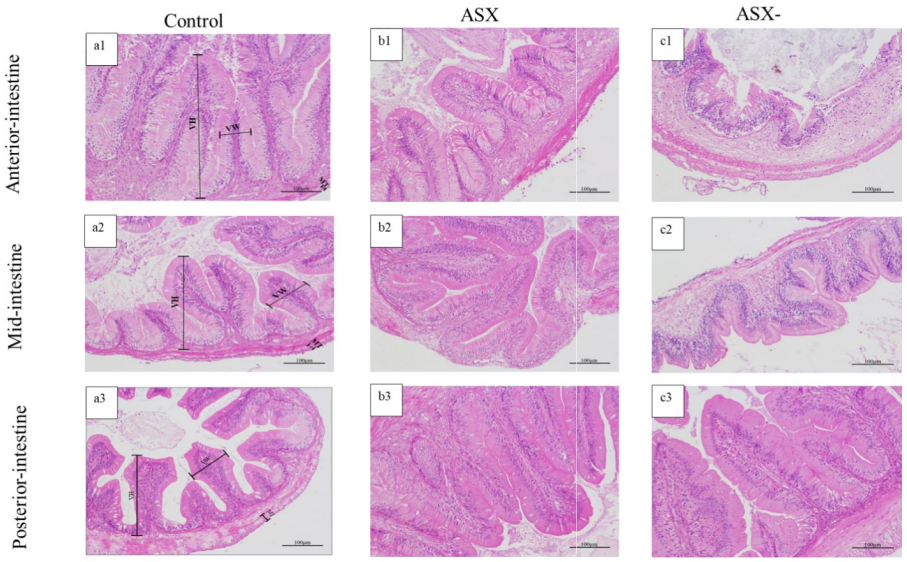

Fig. 7 The anterior intestine, mid-intestine, and posterior intestine (H&E-stained sections) morphology of blood parrotfish showing the VH (villus height); VW (villus width); and MT (muscular thickness) of **a** control, **b** ASX, and **c** ASX- groups (magnification ×200) for 84 days of feeding trial

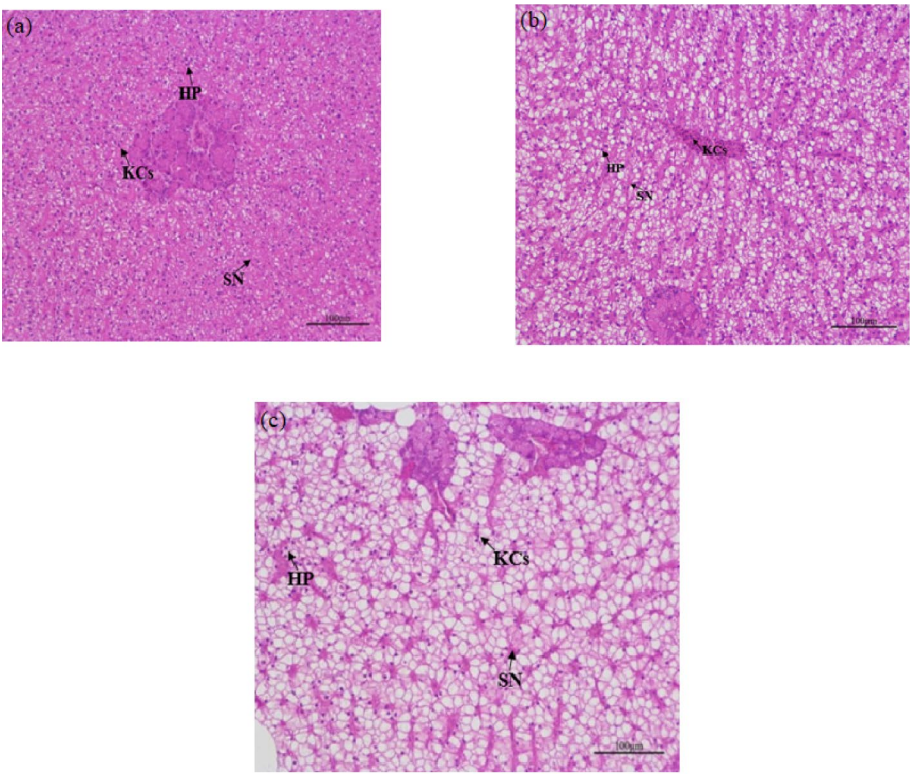

Fig. 8 The liver (H&E-stained sections) morphology of blood parrotfish showing the hepatocytes (HP), sinusoids (SN), and Kupffer cells (KCs) of **a** control, **b** ASX, and **c** ASX- groups (magnification ×200) for 84 days of feeding trial

saturation and deposition of astaxanthin take place in the skin faster than in the muscle which may be due to the transforming ability of the alimentary tract to ingest and transform carotenoids, thereby storing it in the skin of blood parrotfish though the exact mechanism of this action is not known. This is similar to the results obtained for koi carp (*Cyprinus carpio*) that were fed beetroot, carrot peel, tomato peel powder, and powder mixture for 45 days (Maiti et al. 2017) and 56 days in blood parrotfish (Li et al. 2016a, b).

This study showed that synthetic astaxanthin is effective in concentrating the pigment cells (erythrophores). Erythrophores are red pigment cells that contain carotenoid vesicles filled with carotenoids (Ligon and Mccartney 2016). Skin color or concentration of pigment cells may be dependent on the duration of the feeding trial. Nevertheless, color comes from carotenoid precipitation in the fish tissue (skin) which contains chromatophores (Chatzifotis et al. 2011). This result was also buttressed by the redness (a^*) value (Fig. 1a). Astaxanthin concentrations of the scale, skin, and intestine of blood parrotfish were significantly higher in the ASX group than in the control and ASX- groups, which indicates adequate absorption and uptake of the carotenoid. Our skin and scale carotenoid concentration results are in accordance with the studies of Song et al. (2017) on discus fish (*Symphysodon* spp.), Gouveia and Rema (2005) on goldfish (*Carassius auratus*), and Kalinowski et al. (2007) on red porgy (*Pagrus pagrus*). However, astaxanthin concentration of the

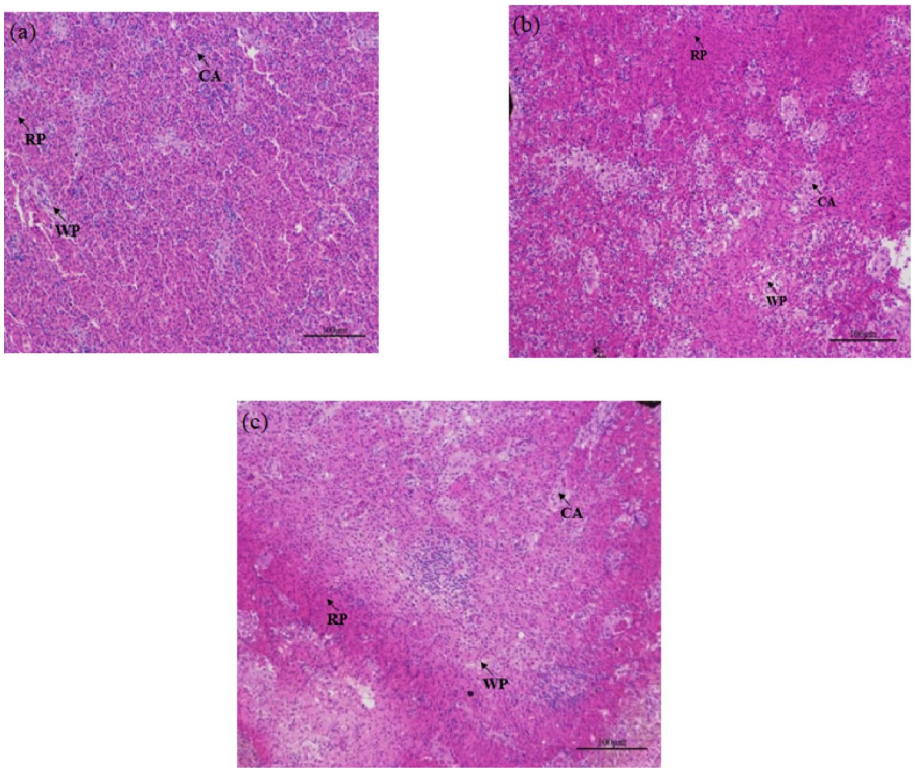

Fig. 9 Spleen (H&E-stained sections) morphology of blood parrotfish showing the white pulp (WP), the red pulp (RP), and central arteriole (CA) of **a** control, **b** ASX, and **c** ASX- groups (magnification ×200) for 84 days of feeding trial

liver was higher in the ASX- group followed by the control and ASX groups, suggesting a higher rate of carotenoid conservation in the ASX- group. Also, the fish may probably have the ability to store astaxanthin after withdrawal. The uptake and transport efficiency can affect carotenoid accumulation (Ho et al. 2013).

Dietary astaxanthin actuates increase in the HDL-C concentration thereby regulating LDL-C concentration in blood plasma and liver which indicates that astaxanthin could help maintain tissue integrity observed in the ASX group. This can be attributed to continuous dietary astaxanthin administration over a long period of time. Furthermore, astaxanthin can swiftly influence the production of HDL-C which is a transporter of long-chain polyunsaturated fatty acid that plays a vital role in carotenoid metabolism before their deposition in the integument of the fish. This present study concurred with a previous study of Aas et al. (1999) that observed a significant effect of ^{14}C-astaxanthin in Atlantic salmon (*Salmo salar* L.) for lipoproteins (HDL and LDL) transport. However, they suggested that the majority of carotenoid transportation may be associated with serum albumin. Moreover, the observation of the effect of carotenoids on blood serum lipoproteins was also documented in rainbow trout (*Oncorhynchus mykiss*) (Salvador et al. 2009). In addition, dietary astaxanthin positively enhanced HDL-C (skin, liver, and intestine) and LDL-C (skin and intestine) of blood

parrotfish but decreased the activity of liver LDL-C compared to the ASX- and control groups. The enhancement may probably be because astaxanthin has been absorbed in the free state and biotransformed by oxidation and reduction. This is in agreement with the results of Choubert and Heinrich (1993) and Aas et al. (1999). Furthermore, when the activity for liver LDL-C increases, it can cause lipid peroxidation which can cause cell death of the fish. Astaxanthin is an antioxidant with a protective effect on lipid peroxidation (Wang et al. 2006; Xie et al. 2017; Li et al. 2018b).

Previous studies have been reported on the mechanisms responsible for carotenoid absorption in man and mammal gut (Erdman et al. 1993; Parker 1996; Furr and Clark 1997; Van den Berg 1999) but limited or no information related to activities in blood parrotfish. The increased VH, VW, and MT in the ASX group of this present study indicate that astaxanthin improves intestinal health and surface area for nutrient uptake of the fish. Torrissen et al. (1989) and White et al. (2002) demonstrated that absorption of carotenoids occurs primarily at the proximal and mid-intestine of salmonids though there is a need for further research to ascertain this hypothesis. In addition, several literatures have reported that absorption of dietary nutrients takes place rapidly in the anterior or posterior intestine due to the higher number and length of the intestinal villi (Collie 1985; Buddington and Diamond 1987; Dabrowski 1990; Bakke-McKellap et al. 2000; Jutfelt et al. 2007; Firdaus-Nawi et al. 2013).

To maintain the normal physiological health status of fish, the liver integrity and morphological structure must be in a good state because of their metabolic role in nutrient metabolism (Yusuf et al. 2021). Johnson and Bergman (1984) reported that a histological approach can be used to evaluate the impact of food additives. The supplementation of dietary carotenoid pigment improved positively the structure of liver cells in our present study. Kupffer cells are a spindle-like structure part of the reticular endothelial system called macrophages whereas hepatocytes are round-like structures separated by sinusoids that help detoxify harmful materials. Also, Kupffer cells are attached to the walls of the sinusoids together with the spleen which participate in removing worn-out erythrocytes (Ferri and Sesso 1981). Excessive Kupffer cells along the portal vein lining sinusoid were highly produced in the ASX group because abundant Kupffer cells play a significant role in improving hepatic homeostasis and boosting the fish immune system against disease-causing organisms. Even so, the presence of hepatocyte cells in all the groups is an indication of protein synthesis. These findings were in line with those of Page et al. (2005) for *Oncorhynchus mykiss* fed carotenoid pigment for 3 weeks by improving the structure of the liver, and Segner et al. (1989) for *Oreochromis niloticus* and *Colisa labiosa* fed astaxanthin for 23 weeks by also observing improvement in liver structure histologically. Our histological results show that astaxanthin can positively improve the metabolic effect of blood parrotfish.

More red pulp was present in the spleen of the ASX group which indicates the removal of worn-out erythrocyte cells, whereas the increase in white pulp is an indication of the proliferation of macrophages, thereby promoting the well-being of the fish against microscopic organisms. This corroborates our findings on liver morphology. Milani et al. (2017) stated that carotenoids can stimulate the proliferation of macrophages, and B- and T- lymphocytes in humans. However, liver and spleen histochemistry analysis to identify reticular fibers, glycogen, neutral glycoprotein, ceroid, hemosiderin, and lipofuscins would be necessary to understand the metabolic activities of macrophages (Sales et al. 2017).

Conclusion

In conclusion, dietary astaxanthin improves the concentration of pigment cells, chromatic parameters, villus height, and muscular thickness in blood parrotfish. Astaxanthin increases blood HDL-C and decreases liver LDL-C in blood parrotfish. Furthermore, the fish probably have the mechanism to store astaxanthin in the liver after withdrawal of the astaxanthin-enriched diet. Consequently, this study unfolded more data about the effects of astaxanthin on the coloration and physiology of blood parrotfish.

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Declarations

Competing interests The authors declare no competing interests.

References

Aas GH, Bjerkeng B, Storebakken T, Ruyter B (1999) Blood appearance, metabolic transformation and plasma transport proteins of C-astaxanthin in Atlantic salmon (*Salmo salar* L). Fish Physiol Biochem 21:325–334

Abd El-Gawad EA, Wang HP, Yao H (2019) Diet supplemented with synthetic carotenoids: effects on growth performance and biochemical and immunological parameters of yellow perch (*Perca flavescens*). Front Physiol 10:1–13. https://doi.org/10.3389/fphys.2019.01056

Alishahi M, Karamifar M, Mesbah M (2015) Effects of astaxanthin and Dunaliella salina on skin carotenoids, growth performance and immune response of Astronotus ocellatus. Aquacult Int 23(5):1239–1248. https://doi.org/10.1007/s10499-015-9880-0

AOAC (1995) Official methods of analysis, 16th edn. Association of Official Analytical Chemists, Arlington, p 1094

Bakke-McKellep AM, Nordrum S, Krogdahl A, Buddington RK (2000) Absorption of glucose, amino acids, and dipeptides by the intestines of Atlantic salmon (*Salmo salar* L). Fish Physiol Biochem 22:33–44. https://doi.org/10.1023/A:1007872929847

Ben JD, Mark AB, Geoff LA, Paul LJ (2009) Effects of dietary astaxanthin concentration and feeding period on the skin pigmentation of Australian snapper Pagrus auratus (Bloch & Schneider, 1801). Aquac Res 40:60–68

Bjerkeng B (2008) Carotenoids in aquaculture: fish and crustaceans. In: Britton G, LiaaenJensen HCS, Pfander H (eds) Carotenoids, Natural Functions. Basel, Switzerland

Bjerkeng B, Berg GM (2000) Apparent digestibility coefficients and accumulation of astaxanthin E/Z isomers in Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L). Comp Biochem Physiol 127B:423–432

Buddington RK, Diamond JM (1987) Pyloric ceca of fish: a new absorptive organ. Am J Physiol 252:G65–G76
Cejas JR, Almansa E, Jérez S, Bolaños A, Felipe B, Lorenzo A (2004) Changes in lipid class and fatty acid composition during development in white seabream (Diplodus sargus) eggs and larvae. Comp Biochem Physio - B Biochem Mol Biol 139(2):209–216. https://doi.org/10.1016/j.cbpc.2004.07.010
Chatzifotis S, Vas J, Kyriazi P, Divanach P, Pavlidis M (2011) Dietary carotenoids and skin melanin content influence the colouration of farmed red porgy (*Pagrus pagrus*). Aquacult Nutr 17:e90–e100
Choubert G, Heinrich O (1993) Carotenoid pigments of the green alga *Haematococcus pluvialis*: assay on rainbow trout, *Oncorhynchus mykiss*, pigmentation in comparison with synthetic astaxanthin and canthaxanthin. Aquaculture 112:217–226
CIE (1976) Colorimetry. Publication No. 15. Bureau Central de la CIE, Vienna
Collie NL (1985) Intestinal nutrient transport in coho salmon (*Oncorhynchus kisutch*) and the effects of development, starvation, and seawater adaptation. J Comp Physiol B 156:163–174. https://doi.org/10.1007/BF00695770
Dabrowski K (1990) Absorption of ascorbic acid and ascorbic sulfate and ascorbate metabolism in stomachless fish, common carp. J Comp Physiol B 160:549–561
Elbahnaswy S, Elshopakey GE (2023) Recent progress in practical applications of a potential carotenoid astaxanthin in aquaculture industry: a review. Fish Physiol Biochem. https://doi.org/10.1007/s10695-022-01167-0
Erdman JW, Bierer TL, Gugger ET (1993) Absorption and transport of carotenoids. Annals New York Acad Sci 691:76–85
Ferri S, Sesso A (1981) Ultrastructural study of the endothelial cells in teleost liver sinusoid under normal and experimental conditions. Cell Tissue Res 219:649–657
Firdaus-Nawi M, Zamri-Saad M, Nik-Haiha NY, Bakar Zuki MA, Mohd Effendy AW (2013) Histological assessments of intestinal immuno-morphology of tiger grouper juvenile, Epinephelus fuscoguttatus. SpringerPlus 2(1):1–13. https://doi.org/10.1186/2193-1801-2-611
Flores EE, Chien YH (2011) Chromatosomes in three phenotypes of neocaridina denticulata kemp, 1918: morphological and chromatic differences measured non-invasively. J Crustac Biol 31(4):590–597. https://doi.org/10.1651/11-3457.1
Furr HC, Clark RM (1997) Intestinal absorption and tissue distribution of carotenoids. Nutr Biochem 8:64–377
Gouveia L, Rema P (2005) Effect of microalgal biomass concentration and temperature on ornamental goldfish (*Carassius auratus*) skin pigmentation. Aqua Nutr 11:19–23
Grung M, Metzger P (1994) Algal carotenoids 53; secondary carotenoids of algae 4; secondary carotenoids in the green alga *Botryococcus braunii*, race L, new strain. Biochem Syst Ecol 22(1):25–29
Ha BS, Kang DS, Kim JH, Choi OS, Ryu HY (1993) Metabolism of dietary carotenoids and effects to improve the body color of cultured founder and red seabream. Bull Korean Fish Soc 26:91–101
Ho ALFC, Oshea SK, Pomeroy HF (2013) Dietary esterified astaxanthin effects on color, carotenoid concentrations, and compositions of clown anemonefish, *Amphiprion ocellaris*, skin. Aquacult Int 21:361–374. https://doi.org/10.1007/s10499-012-9558-9
Inayah Y, Qin JQ (2010) Effect of dietary carotenoids on skin color and pigments of false clownfish, Amphiprion ocellaris, cuvier. J World Aquac Soc 41:308–318
Johnson RD, Bergmann HL (1984) Use of histopathology in aquatic toxicology: a critique. In: Cairns VW, Hodson PV, Nriagu JO (eds) Contaminant effects on fisheries. Wiley, New York, pp 19–36
Jutfelt F, Olsen RE, Björnsson BTH, Sundell K (2007) Parr-smolt transformation and dietary vegetable lipids affect intestinal nutrient uptake, barrier function and plasma cortisol levels in Atlantic salmon. Aquaculture 273:298–311. https://doi.org/10.1016/j.aquaculture.2007.10.012
Kalinowski CT, Izquierdo MS, Schuchardt D, Robaina LE (2007) Dietary supplementation time with shrimp shell meal on red porgy (*Pagrus pagrus*) skin colour and carotenoid concentration. Aquaculture 272(1–4):451–457. https://doi.org/10.1016/j.aquaculture.2007.06.008
Katsumata T, Ishibashi T, Kyle D (2014) A sub-chronic toxicity evaluation of a natural astaxanthin-rich carotenoid extract of *Paracoccus carotinifaciens* in rats. Toxicol Rep 1:582–588. https://doi.org/10.1016/j.toxrep.2014.08.008
Kop A (2008) The effect of synthetic and natural pigments on the colour of the cichlids (*Cichlasoma severum Sp*). Aquac Int 16:117–122. https://doi.org/10.1007/s10499-007-9130-1
Li J, Zhu DL, Niu J, Shen SD, Wang G (2011) An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis* Biotechnol Adv 29:568–574
Li M, Rahman MM, Wu B (2016a) Effects of dietary canthaxanthin on growth and body colour of blood parrot cichlid *Amphilophus citrinellus* × *Paraneetroplus synspilus*. https://doi.org/10.1007/s10499-016-0068-z

Li T, He C, Ma Z, Xing W, Jiang N, Li W, Sun X, Luo L (2016b) Effects of different carotenoids on pigmentation of blood parrot (*Cichlasoma synspilum* × *Cichlasoma citrinellum*). J Aquac Res Dev 07(03). https://doi.org/10.4172/2155-9546.1000414

Li F, Huang S, Lu X, Wang J, Lin M, An Y, Wu S, Cai M (2018a) Effects of dietary supplementation with algal astaxanthin on growth, pigmentation, and antioxidant capacity of the blood parrot (*Cichlasoma citrinellum* × *Cichlasoma synspilum*). J Oceanol Limnol 36(5):1851–1859. https://doi.org/10.1007/s00343-019-7172-7

Li M, Rahman MM, Lin YC, Chiu K (2018b) Effect of dietary lipid on growth, expression of canthaxanthin-based coloration, digestive enzymes activities and immunity in blood parrot cichlid *Amphilophus citrinellus* × *Paraneetroplus synspilus*. Aquac Nutr 24(1):277–284. https://doi.org/10.1111/anu.12556

Ligon RA, Mccartney KL (2016) Biochemical regulation of pigment motility in vertebrate chromatophores: a review of physiological color change mechanisms. Curr Zool 62(3):237–252. https://doi.org/10.1093/cz/zow051

Lim KC, Yusoff FM, Shariff M, Kamarudin MS (2018) Astaxanthin as feed supplement in aquatic animals. Rev Aquac 10(3):738–773. https://doi.org/10.1111/raq.12200

Liu F, Shi HZ, Guo QS, Yu YB, Wang AM, Lv F, Shen WB (2016) Effects of astaxanthin and emodin on the growth, stress resistance and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*). Fish Shellfish Immunol 51:125–135

Lu Q, Li H, Zou Y, Liu H, Yang L (2021) Astaxanthin as a microalgal metabolite for aquaculture: a review on the synthetic mechanisms, production techniques, and practical application. Algal Res 54:102178

Maiti M, Bora D, Nandeesha TL, Sahoo S, Adarsh BK, Kumar S (2017) Effect of dietary natural carotenoid sources on colour enhancement of Koi carp, *Cyprinus carpio* L. Int J Fish Aquat Stud 5(4):340–345

March BE, MacMillan C (1996) Muscle pigmentation and plasma concentrations of astaxanthin in rainbow trout, chinook salmon, and Atlantic salmon in response to different dietary levels of astaxanthin. Progr Fish-Cult 58:178–186

Merhan O (2017) The biochemistry and antioxidant properties of carotenoids. Carotenoids 5:51

Milani A, Basirnejad M, Shahbazi S, Bolhassan A (2017) Carotenoids: biochemistry, pharmacology and treatment. Br J Pharmacol 174(11):1290–1324. https://doi.org/10.1111/bph.13625

Nakagawa K, Kiko T, Miyazawa T, Burdeos GC, Kimura F, Satoh A, Miyazawa T (2011) Antioxidant effect of astaxanthin on phospholipid peroxidation in human erythrocytes. Br J Nutr 105:1563–1571

Niu J, Zhao W, Lu DQ, Xie JJ, He XS, Fang HH, Liao SY (2020) Dual-function analysis of Astaxanthin on Golden Pompano (Trachinotus ovatus) and its role in the regulation of gastrointestinal immunity and retinal mitochondrial dysfunction under Hypoxia conditions. Front Physiol 11:1–18. https://doi.org/10.3389/fphys.2020.568462

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*). Anim Feed Sci Technol 276:114916. https://doi.org/10.1016/J.ANIFEEDSCI.2021.114916

Novoveská L, Ross ME, Stanley MS, Pradelles R, Wasiolek V, Sassi JF (2019) Microalgal carotenoids: a review of production, current markets, regulations, and future direction. Mar Drugs 17:640

Page GI, Russell PM, Davies SJ (2005) Dietary carotenoid pigment supplementation influences hepatic lipid and mucopolysaccharide levels in rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol - B Biochem Mol Biol 142(4):398–402. https://doi.org/10.1016/j.cbpb.2005.09.001

Parker RS (1996) Absorption, metabolism, and transport of carotenoids. FASEB J 10:542–551

Poolsawat L, Li X, Xu X, Rahman MM, Boonpeng N, Leng X (2021) Dietary xylooligosaccharide improved growth, nutrient utilization, gut microbiota and disease resistance of tilapia (*Oreochromis niloticus* x *O. aureus*). J Anim Feed Sci Technol 275:1–12. https://doi.org/10.1016/j.anifeedssci.2021.114872

Qin S, Liu GX, Hu ZY (2008) The accumulation and metabolism of astaxanthin in *Scenedesmus obliquus* (Chlorophyceae). Process Biochem 43(8):795–802. https://doi.org/10.1016/j.procbio.2008.03.010

Rahman MM, Khosravi S, Chang KH, Lee SM (2016) Effects of dietary inclusion of astaxanthin on growth, muscle pigmentation and antioxidant capacity of juvenile rainbow trout (*Oncorhynchus mykiss*). Prev Nutr Food Sci 21(3):281–288

Sales CF, Silva RF, Amaral MGC, Domingos FFT, Ribeiro RIMA, Thome RG, Santos HB (2017) Comparative histology in the liver and spleen of three species of freshwater teleost. Neotrop Ichthyol 15(11):1–12. https://doi.org/10.1590/1982-0224-20160041

Salvador AM, Allonds-Damián A, Choubert G, Milicua JCG (2009) Impact of different dietary phospholipid levels on cholesterol and canthaxanthin lipoprotein-serum transport and muscle deposition in rainbow trout. J Agric Food Chem 57(5):2016–2021. https://doi.org/10.1021/jf802954t

Sathyaruban S, Uluwaduge DI, Yohi S, Kuganathan S (2021) Potential natural carotenoid sources for the colouration of ornamental fish: a review. Aquacul Int 29:1507–1528. https://doi.org/10.1007/s10499-021-00689-3

Schuchardt D, Izquierdo MS (2005) Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. 244:223–231. https://doi.org/10.1016/j.aquaculture.2004.11.001

Segner H, Arend P, Von Poeppinghausen K, Schmidt H (1989) The effect of feeding astaxanthin to *Oreochromis niloticus* and *Colisa labiosa* on the histology of the liver. Aquaculture 79(1–4):381–390. https://doi.org/10.1016/0044-8486(89)90480-8

Song H, Wei M, Mou X, Liu Y, Wang X, Liu C, Hu Y (2016) Efficacy of alfalfa saponins on promoting pigmentation by astaxanthin in blood parrotfish (*Vieja synspila* × *Amphilophus citrinellus*). Isr J Aqua-Bamidgeh, IJA_68.2016.1247, 10 pages

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*). Aquac Res 48(4):1359–1367. https://doi.org/10.1111/are.13200

Stokes M, Brill MH (1992) Efficient computation of H°_{ab} Color Res Appl 17:410–411

Storebakken T, No HK (1992) Pigmentation of rainbow trout. Aquaculture 100:209–229

Sui Y, Huang X, Kong H, Lu W, Wang Y (2016) Physiological responses to salinity increase in blood parrotfish (*Cichlasoma Synspilum* ♀ × *Cichlasoma citrinellum* ♂). Springerplus 5:1246. https://doi.org/10.1186/s40064-016-2930-x

Torrissen OJ (1995) Strategies for salmonid pigmentation. J Appl Ichthyol 11:276–281

Torrissen OJ, Hardy RW, Shearer KD (1989) Pigmentation of salmonids - carotenoid deposition and metabolism. CRC Crit Rev Aquat Sci 1:209–225

Van den Berg H (1999) Carotenoid interactions. Nutr Rev 57:1–10

Wang YJ, Chien YH, Pan CH (2006) Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus* Aquaculture 261:641–648. https://doi.org/10.1016/j.aquaculture.2006.08.040

Wang Z, Cai CF, Cao XM, Zhu J-M, He J, Wu P, Ye YT (2018) Supplementation of dietary astaxanthin alleviated oxidative damage induced by chronic high pH stress, and enhanced carapace astaxanthin concentration of Chinese mitten crab *Eriocheir* sinensis. Aquaculture 483:230–237

White DA, Page GI, Swaile J, Moody JA, Davies SJ (2002) Effect of esterification on the absorption of astaxanthin in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aqua Res 33:343–350

Wyszecki G, Stiles WS (1967) Color science. Wiley, New York

Xie J, Chen X, Niu J, Wang J, Wang Y, Liu QQ (2017) Effects of astaxanthin on antioxidant capacity of golden pompano (*Trachinotus ovatus*) in vivo and in vitro. Fish Aquat Sci 20(1):1–8. https://doi.org/10.1186/s41240-017-0052-1

Yadavalli R, Ratnapuram H, Peasari JR, Reddy CN, Ashokkumar V, Kuppam C (2021) Simultaneous production of astaxanthin and lipids from *Chlorella sorokiniana* in the presence of reactive oxygen species: a biorefinery approach. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-021-01276-5

Yusuf A, Huang X, Chen N, Li S, Apraku A, Wang W, David MA (2021) Growth and metabolic responses of juvenile largemouth bass (*Micropterus salmoides*) to dietary vitamin C supplementation levels. Aquaculture 534(July 2020):736243. https://doi.org/10.1016/j.aquaculture.2020.736243

Zhang JJ, Li XQ, Leng XJ, Han ZY, Zhang EG, Wu SL (2012) The study of deposition and degradation of astaxanthin on rainbow trout *Oncorhynchus mykiss*. J Fish China 36:1872–1879

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