



Replacement of Astaxanthin With Lutein in Diets of Juvenile *Litopenaeus vannamei*: Effects on Growth Performance, Antioxidant Capacity, and Immune Response

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An 8-week feeding experiment was conducted to investigate whether diet supplementation of lutein could result in similar growth performance, antioxidant capacity, and immunity of *Litopenaeus vannamei* when compared to dietary astaxanthin. Juvenile *L. vannamei* (initial body weight: 0.64 ± 0.04 g) were fed with one of five isonitrogen and isolipids diets with/without lutein or astaxanthin [control group (C); the lutein (L) groups contained 0, 62.5, 75, 87.5 ppm lutein, respectively, the astaxanthin (A) group contained 50 ppm astaxanthin]. Results showed that dietary supplementation of lutein ranging from 62.5 to 75 ppm resulted in similar growth performance (WGR, SGR, FCR, and SR) of *L. vannamei* compared with the A group ($P > 0.05$). Apart from that, no statistical difference was observed in antioxidant parameters (hemolymph T-AOC, hemolymph MDA, and RNA expression level of GSH-PX, CAT), anti-inflammatory ability (Relish, Rho, and HSP70) and apoptosis-related gene expression (Caspase3) among lutein treatments ranging from 62.5 to 87.5 ppm and the A group ($P > 0.05$). These results indicate that a dose of 62.5–75 ppm of lutein was suitable in the diet of *L. vannamei* for substituting dietary astaxanthin.

Keywords: lutein, *L. vannamei*, astaxanthin, growth performance, antioxidant capacity, immunity

INTRODUCTION

In recent years, there have been two significant factors existing in the shrimp farming industry that may affect the profit of shrimp. Firstly, water environments were deteriorated by plenty of pollutants (such as heavy metals, pesticides, bacteria, and viruses) due to industrialization's development and human activity's involvement, resulting in seriously high stress-induced diseases and low survival rate in the shrimp culture industry (Zhang et al., 2013). In addition, maintaining bright and appropriate body pigmentation is also a significant factor closely related to customers'

preference and shrimp's price because it means freshness and high quality of the merchandise (Shahidi and Brown, 1998; Seidgar, 2015). In fact, shrimp and other crustaceans are incapable of bio-synthesize carotenoids *de novo*, which makes it essential for them to obtain carotenoids from the daily diet for body pigmentation (Boonyaratpalin et al., 2001). Previous studies paid more attention to diet supplementation with astaxanthin (3,3'-dihydroxy- β , β -carotene-4,4'-dione) on crustaceans as it helps to enhance the stress resistance of crustaceans by acting as an effective antioxidant against ROS as well as being a green additive for improving growth performance and skin pigmentation of aquatic animals (Latscha, 1989; Petit et al., 1997; Chien et al., 2003; Seabra and Pedrosa, 2010). However, diet supplementation with astaxanthin seems to considerably increase the shrimp feed cost because of the expensive synthetic process of this carotenoid pigment (Boonyaratpalin et al., 2001). Therefore, other cost-effective additives need to be identified for substituting the astaxanthin in the shrimp diets.

Crustaceans could convert many carotenoids like β -carotene, which are easy to be acquired from vegetable ingredients of the feed, into astaxanthin (Giuliano et al., 2000; Niu et al., 2014). Therefore, these green additives are of high interest in the aquaculture industry (da Costa and Miranda-Filho, 2020). For example, lutein, one of the significantly cheaper natural carotenoid pigments compared to astaxanthin, can be extracted from some vegetables like marigolds (da Costa and Miranda-Filho, 2020). Ettefaghdoost and Haghghi (2021) reported that dietary supplementation with 200 mg/kg lutein could enhance the growth performance, immune capacity and total carotenoid pigment content in different tissues of *Macrobrachium nipponense*.

Litopenaeus vannamei, one of the most profitable shrimp species in south China, is very popular with customers because of its delicious flesh and high nutritional value (Sirirustananun et al., 2011). Previous studies demonstrated that both lutein and astaxanthin play essential roles in growth performance, anti-resistance as well as pigmentation of aquatic animals (Kalinowski et al., 2005; Meiliszka et al., 2017). However, knowledge about whether lutein can become a substitution for astaxanthin remained little understood. In our present study, diet supplementation with/without lutein or astaxanthin was compared regarding the effects of the two carotenoids on growth performance, anti-resistance and metabolism of *L. vannamei*. These results may provide a reference for the efficient diet formulation of *L. vannamei*.

MATERIALS AND METHODS

Experiment Diet

In the current study, the commercial lutein (5% lutein) and astaxanthin (10% astaxanthin) were kindly provided by Guangzhou Leader Bio-Technology Co., Ltd., China. Other ingredients like fish meal were all purchased from Guangzhou Chengyi Co., Ltd., China. Five isonitrogen and isolipids experiment diets were formulated with or without lutein or astaxanthin [control group (C); the lutein (L) groups, L1-L3,

contained 0, 62.5 ppm, 75 ppm, 87.5 ppm lutein, respectively, the astaxanthin (A) group contained 50 ppm astaxanthin] (Table 1). All feeds contained 405 g kg⁻¹ crude protein and 71 g kg⁻¹ crude lipid approximately. The diets were made according to the methods reported by Niu et al. (2012). Briefly, all ingredients presented in the Table 1 and deionized water (250 ml/kg dry ingredients mixture) were thoroughly mixed in the Hobart-type mixer, and then the diets (1.2 mm diameter) were extruded using the pelletizer (South China University of Technology, Guangdong, China). Then, diets were heated for 60 min in a ventilated oven (70°C). Afterward, the diets were stored at -20°C and were kept in dark prior to the feeding trail.

Shrimp and Experimental Conditions

Juvenile *L. vannamei* were obtained and cultured at the Chinese Academy of Fishery Sciences (Lingshui, China). Before the feeding experiment, shrimps were fed with the C group diet for 2 weeks to acclimate to the experiment conditions. At the beginning of the feeding trial, 800 lively shrimps (initial body weight: 0.64 ± 0.04 g) with similar size were distributed randomly into recirculating water systems with 20 cylindrical fiber tanks (300 L). Each diet was randomly assigned to quadruplicate tanks.

TABLE 1 | Ingredients and proximate composition of five experiment diets (g kg⁻¹ diets).

Ingredients	C	L1	L2	L3	A
Fish meal	250	250	250	250	250
Soybean meal	270	270	270	270	270
Peanut meal	120	120	120	120	120
Wheat flour	232.2	232.2	232.2	232.2	232.2
Beer yeast	30	30	30	30	30
Shrimp meal	30	30	30	30	30
Fish oil	10	10	10	10	10
Soybean lecithin	10	10	10	10	10
Soybean oil	10	10	10	10	10
Choline chloride (50Vitamin C phosphate	1	1	1	1	1
Vitamin and mineral premix ^a	20	20	20	20	20
Calcium dihydrogen phosphate	10	10	10	10	10
Astaxanthin ^b	0	0	0	0	0.5
Lutein ^b	0	1.25	1.5	1.75	0
Cellulose	1.75	0.5	0.25	0	1.25
Sum	1000.00	1000.00	1000.00	1000.00	1000.00
Nutrient levels (g kg⁻¹)					
Moisture	74.5	79.2	76.6	73.7	75.8
Crude lipid	71.2	70.3	69.7	72.8	70.8
Crude protein	405.2	408.3	406.9	404.1	404.8

^aComposition of vitamin and mineral mixture (kg-1 of mixture): vitamin A, 250,000 IU; riboflavin, 750 mg; pyridoxine HCL, 500 mg; cyanocobalamin, 1 mg; thiamin, 500 mg; menadione, 250 mg; folic acid, 125 mg; biotin, 10 mg; a-tocopherol, 3,750 mg; myo-inositol, 2,500 mg; calcium pantothenate, 1,250 mg; nicotinic acid, 2,000 mg; vitamin D3, 45,000 IU; vitamin C, 7,000 mg; Zn, 4,000 mg; K, 22,500 mg; I, 200 mg; NaCl, 2.6 g; Cu, 500 mg; Co, 50 mg; FeSO₄, 200 mg; Mg, 3,000 mg; Se, 10 mg.

^bSupplied by Guangzhou Leader Bio-Technology Co., Ltd., China.

Shrimps were fed to apparent satiation three times (at 6:00, 12:00, and 18:00; with 5–8% body weight) daily for 8 weeks.

During the feeding trial, the water temperature was maintained 26.9–28.2°C, pH 7.6–7.8, salinity 30–32‰, dissolved oxygen > 7 mg/L, total ammonia nitrogen < 0.1 mg/L, and sulfide < 0.05 mg/L.

Sample Collection

At the end of the feeding trial, shrimps were starved for 24 h and then weighed. The total number of shrimps in each tank was counted at the same time. Afterward, eight shrimps from each tank were randomly collected and anesthetized (MS-222, Sigma, St. Louis, MO, United States) to collect the hemolymph samples. Subsequently, shrimps were dissected and hepatopancreas samples were removed for enzyme and RNA expression analysis. The hemolymph sample was centrifuged (7,100 g, 10 min, 4°C) and the hemolymph was separated for antioxidant parameter analysis. All samples were collected rapidly and frozen in liquid nitrogen until analysis.

Proximate Composition Analysis

Proximate compositions of diets were assayed using the standard methods of AOAC (Horwitz et al., 2010). Briefly, diets crude protein content ($N \times 6.25$) was detected using the Kjeldahl method (1030- Autoanalyzer; Tecator, Höganäs, Sweden). Diet crude lipid was detected by the Soxhlet extractor method (Soxtec System HT6, Tecator, Sweden). Diets moisture was analyzed by drying the diets in oven at 105°C to constant weight.

Detection of Hepatopancreas and Hemolymph Antioxidant Parameters

Hepatopancreas samples were homogenized and centrifuged according to the methods of Fang et al., 2021b). Briefly, hepatopancreas samples and phosphate buffer (1:10) were mixed and homogenized, and then centrifuged for 10 min (4°C, 1,200 g), and the supernatants were collected. Supernatants, as well as hemolymph samples, were determined for antioxidant parameters, including superoxide dismutase (SOD) (A001–1), total antioxidant capacity (T-AOC) (A015–2-1) and malondialdehyde (MDA) (A003–1). The analysis of antioxidant parameters was carried out following the kits' instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Hepatopancreas RNA Extraction and Expression Analysis

RNA extraction and Real-time PCR were following the methods reported in our previous articles (Fang et al., 2019). Briefly, total RNA was extracted from the hepatopancreas using Trizol® reagent (Invitrogen, United States) following the manufacturer's instructions. 1% agarose gel electrophoresis and spectrophotometer (NanoDrop 2,000, Thermo Fisher, United States) were used to determine RNA quality and concentration, respectively. Afterward, cDNA was synthesized using the PrimeScript™ RT reagent kit (Takara, Japan), following the manufacturer's instructions. Real-time PCR for the target genes was performed using an SYBR® Premix Ex Taq™

II (Takara, Japan) and quantified on the LightCycler 480 (Roche Applied Science, Basel, Switzerland).

The primers used in the current study are presented in Table 2.

Statistical Analysis

All data in the current study are presented as means \pm standard error (SE). Data were analyzed by SPSS 22.0 (SPSS, Chicago, IL, United States) and followed by one-way analysis of variance (ANOVA) and Duncan's test. $P < 0.05$ was regarded as significant difference among groups.

RESULTS

Growth Performance

As shown in Table 3, diet supplementation with a suitable level of lutein or astaxanthin significantly altered the growth performance of *L. vannamei*. Significantly higher weight gain rate (WGR) and specific growth rate (SGR) of *L. vannamei* were obtained in the L1, L2, and A groups compared to the C group ($P < 0.05$), while no statistical difference was found between the C and L3 groups ($P > 0.05$). At the same time, data in the A group showed no significant difference compared with the L1 and L2 groups ($P > 0.05$). Feed conversion ratio (FCR) of *L. vannamei* in the A group was significantly lower than that in the C group ($P < 0.05$) and no statistical difference was observed compared

TABLE 2 | Sequences of primers used for real-time quantitative PCR.

Gene	Primer sequence (5'-3')
EF1a-F	TGGCTGTGAACAAGATGGAC
EF1a-R	AGATGGGGATGATTGGGACC
SOD-F	CCGTGCAGATTACGTGAAGG
SOD-R	GTCGCCACGAGAAGTCAATG
GSH-PX F	GGCACCAGGAGAACAACACTAC
GSH-PX R	CGACTTTGCCGAACATAAC
CAT-F	TACTGCAAGTTCCATTACAAGACG
CAT-R	GTAATTCCTTTGGATTGCGGTCA
HSP70-F	CAACGATTCTCAGCGTCAGG
HSP70-R	ACCTTCTTGTGCGAGGCCGTA
Caspase 2-F	ATGGCTCGTGGTTCAATCAG
Caspase 2-R	CATCAGGGTTGAGACAATACAGG
Caspase 3-F	AGTTAGTACAACAGATTGAGCG
Caspase 3-R	TTGTGGACAGACAGTATGAGGC
Relish-F	CTACATTCTGCCCTTGACTCTGG
Relish-R	GGCTGGCAAGTCGTTCTCG
Rho-F	GTGATGGTGCTGTGGTAAA
Rho-R	GCCTCAATCTGTGTCATAGCTCTC
Chymotrypsin-F	GGCTCTCTTCATCGACG
Chymotrypsin-R	CGTGAGTGAAGAAGTCGG
Trypsin-F	TCCAAGATCATCCAACACGA
Trypsin-R	GACCCGTAGCGGGGAATATC
HK-F	AGTCCGACGAAACAGGAAGTT
HK-R	CGCTCTTCTGGCACATGATA
FAS-F	GCGTGATAACTGGGTGTCTCT
FAS-R	ACGTGTGGGTTATGGTGGAT

TABLE 3 | Growth performance of *L. vannamei* fed diet supplementation with/without lutein or astaxanthin for 56 days.

	C	L1	L2	L3	A
IBW ^a	0.64 ± 0.01	0.61 ± 0	0.64 ± 0.01	0.66 ± 0.01	0.63 ± 0.01
FBW ^b	5.98 ± 0.03 ^a	6.44 ± 0.08 ^c	6.34 ± 0.06 ^{bc}	6.14 ± 0.09 ^{ab}	6.5 ± 0.08 ^c
WGR (%) ^c	828.31 ± 15.07 ^a	950.96 ± 10.36 ^c	890.33 ± 2.3 ^b	832.82 ± 3.01 ^a	926.69 ± 17.54 ^{bc}
SGR (%) ^d	3.98 ± 0.03 ^a	4.2 ± 0.02 ^c	4.09 ± 0 ^b	3.99 ± 0.01 ^a	4.16 ± 0.03 ^{bc}
FCR ^e	1.24 ± 0.03 ^a	1.19 ± 0.02 ^{ab}	1.18 ± 0.05 ^{ab}	1.18 ± 0.07 ^{ab}	1.12 ± 0.02 ^b
SR (%) ^f	96.25 ± 1.25	94.38 ± 2.13	96.67 ± 2.2	98.13 ± 2.77	96.88 ± 0.63

^aIBW (g per shrimp), initial body weight.

^bFBW (g per shrimp), final body weight.

^cWeight gain rate (WGR, %) = 100 × (final body weight–initial body weight)/initial body weight.

^dSpecific growth rate (SGR, % day⁻¹): 100 × (Ln final shrimp weight–Ln initial shrimp weight)/the experimental duration in days.

^eFeed conversion ratio (FCR) = dry diet fed/wet weight gain.

^fSurvival rate (SR) (%) = 100 × (final number of shrimp)/(initial number of shrimp).

Values are mean ± SE (n = 4). Means in the same row with different superscripts are significantly different (P < 0.05).

with the other three lutein-supplemented groups (P > 0.05). The survival rate (SR) of *L. vannamei* ranged from 94.38 to 98.13% after 8 weeks of feeding treatment (P > 0.05).

Antioxidant Capacity

The hepatopancreas and hemolymph antioxidant parameters of *L. vannamei* fed with/without dietary lutein or astaxanthin are shown in **Table 4**. Four carotenoid pigments-supplemented groups (L1-L3, A) all had significantly lower enzyme activity of hepatopancreas T-SOD, lower level of hemolymph T-AOC and lower concentration of hemolymph MDA than the C group (P < 0.05). Meanwhile, no significant difference in hemolymph T-AOC and MDA of *L. vannamei* was found among the L1-3 and A groups (P > 0.05). Although there was no statistical difference in hepatopancreas MDA among five experiment groups (P > 0.05), the MDA value in four carotenoid pigment-supplied groups was still relatively lower than the control group.

RNA Expression of Genes Related to Immunity

RNA expression levels of antioxidative genes (SOD, GSH-PX, and CAT) in the hepatopancreas of *L. vannamei* are presented in **Figure 1**. The RNA expression levels of hepatopancreas GSH-PX and CAT in four carotenoid pigments-supplemented groups showed significantly lower values compared to the C group (P < 0.05), and data were not statistically different among these four groups (P > 0.05). Compared to the C group, the RNA expression level of SOD was no statistically different in *L. vannamei* fed with/without dietary lutein or astaxanthin (P > 0.05).

Hepatopancreas RNA expression of anti-inflammatory genes (Relish, Rho, HSP70) of *L. vannamei* exposed to different diet treatments was shown in **Figure 2**. The RNA expression level of HSP70 in the A group was significantly lower than that in the C group (P < 0.05), and no statistical difference was observed compared with the other three lutein groups (P > 0.05). Significantly lower data of Relish has been observed in four carotenoid pigments-supplemented groups compared to the C group (P < 0.05). However, regarding the RNA expression level

of Rho, no statistical difference was found among all the groups (P > 0.05).

As shown in **Figure 3**, the hepatopancreas RNA expression level of Caspase 3 in four carotenoid pigments groups were significantly lower compare to the C group (P < 0.05), and the data were not statistically different among these four groups (P > 0.05). Apart from that, significantly lower RNA expression level of Caspase 2 was found in the L3 and A groups than in C, L1 and L2 groups (P < 0.05).

RNA Expression of Genes Related to Digestive and Metabolic Enzymes

As shown in **Figure 4**, diet supplementation with lutein or astaxanthin was unable to alter the hepatopancreas RNA expression level of digestive enzyme genes (chymotrypsin and trypsin) of *L. vannamei* (P > 0.05).

Hepatopancreas RNA expression levels of metabolic enzyme genes [Hexokinase (HK) and Fatty acid synthase (FAS)] of *L. vannamei* fed diet supplemented with/without lutein or astaxanthin were shown in **Figure 5**. Regarding the RNA expression level of HK, the data in the L1 and L2 groups were significantly higher than that of the C and A groups (P < 0.05). In addition, the RNA expression level of FAS in the L2 and A groups was higher than that in the C group (P < 0.05), and statistical difference was not found between the L2 and A group (P > 0.05).

DISCUSSION

Diet supplementation with carotenoid pigment could improve the growth performance of the aquatic animals. For example, dietary astaxanthin supplementation could optimize the growth performance of *Marsupenaes japonicus* (Wang et al., 2018), *Paralithodes camtschaticus* (Daly et al., 2013), *Trachinotus ovatus* (Fang et al., 2021a) and *Micropterus salmoides* (Xie S. et al., 2020). In addition, diet supplementation with β-carotene also enhanced the growth performance of *Penaeus monodon* (Niu et al., 2014), *Piaractus mesopotamicus* (Bacchetta et al., 2019) and *Oreochromis niloticus* × *O. aureus* (Hu et al., 2006). Similar results were shown in the present study. Carotenoid

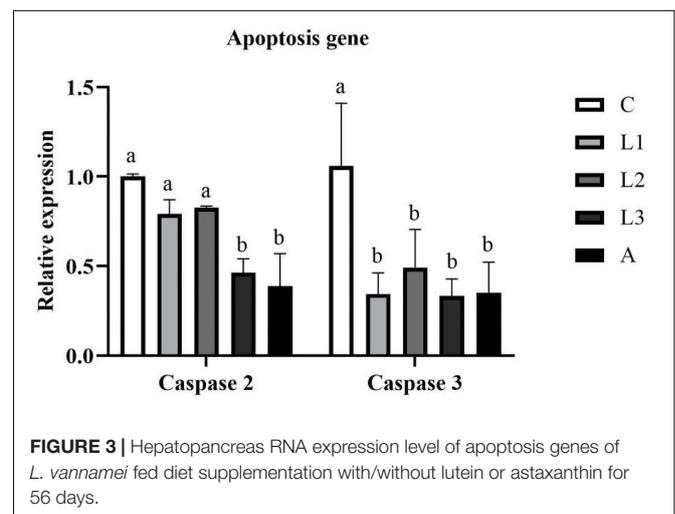
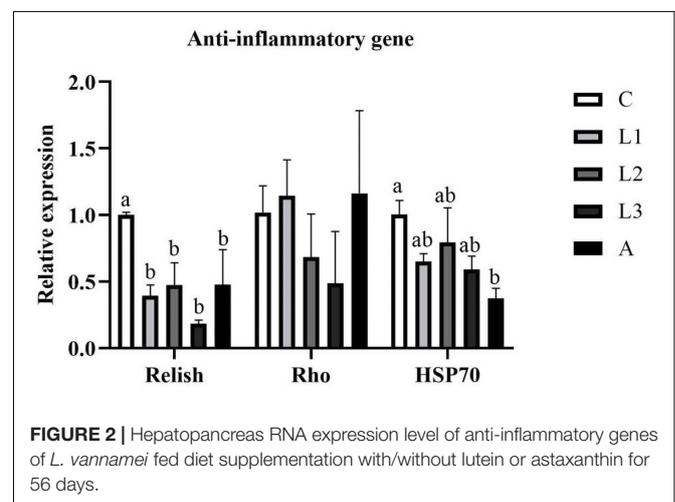
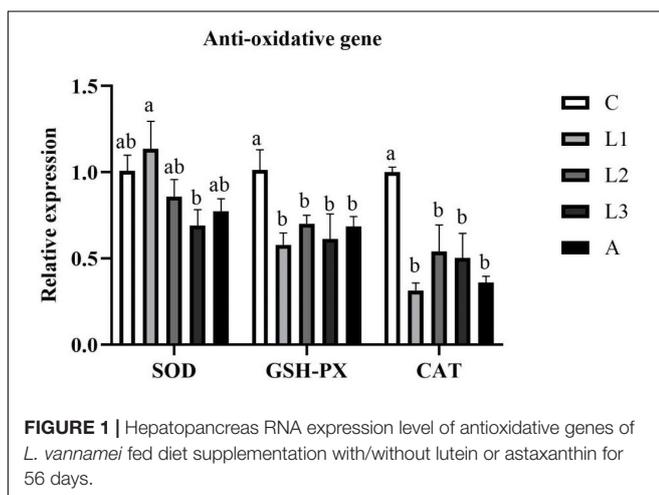
TABLE 4 | Hepatopancreas and hemolymph antioxidant parameter of *L. vannamei* fed diet supplementation with/without lutein or astaxanthin for 56 days.

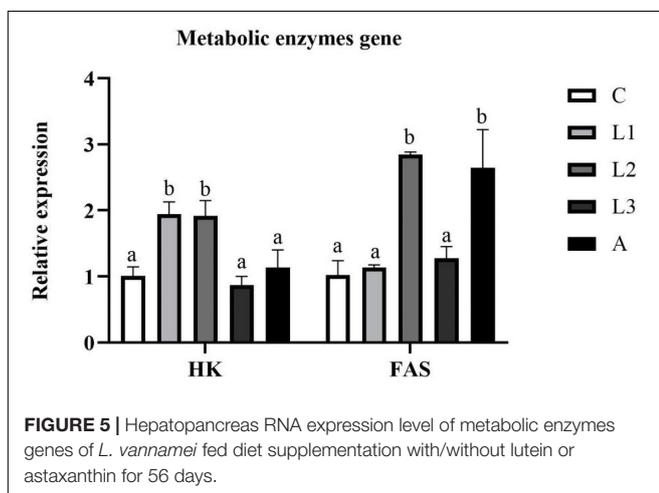
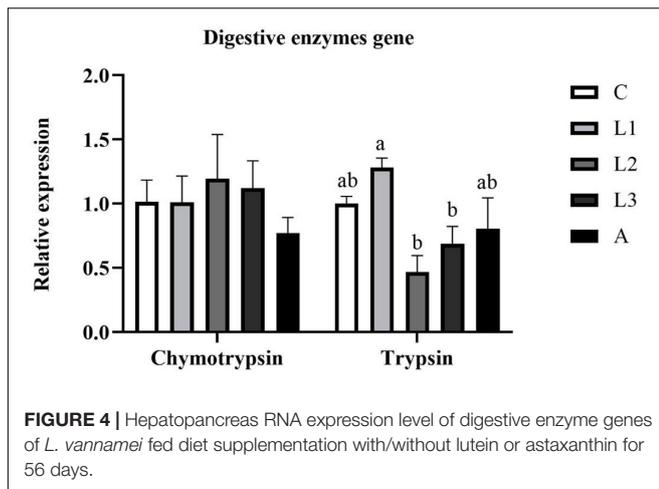
	C	L1	L2	L3	A
Hepatopancreas					
T-SOD (U/mgprot)	10.4 ± 0.88 ^a	7.79 ± 0.34 ^b	6.3 ± 0.4 ^{bc}	7.52 ± 0.71 ^b	5.3 ± 0.19 ^c
T-AOC (mgprot/ml)	0.27 ± 0.01	0.17 ± 0.03	0.24 ± 0.09	0.22 ± 0.02	0.16 ± 0.02
MDA (nmol/mgprot)	1.26 ± 0.03	1.13 ± 0.09	0.89 ± 0.24	1.03 ± 0.13	0.81 ± 0.07
Hemolymph					
T-SOD (U/mgprot)	273.75 ± 6.08 ^a	228.35 ± 8.56 ^c	251.38 ± 2.01 ^{abc}	241.72 ± 6.46 ^{bc}	257.08 ± 10.09 ^{ab}
T-AOC (mgprot/ml)	3.7 ± 0.12 ^a	2.59 ± 0.19 ^b	3 ± 0.15 ^b	2.59 ± 0.19 ^b	2.84 ± 0.25 ^b
MDA (nmol/mgprot)	8.27 ± 1.04 ^a	3.92 ± 0.28 ^b	4.69 ± 0.74 ^b	4.15 ± 0.41 ^b	3.54 ± 0.13 ^b

Values are mean ± SE (n = 4). Means in the same row with different superscripts are significantly different (P < 0.05).

pigment-supplemented groups except for the L3 group all showed significantly higher growth performance (WGR and SGR) of *L. vannamei* than the C group. There were two main reasons for why carotenoid pigments could improve the growth performance of crustaceans. Firstly, the carotenoid pigment could regulate the metabolism of aquatic animals and thus promote the digestion of nutrients (Baron et al., 2008; Zhang et al., 2013). In addition, the carotenoid pigment could shorten the molt cycle interval of crustaceans and regulate the NADPH metabolism for reducing energy consumption, resulting in enhanced growth performance (Hertrampf and Piedad-Pascual, 2012; Mao et al., 2017). Meanwhile, in the current study, similar growth performances (WGR and SGR), FCR, and SR of *L. vannamei* were found in the L1-2 groups and A group, indicating that diet supplementation with lutein ranging from 62.5 to 75 ppm could substitute astaxanthin in the diets of *L. vannamei*. However, previous studies also showed that carotenoid pigment was unable to alter the growth performance of aquatic animals. For example, dietary astaxanthin could not improve the growth performance of *Portunus trituberculatus* (Han et al., 2018), *Eriocheir sinensis* (Jiang et al., 2020), *Symphysodon* spp. (Song et al., 2017), and *Pagrus pagrus* (Nogueira et al., 2021). These results might be attributed to the culture environment, aquatic animal species and carotenoid pigment doses.

Nutrient digestion and metabolism capacity in aquatic animals are also crucial parameters for evaluating a diet additive. In the present study, no significant differences in RNA expression level of Chymotrypsin and Trypsin were found between the four carotenoid pigment treatment groups and the C group, indicating that dietary carotenoid pigment was unable to alter the protein digestive capacity of *L. vannamei*. In terms of





metabolic enzyme genes, HK is a critical rate-limiting enzyme in regulating the glycolysis cycle; FAS plays a significant role in catalyzing the synthesis of the fatty acids (Chirala and Wakil, 2004; Guo et al., 2008). In the present study, RNA expression level of HK in the L1 and L2 groups was higher than in other groups, indicating that diet supplementation of lutein ranging from 62.5 to 75 ppm could improve the capacity of carbohydrate metabolism. In addition, RNA expression level of FAS in the L2 and A groups was significantly higher than in other groups, meaning that lutein and astaxanthin could improve the fat synthesis ability of *L. vannamei*.

During the feeding trial, the shrimp might be affected by several uncontrollable external factors, such as water temperature change, bacterial contaminations in aquatic water and feed, as well as stress caused by the feeding process. In this situation, oxidative burst would occur in cells to produce numerous reactive oxygen species (ROS) to protect the organism from oxidative stress (Wilhelm Filho, 2007; Banh et al., 2016). However, ROS overproduction may cause DNA damage and other severe detriments in cells (Piconi et al., 2006; de Souza Grinevicius et al., 2016). If cells were exposed to excess ROS, they would

activate the antioxidant system and upregulate the expression of antioxidant genes and enzymes, like SOD, GSH-PX, and CAT (Ighodaro and Akinloye, 2018). In addition, T-AOC was also a significant parameter to reflect the antioxidant capacity of crustaceans (Dai et al., 2018). Since carotenoid pigment contains ionone rings with hydroxyl and keto, they could remove ROS and protect cells from various environmental stress (Chien et al., 2003). In the current study, compared to the control group, significantly lower levels of hepatopancreas T-SOD activity, hemolymph T-AOC and RNA expression of GSH-Px and CAT were observed in four carotenoids pigments-supplemented groups, indicating lutein and astaxanthin could act as an antioxidant for scavenging ROS and thus protecting cells from oxidative stress. As a result, the cells unnecessarily stimulate the antioxidant system and produce more antioxidant enzymes. These results were similar to the previous results reported by Zhang et al. (2013), Xie J. et al. (2020), and Etefaghdoost and Haghighi (2021). MDA is a product of lipid peroxidation, which can be used to evaluate the damage degree of cell structure and function (Çilingir Yeltekin and Oğuz, 2018). The present study demonstrated that significantly lower hemolymph MDA was obtained in four carotenoid pigment treatment groups than the C group, indicating lutein and astaxanthin could improve the antioxidant capacity of *L. vannamei*. No significant statistical difference in hemolymph T-AOC, MDA, and hepatopancreas RNA expression level of GSH-PX, CAT between dietary lutein treatment groups (L1-3) and the astaxanthin group was found in the present study, indicating that dietary supplementation with lutein ranging from 62.5 to 87.5 ppm could obtain similar antioxidant effects in *L. vannamei* compared to astaxanthin.

The antioxidant property of lutein and astaxanthin had been widely reported (Zhang et al., 2013; Rahman et al., 2016; Song et al., 2017). However, few studies focused on anti-inflammation and other immune functions of these carotenoid pigments. The NF- κ B pathway is an essential moderator related to inflammatory responses, and Relish as well as Rho are two essential NF- κ B family proteins (Ko et al., 2017; Xie et al., 2018). In addition, HSP70 could also exert the anti-inflammatory property by stimulating its IL-10 producing T cells (Wendling et al., 2000; Tanaka et al., 2007). Lower expression of inflammatory cytokines or higher expression of anti-inflammatory cytokines can suppress the inflammatory response (Zhao et al., 2020). In the present study, significantly lower RNA expression level of Relish was found in four carotenoid pigment treatment groups than in the C group, indicating lutein and astaxanthin could improve the anti-inflammation capacity of *L. vannamei* by regulating the NF- κ B pathway. Caspases, which belong to the cysteine proteases family, are essential regulators of programmed cell death (apoptosis) (Taylor et al., 2008). In general, Caspase 2 was a factor which mainly responded to stress-induced apoptosis, while Caspase 3 frequently acted as a proenzyme and was activated to destroy various specific cellular structures and proteins (Dorstyn et al., 2012; Gao et al., 2013). The present study demonstrated significantly lower RNA expression level of Caspase 3 in four carotenoid pigment treatment groups than in the control group, indicating diet supplementation of lutein or astaxanthin could

inhibit the apoptosis of *L. vannamei*. In addition, no statistical differences in Relish, HSP70 and Caspase 3 were observed among L1-L3 and A groups, meaning that diet supplementation of lutein ranging from 62.5 to 87.5 ppm could stimulate similar immune response in *L. vannamei* compared to diet supplemented with astaxanthin.

CONCLUSION

Overall, diet supplementation of lutein ranging from 62.5 to 75 ppm could result in similar growth performance, antioxidant capacity and immune response of *L. vannamei* compared with diet supplemented with 50 ppm astaxanthin. Therefore, dietary lutein was suggested to be suitable in the diet of *L. vannamei* for substituting astaxanthin.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

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ETHICS STATEMENT

The animal study was reviewed and approved by Experimental Animal Ethics Committee of Sun Yat-sen University.

AUTHOR CONTRIBUTIONS

HF, YL, and LT designed the study. HZ provided relative experiment material. HF, XH, and JN analyzed data. HF carried out the experiment and wrote this manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: HZ was employed by company Guangzhou Leader Bio-Technology Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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