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Effects of Three Sources of Astaxanthin on the Growth, Coloration, and Antioxidant Capacity of Rainbow Trout (*Oncorhynchus mykiss*) during Long-Term Feeding

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Abstract: Astaxanthin is an important pigment for the rainbow trout *Oncorhynchus mykiss*. This study was conducted to investigate the effects of different sources of dietary astaxanthin on the growth, coloration, and antioxidant capacity of the commercial-sized *O. mykiss* during long-term feeding. *Haematococcus pluvialis* (HP), yeast *Phaffia rhodozyma* (PR), and synthetic astaxanthin (SA) were added to the basic feed (no astaxanthin, NA) to prepare the isonitrogenous and isolipidic experimental diets; the actual astaxanthin content values in the diets were 31.25, 32.96, and 31.50 mg/kg, respectively. Eighteen hundred *O. mykiss*, averaging 670 ± 20 g, were randomly divided into four groups and then fed with the experimental diet for four months. Dietary supplementation of *P. rhodozyma* and synthetic astaxanthin had no significant effects on the growth and tissue indexes of *O. mykiss*. In contrast, dietary supplementation with astaxanthin from *H. pluvialis* significantly increased the weight gain rate after four months of feeding. The fillet lightness of *O. mykiss* in the PR and SA was statistically lower than that in the NA and HP; the redness and astaxanthin content of fillet in the HP, PR, and SA groups were statistically higher than those in the NA. The total antioxidant capacity of the liver and serum in the HP was statistically higher than that in other diet groups, and a higher liver total superoxide dismutase activity was detected in the HP compared with the PR. Dietary supplementation of astaxanthin significantly increased the glutathione peroxidase activity in the liver and serum, and the highest serum glutathione peroxidase activity was detected in the HP, while dietary astaxanthin significantly decreased the malondialdehyde content in the liver and serum. Dietary supplementation of PR significantly increased the fillet ash content, while the highest fillet total lipid content was detected in the HP. Dietary astaxanthin significantly improved fillet redness and antioxidant capacity, among which *H. pluvialis* astaxanthin has greater effects on improving weight gain, antioxidant capacity, and fillet total lipid content.

Keywords: astaxanthin; *Oncorhynchus mykiss*; growth performance; coloration; antioxidant capacity

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1. Introduction

Rainbow trout *Oncorhynchus mykiss* is an important commercial fish species, which is favored by consumers because its fillet is tender and has high nutritional value [1,2]. The fillet is the main edible part of the *O. mykiss*, and the fillet color depth is an important factor affecting consumers' purchasing desire [3], which directly affects its market price [4,5]. Previous studies have shown that the fillet color of *O. mykiss* is closely related to the carotenoid composition

and content (especially astaxanthin) [2]. However, because fish cannot synthesize astaxanthin de novo, they can only obtain astaxanthin from their diets [6,7]; hence, it is necessary to additionally supplement the astaxanthin in the feed.

Dietary astaxanthin sources generally include natural and synthetic astaxanthin [8,9]. Natural astaxanthin is mainly obtained from the green alga *Haematococcus pluvialis* [10], yeast *Phaffia rhodozyma* [8], *Paracoccus bacterium* [11], and the Antarctic krill *Euphausia superba* [4]. Different sources of astaxanthin have different optical isomers and geometric isomers [12]; astaxanthin in both *H. pluvialis* and *P. carotinifaciens* is a 3S,3′S isomer, astaxanthin in *P. rhodozyma* is a 3R,3′R isomer, while synthetic astaxanthin consists of the three optical isomers (3S,3′S, 3R,3′S, and 3R,3′R) in a 1:2:1 ratio [9,13]. Natural astaxanthin mostly has an all-trans isomer (*E*), while synthetic astaxanthin has cis-isomers (*Z*) [14]. Synthetic astaxanthin is currently the main source of astaxanthin in the aquatic feed industry due to its relatively cheaper price and wide accessibility, and it has been widely used in the feeds of salmon and trout [2,7]. The unit price of *P. rhodozyma* and *P. carotinifaciens* is moderate, while the unit price of *H. pluvialis* astaxanthin is relatively high [11,15]. However, there are potential benefits to exploring natural sources, such as *H. pluvialis* and *P. rhodozyma*. Natural astaxanthin sources may contain specific isomeric compositions that confer unique physiological benefits compared to synthetic astaxanthin [9]. Research suggests that the isomeric forms found in natural sources could have superior antioxidant and health-promoting properties [16]. There is a growing trend towards natural and sustainably sourced ingredients in food production, including aquafeed [17]. Consumers may perceive products derived from natural sources as healthier and more environmentally friendly, leading to a higher demand and potentially increased market value [9]. Regulatory bodies in some regions may impose restrictions or guidelines on the use of synthetic additives in animal feed. Utilizing natural sources may offer regulatory advantages and align more closely with evolving food safety and sustainability standards.

Astaxanthin plays important roles in regulating color and improving antioxidant, antistress, and immune functions in aquatic animals [18–20]; different sources of astaxanthin have different physiological functions [7,8,21–25]. Although previous studies have investigated the effects of different sources of astaxanthin on the coloration and antioxidant and immune function of small-size *O. mykiss* [26–29], to date there is a lack of comprehensive studies on commercial-sized *O. mykiss* after long-term feeding. Therefore, these data are not conducive to comprehensively evaluating the effects of different sources of dietary astaxanthin on commercial-sized *O. mykiss*. This study aims to investigate the effects of adding *H. pluvialis*, *P. rhodozyma*, and synthetic astaxanthin into the basic feed on the growth performance, coloration, antioxidant capacity, and fillet biochemical composition of the *O. mykiss*. These study results could provide a theoretical basis for evaluating the efficacy, selection, and application of different sources of astaxanthin in the feed of *O. mykiss*.

2. Materials and Methods

2.1. Ethics Statement

The Animal Welfare and Ethical Committee of Shanghai Ocean University approved all of the experimental protocols and guidelines (ethic code: SHOU-DW-2020–152, date: 22 March 2020). Fish were maintained in effectively aerated water, anesthetized with eugenol before sampling, and their viscera were extracted according to the Guidelines for the Care and Use of Laboratory Animals in China.

2.2. Experimental Diets

The *O. mykiss* commercial feed (crude protein: 42% dry diet; total lipids: 24% dry diet) from Officially Aller Aqua (Qingdao) Co., Ltd., Qingdao, China, was used as the basic feed. *H. pluvialis* astaxanthin (donated by Yunnan Alphy Biotech Co., Ltd., Chuxiong, China), *P. rhodozyma* astaxanthin (purchased from Xiamen Junhecheng Biotech Co., Ltd., Xiamen, China), and synthetic astaxanthin (Carophyll pink™, purchased from DSM Vitamins (Shanghai) Co., LTD., Shanghai, China) were added to the basic feed, and we prepared three experimental diets, defined as the HP, PR, and SA groups, respectively.

Currently, the astaxanthin dose of the fattening feed in commercial-sized rainbow trout is approximately 30 mg/kg in China. In this study, this dose was selected as the added amount of astaxanthin in the experimental diets. Meanwhile, the actual astaxanthin content values of four experimental diets were detected, which were 31.25, 32.96, and 31.50 mg/kg, respectively. The diameter of the experimental feed was 6 mm. After processing and packaging, all experimental dies were stored in a refrigerator at −20 °C until use. The proximate composition and actual astaxanthin content of experimental diets are shown in Table 1. Since the feed formulations (the proportion of ingredients) involve the commercial confidentiality of the Officially Aller Aqua (Qingdao) Co., Ltd., they could not be shown in this paper.

Table 1. Proximate composition and astaxanthin content of experimental diets.

Items	NA	HP	PR	SA
Moisture (% dry diet)	9.37	10.13	9.35	9.58
Crude protein (% dry diet)	42.37	42.02	42.85	42.81
Total lipids (% dry diet)	24.04	24.71	24.53	24.54
Ash (% dry diet)	6.91	6.88	7.03	6.80
Astaxanthin (mg/kg dry diet)	0	31.25	32.96	31.50

NA: no astaxanthin; HP: *Haematococcus pluvialis*; PR: *Phaffia rhodozyma*; SA: synthetic astaxanthin.

2.3. Experimental Setup and Culture Management

Eighteen hundred *O. mykiss* (initial body weight: 670 ± 20 g, body length: 34.04 ± 2.55 cm) were obtained from Qinghai Minze Longyangxia Ecological Hydroponics Co., Ltd. (Qinghai, China) for the feeding trial. The experiment was conducted on the Longyangxia Reservoir. These *O. mykiss* were randomly divided into 12 cages (length × width × height = 4 m × 4 m × 5 m), and each cage was stocked with 150 *O. mykiss*. In this study, there were a total of 4 diet treatments, and each treatment was set up with 3 replicates. All fish acclimated to the culture environment and were fed with a control diet (NA) for one week. The feeding trial started on 3 June 2020 and lasted for 4 months. During the feed trial, all experimental fish were hand-fed to apparent satiation twice (8:00 and 16:00) daily with the respective diet. The number of dead fish in each cage was checked and recorded daily. During the experiment, the water temperature and dissolved oxygen level were monitored all day by the automatic monitoring system. The water dissolved oxygen level was 6–9 mg/L, and the water temperature was 12–19 °C.

2.4. Sample Collection and Color Parameter Measurement

At the beginning of the experiment, 12 fish were randomly sampled and dissected, and the fillets on both sides (10 cm × 6 cm) were dissected. The fillet visual color was evaluated using the DSM Color Card (SalmoFan™ Lineal, purchased from DSM Vitamins (Shanghai) Co., LTD., Shanghai, China), and a score of 28 or greater was considered to be an intensity of color suitable for marketed fish [30]. The color value (*L*a*b*) of the fillet surface of the *O. mykiss* was measured by using a colorimeter (CR-400, Konica Minolta, Marunouchi, Tokyo, Japan) under incandescent light in the laboratory [*L*a*b*, *L** represents the lightness value, ranging from 0 (black) to 100 (white); *a** represents the color saturation of the red–green axis, where −*a** is green, +*a** is red; *b** represents the color saturation of the blue–yellow axis, where −*b** is blue, +*b** is yellow)]. The measured position of fillets for the *L*a*b* value of *O. mykiss* fillet is shown in Figure 1.

The fillet chromaticity value was measured with the DSM card score (Salmo Fan™ value > 28), and then the fillets were stored in a Ziplock bag at −40 °C for the later astaxanthin analysis. The feed amount of the experimental fish was counted every month, and 30 *O. mykiss* from each cage were randomly captured and weighed; then, 3 fish were randomly selected and weighed with an electronic scale (precision = 0.01 kg), and the body length was measured with a ruler (precision = 0.01 cm), which was used to calculate the growth performance indexes. The *O. mykiss* was dissected, the color value (*L*a*b*) and DSM card score of the fillets were measured, all the viscera were taken out and weighed, and then the liver was carefully stripped and weighed to calculate the viscerosomatic index

(VSI) and hepatosomatic index (HSI). The weight of each piece of fillet of fish was weighed to calculate the meat yield (MY); each fillet was cut into several pieces (10 cm × 6 cm) and stored in a −40 °C refrigerator for later astaxanthin content determination.

Figure 1. The measure points of a fillet for *L*a*b* values of *O. mykiss*. ①, ②, and ③ indicates the anterior, middle and posterior parts on the fillet surface, respectively.

In the fourth month of the feeding trial, all experimental fish fasted for 24 h, and the survival rate (SR) of *O. mykiss* in each experimental group was counted. Three *O. mykiss* were randomly sampled from each cage. About 3 mL blood was drawn from the caudal vein of *O. mykiss* and divided into three 1.5 mL non-enzyme centrifuge tubes. The serum was obtained after centrifugation (5000 rpm for 10 min at 4 °C) and stored at −40 °C for the later determination of antioxidant indices. The *O. mykiss* was dissected, all viscera were taken out and weighed, and then the liver was weighed. The liver samples were stored in a Ziplock bag at −40 °C for later determination of antioxidant indices. One side of the fillets of each fish was cut and weighed, and its color values were determined. The fillet samples were placed in a Ziplock bag and stored in a refrigerator at −40 °C for the later astaxanthin content detection. The survival rate (SR), weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and meat yield (MY) were calculated using the following formulas:

$$SR\ (\%) = 100 \times F_f/F_i$$

$$WGR\ (\%) = 100 \times (W_f - W_i)/W_i$$

$$SGR\ (\%/d) = 100 \times (\mathrm{Ln}\ W_f - \mathrm{Ln}\ W_i)/D_t$$

$$FCR = F_t/(W_f - W_i)$$

$$CF\ (g/cm^3\%) = 100 \times W_b/L^3$$

$$VSI\ (\%) = 100 \times W_v/W_b$$

$$HSI\ (\%) = 100 \times W_h/W_b$$

$$MY\ (\%) = 100 \times 2 \times M_m/M_b$$

where F_i is the number of fish at the beginning of the experiment, F_f is the number of fish at the end of the experiment, W_i is the average weight of *O. mykiss* at the beginning of the experiment, W_f is the average weight at the end of the experiment, D_t is the total number of days of the experiment, F_t is the total feeding amount, W_b is the body weight, L is the body length, W_v is the weight of the viscera, W_h is the weight of the hepatopancreas, and M_m is the weight of one side fillet.

2.5. Proximate Composition and Astaxanthin Content Analysis

The fillet moisture content of *O. mykiss* was determined by the freeze-drying method [31], while the moisture content of experimental feeds was determined by the drying method at 105 °C. The contents of crude protein and crude ash in the fillets and experimental feeds

were determined according to the standard method of the Association of Official Analytical Chemists [32]. The total lipid content of fillets and experimental feeds was detected according to the method described by Folch et al. [33]. The determination of the fillet's astaxanthin content referred to the methods of Gao et al. [34] and the standard methods for the aquatic industry of the People's Republic of China [35].

2.6. Antioxidant Capacity Indexes Determination

The liver samples from the −40 °C refrigerator were thawed at 4 °C. Approximately 0.1 g liver tissue plus a 5-fold volume (*v*/*w*) of ice-cold physiological saline solution were added to a 5 mL centrifuge tube and homogenized with a micro-homogenizer (Shanghai Huxi Industrial Co., Ltd., Shanghai, China; HR-6). The homogenate was centrifuged at 2000 rpm for 20 min at 4 °C, and the supernatant was collected for later analysis of antioxidant indices. The serum was thawed at 4 °C before testing. The total antioxidant capacity (T-AOC, A015-1-2), total superoxide dismutase (T-SOD, A001-1-2), malondialdehyde (MDA, A003-1-2), and glutathione peroxidase (GSH-Px, A005-1-2) in the liver homogenate supernatant and serum were determined by using commercial test kits purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

2.7. Data Analysis

SPSS (version 19.0, SPSS Inc., Chicago, IL, USA) software was used for statistical analysis of experimental data, and all data were expressed as mean ± standard error. All data were tested for homogeneity of variance using Levene's method, and when the homogeneity of variance was not met, arc sine or square root processing was performed on the percentage data. One-way ANOVA was used for the analysis of variance, and Duncan's multiple comparisons were performed when the difference was significant ($p < 0.05$).

3. Results

3.1. Growth Performance and Tissue Indexes

The survival rates, body weight, weight gain rate, specific growth rate, and feed conversion ratio of *O. mykiss* are shown in Figures 2 and 3. There was no significant difference in the survival rate (SR) from 0 to 1, 1–2, 2–3, 3–4, and 0–4 months (Figure 2), body weight (BW) in the first, second, third, and fourth months (Figure 3A), the weight gain rate (WGR) from 0 to 1, 1–2, 2–3, and 3–4 months (Figure 3B), and the specific growth rate (SGR, Figure 3C), feed conversion ratio (FCR, Figure 3D) among the four diet groups. Statistically higher WGR was recorded in the HP group compared to NA from 0–4 months ($p < 0.05$, Figure 3B).

The tissue indexes of *O. mykiss* are shown in Figure 4. In the first and second months of the feeding trial, there were no significant differences in the condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), or meat yield (MY) of *O. mykiss* among the four diet groups. In the third month, the VSI of *O. mykiss* in the SA was statistically higher than that in other diet groups ($p < 0.05$), while a higher MY was recorded in the NA and PR groups ($p < 0.05$). In the fourth month, the HSI of the NA and HP groups was statistically higher than that of the SA group ($p < 0.05$), while there were no significant differences in the CF, VSI, and MY among the four diet groups.

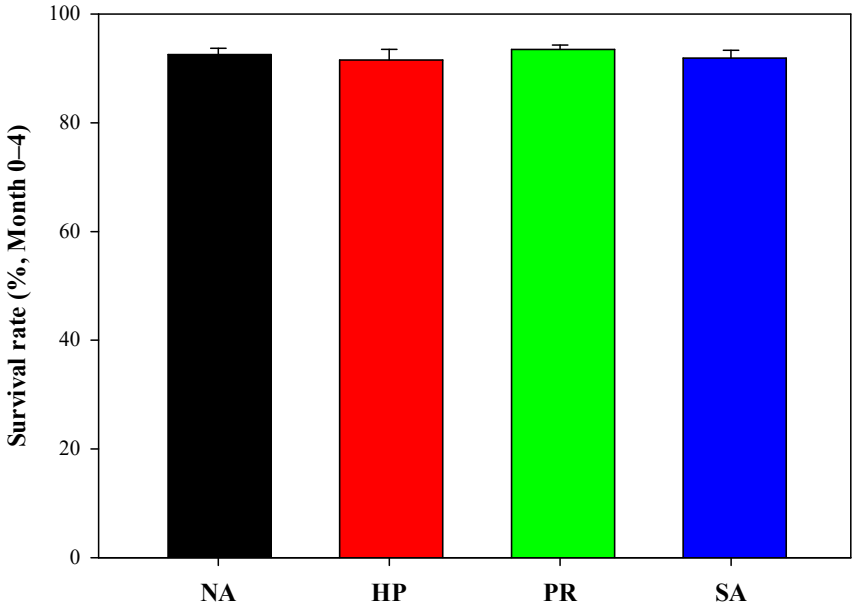

Figure 2. The survival rates of *O. mykiss* fed three sources of dietary astaxanthin. Data are presented as mean ± SE (n = 3). Black bar represents the control diet group (no astaxanthin, NA); red bar represents the *Haematococcus pluvialis* diet group (HP); green bar represents the yeast *Phaffia rhodozyma* diet group (PR); blue bar represents the synthetic astaxanthin diet group (SA).

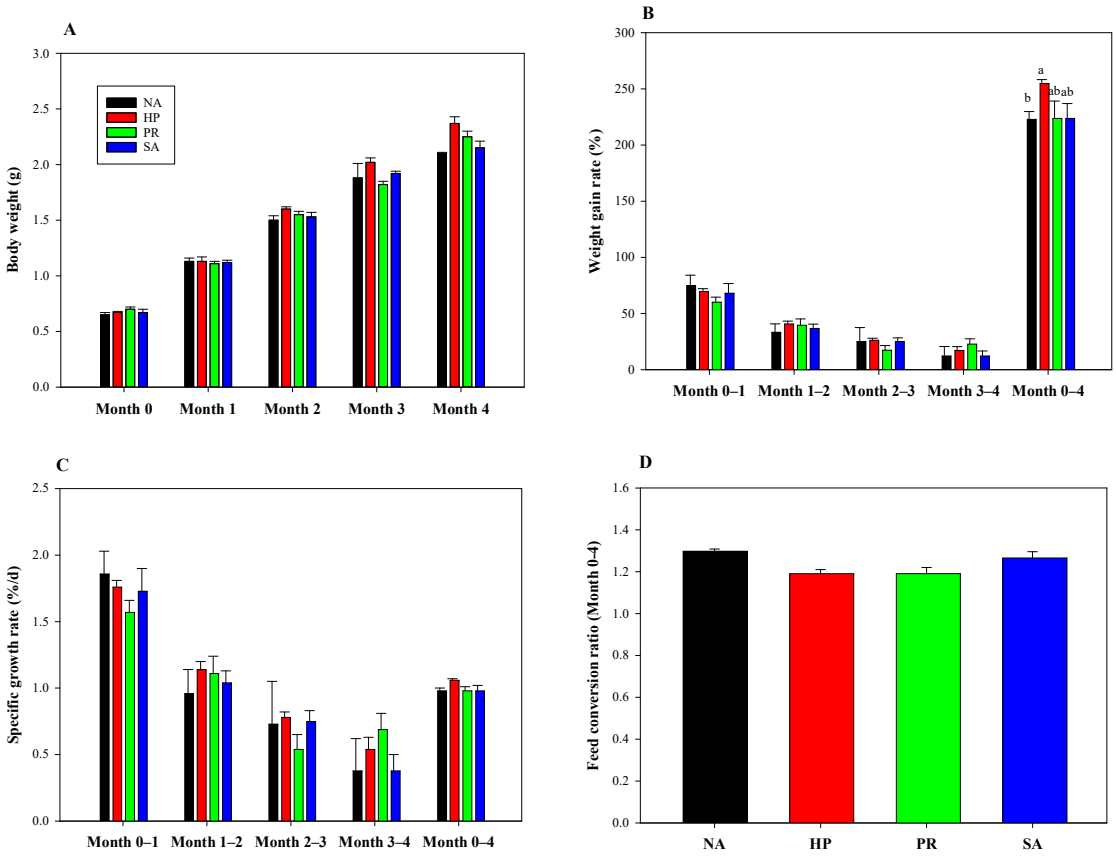

Figure 3. The body weight, weight gain rate, specific growth rate, and feed conversion ratio of *O. mykiss* fed three sources of dietary astaxanthin. Data are presented as mean ± SE (n = 3). The bars without a same letter are significantly different ($p < 0.05$). (**A**): body weight; (**B**): weight gain rate; (**C**): specific growth rate; (**D**): feed conversion ratio.

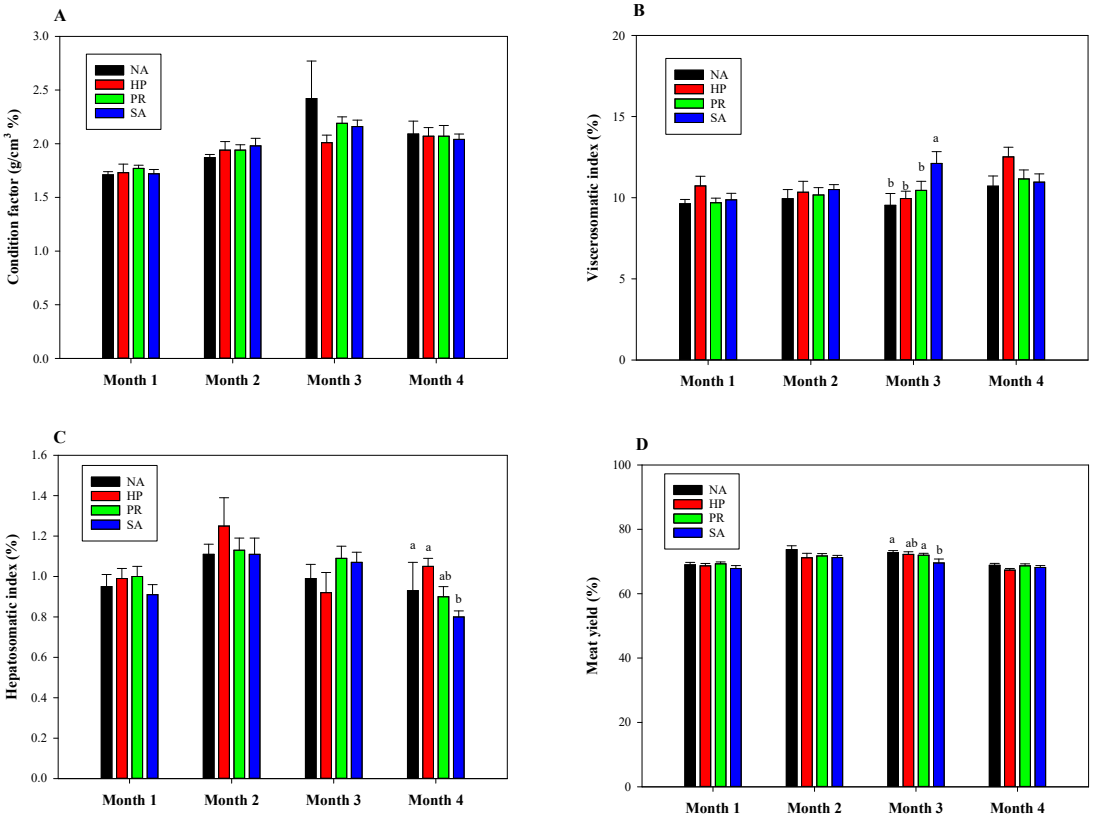

Figure 4. The condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and meat yield (MY) of *O. mykiss* fed three sources of dietary astaxanthin. Data are presented as mean ± SE (n = 3). The bars without a same letter are significantly different ($p < 0.05$). (**A**): condition factor; (**B**): viscerosomatic index; (**C**): hepatosomatic index; (**D**): meat yield.

3.2. Color Value and Astaxanthin Content of Fillet

There were striking differences in the color of fillets between the control group (NA) and the three astaxanthin-supplemented diet groups. The visible redness of fillets was improved by the dietary astaxanthin (Figure 5). The fillet color value of *O. mykiss* is shown in Figure 6. In the first month, dietary supplementation of astaxanthin significantly reduced the L^* and b^* of the fillet and increased the a^*, DSM SalmoFan™ scores of the fillet ($p < 0.05$). In the second month, the fillet L^* of *O. mykiss* in the HP and PR groups was statistically higher than that in the NA group ($p < 0.05$), but not significantly different from the SA; dietary astaxanthin significantly increased the a^*, b^*, DSM SalmoFan™ scores of the fillet ($p < 0.05$). In the third month, dietary supplementation of astaxanthin significantly decreased the fillet L^* of *O. mykiss*, while increasing the fillet a^* and DSM SalmoFan™ scores ($p < 0.05$). In the fourth month, the fillet L^* of *O. mykiss* in the NA and HP was statistically higher than that in the PR and SA, while the fillet a^* and DSM SalmoFan™ scores in the astaxanthin-supplemented groups were statistically higher than those in the NA group ($p < 0.05$), but no significant differences were found for the fillet a^* and DSM SalmoFan™ scores among the HP, PR, and SA groups ($p < 0.05$).

Figure 5. Colors of fillets of *O. mykiss* fed three sources of dietary astaxanthin.

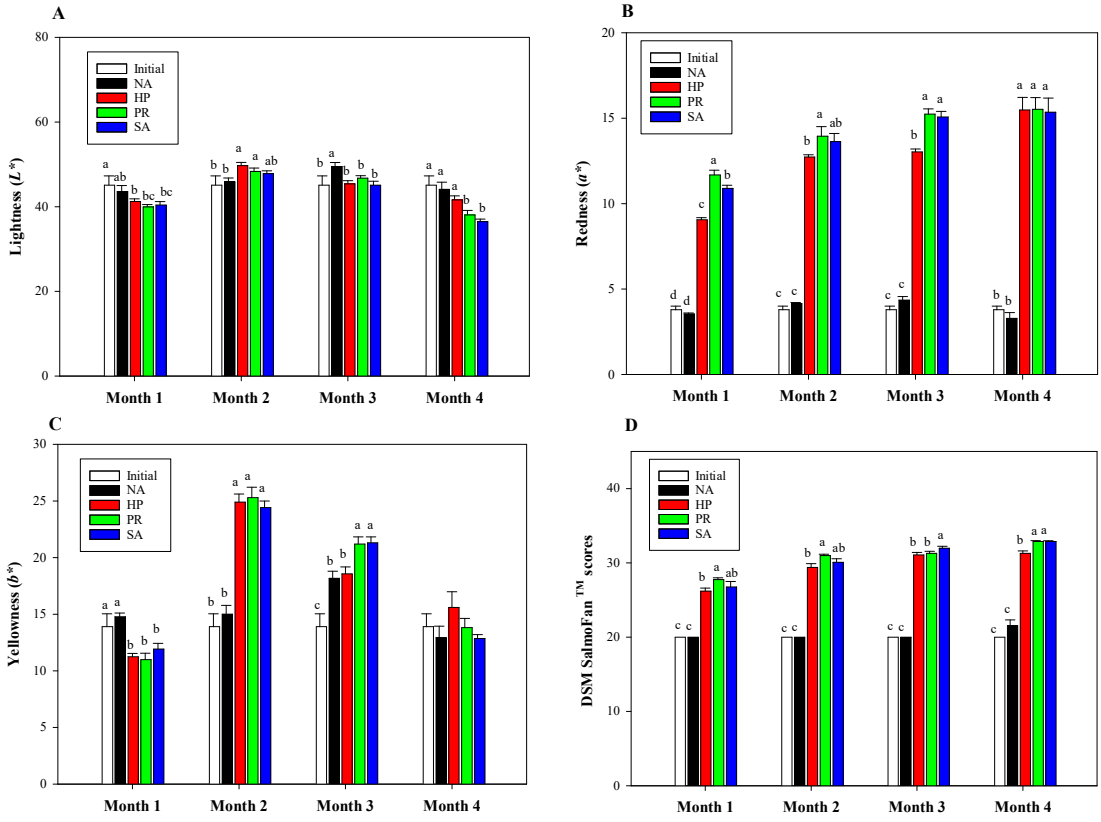

Figure 6. The color values (*L*a*b*) and DSM SalmoFan™ scores of fillets of *O. mykiss* fed three sources of dietary astaxanthin. Data are presented as mean ± SE (n = 3). The bars without a same letter are significantly different ($p < 0.05$). (**A**): lightness (*L**); (**B**): redness (*a**); (**C**): yellowness (*b**); (**D**): DSM SalmonFan™ scores.

The fillet astaxanthin content of the *O. mykiss* is shown in Figure 7. In the first month, the fillet astaxanthin content in the PR and SA groups was statistically higher than that in the NA and HP groups ($p < 0.05$). In the second month, the highest fillet astaxanthin

content was detected in the PR compared to the NA and HP groups ($p < 0.05$). In the third month, the fillet astaxanthin content in the PR and SA was significantly higher than that in the NA and HP groups ($p < 0.05$), and the highest value was detected in the SA. In the fourth month, the fillet astaxanthin content in the astaxanthin-supplemented groups was statistically higher than that in the NA ($p < 0.05$), and no significant difference was found among the three resources of astaxanthin groups.

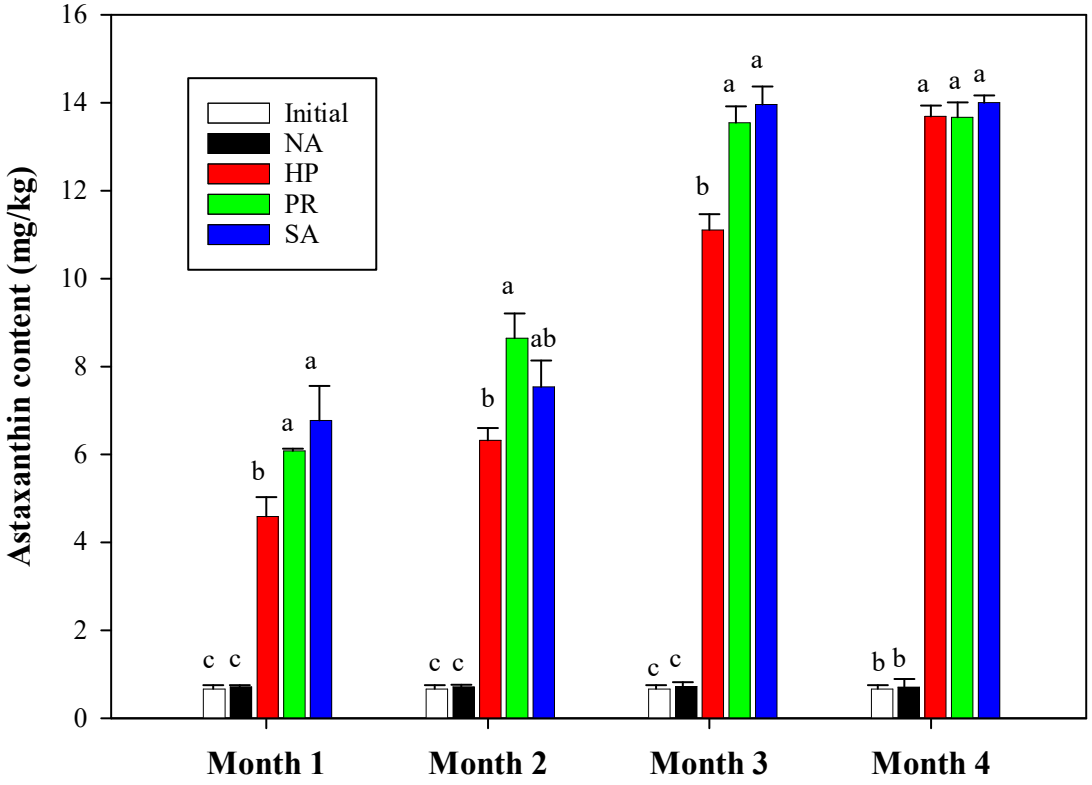

Figure 7. The fillet astaxanthin content of the *O. mykiss* fed three sources of dietary astaxanthin. Data are presented as mean ± SE (n = 3). The bars without a same letter are significantly different ($p < 0.05$).

3.3. Antioxidant Capacity Indices of Liver and Serum

The antioxidant capacity indices of the liver and serum are shown in Table 2. For the liver, the total antioxidant capacity (T-AOC) in the HP was statistically higher than that in the other diet groups ($p < 0.05$), and the highest total superoxide dismutase (T-SOD) activity was detected in the HP. The glutathione peroxidase (GSH-Px) activity in the HP, PR, and SA groups was statistically higher than that in the NA group ($p < 0.05$), while there were no significant differences among the three astaxanthin-supplemented groups ($p > 0.05$). Dietary supplementation of astaxanthin significantly reduced the liver malondialdehyde (MDA) content ($p < 0.05$), while no significant differences were found among the three astaxanthin-supplemented diet groups. For the serum, the highest levels of serum T-AOC and GSH-Px were detected in the HP group ($p < 0.05$), while the lowest serum T-SOD in the HP group was statistically lower than that in other groups ($p < 0.05$). The serum MDA content in the NA group was significantly higher than that in the other three groups ($p < 0.05$), but there was no significant difference among the three astaxanthin-supplemented diet groups.

Table 2. Effects of different sources of astaxanthin on the antioxidant capacity of *O. mykiss*.

Items	NA	HP	PR	SA
Liver				
T-AOC (U/mg protein)	0.64 ± 0.07 ^{c}	1.58 ± 0.07 ^{a}	0.76 ± 0.12 ^{bc}	1.02 ± 0.14 ^{b}
T-SOD (U/mg protein)	20.66 ± 1.12 ^{ab}	22.41 ± 0.89 ^{a}	18.54 ± 0.65 ^{b}	20.32 ± 1.11 ^{ab}
GSH-Px (U/mg protein)	20.99 ± 1.11 ^{b}	27.54 ± 1.73 ^{a}	29.97 ± 1.60 ^{a}	30.16 ± 1.77 ^{a}
MDA (nmol/mg protein)	1.30 ± 0.07 ^{a}	0.64 ± 0.05 ^{b}	0.83 ± 0.06 ^{b}	0.83 ± 0.05 ^{b}
Serum				
T-AOC (U/mL)	8.52 ± 0.41 ^{c}	16.43 ± 0.32 ^{a}	11.74 ± 0.51 ^{b}	11.06 ± 0.54 ^{b}
T-SOD (U/mL)	103.03 ± 1.99 ^{a}	84.51 ± 2.03 ^{b}	103.18 ± 2.09 ^{a}	103.97 ± 2.37 ^{a}
GSH-Px (U/mL)	97.30 ± 4.45 ^{b}	132.47 ± 5.29 ^{a}	96.52 ± 2.54 ^{b}	98.00 ± 5.07 ^{b}
MDA (nmol/mL)	33.75 ± 1.50 ^{a}	16.63 ± 0.76 ^{b}	18.70 ± 0.25 ^{b}	19.15 ± 0.20 ^{b}

Data are presented as mean ± SE (n = 3). NA: no astaxanthin; HP: *Haematococcus pluvialis*; PR: *Phaffia rhodozyma*; SA: synthetic astaxanthin. Values within the same row with different letters mean a significant difference ($p < 0.05$). T-AOC: total antioxidant capacity; T-SOD: total superoxide dismutase; GSH-Px: glutathione peroxidase; MDA: malonaldehyde.

3.4. Biochemical Composition of Fillet

The fillet biochemical composition of *O. mykiss* is shown in Table 3. The moisture and ash content of fillets in the PR group was statistically higher than those in other diet groups ($p < 0.05$), while no significant difference was found for the fillet crude protein content among the four diet groups. The total lipids content of the fillet in the HP was statistically higher than that in the NA and PR groups ($p < 0.05$) but was not significantly different from the SA group.

Table 3. Effects of different sources astaxanthin on the proximate composition of fillet of *O. mykiss*.

Items	NA	HP	PR	SA
Moisture (% wet fillet)	65.36 ± 0.47 ^{bc}	67.76 ± 1.10 ^{b}	69.06 ± 0.84 ^{a}	63.82 ± 0.30 ^{c}
Crude protein (% wet fillet)	22.48 ± 1.00	21.58 ± 0.92	21.78 ± 0.29	22.14 ± 0.39
Total lipids (% wet fillet)	7.52 ± 0.95 ^{b}	10.63 ± 0.12 ^{a}	7.57 ± 0.73 ^{b}	9.00 ± 0.59 ^{ab}
Ash (% wet fillet)	1.88 ± 0.09 ^{b}	2.03 ± 0.06 ^{b}	2.45 ± 0.07 ^{a}	2.09 ± 0.16 ^{b}

Data are presented as mean ± SE (n = 3). NA: no astaxanthin; HP: *Haematococcus pluvialis*; PR: *Phaffia rhodozyma*; SA: synthetic astaxanthin. Values within the same row with different letters mean a significant difference ($p < 0.05$).

4. Discussion

Previous studies have reported the effects of dietary astaxanthin on the growth performance, feed coefficient ratio, and survival rate of salmonids. The study of Christiansen et al. [36] showed that dietary supplementation of 36 and 190 mg/kg synthetic astaxanthin significantly improved the weight gain rate and survival rate of juvenile *O. mykiss* (initial body weight: 1.75 g). In the study of Tao et al. [37], dietary supplementation of 40 and 80 mg/kg synthetic astaxanthin significantly increased the weight gain rate of *O. mykiss* (initial body weight: 850 g) and reduced the feed coefficient ratio. Our study showed that dietary supplementation of 30 mg/kg *H. pluvialis* astaxanthin significantly improved the weight gain rate of *O. mykiss* (initial weight: 600 g) in the fourth month of the feeding trial, and may be due to astaxanthin-promoted nutrient assimilation, intermediary metabolism in cells, antioxidant potential, and hemolymph cell level [38], while dietary *P. rhodozyma* and synthetic astaxanthin had no significant effects on the growth performance and meat yield during the feeding trial. On the contrary, the study by Zhang et al. [39] showed that dietary supplementation of 100 mg/kg of synthetic astaxanthin and natural astaxanthin derived from *Adonis amurensis* and *H. pluvialis* to the feed has no significant effects on the growth performance of juvenile *O. mykiss* (initial body weight: 6.28 g). Yanar et al. [40] reported that dietary supplementation of 70 mg/kg red pepper *Capsicum annum* and marigold *Tagetes erecta* astaxanthin had no significant effect on the final body weight *O. mykiss* (initial body weight: 120.57 g) after a 60-day feeding trial. Dietary supplementation of 100 mg/kg synthetic astaxanthin, canthaxanthin, and β-carotene also has no significant effect on the growth performance of *O. mykiss* (initial body weight: 140 g) [41]. To

sum up, astaxanthin has different effects on the growth performance of *O. mykiss* in different studies, which may be related to various factors, such as the cultural environment, the content of dietary astaxanthin, fish size, and feeding ways (satiation or quantification), etc. However, the specific reasons still need to be further studied.

Color is an important indicator evaluating the fillet sensory characteristics of *O. mykiss* [42]. The fillet visual color will affect consumers' purchasing desire and the market price for *O. mykiss* [43]. When purchasing salmon and trout, color is one of the first things a consumer will consider; to a consumer, the color generally indicates species, age, origin, price, expected flavor/texture, freshness, and quality. Consumers perceive that redder salmon or trout is equated to these characteristics: fresher, better flavor, higher quality, and higher price [44,45]. Previous studies have shown that the fillet chroma value (*L*a*b*) of salmon and trout is related to the dietary carotenoid content, especially astaxanthin [46]. The redness value (*a**) and yellowness value (*b**) of the fillet were positively correlated with the dietary carotenoids content, while the lightness value (*L**) was negatively correlated with the dietary carotenoids content [2]. In this study, dietary supplementation of astaxanthin could significantly increase the redness value (*a**) and astaxanthin content of the fillet in the *O. mykiss*, and the DSM SalmoFan™ scores of the fillet was greater than 28 in the second month of the feeding trial in the astaxanthin-supplemented groups, which met the standard of commercial fish [30,47]. In the first, second, and third months of the feeding trial, *a** and astaxanthin content of the fillet in the PR and SA groups were statistically higher than those in the NA and HP groups. However, in the fourth month of the feeding trial, there was no significant difference in the *a** and astaxanthin content of *O. mykiss* fillets among the three astaxanthin-supplemented groups. Such results indicated that the pigmentation rate or astaxanthin deposition rate of the *P. rhodochrous* and synthetic astaxanthin is faster compared with the *H. pluvialis* astaxanthin. The possible reasons are as follows: (1) astaxanthin in *H. pluvialis* mostly exists in an esterified form, while the synthetic astaxanthin and *P. rhodozyma* astaxanthin mainly exist in a free form [48]; the free form of astaxanthin may be directly absorbed and accumulated in the fillet [49,50], while the esterified form of astaxanthin in the *H. pluvialis* needs to be decomposed before combining with fillet-related pigment cells [51,52]. (2) The green alga *H. pluvialis* has a cell wall, and the intracellular astaxanthin might be utilized after enzymatically breaking down for *O. mykiss* [6,53,54]; therefore, the breaking rate of *H. pluvialis* cell wall will also affect the utilization of astaxanthin in *O. mykiss*.

This study showed that dietary supplementation of three sources of astaxanthin could improve the T-AOC in the liver and serum of *O. mykiss*, and the highest value was detected in the *H. pluvialis* astaxanthin-supplemented group. Such results suggested that the dietary supplement of different sources of astaxanthin can improve the antioxidant capacity of *O. mykiss*, and the effect of *H. pluvialis* astaxanthin is better. Previous studies have shown that dietary astaxanthin reduced the dependence of Atlantic salmon *Salmo salar* on endogenous antioxidant enzymes, such as CAT and T-SOD [55]. This study also found that feeding *H. pluvialis* astaxanthin significantly reduced the T-SOD activity in the serum of *O. mykiss*, which was consistent with the findings of Rahman et al. [2] and Zhang et al. [56]. This may be because the astaxanthin participated in the antioxidant process as a non-enzymatic system component, effectively scavenging excess oxygen free radicals before endogenous antioxidant enzymes were activated [2], thereby reducing the reaction substrates of antioxidant enzymes, which ultimately led to a decrease in the activity of antioxidant enzymes [57]. In this study, the highest GSH-Px activity, and the lowest MDA content of the serum and liver were detected in the *H. pluvialis* astaxanthin-supplemented group. This may be because GSH-Px, as an important peroxide-decomposing enzyme, can prevent membrane lipid peroxidation caused by free radicals in the body, and scavenge MDA and various peroxidation products [58]. In conclusion, dietary supplementation of different sources of astaxanthin can significantly improve the antioxidant capacity of *O. mykiss*, and *H. pluvialis* astaxanthin has a better effect. Previously, in human umbilical vein endothelial cells (HUVECs), natural astaxanthin exerted its antioxidant effect by in-

ducing the production of ROS to activate the Nrf2 signaling pathway; however, synthetic astaxanthin had no effect on the mRNA expression of Nrf2-related target genes in Huh7 cells [59,60]. The study of Ding et al. (2023) showed that the antioxidant capacities of different sources of astaxanthin were different, which may be related to the different scavenging ability of astaxanthin on intracellular ROS. Therefore, it is necessary to compare and study the difference in antioxidant capacity of astaxanthin from different sources, different configurations, or existing forms through in vitro or in vivo experiments, and to further analyze its specific mechanism [61].

Previously, studies on the effects of dietary astaxanthin on fish mainly focused on the survival rate, growth performance, coloration, antioxidant activity, immunity, and anti-stress ability [62–66], while there are relatively few studies on the biochemical composition. Amar et al. [41,65,66] reported that various dietary synthetic carotenoids had no significant effects on the biochemical composition of fillets in the *O. mykiss*. The study of Yanar et al. [3] showed that dietary supplementation of 70 mg/kg synthetic astaxanthin, *Capsicum annum*, and *Tagetes erecta* astaxanthin had no significant effects on the contents of moisture, ash, crude protein, and total lipids of fillet in the *O. mykiss* with an initial body weight of 120.57 g after 60-day feeding. Similarly, Rebolé et al. [1] reported that dietary supplementation of different sources of astaxanthin had no significant effects on the fillet biochemical composition of *O. mykiss* with an initial body weight of 168 g after one month of feeding. However, our study showed that dietary *H. pluvialis* astaxanthin significantly increased the total lipids content of fillet in the *O. mykiss*, which was similar to the findings of the shrimp [19] and crab [10,67]. A possible reason for this is that dietary *H. pluvialis* astaxanthin promotes the synthesis of vitamin A in the body [68,69], thereby improving lipid metabolism [50], but the specific reasons remain to be further studied.

5. Conclusions

Dietary supplementation of *P. rhodozyma* and synthetic astaxanthin had no significant effects on the growth performance and meat yield of *O. mykiss*, while dietary *H. pluvialis* astaxanthin improved the weight gain rate. Dietary astaxanthin significantly increases the fillet astaxanthin content, redness, and antioxidant capacity. Among these, *H. pluvialis* astaxanthin had greater effects on improving the weight gain rate, antioxidant capacity, and fillet total lipid content. In the first three months of culturing, the coloring efficiency and speed of *P. rhodozyma* and synthetic astaxanthin were higher than that of *H. pluvialis*; however, at the fourth month, the coloring effect of the three sources of astaxanthin was not different. The difference in the efficacy of astaxanthin from different sources may be related to their bioavailability to rainbow trout and their differences in physiological and metabolic characteristics. Therefore, the physiological effects, synergies, and metabolic mechanisms of astaxanthin from different sources, dosages and configurations or the long-term effects beyond the four-month feeding period will be future research directions.

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