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Application of Astaxanthin-Added Feed in *Macrobrachium nipponense* Culture

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Research Article

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Abstract

To investigate the effects of astaxanthin-supplemented feed on the growth performance, hatching success, and coloration of *Macrobrachium nipponense*, two types of feed were evaluated: one with added astaxanthin (experimental group) and one regular (control group). Two separate aquaculture trials were conducted—one in autumn (August 2023) and another in spring (March 2024). In the autumn experiment, the experimental group prawns at Canlin Family Farm exhibited a 3.6% increase in average body length compared to the control group. At Jiangsu Provincial Breeding Base for *M. nipponense*, the experimental group showed a 10.3% increase in average body weight relative to the control group. Additionally, the experimental group prawns demonstrated a 1.8% increase in average body length and a 10.3% increase in average body weight compared to the control group.

In the spring experiment, after one month of feeding, the experimental group prawns exhibited significantly greater body length and weight compared to the control group ($P < 0.05$), with an average body length increase of 4.6% and an average body weight increase of 11.0% . After two months of feeding, the experimental group showed even more pronounced differences, with an average body length increase of 15.9% and an average body weight increase of 63.7% compared to the control group. The prawns in the experimental group exhibited a deeper coloration compared to those in the control group, indicative of increased pigment accumulation. Furthermore, the experimental group had a higher proportion of brooding females and a greater broodiness rate than the control group. The results indicate that the inclusion of astaxanthin in the feed can increase its nutritional content, enhance the weight gain rate and broodiness rate of the *M. nipponense* prawn, and significantly improve the coloration of the prawn.

Keywords: Astaxanthin; *Macrobrachium nipponense*; Growth Performance; Coloring

Introduction

Macrobrachium nipponense is naturally distributed across several regions in East Asia, including China, Japan, Korea, Vietnam, Myanmar, and Taiwan [1-3]. Its culture began in Southeast Asian countries in the 1990s.*Macrobrachium nipponense* has a high consumption in China because of its good taste and high nutritional value. Despite its small size,

Macrobrachium nipponense exhibits notable adaptability to environmental stressors, which contributes to its viability in aquaculture [4].

According to statistics, the production of *Macrobrachium nipponense* in 2021 was approximately 230 thousands tons in China, making it the greatest among the world. Currently, however, aquaculture farmers usually use feeds for *Macrobrachium rosenbergii* or *Litopenaeus vannamei* to culture *Macrobrachium nipponense*, resulting in problems such as weak palatability and low absorption [5].

Astaxanthin is a ketone carotenoid with strong biological activities such as antioxidant, anti-inflammatory, anti-tumor, neuroprotective, and coloring properties [6]. It widely presents in the bodies of prawn and crabs and plays a very important role. It cannot be synthesized and can only be obtained through dietary intake. Studies have shown that adding astaxanthin to feed has positive effects on crustaceans, such as improving pigmentation, reproductive ability, and immunity [7-9]. At the same time, astaxanthin has a coloring effect, which can improve the muscle and skin color of farmed fish, enhance meat quality and commercial value, and is widely used in ornamental fish feed to meet the demand of ornamental fish for bright body colors [10]. Wang JH, et al. [11] stated that adding 400 mg/kg astaxanthin to feed can effectively enhance the growth performance, coloring effect, and antioxidant capacity of *Cyprinus carpio*. Li, et al. [12] found that supplementing astaxanthin in the feed can improve the growth performance and antioxidant capacity of blood parrots. The study by Cheng Y, et al. [13] indicates that adding 400 mg/kg astaxanthin to feed can significantly improve the growth performance and non-specific immunity of *Procambarus clarkii*.

Former research on *Litopenaeus vannamei* has shown that adding 50mg/kg astaxanthin to feed can enhance the stress response of juvenile prawns, promote their growth rate, survival rate, and feed conversion rate [14]. The study by Jian X, et al. [15] showed that adding astaxanthin to the feed of *Penaeus vannamei* can result in an increase in survival rate, as well as a significant increase in weight gain rate, specific growth rate, and phenoloxidase activity. However, the effect of astaxanthin-added feed on *Macrobrachium nipponense* remains unknown.

The aim of this study was to investigate the effects of astaxanthin-supplemented feed on the growth performance, hatching success, and coloration of *Macrobrachium nipponense*, providing insights for its application in compound feed.

Materials and Methods

Experimental Site

Aquaculture experiments were conducted at the Jiangsu Provincial Breeding Base for *M. nipponense* and at Canlin Family Farm, Hushu Street, Nanjing City. Two comparable ponds, each measuring 5 mu with flat bottoms, leveled and disinfected silt, and separate water intake and drainage systems, were designated as the experimental and control ponds.

Experimental Animals

The *M. nipponense* prawns used in this experiment were all from the same batch, with body lengths of 3.6-3.8 cm and weights of 0.7-0.9 g, belonging to the "Taihu No.2" *M. nipponense* prawns. Healthy juvenile prawns with plump bodies, translucent appearance, firm and elastic muscles, strong activity, swift swimming, smooth body surfaces, and no injuries or diseases were randomly selected. Approximately 50,000 prawns were collected from each group. The autumn breeding experiment began in early July 2023, and the spring breeding experiment in late February 2024.

Experimental Feed

The control group feed was a regular *M. nipponense* prawn compound feed from Jiangsu Changshou Group Nanshan Feed Co., Ltd., with the main components including imported fish meal, imported fish oil, prawn meal, fermented soybean oil, high-gluten flour, yeast extract, soybean phospholipid oil, vitamins, and trace elements. The composition of the feed is listed in Table 1.

Nutrient Composition	Regular *M. nipponense* prawn compound feed (%)
crude protein	≥38.0
crude fat	≥5.0
Ash	≤18.0
Crude fiber	≤6.0
total phosphorus	≥1.0
Lysine	≥1.9
water	≤12.0

Table 1: Content of feed ingredients.

The experimental group feed was the "Taihu No.2" *M. nipponense* prawn specialty feed developed by the Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences, this feed adds astaxanthin on top of the regular *M. nipponense* compound feed (containing 0.2% astaxanthin-rich Phaffia rhodozyma, with an astaxanthin content of 0.5%) [16].

Feeding

The experimental group was fed the diet, while the control group was fed regular *M. nipponense* prawn feed. Both groups strictly adhered to the experimental requirements for feeding, with daily feeding amounts ranging from 5% to 8% of body weight (adjusted based on feeding and growth conditions, to be fully consumed within 1 h). Feeding was also adjusted according to weather and water quality factors. The autumn breeding experiment began in August 2023 and the spring breeding experiment in March 2024.

Daily Management

Ponds were inspected daily and oxygenation equipment was promptly activated to prevent oxygen deficiency. Potassium bisulfate was used every 20 days for substrate improvemen to enhance the bottom environment and reduce the risk of parasites. Lactobacillus was added once or twice to the entire pond to regulate water quality and maintain water stability.

Collection of Growth Parameters

After the aquaculture experiments, *M. nipponense* prawns were fasted for 24 h before sampling. Thirty prawns were randomly selected for each pond to measure body length and weight, and growth parameters were collected for both the experimental and control groups. Each prawn was placed on filter paper for several minutes (to remove excess water) and then weighed with an accuracy of 0.1 g (Weight).

Subsequently, we measured the carapace length and width of each individual using a vernier caliper (accuracy ± 0.01 mm). The body length is the length of the prawn from the base of the eyestalk to the end of the caudal segment of the prawn.

The autumn breeding experiment collected data on November 21, 2023, and the spring breeding experiment began sampling on March 27, with subsequent sampling every week eight times.

Statistical Analysis

T-test were employed to compare the differences between the experimental and control group. A difference of $P < 0.05$ was considered to be of significance. All statistical analyses were carried out with SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Growth Performance

The autumn breeding experiment: the body length and weight of *M. nipponense* prawns in the experimental group at Canlin Family Farm were higher than those in the control group ($P>0.05$). The average body length (5.7±0.5 cm) of the experimental group was 3.6% higher than that of the control group (5.5±0.8 cm), and the average weight (3.2±0.9 g) was 10.3% higher. Similarly, at the Jiangsu Provincial Breeding Base for *M. nipponense*, the experimental group's average body length (5.6±0.8 cm) was 1.8% higher, and the average weight (3.2±1.5 g) was 10.3% higher (Table 2).

	Canlin Family Farm		Jiangsu Provincial Breeding Base for *M. nipponense*	
	Experimental group	**Control group**	**Experimental group**	**Control group**
Body Length/cm	5.7±0.0.5[a]	5.5±0.8[a]	5.6±0.8[c]	5.5±0.8[c]
Weight/g	3.2±0.9[a]	2.9±1.2[a]	3.2±1.5[c]	2.9±1.3[c]

Note: Different letters of the same row indicate significant differences ($P<0.05$), while the same letter indicates no significant differences ($P>0.05$).

Table 2: Comparison of average body length and weight of *M. nipponense* prawns in the autumn breeding experiment.

The spring breeding experiment: Initial sampling showed no significant difference in body length and weight between the experimental and control groups. However, by the third sampling (one month after feeding), the experimental group's body length and weight were significantly higher than the control group's ($P < 0.05$). The average body length (5.05±0.71 cm) was 4.6% higher, and the average weight (2.43±1.37 g) was 11.0% higher. By the final sampling (two months after feeding), the experimental group's body length (6.18±0.67 cm) was 15.9% higher, and the weight (5.32±1.90 g) was 63.7% higher (Table 3).

Sampling stage/number	Body length/cm		Weight/g	
	Experimental group	Control group	Experimental group	Control group
0	3.80±0.43[a]	3.59±0.48[a]	0.70±0.26[c]	0.71±0.32[c]
1	3.81±0.41[a]	3.75±0.42[a]	0.84±0.33[c]	0.85±0.41[c]
2	3.96±0.78[a]	3.98±0.71[a]	1.09±0.70[c]	1.08±0.56[c]
3	5.05±0.71[a]	4.83±0.62[b]	2.43±1.37[c]	2.19±0.91[d]
4	5.52±0.69[a]	5.19±0.67[b]	3.36±1.25[c]	2.69±1.14[d]
5	5.44±0.66[a]	5.31±0.73[b]	3.02±1.24[c]	2.75±1.30[d]
6	5.67±0.60[a]	5.33±0.61[b]	3.88±1.43[c]	2.96±1.24[d]
7	6.18±0.67[a]	5.33±0.98[b]	5.32±1.90[c]	3.25±1.97[d]

Note: Different letters of the same row indicate significant differences ($P<0.05$), while the same letter indicates no significant differences ($P>0.05$).

Table 3: Comparison of average body length and weight of *M. nipponense* prawns in the spring breeding experiment.

Research has shown that astaxanthin has a promoting effect on the growth of aquatic animals and can be used as a feed additive [17]. When astaxanthin is added to the feed, compared to the control group, the experimental group's feed coefficient significantly decreases, and both body length and weight increase. This is consistent with the findings in studies on *Litopenaeus vannamei* [18] and *Penaeus japonicus* [19], where the addition of astaxanthin in feed can enhance the antioxidant capacity of cultured animals and promote growth. The significant impact of astaxanthin in this experiment may be due to the relatively high dosage added, with an optimal addition level of 400 mg/kg for astaxanthin in feed for *Procambarus clarkii* [20], while this experiment added 1000 mg/kg of astaxanthin. However, this does not prove that higher astaxanthin content leads to faster prawn growth. Excessive doses of astaxanthin may not necessarily benefit growth performance, as they can inhibit the expression of certain crucial genes or promote abnormal expression of other important genes, potentially disrupting normal growth metabolism [21].

Color

Upon observation of various groups of *M. nipponense* prawns, it was found that in terms of body color, the experimental group of *M. nipponense* prawns fed with astaxanthin feed exhibited a deeper body color compared to the control group, with more pigment accumulation and sedimentation (Figures 1, 3 & 5). Processing the experimental and control groups using the Photoshop color picker, revealed that the experimental group of *M. nipponense* prawns (Figure 4) had R, G, and B values of 55, 45, and 27, respectively, while the control group of *M. nipponense* prawns (Figure 2) had R, G, and B values of 122, 100, and 49, respectively (lower RGB values indicate a deeper color). Through numerical comparison, it was found that the RGB values of the experimental group were lower than those of the control group, indicating that the experimental group with astaxanthin had a deeper color than the control group.

Figure 1: Autumn breeding of *M. nipponense* prawns (Top: Control group, Bottom: Experimental group).

Figure 2: Autumn breeding of *M. nipponense* prawns Boiled (Left: Control group, Right: Experimental group).

Figure 3: Spring breeding of *M. nipponense* prawns (Experimental group).

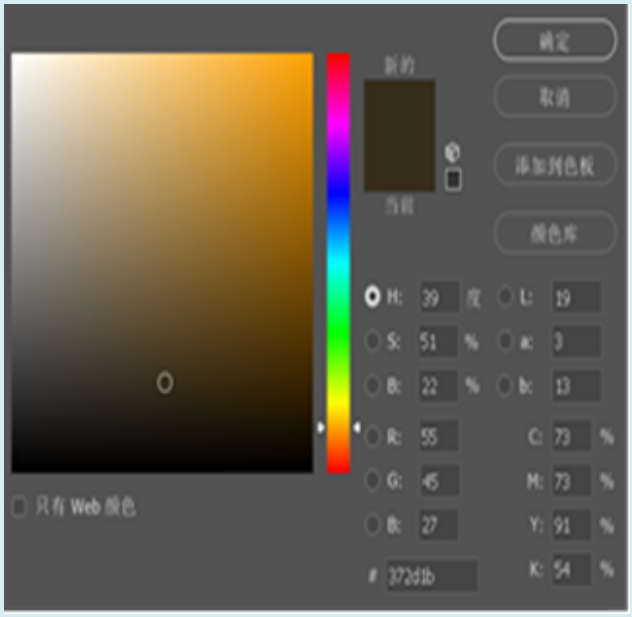

Figure 4: Colorimetric Values of *M. nipponense* prawns in the autumn breeding (Experimental group).

Figure 5: Spring breeding of *M. nipponense* prawns (Control group).

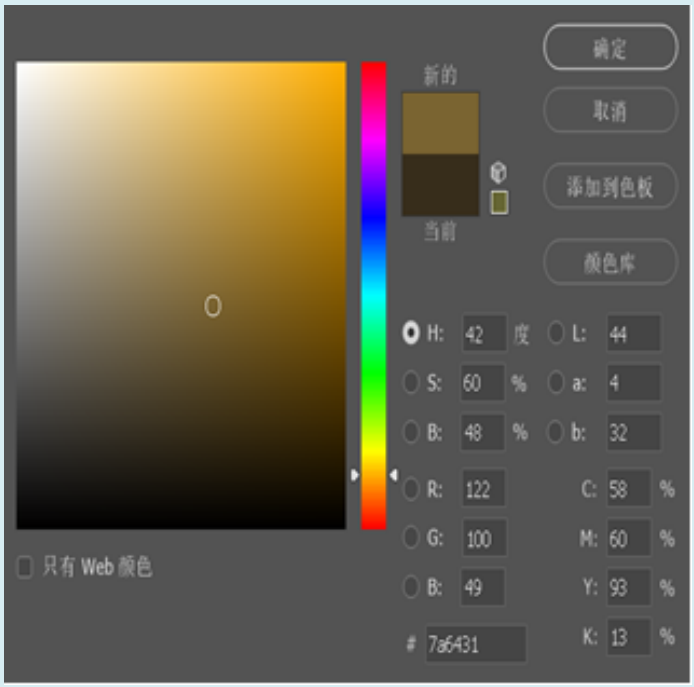

Figure 6: Colorimetric Values of *M. nipponense* prawns in the Spring breeding (Control group).

Figure 7: Boiled *M. nipponense* prawns in the spring breeding (Left: Experimental group, Right: Control group).

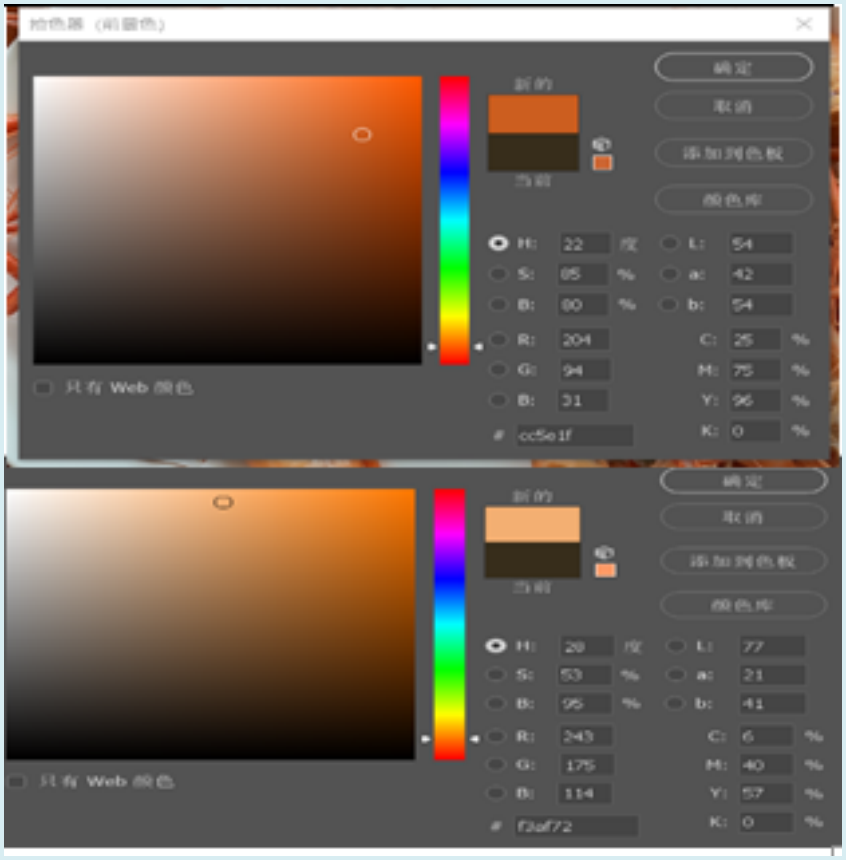

Figure 8: Colorimetric Value Detection of *M. nipponense* prawns in the Spring breeding (Top: Experimental group, Bottom: Control group).

Upon observation of the two groups of *M. nipponense* prawns after boiling, it was found that the experimental group of J *M. nipponense* prawns exhibited a more pronounced orange-red color with a brighter hue (Figures 2 & 7). This indicates that astaxanthin can promote pigment accumulation in the bodies of *M. nipponense* prawns, thereby enhancing their color intensity and making their body color more vibrant. By processing the experimental and control groups of boiled prawns using the Photoshop color picker, we found that the astaxanthin-fed group of *M. nipponense* prawns (Figure 8) had R, G, and B values of 204, 94, and 31, respectively, whereas the control group of *M. nipponense* prawns (Figure 8) had R, G, and B values of 243, 175, and 114, respectively (lower RGB values indicate a deeper color). Through numerical comparison, it was found that the RGB values of the experimental group were lower than those of the control group, indicating that the experimental group with added astaxanthin had a deeper color than the control group.

Aquatic animals generally have a limited ability to synthesize astaxanthin de novo [22]. In prawns and other crustaceans, astaxanthin is the predominant pigment, constituting 86% to 98% of total carotenoids, and is crucial for their coloration [23]. Although crustaceans cannot produce astaxanthin on their own, they can convert exogenous carotenoids from their diet into astaxanthin or directly store dietary carotenoids, including astaxanthin, to achieve appropriate coloring [24,25]. Thus, feed composition significantly influences crustacean coloration. This study demonstrates that astaxanthin enhances the coloration of *M. nipponense*, making the liver and pancreas more vivid and increasing their ornamental value and market appeal.

Benefit

From November to December 2023, the autumn breeding experiment recorded sales data and actual yield comparisons for both experimental and control groups (Table 4).

	Yield/kg	Yield per mu/kg	Commodity prawn yield/kg	Commodity prawn Yield per mu /kg	Revenue/¥	Average revenue per mu/¥
Experimental group	755	151	272	54	36220	7244
Control group	698	139	202	40	31040	6208

Table 4: Autumn breeding Experiment Yield Comparison - Jiangsu Provincial Breeding Base for *M. nipponense.*

	Yield/kg	Yield per mu/kg	Commodity prawn yield/kg	Commodity prawn yield per mu /kg	Revenue/¥	Average revenue per mu/¥
Experimental group	1357	167	553	68	68352	8438
Control group	968	158	338	55	45916	7527

Table 5: Autumn breeding Experiment Yield Comparison - Canlin Family Farm.

Data analysis using SPSS 20.0 Tables 4 & 5 showed that both the yield and average revenue per mu were higher in the experimental group than in the control group. The experimental group's total yield and average revenue per mu increased by 7.5% and 14.3% at the Jiangsu Provincial Breeding Base for *M. nipponense*, and by 5.3% and 12.1% at Canlin Family Farm.

Hatching

	Sampling count/individuals	Broodstock/individuals	Holding egg rate/%
Experimental group	70	40	67.5
Control group	70	47	59.6

Table 6: Hatching of *M. nipponense* prawns.

As shown in Table 6, on May 17, approximately 70 *M. nipponense* prawns from each group were sampled and their holding egg rate are calculated. The experimental group had 40 broodstocks, with 27 egg-bearing, shows a holding egg rate of 67.50%. The control group had 47 broodstocks, with 28 egg bearing, shows a holding egg rate of 59.57%. The experimental group had a higher holding egg rate than that of the control group.

Research has shown that adding astaxanthin to feed can protect the ovarian development of female prawns and enhance their reproductive ability. The content of astaxanthin in prawn can affect the quality of juvenile embryos, and a shortage of astaxanthin can lead to a decrease in juvenile quality [26].

Astaxanthin can enter the nucleus as an active substance, be received by hormone receptors as a precursor, activate sex hormones, and promote accelerated development of oocytes [27]. Wang ZX, et al. [28] have shown through research that adding an appropriate amount of astaxanthin to feed can increase the yolk protein content of parent shrimp ovaries, hatching rate of fertilized eggs, metamorphosis rate of larvae, and the number of nauplii and flea like larvae, thereby improving the reproductive performance of female prawn. In this experiment, astaxanthin can increase the egg holding rate of female prawn and effectively improve the reproductive ability of *M. nipponense*, which is consistent with the above research.

Conclusion

Astaxanthin, a natural nutrient, is widely utilized in aquaculture due to its unique properties. The results of this experiment indicate that astaxanthin-supplemented feed enhances coloration and improves the growth and hatching ratio of *M. nipponense*. Future research will focus on investigating the impact of astaxanthin-supplemented feed on the nutritional composition and flavor of *M. nipponense*.

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