

Research Article

Dietary Supplement of Microalgal Astaxanthin Extraction Improved Shell Pigmentation and Nutritional Value of *Litopenaeus vannamei* in an Indoor Industrial Aquaculture System

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Blue-shell syndrome is associated with low levels of astaxanthin (Ax) deposition in cultured shrimp. Such a syndrome is a key issue in indoor industrial shrimp aquaculture. In this study, we investigated the effect of dietary supplementation with microalgal Ax extract (MAAE), extracted from the microalgae, *Haematococcus pluvialis*, on shell pigmentation and nutritional value of shrimp *Litopenaeus vannamei*. Dietary supplementation with MAAE (20 mg kg^{-1}) effectively improved shrimp pigmentation. In addition to increased growth and nutritional value, shrimp muscles had higher free amino acid concentrations (Asp, Ser, Ala, Val, and His) and lower fatty acid concentrations (except for C_{18:0}, C_{18:1n9c}, C_{20:0}, C_{20:2}, and C_{24:1}) compared to those in the control group. In conclusion, this study indicated that a relatively low dose of MAAE at 20 mg kg⁻¹ may improve the growth and nutritional value of shrimp in an indoor industrial shrimp aquaculture system.

1. Introduction

Shrimp is one of the most important species in the global fishery and aquaculture industry, among which the Pacific white shrimp, *Litopenaeus vannamei*, has the highest productivity level [1]. In 2019, the production of marine *L. vannamei* was approximately 1.14 million tons in China [2], and this was due to its rapid growth rate and high yield and nutritive value [1]. *L. vannamei* can be cultured in an intensive or even a super-intensive culture system. However, such systems have been associated with blue-shell syndrome (characterized by a white body). Body color of live shrimp is an important quality criterion in markets, with the red

color being preferred [3]. Flesh quality is also an important factor and includes physical properties, nutritive composition, and special flavors [4]. It is difficult for crustaceans to biosynthesize carotenoids in intensive indoor aquaculture systems. However, they can metabolize dietary carotenoids to astaxanthin (Ax) and deposit these molecules in their tissues [5]. Therefore, dietary supplementation with carotenoids is a proposed method to improve the coloration and nutritional value of shrimp [6].

Ax is an important carotenoid pigment present in aquatic species such as salmon, shrimp, and crab [7] and often increases a red color in aquatic animals, such as shrimp. Ax can be extracted from natural sources, such as

algae and yeast, and the green microalga, *Haematococcus pluvialis*, has been suggested as a suitable source [8, 9]. Previous studies have shown that Ax can enhance maturation, increase immune responses, and reduce the stress associated with high ammonia levels in shrimp [10–12]. Most studies have focused on the effect of Ax at the experimental level, whereas few studies have been conducted at the commercial level [13, 14].

Therefore, we extracted microalgal Ax (MAAE) from *Haematococcus pluvialis* and explored how it affects the body color and nutritional value of *L. vannamei* in an indoor industrial aquaculture system when given as a dietary supplement.

2. Materials and Methods

2.1. MAAE and Preparation of Shrimp Diet. The MAAE in oil form was provided by Bioalgo (WF) Co. Ltd., Shandong, China. In summary, an Ax mixture (Ax content of 0.2%) was prepared from mature *H. pluvialis* spores, which were collected, dried, and ground.

Ax concentrations in the MAAE were determined by a high-performance liquid chromatography (HPLC) method, with a YMC carotenoid column (4.6 mm ×250 mm ×5 μ m), as described for the detection of Ax esters in the United States Pharmacopeia [15]. The chromatographic conditions were as follows: absorbance of the UV detector was set at 474 nm; column temperature was 30°C; flow rate was 1.0 mL·min⁻¹; the sample size was 20 μ L; and the mobile phase comprised of methanol, methyl tert-butyl ether, and 1% phosphoric acid solution.

The diet for experimental shrimp is prepared according to the feed formula shown in Table 1. The baseline diet followed the formula of the commercial feed of Tongwei Co., Ltd. Ax was supplemented into the basal diet at a dose of 20mgkg⁻¹, which was the optimal dose determined in our pre-experiment. Because the concentration of Ax in the MAAE was 0.2%, the amount of MAAE added to the feed was calculated to be $10 \text{ g} \cdot \text{kg}^{-1}$. The MAAE was added directly to the feed and mixed for 1h before use.

2.2. Experimental Design. The study was conducted at the Dongying Delta Aquaculture Breeding Co. Ltd. $(37^{\circ}44'N, 118^{\circ}92'E)$ in the Shandong Province of China in 2020. Six concrete tanks $(8.0 \text{ m} \times 4.0 \text{ m} \times 1.3 \text{ m})$ were used, with the maximum indoor illumination of approximately 1900 lux. Disinfected seawater, with a salinity of 29, was heated to 28°C before being added to culture tanks up to a depth of 1 m. Twenty percent of the water is exchanged each day during the experiment, to prevent the deterioration of water quality, such as the accumulation of ammonia and nitrite, which threatens the shrimp.

Juveniles of *L. vannamei* with similar body weight $(4.46 \pm 0.9 \text{ g})$ were used in the experiment and stocked at an initial density of 320 ind./m³. Six shrimp culture tanks were randomly and equally allocated into the MAAE group (which included shrimp fed the MAAE diet) and the control group (which included shrimp fed a basal diet). Shrimps were fed five times per day with an interval of 4 h, starting

TABLE 1: The composition of the experimental feeds $(g kg^{-1})$.

Composition	Treatment and content				
Composition	MAAE	Control			
Fish meal	170	170			
Soybean meal	261	261			
Soybean oil	15	15			
Fish oil	5	5			
Vitamin mix ^a	12	12			
Mineral mix ^b	17	17			
MAAE	0.02	—			
Crude protein	420	420			
Crude lipid	60	60			
Crude ash	150	150			

Note: ^aVitamin mix (/kg feed): V_A , 300000 IU; V_{B2} , 480 mg; V_{B6} , 360 mg; B_{12} , 1.2 mg; V_{B1} , 20.0 mg; V_k , 20 mg; folic acid, 170 mg; biotin, 10 mg; VE, 3000 IU; inositol, 8000 mg; calcium pantothenate, 800 mg; niacin, 200 mg; choline chloride, 8000 mg; and VD, 40000 IU. ^bMineral mix (/kg feed): ZnSO₄·7H₂O 0.817 g; CaCO₃ 3.28 g; NaH₂PO₄ 2.96 g; KH₂PO₄ 6.752 g; CaCl₂ 1.3328 g; MgSO₄·7H₂O 1.6 g; KCl 0.448 g; AlCl₃·6H2O 0.0192 g; MnSO₄·(4/6) H₂O 0.229 g; CuCl₂ 0.52 g; FeSO₄·7H₂O 1.8 g; CoCl₂ 0.0282 g; and KI 0.031 g.

at 05:00 and ending at 21:00. Discharge of sewage was carried out 1 h after every feed.

2.3. Measurement of Water Quality and Shrimp Growth. The dissolved oxygen (DO), pH, temperature, and salinity of the rearing water in the experimental tanks were measured from 8:00-to 9:00 every day using a YSI portable multiparameter water quality meter (Aqua TROLL 400; in situ, USA). Rearing water was sampled every five days, and the concentrations of nitrate nitrogen (NO2-N) and total ammonia nitrogen (TAN) were measured using spectrophotometry according to the method described by Bendschneider and Robinson [16]. Standard methods, as described by Eaton et al. [17], were used to examine water and wastewater after filtration through a 0.22- μ m pore diameter filter membrane. After the experiment began, 50 shrimps were randomly selected from each tank every 10 days for the measured body weight. Growth performance, feed conversion rate, and the survival rate of shrimp were assessed in each group at the end of the experiment.

2.4. Measurement of Shrimp Body-Color. Twenty live shrimps were randomly selected from the MAAE and control groups, and three cooked shrimps from each group were selected for body color photography analysis. The shrimps were cooked by steaming until the core temperature reached 85°C and then cooled rapidly in ice water for 1 min [18]. Colorimetric cards made of cyan, magenta, yellow, and key (black) (CMYK) and red, green, and blue (RGB) were used to compare the body colors of shrimp [19]. RGB and CMYK were two ways of representing colors. CMYK, also known as print color mode, produces different colors by adding different proportions of four colors (cyan, magenta, yellow, and key); RGB is a color standard in industry; a variety of colors is obtained by changing three color channels (red, green, and blue) and superimposing them on each other. When using CMYK and RGB to evaluate



FIGURE 1: Chromatograms of all trans Ax, 9-cis-Ax, 13-cis-Ax in the standard (a) and MAAE (b) absorption peak at 474 nm. P_{13-cis} , P_{trans} , P_{9-cis} , and P_{IS} are the peak areas of 13-cis, trans, 9-cis Ax, and internal standard, respectively.

the body color of shrimp, the number after different letters indicates the percentage of each color component.

2.5. Measurement of Shrimp Muscle Texture and Nutritional Composition. Nine live shrimps were randomly selected from the MAAE and control groups to analyze muscle texture and nutritional composition after the feeding trial. Fresh shrimp muscle was isolated after removal of the head and shell, and muscle texture was measured using a TMS-Pro texture analyzer equipped with a 25 N gravity sensor. Measurement conditions were as follows: ambient temperature of approximately 23°C; circular probe measuring 8 mm; compression rate of 30 mm/min; and shape variable of 30%. The measurement indices included hardness, chewiness, gumminess, springiness, cohesiveness, and adhesiveness. Shrimp muscle concentrations of free amino acids were determined using an automatic high-speed amino acid analyzer (LA8080, AminoSAAYA, Hitachi), and the concentrations of 37 fatty acids were determined using a gas chromatograph (Agilent 7890A, Agilent Technologies).

2.6. Statistical Analysis. A randomized study design was applied, and all data were analyzed using an independent samples *t* test. Statistical significance was determined at p < 0.05 for the comparison of mean values. Data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS statistics version 22.0 (SPSS Inc.).

3. Results

3.1. Ax Content in MAAE. The chromatograms of all-trans-Ax, 9-cis-Ax, and 13-cis-Ax in the standard material and MAAE are shown in Figure 1. The total Ax content in MAAE was calculated based on the peak area ratio of the total Ax to the standard solution according to the formula described in the United States Pharmacopeia [15]. The Ax content in MAAE was calculated to be 0.2%.

3.2. Water Quality. During the 30-day experimental period, the basic water quality of the two groups is as follows: MAAE: temperature $28.46 \pm 0.59^{\circ}$ C; salinity 29.06 ± 0.84 ; DO 5.62 ± 0.29 mgL⁻¹; pH 7.91 ± 0.97 , and control group: temperature $28.31 \pm 0.43^{\circ}$ C; salinity 28.31 ± 0.43 ; DO 28.31 ± 0.43 mgL⁻¹; pH 7.97 ± 0.07 . The concentrations of ammonia nitrogen and nitrite in the water column of the two experimental groups were lower than 4.5mgL⁻¹ and 3.5mgL⁻¹, respectively. These water quality indicators were within the range suitable for shrimp growth.

3.3. Growth Performance and Feed Utilization

3.3.1. Growth Performance. The differences in the body weights between the MAAE and control groups are shown in Figure 2. The shrimps in the MAAE and control groups both showed significant growth during the experimental period. The average live body weight of the shrimp fed the MAAE diet was significantly higher than that of the shrimp fed the control diet on day 20 (p < 0.05), but not on days 10 and 30 (p > 0.05).

3.3.2. Feed Utilization. The feed utilization of the control and MAAE groups is shown in Table 2. The final body weight, weight gain, net protein utilization, and survival rate were higher in the MAAE group than in the control group, whereas the feed conversion ratio was lower than that in



FIGURE 2: The body weight change of shrimp cultured with or without MAAE.

the control group, but the differences were not significant (p > 0.05).

3.4. Body Color. The body color of the shrimps before and after cooking is shown in Figure 3. There were significant differences in body color between the MAAE and control groups. The body color of live shrimps from the MAAE group represented a sparrow tea color (R143 G90 B60, C52 M69 Y73 K15), whereas that from the control group represented a spring tea color (R123 G161 B168, C26 M4 Y0 K34). The body color of cooked shrimps from the MAAE and control groups represented a masicotite color (R240 G94 B28, C0 M72 Y90 K0) and thin persimmon color (R236 G184 B138, C0 M34 Y52 K0), respectively (Figure 3).

3.5. Muscle Texture and Nutritional Constitution

3.5.1. Muscle Texture. The muscles of shrimp are collected after 30 days of culture, and their muscle texture properties are shown in Table 3. The springiness of shrimp muscle was slightly higher in the MAAE group than in the control group. Although the hardness, chewiness, gumminess, and adhesiveness of shrimp muscle were slightly lower in the MAAE group than in the control group, this difference was not statistically significant (p > 0.05).

3.5.2. Concentrations of Free Amino Acids. Table 4 shows the composition of free amino acids in the shrimp muscle. The concentrations of Asp, Ser, Ala, Val, and His, were significantly higher in the MAAE group than in the control group (p < 0.05). In contrast, the concentrations of Thr, Glu, Cys, IIe, and Pro were significantly lower in the MAAE group than in the control group (p < 0.05), and there were no significant differences in the concentrations of Gly, Leu, Tyr, Phe, Lys, and Arg between the two groups (p > 0.05). The essential, nonessential, and total amino acids were also calculated and compared, and no significant difference was observed between groups.

3.5.3. Fatty Acids Concentrations. The fatty acid concentrations in shrimp muscle are shown in Table 5. Fifteen fatty acids were detected. The concentrations of $C_{18:0}$, $C_{18:1n9c}$, $C_{20:0}$, $C_{20:2}$, and $C_{24:1}$ did not differ between the two groups (p > 0.05), whereas the concentrations of other fatty acids ($C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:2n6c}$, $C_{18:3n3}$, $C_{20:1}$, $C_{22:1n9}$, $C_{20:5n3}$, and $C_{22:6n3}$) were significantly lower in the MAAE group than in the control group (p < 0.05).

4. Discussion

Microalgae are expected to have a high yield of Ax for extraction if their cell walls are destroyed. Before our Ax extraction, the cell wall of *H. pluvialis* was destroyed by cryogenic crushing to obtain high yield. Ambati et al. [20] reported that *H. pluvialis* is a unicellular freshwater microalga found in many habitats worldwide and is a suitable natural source of Ax. Fukami et al. [21] report that the current sources of Ax products include natural and synthetic types. However, Zhang et al. [22] have demonstrated that natural Ax is more effective and cheaper than synthetic Ax. Therefore, given the emphasis on natural carotenoids in the food and feed industries, this study used natural Ax extracted from *H. pluvialis* to investigate its potential application at the industrial scale.

Several studies have investigated the effects of Ax supplementation on aquatic animals. Storebakken et al. [23] reported that the growth rate of rainbow trout (Oncorhynchus mykiss) was significantly higher than other groups after feeding with dietary Ax (57 mg kg⁻¹) at 5°C and 15°C. Christiansen et al. [24] reported that there was a tendency to higher survival in the groups fed the diets containing Ax when compared with the groups fed the nonsupplemented diets for *Salmo salar*. Cui et al. [25] reported that dietary supplementation with 100 mg kg^{-1} Ax increased the specific growth rate of Cyprinus carpio, while excessive Ax supplementation slowed down the growth rate. Wang et al. [26] also reported that dietary supplementation with Ax at doses of 60 mg kg⁻¹ and 90 mg kg⁻¹ increased the specific growth rate of Apostichopus japonicus by 113.33% and 66.67%, respectively. Pei et al. [13] demonstrated that dietary supplementation with 100 mg kg⁻¹ Ax significantly improved the specific growth rate and increased the survival rate of L. vannamei. Moreover, Robert et al. [27] demonstrated that the addition of 10 mg kg⁻¹ Ax improved the spawning rate of the striped jack Pseudocaranx dentex. Wade et al. [28] reported that an Ax concentration of 100 mg kg^{-1} is the optimal supplementation level for crustaceans, but it raises feed costs by 25-30%. Our pre-experiment showed that dietary supplementation with the MAAE at 60 mg kg^{-1} for 7 days reduced the blue-shell syndrome of L. vannamei by over 60%, which was over three-fold higher than that of the control treatment (data not shown). Therefore, although the concentration of dietary MAAE supplementation was relatively low (20 mg kg^{-1}) in this study, it showed a significant increase in the specific growth rates of the cultured shrimps. This finding is consistent with results reported by Segner et al. [29] and Amar et al. [30], which showed a positive role for Ax supplementation in the intermediary metabolism of aquatic animals, with improvement in nutrient utilization and growth.

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TABLE 2: Performance of	shrimp (initial we	ight and	length	n: 4.46 ± 0.9 g	7.20 ± 1.13 cm	ı) fec	l wit	h MAAE oi	r control	diet i	for 30 d	lays.
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	FBW	WG	NPU	FCR	Survival
MAAE	16.37 ± 2.33^{a}	258 ± 60^{a}	1.20 ± 0.25^{a}	$0.43\pm0.09^{\rm a}$	98%
Control	15.36 ± 2.44^{a}	244 ± 54^{a}	1.08 ± 0.26^{a}	0.47 ± 0.11^{a}	97%

Abbreviations: FBW: final body weight (g); WG: weight gain (%) = [(final body weight – initial body weight)/initial body weight] × 100. NPU: net protein utilization ratio (%) = (crude protein of muscle/crude protein into feed) × 100. FCR: feed coefficient ratio (%) = (feed consumption/weight gain) × 100. Note: fifty shrimps were randomly selected to measure the body length and body weight for calculation. Data with different superscripts in the same column indicate significant differences (p < 0.05).



FIGURE 3: Body color of shrimp fed with MAAE or control diet for 30 days before (a) and after cooking (b).

	Hardness/N	Chewiness/mJ	Gumminess/N	Springiness/mm	Cohesiveness	Adhesiveness/mJ
MAAE	3.20 ± 0.43^{a}	2.69 ± 1.19^{a}	1.33 ± 0.41^{a}	$1.97\pm0.37^{\rm a}$	0.41 ± 0.09^{a}	0.105 ± 0.062^{a}
Control	3.71 ± 0.65^a	3.05 ± 1.27^a	$1.53\pm0.43^{\rm a}$	1.95 ± 0.25^{a}	0.41 ± 0.05^a	0.112 ± 0.056^a

TABLE 3: Muscle texture properties of shrimp fed with MAAE or control diet for 30 days.

Note: data with different superscripts in the same column indicate significant differences (p < 0.05).

Ax can be deposited in tissues when it enters animals. It can nonspecifically bind to hemoglobin to improve animal color. Cui et al. [25] added Ax to the feed have shown that the pigmentation of koi fish (*Cyprinus carpio*) skin was significantly improved. Similarly, Chen et al. [31] reported that dietary Ax supplementation resulted in ideal pigmentation levels in Chinese goldfish. In crustaceans, pigmentation is mainly localized to the shell, gonads, and hepatopancreas, with little pigmentation in the meat. Shrimp shells are usually red during ripening. This study showed that with added MAAE in the feed, shrimp had a more pronounced red color than those fed the control diet in an indoor-cultured system. It is consistent with that reported by Zhi et al. [32], who showed that the addition of Ax-rich algal powder to the diet could enhance shrimp pigmentation.

Espe et al. [33] demonstrated that flesh quality characteristics such as nutritional level, flavor, and texture are determined by biological factors such as muscular organization, protein, amino acid, and lipid concentrations. Thomas et al. [34] reported that the foundation of quality characteristics is the selective retention of nutrients and biochemical substances from the feed. For instance, Hagen et al. [7] reported that dietary fat and protein levels, as well as their sources, affect the nutritional value and texture of Atlantic cod. Lie et al. [35] reported that the texture profile is associated with consumer acceptance and can be assessed by measuring muscle hardness, chewiness, springiness, and cohesiveness. However, in this study, the texture characteristics of flesh from the MAAE group were not significantly different compared to that of the control group. This may be due to the relatively low supplementation level or short experimental period.

Ma et al. [36] reported that free amino acids, the main water-soluble flavor precursors in muscle, are often used as flesh quality indices of crustaceans. Generally, meat has a sweet taste when it contains abundant free Gly, Ala, Ser, Thr, Lys, and Pro and is full of umami taste when it contains a high content of glutamic and aspartic acids. Lopez-Cervantes et al. [37] reported that Glu and Asp are amino acids that are directly related to umami taste. In this study, the addition of the MAAE significantly increased the Asp content in shrimp meat, indicating the enhancement of shrimp meat flavor. Wade et al. [14] found that Ax monoesters are enriched with saturated fatty acids, whereas Ax diesters are enriched with monounsaturated and polyunsaturated fatty acids. In our study, the addition of MAAE reduced the content of 10 fatty acids in shrimp meat, but there was no significant difference in the levels of the other five fatty acids.

TABLE 4: Muscle amino acids profile in shrimp fed with MAAE diet or control diet for 30 days (g 100 g^{-1}).

	MAAE	Control
Asp	0.0756 ± 0.0355^{a}	$0.0586 \pm 0.0300^{\rm b}$
Thr	0.203 ± 0.017^{b}	0.247 ± 0.030^a
Ser	$0.030 \pm 0.002^{\rm a}$	0.025 ± 0.032^b
Glu	0.103 ± 0.005^{b}	0.111 ± 0.027^{a}
Gly	1.05 ± 0.13^{a}	$1.10\pm0.87^{\rm a}$
Ala	0.366 ± 0.080^{a}	0.278 ± 0.086^b
Cys	$0.0028 \pm 0.0003^{\rm b}$	0.0050 ± 0.0027^{a}
Val	0.0141 ± 0.0167^{a}	$0.0049 \pm 0.0009^{\rm b}$
Met	ND	ND
IIe	$0.012 \pm 0.001^{\mathrm{b}}$	0.019 ± 0.006^{a}
Leu	0.029 ± 0.086^{a}	0.037 ± 0.016^{a}
Tyr	0.031 ± 0.007^{a}	0.037 ± 0.018^a
Phe	0.015 ± 0.003^{a}	0.019 ± 0.016^{a}
Lys	$0.040 \pm 0.010^{\mathrm{a}}$	0.048 ± 0.013^a
His	0.043 ± 0.006^{a}	$0.035 \pm 0.003^{\mathrm{b}}$
Arg	$0.678 \pm 0.016^{\mathrm{a}}$	0.677 ± 0.033^{a}
Pro	$0.41\pm0.02^{\rm b}$	$0.52\pm0.10^{\rm a}$
EAA	$1.24\pm0.10^{\rm a}$	1.94 ± 0.20^{a}
NEAA	$7.88\pm0.18^{\rm a}$	$8.30\pm0.28^{\rm a}$
Total	9.12 ± 0.16^a	10.24 ± 0.18^{a}

Note: Different letters indicate significant differences between treatments in the same line (p < 0.05). Abbreviations: ND: no ingredients detected (<0.0075); EAA: essential amino acid, the sum of Lys, Phe, Met, Thr, IIe, Leu, Val, His; NEAA: nonessential amino acid, the sum of the other amino acids.

TABLE 5: Fatty acids profile in muscle of shrimp fed with MAAE diet or control diet for 30 days (g 100 g^{-1}).

	MAAE	Control
C _{16:0}	0.128 ± 0.012^{b}	0.148 ± 0.009^{a}
C _{16:1}	0.0044 ± 0.0006^{b}	0.0059 ± 0.0005^{a}
C _{17:0}	$0.0090 \pm 0.0008^{\mathrm{b}}$	$0.0100 \pm 0.0005^{\rm a}$
C _{18:0}	0.1075 ± 0.0099^{a}	0.1101 ± 0.0059^{a}
C _{18:1n9c}	0.093 ± 0.008^{a}	0.096 ± 0.007^{a}
C _{18:2n6c}	$0.098 \pm 0.012^{\mathrm{b}}$	0.107 ± 0.006^{a}
C _{20:0}	$0.0072 \pm 0.0027^{\mathrm{a}}$	0.0077 ± 0.0004^{a}
C _{18:3n3}	$0.0050 \pm 0.0010^{\mathrm{b}}$	0.0062 ± 0.0004^{a}
C _{20:1}	$0.0050 \pm 0.0049^{\mathrm{b}}$	0.0059 ± 0.0004^{a}
C _{20:2}	0.0090 ± 0.0010^{a}	$0.0088 \pm 0.0030^{\mathrm{a}}$
C _{22:0}	0.0055 ± 0.0032^{a}	$0.0053 \pm 0.0030^{\mathrm{a}}$
C _{22:1n9}	$0.0190 \pm 0.0037^{\mathrm{b}}$	0.0216 ± 0.0012^{a}
C _{20:5n3}	$0.0507 \pm 0.0120^{\mathrm{b}}$	0.0622 ± 0.0038^{a}
C _{24:1}	0.0013 ± 0.0019^{a}	$0.0012 \pm 0.0018^{\rm a}$
C _{22:6n3}	0.0834 ± 0.0205^{b}	0.1033 ± 0.0053^{a}

Note: Different letters indicate significant differences between treatments in the same line (p < 0.05).

This indicates that dietary supplementation with MAAE at low doses may reduce the content of some fatty acids in shrimp muscle.

5. Conclusion

Dietary supplementation of MAAE at relatively low doses of 20 mg kg^{-1} in indoor industrial shrimp aquaculture could improve the specific growth rate and shell pigmentation of shrimps, thereby decreasing the occurrence of blue-shell syndrome. The dosage of the MAAE effectively increased the content of flavor-related amino acids and significantly reduced the levels of fatty acids in shrimp muscle. Therefore, low doses of Ax at 20 mg kg^{-1} may be adopted as an effective supplementation concentration to overcome blue-shell syndrome and improve the nutritional value of shrimps in indoor industrial aquaculture systems.

Data Availability

The data that support the findings of this study are available from the corresponding author.

Conflicts of Interest

The authors have no conflict of interest to declare.

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