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Effects of Diets with Varying Astaxanthin from *Yarrowia lipolytica* Levels on the Growth, Feed Utilization, Metabolic Enzymes Activities, Antioxidative Status and Serum Biochemical Parameters of *Litopenaeus vannamei*

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Abstract: *Litopenaeus vannamei* was divided into seven groups (defined as diets A0–A6) and fed with diets respectively containing 0, 0.5, 1, 2, 4 and 8 g/kg *Yarrowia lipolytica* (astaxanthin content: 1.5%) and 3 g/kg *Haematococcus pluvialis* (astaxanthin content: 2%). After an eight-week feeding trial, the results reflected that different levels of *Y. lipolytica* and *H. pluvialis* could significantly increase the weight gain rate of *L. vannamei* ($p < 0.05$). The condition factor and weight gain rate of group A4 were significantly higher than those of the other groups ($p < 0.05$); the HSI significantly decreased with the increase of *Y. lipolytica* ($p < 0.05$). The addition of *Y. lipolytica* to the diet had significant effects on total protein (TP), albumin (ALB), glutathione peroxidase (GSH-Px), malonaldehyde (MDA) and total antioxidant capacity (T-AOC) ($p < 0.05$). The total protein and albumin of the A5 and A6 groups were significantly higher than those of the other groups ($p < 0.05$). The GSH-Px activity of the A5 group was the highest and the T-AOC of the A0 group was the lowest. Inducible nitric oxide synthase (I-NOS) increased with the addition of *Y. lipolytica* ($p < 0.05$). *Y. lipolytica* inclusion had no negative effect on physiological and biochemical parameters and some serum immune and antioxidant indexes ($p > 0.05$). Astaxanthin in *Y. lipolytica* had an obvious effect on body color. After cooking, the body color of the shrimp deepened with increasing *Y. lipolytica* content. The red body color of *L. vannamei* was significantly improved by adding yeasts hydrolysate 2~8 g/kg to the diet. According to the regression analysis between the level of *Y. lipolytica* added to the diets and the weight gain rates, the optimal level of *Y. lipolytica* is 4.64 g/kg.

Keywords: *Litopenaeus vannamei*; *Yarrowia lipolytica*; *Haematococcus pluvialis*; astaxanthin; growth performance; pigmentation deposit; antioxidation

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1. Introduction

Litopenaeus vannamei, also known as King Prawn or Pacific White Shrimp [1], is a kind of shrimp in the East Pacific Ocean, which is usually caught or raised as food. Around the beginning of the millennium, Asia introduced this species into its aquaculture business. China, Vietnam, India and other countries have also become major producers. The marine fishing and aquaculture of *L. vannamei* are affected by weather changes and diseases [2]. The deteriorating water environment has seriously affected the shrimp industry due to stress and diseases in recent years [3,4]. Therefore, in the process of shrimp culture, it is very important to develop feed additives to improve the resistance and survival rate of shrimp for better growth and development.

Astaxanthin is a kind of ketone carotene with many uses, including as a dietary supplements and food dye [5,6]. Due to the presence of ketone functional groups and hydroxyl groups, the compound has a variety of biological properties, including antioxidation, immune regulation, anti-inflammation, disease prevention and coloring [7]. Astaxanthin

is produced when algae are stressed due to lack of nutrition, increased water salinity or inordinate sunshine levels. Algae-eating animals such as red snapper, salmon, flamingo, red trout and crustaceans (i.e., shrimp, krill, crab, lobster, and crayfish) subsequently reflect red–orange pigmentation to varying degrees due to the presence of carotenoids.

Astaxanthin has been used to feed animals because of its strong antioxidant activity and safety, and because this pigment improves the sensory properties of animal products. Animals cannot synthesize carotenoids by themselves, which is why they must eventually obtain the pigment from their diets [8,9]. However, production of carotenoids through natural producers such as plants and algae faces the challenges of limited production and high planting and mining costs. Astaxanthin production through metabolically engineered artificial microbial cell factories is another promising strategy to overcome these limitations [9]. *Yarrowia lipolytica* is an aerobic, dimorphic, non-pathogenic ascomycete yeast, which has been isolated from various marine and coastal environments [10]. Due to its essential fatty acid profile, *Y. lipolytica* has been used as a dietary supplement in aquaculture [11] and is a potential commercial source of astaxanthin. However, few studies have focused on the effects of *Y. lipolytica* on *L. vannamei*. Therefore, the purpose of this work was to study the effects of astaxanthin from *Y. lipolytica* on the physiological, metabolic and hematological responses of *L. vannamei* to evaluate the effectiveness of this feed additive.

2. Materials and Methods

2.1. Experimental Diets

The *Y. lipolytica* (astaxanthin content 1.5%) was provided by Shandong Jincheng Biological Pharmaceutical Co. Ltd., China. Seven isonitrogenous (40.4% crude protein) and isoenergetic (17.3 kJ g^{-1} gross energy) diets with varying astaxanthin contents were formulated as shown in Table 1. Groups A0, A1, A2, A3, A4, and A5 had 0, 7.5, 15, 30, 60 and 120 mg/kg astaxanthin, respectively, from *Y. lipolytica*, while group A6 was supplemented with 60 mg kg^{-1} astaxanthin from *Haematococcus pluvialis* (astaxanthin content 2.0%). The content of astaxanthin in the A6 and A4 groups was the same. Fishmeal, soya protein concentrate, fermented soybean meal and wheat gluten meal were used as the major protein ingredients, while fish oil served as the main lipid source. Crystalline DL-methionine and L-arginine were added to maintain adequate and balanced levels of these essential amino acids according to the recommended level from previous studies.

Diet ingredients were crushed through an 80 mesh sieve (178-μm). After mixing evenly, the mixture was made into granular diets with a particle size of 1.0 mm and length of 2.5~4.0 mm by feed granulation mechanism. After steam curing for 10 min, they were dried indoors under air conditioning (24 °C), dehumidified by dehumidifier and fan blowing for 72 h, and then stored in a −20 °C freezer until use.

2.2. Experimental Shrimp and Feeding Trial

Juvenile *L. vannamei* (0.23 ± 0.02 g) provided by Wenzhou Qingjiang Base of Zhejiang Institute of Marine Aquaculture were used in this study. They were from the same batch, healthy and consistent in size. The feeding trial was conducted at the Marine Fisheries Research Institute of Zhejiang Province in Zhoushan, China. The shrimp were kept in an acclimatization tank (10 m × 2 m × 4.5 m) in a seawater recirculation system for two weeks. A total of 2100 healthy and disease-free juvenile shrimp of similar size were weighed and randomly divided into 42 tanks (400 L water volume) with 50 in each tank. The tanks were randomly divided into 7 groups of 6 replicates. Each diet was fed to 6 tanks of shrimp for 8 weeks. The quality parameters in the water (temperature 26 ± 2 °C; salinity 26~29 g L^{-1}; dissolved oxygen ≥5 mg L^{-1}; pH 7.6~7.8; and ammonia nitrogen <0.1 mg L^{-1}) were recorded daily and maintained throughout the acclimation and experimental periods. During the first four weeks, the shrimp were fed manually four times a day (6:00 h, 10:00 h, 14:00 h and 18:00 h), and three times a day (8:00 h, 12:00 h and 16:00 h) in the last four weeks, and observed every day to ensure that they ate the feed within two hours after feeding.

Table 1. Formulation and proximate composition of the experimental diets.

Ingredients (%)	A0	A1	A2	A3	A4	A5	A6
Fishmeal	22	22	22	22	22	22	22
Peeled soybean meal	8	8	8	8	8	8	8
Fermented soybean meal	13	13	13	13	13	13	13
Soy protein concentrate	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Squid liver powder	4	4	4	4	4	4	4
Chicken meal	6	6	6	6	6	6	6
High gluten flour	22	22	22	22	22	22	22
Fish oil	2	2	2	2	2	2	2
Soy phospholipids	2	2	2	2	2	2	2
L-lysine	0.2	0.2	0.2	0.2	0.2	0.2	0.2
DL-methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Taurine	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Sodium carboxymethyl cellulose	0.50	0.50	0.50	0.50	0.50	0.5	0.50
Carrageenan	0.20	0.20	0.20	0.20	0.20	0.2	0.20
Calcium dihydrogen phosphate	2.1	2.1	2.1	2.1	2.1	2.1	2.1
Vc phosphate	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin mixture	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mineral premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Zeolite powder	3	3	3	3	3	3	3
Antioxidant	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Antifungal agent	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Yarrowia lipolytica (astaxanthin 1.5%)	0	0.05	0.1	0.2	0.4	0.8	0
Haematococcus pluvialis (astaxanthin 2.0%)	0	0	0	0	0	0	0.4
Beer yeast	0.96	0.93	0.9	0.84	0.72	0.48	0.72
Alpha cellulose	2.07	2.05	2.03	1.99	1.91	1.75	1.91
Total	100	100	100	100	100	100	100
Astaxanthin (mg kg^{-1})	0	7.5	15	30	60	120	60
Proximate analysis (%)							
Crude protein	39.55	39.04	39.09	39.54	38.97	39.26	39.65
Crude lipid	7.31	7.09	7.85	7.60	7.51	7.44	7.54
Gross energy (kJ g^{-1})	16.93	16.72	17.03	17.04	16.87	16.91	17.04
Gross phosphorus	0.82	0.78	0.86	0.81	0.85	0.82	0.80

2.3. Methods of Sample Collection and Analysis

Before the start of the feeding experiment, 60 juvenile shrimp of the same size were randomly selected, weighed and stored in the −20 °C freezer for body composition analysis. At the end of the 8-week culture period, the test shrimp were starved for 24 h and then paralyzed with ice water. The shrimp were counted to obtain the survival rate (SR) and batch-weighed to obtain the data for weight gain rate (WGR) and feed conversion rate (FCR). After that, 10 shrimp were randomly weighed and measured respectively to obtain the conditioning factor (CF). Three shrimp were randomly selected for the analysis of whole-body composition, and the hemolymph of other shrimp was taken from the heart. The hemolymph was left to settle at 4 °C for 2 h, centrifuged at 8000 r min^{-1} for 15 min, and the supernatant was taken and stored in a refrigerator at −20 °C. The viscera of shrimp were taken after dissection on an ice plate, and the hepatopancreas and intestine were separated. The hepatopancreas was weighed to obtain the hepatosomatic index (HSI) and the intestine was divided into midgut and hindgut.

The proximate compositions of diets, shrimp whole-body and muscle were analyzed according to the standard protocols of the Association of Official Analytical Chemists (AOAC, 1995). The moisture content was determined by drying ground samples in an oven for 24 h. The Kjeldahl method was used to determine the crude protein, the Soxhlet extraction method was used to determine the crude fat, and the ash content was calculated after burning the sample at 550 °C for 6 h.

The supernatants of liver and intestine were obtained according to the method described by Xu et al. (2021) [12]. Diagnostic reagent kits (from Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used to determine the contents of serum nitric oxide synthase (i-NOS), triglycerides (TG), total antioxidant capacity (TAOC), total cholesterol (TCH) and total protein (TP), and the activities of gastrointestinal digestive enzyme, liver alanine aminotransferase (ALT), aspartate aminotransferase (AST), cyclooxygenase (COX), fatty acid synthetase (FAS), caspase and Nuclear Factor κB (NF-κB). The activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) content in serum and liver were assayed as reported by Wang et al. (2020) [13].

Three shrimp from each tank were collected into transparent polyethylene bags and sealed. They were then heated in a 100 °C constant temperature water bath for 10 min, taken out and cooled for 5 min. Filter paper was used to wipe the water before photos taken to observe the body color. The chromatism(a*) of the shrimp body was detected by colorimeter.

The astaxanthin content in the test diet and shrimp was detected by high performance liquid chromatography (HPLC).

2.4. Statistical Analysis

SPSS 20.0 software was used for all statistical analysis, and the results were presented as the means ± SD. After one-way ANOVA was performed on the data, Duncan multiple comparison was used to test the significant difference of the mean at p level 0.05. The WGR and astaxanthin content of the whole shrimp were analyzed by regression method. Quadratic regression analysis was adopted to fit the trendline. The Shapiro Wilk test was used to verify the normality, and the Bartlett test was used to verify the homogeneity of variance.

3. Results

3.1. Growth Performance and Morphological Index

The growth performance and morphological indices of *L. vannamei* fed different *Y. lipolytica* and *H. pluvialis* are shown in Table 2. Different levels of *Y. lipolytica* and *H. pluvialis* significantly increased the weight gain rate of *L. vannamei* ($p < 0.05$). They also had a significant effect on the condition factor and HSI of *L. vannamei* ($p < 0.05$). There was no significant difference in survival rate and body length ($p > 0.05$). The quadratic regression ($y = -26.25x^2 + 243.64x + 2563.1$, $R^2 = 0.9519$) of WG against *Y. lipolytica* levels in the diets is presented in Figure 1. Xpot in the figure is the content of *Y. lipolytica* in the ration at the highest time of WG. According to the calculation of the fitting curve formula, the curve reaches the peak on the horizontal axis at 4.64 g kg^{-1}. Thus, the weight gain rate of *L. vannamei* reached a maximum at 4.64 g kg^{-1} *Y. lipolytica* inclusion level. The feeding rate, feed conversion rate, protein efficiency and protein deposition rate were not significantly influenced by various levels of *Y. lipolytica* and *H. pluvialis* ($p > 0.05$).

Table 2. Effects of *Yarrowia lipolytica* on growth performance and feed utilization of *Litopenaeus vannamei* [1].

Index [1]	A0	A1	A2	A3	A4	A5	A6
SR [2]	96.33 ± 4.80	99.60 ± 0.89	95.33 ± 5.75	95.67 ± 4.63	96.80 ± 3.03	96.67 ± 4.68	97.00 ± 2.76
WGR [3]	2502.43 ± 160.01 ^{c}	2717.21 ± 62.32 ^{b}	2839.99 ± 146.13 ^{b}	2918.19 ± 131.99 ^{ab}	3102.94 ± 51.20 ^{a}	2836.46 ± 181.21 ^{b}	2876.05 ± 143.69 ^{b}
FBL [4]	9.08 ± 0.67	9.25 ± 0.56	9.22 ± 0.54	9.34 ± 0.52	9.50 ± 0.53	9.61 ± 0.52	9.48 ± 0.55
CF [5]	0.81 ± 0.02 ^{b}	0.80 ± 0.02 ^{b}	0.82 ± 0.1 ^{b}	0.81 ± 0.01 ^{b}	0.84 ± 0.01 ^{a}	0.82 ± 0.01 ^{b}	0.78 ± 0.01 ^{c}
HIS [6]	4.99 ± 0.44 ^{ab}	5.50 ± 0.60 ^{a}	4.80 ± 0.35 ^{bc}	4.75 ± 0.39 ^{bc}	4.35 ± 0.68 ^{cd}	4.04 ± 0.41 ^{d}	4.84 ± 0.63 ^{bc}
FR [7]	4.46 ± 0.60	4.47 ± 0.83	4.49 ± 0.11	4.72 ± 0.36	4.26 ± 0.28	4.36 ± 0.29	4.32 ± 0.29
FCR [8]	1.23 ± 0.16	1.23 ± 0.23	1.23 ± 0.03	1.30 ± 0.10	1.17 ± 0.08	1.20 ± 0.08	1.19 ± 0.08
PER [9]	2.05 ± 0.29	2.08 ± 0.42	2.01 ± 0.05	1.92 ± 0.14	2.13 ± 0.14	2.08 ± 0.14	2.10 ± 0.15
PPV [10]	36.33 ± 5.02	37.75 ± 7.57	36.56 ± 0.95	35.65 ± 2.68	39.12 ± 2.60	38.97 ± 2.69	39.10 ± 2.67

[1] Mean values ± standard deviation (SD) are presented for each group ($n = 6$); values with different superscripts in the same row differ significantly ($p < 0.05$). [2] SR (survival rate, %) = 100 × (final shrimp number/initial shrimp number). [3] WGR (weight gain rate, %) = 100 × (final body weight–initial bodyweight)/initial body weight. [4] FBL (final body length, cm). [5] CF (conditioning factor) = (final body weight/final body length^3) × 100. [6] HSI (hepatosomatic index) = 100 × (liver weight/body weight). [7] FR (feeding rate, %/day) = dry feed intake/[(final weight + initial weight)/2]/days × 100. [8] FCR (feed conversion rate) = dry feed intake/weight gain. [9] PER (protein efficacy ratio) = weight gain/total protein intake. [10] PPV (protein productive value, %) = protein gain/total protein intake × 100.

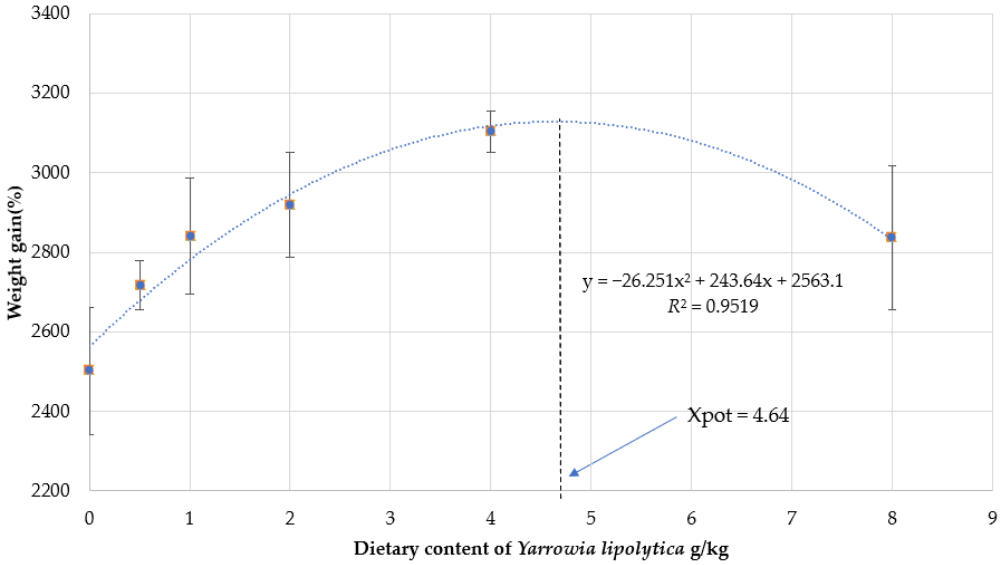

Figure 1. The relationship between the weight gain rate of shrimp and the content of *Yarrowia lipolytica* in feed based on quadratic regression analysis.

The astaxanthin content in whole shrimp (Figure 2) increased with increasing dietary astaxanthin content. Compared with the control group, astaxanthin content in the supplemented groups was significantly higher ($p < 0.05$). The whole body astaxanthin content correlated positively with the dietary levels, as the 120 mg kg^{-1} dietary inclusion group had the highest content.

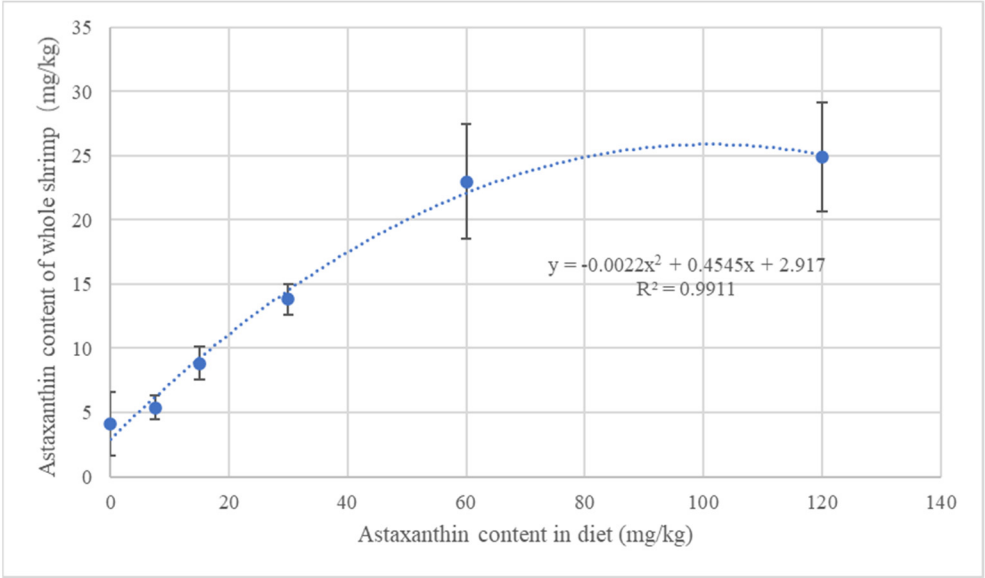

Figure 2. The relationship between the astaxanthin content of whole shrimp and astaxanthin content in diet based on quadratic regression analysis.

3.2. Effect on Body Color of Shrimp

Figure 3 shows the relationship between shrimp chromatism a* and astaxanthin content in the diet. The larger the value of a*, the redder the color. It can be seen that with the increase of astaxanthin content in the diet, a* also increases.

The pictorial results are shown in Figure 4. A high dose of *Y. lipolytica* (2~8 g/kg) in the diet significantly improved shrimp shell color (red) by colorimeter. The body color of the *H. pluvialis* group was not significantly improved.

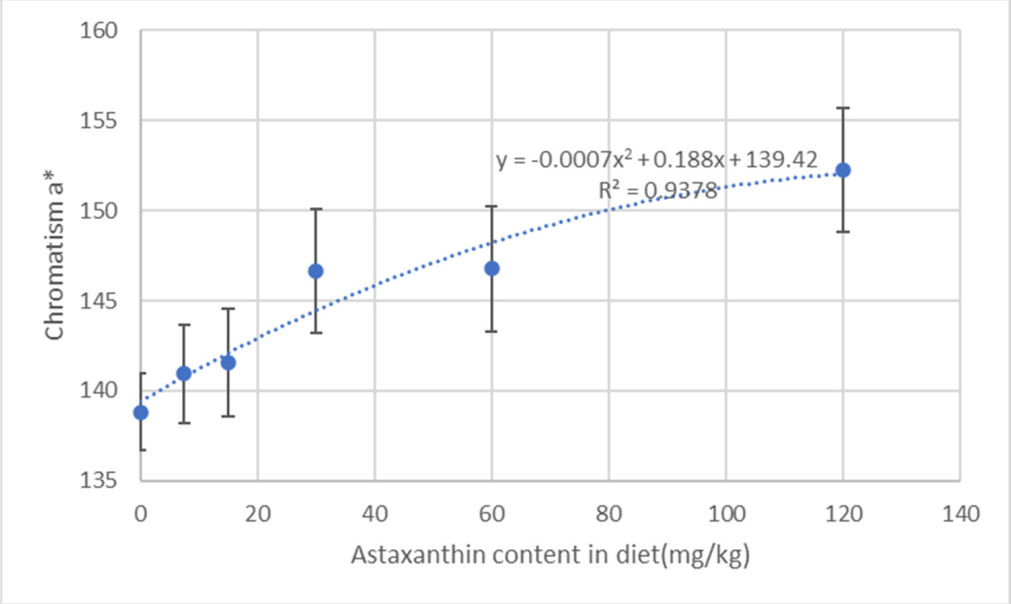

Figure 3. The relationship between the chromatism a* of shrimp and astaxanthin content in diet based on quadratic regression analysis.

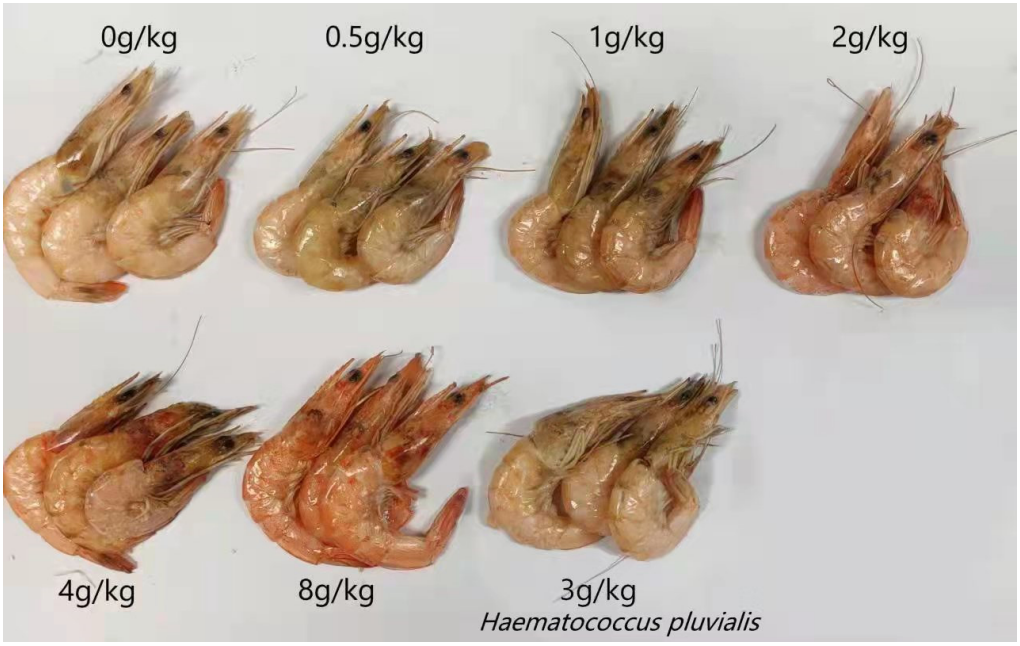

Figure 4. Comparison of body color of boiled *Litopenaeus vannamei* after 8-week growth trial.

3.3. Proximate Composition of the Whole Body and Muscles

As presented in Table 3, different amounts of *Y. lipolytica* added to the feed had significant effects on the moisture content of whole body, and the protein content of the muscle, of *L. vannamei* ($p < 0.05$). There was no significant influence on crude fat and ash ($p > 0.05$).

Table 3. Effects of *Yarrowia lipolytica* on the whole shrimp and muscle composition of *Litopenaeus vannamei* (%) [1].

Index [1]	A0	A1	A2	A3	A4	A5	A6
Whole Shrimp							
Moisture	75.78 ± 0.10 [a]	75.14 ± 0.14 [bc]	75.37 ± 0.18 [b]	74.95 ± 0.13 [c]	75.09 ± 0.18 [bc]	74.78 ± 0.11 [c]	74.69 ± 0.12 [c]
Crude protein	17.05 ± 0.21	17.53 ± 0.14	17.56 ± 0.23	17.97 ± 0.08	17.78 ± 0.16	18.10 ± 0.06	17.58 ± 0.12
Crude lipid	0.96 ± 0.02	1.01 ± 0.05	1.01 ± 0.05	0.95 ± 0.06	0.94 ± 0.04	1.01 ± 0.03	0.99 ± 0.03
Ash	3.42 ± 0.05	3.36 ± 0.05	3.40 ± 0.04	3.30 ± 0.09	3.90 ± 0.08	3.42 ± 0.06	3.32 ± 0.05
Muscle							
Moisture	76.08 ± 0.07	76.07 ± 0.13	75.54 ± 0.15	75.80 ± 0.15	76.53 ± 0.19	75.91 ± 0.15	75.81 ± 0.16
Crude protein	22.13 ± 0.14	22.19 ± 0.02	22.20 ± 0.16	22.25 ± 0.07	22.31 ± 0.20	22.50 ± 0.14	22.40 ± 0.14
Crude lipid	0.38 ± 0.01	0.40 ± 0.02	0.41 ± 0.03	0.39 ± 0.01	0.40 ± 0.02	0.41 ± 0.02	0.40 ± 0.02
Ash	1.56 ± 0.07	1.52 ± 0.04	1.51 ± 0.10	1.64 ± 0.10	1.54 ± 0.08	1.72 ± 0.03	1.65 ± 0.03

[1] Mean values ± standard deviation (SD) are presented for each group (n = 6); values with different superscripts in the same row differ significantly ($p < 0.05$).

3.4. Serum Biochemical and Antioxidative Indexes

The effects of *Y. lipolytica* on the main serum physiological and biochemical indexes of *L. vannamei* are shown in Table 4. The total protein and albumin of the A5 and A6 groups were significantly higher than those in other groups. The GSH-Px activity of A5, and the MDA content of A1 groups were the highest, whilst the A1 group had the lowest T-AOC. The total protein and albumin contents, and GSH-Px activity, increased with the increasing inclusion of *Y. lipolytica* and reached the highest values in the group A5. MDA content decreased with the increase of *Y. lipolytica*, and the T-AOC of group A4 was higher than that of the other groups. No significant differences were observed in the other physiological and biochemical indexes among the groups ($p > 0.05$).

Table 4. Effects of *Yarrowia lipolytica* on main biochemical, immune and antioxidant indexes in serum of *Litopenaeus vannamei*.

Index [1]	A0	A1	A2	A3	A4	A5	A6
TP [2]	20.80 ± 1.49 [c]	27.22 ± 4.33 [b]	27.99 ± 2.82 [b]	25.38 ± 4.44 [b]	28.46 ± 6.85 [b]	34.74 ± 2.13 [a]	35.05 ± 1.81 [a]
ALB [3]	10.12 ± 0.84 [c]	12.39 ± 1.36 [b]	12.34 ± 0.71 [b]	11.94 ± 1.66 [b]	12.80 ± 2.40 [b]	14.53 ± 0.49 [a]	15.44 ± 0.99 [a]
SOD [4]	19.80 ± 4.87	21.69 ± 3.94	19.84 ± 1.72	21.10 ± 4.78	19.92 ± 6.44	19.54 ± 3.22	19.62 ± 5.87
MDA [5]	12.48 ± 2.25 [a]	10.69 ± 1.04 [b]	9.58 ± 2.36 [b]	9.18 ± 3.53 [c]	9.11 ± 2.57 [c]	7.97 ± 1.14 [d]	9.10 ± 2.01 [c]
GSH-Px [6]	127.51 ± 17.72 [c]	140.07 ± 38.40 [b]	153.80 ± 41.35 [b]	153.09 ± 41.48 [b]	153.85 ± 56.20 [b]	181.29 ± 42.21 [a]	156.93 ± 19.64 [b]
CAT 7	0.68 ± 0.19	0.72 ± 0.13	0.72 ± 0.17	0.78 ± 0.19	0.73 ± 0.29	0.65 ± 0.13	0.63 ± 0.19
T-AOC [8]	0.19 ± 0.04 [c]	0.24 ± 0.04 [b]	0.25 ± 0.03 [b]	0.29 ± 0.13 [b]	0.34 ± 0.15 [a]	0.31 ± 0.05 [b]	0.30 ± 0.06 [b]
LZM [9]	254.39 ± 32.22	238.60 ± 81.99	263.16 ± 23.06	201.75 ± 28.57	208.77 ± 21.49	233.33 ± 47.27	228.07 ± 45.96
AKP [10]	0.34 ± 0.16	0.28 ± 0.08	0.35 ± 0.16	0.25 ± 0.05	0.27 ± 0.08	0.29 ± 0.04	0.29 ± 0.06
PPO [11]	9.06 ± 1.08	9.78 ± 2.66	9.17 ± 3.72	9.56 ± 1.61	10.50 ± 5.18	12.50 ± 3.90	11.83 ± 3.05
AST [12]	1.81 ± 0.70	1.96 ± 0.42	1.86 ± 0.35	1.83 ± 0.91	2.12 ± 1.49	1.93 ± 0.69	1.84 ± 0.59
TG [13]	0.86 ± 0.19	0.76 ± 0.21	0.79 ± 0.15	0.61 ± 0.17	0.62 ± 0.16	0.70 ± 0.16	0.75 ± 0.13
TC [14]	1.38 ± 0.22	1.47 ± 0.38	1.44 ± 0.32	1.22 ± 0.46	1.32 ± 0.37	1.42 ± 0.31	1.26 ± 0.28

[1] Mean values ± standard deviation (SD) are presented for each group (n = 6); values with different superscripts in the same row differ significantly ($p < 0.05$). [2] TP (total protein, g L^{-1}). [3] ALB (albumin, g L^{-1}). [4] SOD (superoxide dismutase, U mL^{-1}). [5] MDA (malonaldehyde, nmol mL^{-1}). [6] GSH-Px (glutathione peroxidase, U mL^{-1}). [7] CAT (catalase, U mL^{-1}). [8] T-AOC (Total antioxidant capacity, mmol L^{-1}). [9] LZM (lysozyme, U mL^{-1}). [10] AKP (alkaline phosphatase, king unit 100 mL^{-1}). [11] PPO (polyphenol oxidase, U mL^{-1}). [12] AST (aspartate aminotransferase, U L^{-1}). [13] TG (triglyceride, mmol L^{-1}). [14] TC (total cholesterol, mmol L^{-1}).

3.5. Immune and Antioxidant Indexes of Hepatopancreas

The effects of *Y. lipolytica* on hepatopancreatic immunity and antioxidant indexes of *L. vannamei* are shown in Table 5. The content of nitric oxide synthase increased significantly with the increase of *Y. lipolytica* ($p < 0.05$). The activity of caspase9 in A3 and A4 groups were significantly lower than that in the other groups, and the activity of GSH-Px in A3 group was significantly lower than that in the other groups ($p < 0.05$).

Table 5. Effects of *Yarrowia lipolytica* on antioxidation and immune indexes in hepatopancreas of *Litopenaeus vannamei*.

Index [1]	A0	A1	A2	A3	A4	A5	A6
MDA [2]	3.82 ± 1.21	3.55 ± 0.77	3.69 ± 1.53	3.42 ± 0.29	3.89 ± 0.90	4.44 ± 1.04	4.56 ± 0.92
CAT [3]	0.15 ± 0.02	0.24 ± 0.08	0.23 ± 0.10	0.18 ± 0.06	0.23 ± 0.09	0.27 ± 0.07	0.18 ± 0.05
SOD [4]	9.04 ± 1.21	9.51 ± 1.70	10.96 ± 1.05	7.86 ± 1.18	8.84 ± 1.76	8.58 ± 2.07	10.25 ± 2.41
GSH-Px [5]	63.22 ± 9.54 ^{b}	62.98 ± 6.69 ^{b}	65.31 ± 12.71 ^{b}	56.20 ± 8.35 ^{c}	71.43 ± 16.60 ^{b}	59.03 ± 4.47 ^{b}	85.60 ± 15.66 ^{a}
i-NOS [6]	1.75 ± 0.34 ^{c}	2.70 ± 0.40 ^{b}	2.37 ± 0.57 ^{bc}	2.76 ± 0.67 ^{b}	2.94 ± 0.28 ^{b}	3.84 ± 0.38 ^{a}	3.05 ± 0.84 ^{b}
NK-κB [7]	0.42 ± 0.05	0.40 ± 0.02	0.41 ± 0.02	0.38 ± 0.10	0.41 ± 0.09	0.44 ± 0.03	0.49 ± 0.06
COX-2 [8]	2.67 ± 0.23	2.37 ± 0.15	2.31 ± 0.27	2.28 ± 0.45	2.44 ± 0.70	2.33 ± 0.08	2.75 ± 0.39
Caspase-3 activation [9]	0.61 ± 0.11	0.64 ± 0.14	0.73 ± 0.12	0.54 ± 0.21	0.55 ± 0.25	0.51 ± 0.30	1.02 ± 0.66
Caspase-9 activation [10]	0.70 ± 0.16 ^{a}	0.68 ± 0.18 ^{a}	0.61 ± 0.16 ^{a}	0.40 ± 0.17 ^{c}	0.31 ± 0.17 ^{c}	0.44 ± 0.09 ^{b}	0.50 ± 0.06 ^{b}

[1] Mean values ± standard deviation (SD) are presented for each group (n = 6); values with different superscripts in the same row differ significantly ($p < 0.05$). [2] MDA (malonaldehyde, nmol mL^{-1}). [3] CAT (catalase, U mL^{-1}). [4] SOD (superoxide dismutase, U mL^{-1}). [5] GSH-Px (glutathione peroxidase, U mL^{-1}). [6] i-NOS (Inducible nitric oxide synthase, U mL^{-1}). [7] NK-κB (nuclear transcription factor-κB, ng mgprot^{-1}). [8] COX-2 (cyclooxygenase-2, ng mgprot^{-1}). [9] Caspase-3 activation: when the substrate is saturated, 1 nmol AC ietd PNA can be sheared at 37 °C to produce 1 nmol PNA of Caspase-3. [10] Caspase-9 activation: when the substrate is saturated, 1 nmol AC ietd PNA can be sheared at 37 °C to produce 1 nmol PNA of caspase-9.

3.6. Gastrointestinal Digestive Enzyme Activities

The effect of *Y. lipolytica* on the intestinal digestive enzyme activity of *L. vannamei* is shown in Table 6. There was no significant difference in intestinal digestive enzymes among the groups ($p > 0.05$).

Table 6. Effects of *Yarrowia lipolytica* on digestive enzyme activities in intestinal tissues of *Litopenaeus vannamei* (n = 6).

Index [1]	A0	A1	A2	A3	A4	A5	A6
Trypsin (U mgprot^{-1})	12.77 ± 1.82	11.19 ± 1.11	11.76 ± 1.10	12.09 ± 1.07	11.10 ± 1.99	12.64 ± 1.65	12.38 ± 1.40
Lipase (U gprot^{-1})	5.88 ± 0.94	5.84 ± 0.91	5.05 ± 1.46	6.30 ± 0.84	5.80 ± 1.50	6.59 ± 1.05	5.85 ± 2.07
Amylase (U mgprot^{-1})	7.01 ± 1.91	7.54 ± 1.38	7.60 ± 1.06	6.20 ± 1.05	6.92 ± 1.99	8.60 ± 1.19	8.28 ± 1.13

[1] Mean values ± standard deviation (SD) are presented for each group (n = 6); values with different superscripts in the same row differ significantly ($p < 0.05$).

4. Discussion

In this study, the survival rate of all treatments of *L. vannamei* was higher than 95%, and there was no significant difference among all treatments, which showed that *L. vannamei* could grow normally and achieve good survival at the level of 0–120 mg/kg astaxanthin. A significant correlation between tissue carotenoid concentration and survival has been observed [14]. Wade et al. (2017) [15] believe that when the content of carotenoids in the body is higher than a certain level, the survival rate will not be affected. Otherwise, it will be damaged below this level [16]. Therefore, higher level of tissue carotenoid beyond the minimum requirement can promote growth.

The WG improved with increasing *Y. lipolytica* contents in the diets up to A4 (4 g/kg) and then reduced, with shrimp fed the A4 diet showing the best growth performance. Previous studies have shown that dietary synthetic astaxanthin and algal astaxanthin have beneficial effects on various species of shrimp. There is evidence that dietary astaxanthin can increase body weight and improve survival rate in *L. vannamei* [16,17], *Macrobrachium rosenbergii* [18], *Paralithodes camtschaticus* [19], *Procambarus clarkii* [20], and *Marsupenaeus japonicus* [21]. A study showed that in sea water culture, when fed a diet supplemented with 50 mg/kg astaxanthin, *L. vannamei* had higher growth (10%) and survival (17%) [16]. Current research supports the positive effects of astaxanthin supplements on the growth and survival performance of *L. vannamei* [22]. Carotenoids have been shown to improve the efficient use of nutrients, and to play a significant role in the intermediate metabolism of aquatic animals, thus promoting growth [2,23]. In addition, astaxanthin can shorten the molting cycle interval of crustaceans and inhibit nicotinamide adenine dinucleotide phosphate (NADPH) in

carotenoids, thus reducing energy consumption and accelerating the optimal growth of these aquatic animals [24,25].

The results showed the ability of *Y. lipolytica* to reduce the hepatosmatic index of *L. vannamei* beyond a 7.5 mg/kg dietary inclusion level. At the same astaxanthin level, the hepatosmatic index of A6 was significantly higher than that of A4. This indicates that the addition of *Y. lipolytica* can inhibit the accumulation of lipids to a certain extent. Astaxanthin has a similar effect on fish, and can improve lipid metabolism and reduce oxidative stress and apoptosis induced by fat rich diet as shown in *Micropterus salmoides* [26].

After high temperature treatment, the body color of *L. vannamei* became darker red with the increase of *Y. lipolytica* content. The body color and flesh color of aquatic animals depends on the intake of carotenoids. Astaxanthin can be used for pigmentation of fish in aquaculture [27,28]. People's interest in natural pigments stems from the increasing consciousness and the trend of consumption concepts towards natural products [29]. Astaxanthin is very important in the culture of crustaceans such as the giant tiger prawn (*Penaeus monodon*) [30]. This carotenoid can reverse the blue syndrome found in cultured shrimp lacking pigment. A diet containing 50–100 g/kg astaxanthin recovered the pigmentation of shrimp within 4 weeks [7]. There was no dramatic difference in the whole-body components of *L. vannamei* among the treatments, indicating that astaxanthin did not affect the shrimp body composition. This is in accordance with the results of Niu et al. (2009) [17]. However, the astaxanthin content of shrimp increased significantly with increasing dietary astaxanthin supplementation, similar to the trend in growth, antioxidative ability and immune response [15,31].

The content of serum total protein (TP) is a sensitive indicator reflecting the protein absorption and metabolism of animals [32,33]. The TP and ALB (Albumin) of the treatments with *Y. lipolytica* and *H. pluvialis* was significantly higher than that of A0. This is consistent with other studies, which supplemented astaxanthin in diets of *Procambarus clarkii* [20], *L. vannamei* [16,17,34], and *Marsupenaeus japonicus* [21]. The TP of group A6 was higher than that of other treatments, indicating that astaxanthin from *H. pluvialis* promoted better protein absorption.

Increasing the dietary astaxanthin level remarkably increased T-AOC compared with the control group, and T-AOC in the A4 group was higher than that in other groups. This is consistent with previous studies on *Penaeus monodon* [21,30] and *L. vannamei* [24]. T-AOC refers to the total antioxidant level, and is composed of various antioxidant enzymes and substances such as vitamin C, vitamin E and carotene, which protect cells and the body from oxidative stress caused by reactive oxygen free radicals. MDA comes from lipid oxidation and is an important indicator of oxidative harm in the body. The content of MDA in the serum presented a downward trend from A0 to A5, suggesting that the oxidized lipids were decreasing with the increase in *Y. lipolytica* level in the diets. The decrease of MDA content in serum is closely related to the increase of GSH-PX activity [33]. GSH-Px can effectively remove all organic lipid peroxides [35]. In this experiment, dietary astaxanthin markedly increased T-AOC and decreased MDA in serum, which supported the results of GSH-Px, indicating that the shrimp fed astaxanthin supplement had lower oxidative stress. There were no significant differences in the activities of SOD and CAT in the serum and liver, which can be attributed to different antioxidant enzymes having competitive responses to different degrees of antioxidant stress [36]. I-NOS is a key variable of oxidative stress, and the results showed the ability of dietary astaxanthin to increase the activity. This is consistent with previous studies on mice [37].

Caspase is a group of proteinases with analogous structure in the cytoplasm. It is implicated in apoptosis of eukaryotic cells and plays an important role in the regulation of cell growth, differentiation and apoptosis. Caspase-9 can be activated by signal stimulation through self-splicing, which then causes a Caspase cascade reaction. With the addition of *Y. lipolytica*, Caspase-9 activity decreased significantly, indicating that astaxanthin can reduce apoptosis to some extent, consistent with studies reported on mice [38]. These findings

suggest that, while the shrimp pigmentation increased with increasing dietary astaxanthin concentration, some antioxidant indices may not be affected in a dose dependent manner.

5. Conclusions

Under the experimental conditions, the growth of *L. vannamei* was significantly promoted by adding different levels of *Y. lipolytica* yeast to the diet, and the body color (red) of *L. vannamei* was significantly improved by adding high doses (2~8 g/kg) of *Y. lipolytica* yeast. The addition of *Y. lipolytica* in the feed improved some antioxidant indexes, and no adverse effects on the feed utilization, serum biochemical indexes and immune parameters of *L. vannamei* were observed. It is concluded that *Y. lipolytica* can be used as an effective feed additive for *L. vannamei*, with an optimal dose of 0.5~8.0 g/kg. By regression analysis of weight gain rate, the best recommended dose of *Y. lipolytica* in the diet of *L. vannamei* is 4.64 g/kg.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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