

## Research Article

# Effects of dietary lycopene on water quality parameters, antioxidant status, and digestive enzymes activities in the oriental river prawn, *Macrobrachium nipponense*

Ettefaghdoost M.<sup>1\*</sup>, Babakhani A.<sup>1</sup>, Alaf Noveirian H.<sup>1</sup>

<sup>1</sup>Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran

\*Correspondence: ettefaghdoost@phd.guilan.ac.ir

Keywords	Abstract
Antioxidant activity, Carotenoids, Diets, Immunity, Oriental river prawn	Lycopene is a major carotenoid pigment recognized for its potent antioxidant properties and its role as an essential micronutrient in aquafeeds, contributing to enhanced antioxidant activity, immune response, and feed efficiency. This study evaluated the effects of dietary lycopene supplementation on growth performance, antioxidant capacity, and digestive enzyme activities in the oriental river prawn ( <i>Macrobrachium nipponense</i> ). A total of 225 prawns with an average initial weight of $1.40 \pm 0.07$ g were fed five formulated diets containing 0 (control), 50, 100, 150, and 200 mg kg <sup>-1</sup> lycopene for 56 days. At the end of the feeding trial, hepatopancreas and intestinal tissues were collected for the analysis of growth performance, antioxidant indicators, and digestive enzyme activities using standard biochemical kits and spectrophotometric methods. The results showed that lycopene supplementation significantly improved growth performance and total antioxidant capacity, while the levels of superoxide dismutase, catalase, and malondialdehyde were significantly reduced compared with the control group ( $p < 0.05$ ). Digestive enzyme activities were also influenced by lycopene levels, with the highest activities observed in prawns fed 200 mg kg <sup>-1</sup> lycopene and the lowest in the control group ( $p < 0.05$ ). Overall, these findings suggest that a dietary inclusion of 200 mg kg <sup>-1</sup> lycopene is optimal for enhancing antioxidant status and digestive enzyme activity in <i>M. nipponense</i> juveniles.
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## Introduction

Optimal diet formulation is a key factor influencing growth performance, immune responses, and resistance to pathogens in aquatic species. A well-balanced diet not only enhances the production of high-quality products but also improves the resilience of cultured organisms to environmental stressors (Jescovitch *et al.*, 2018). The expansion of prawn aquaculture largely depends on dietary additives in addition to core nutrients such as carbohydrates, proteins, and lipids, since basic formulations alone cannot meet all the nutritional and physiological requirements necessary for optimal growth and immune function (Flegel, 2019).

Among various dietary additives, carotenoids are lipid-soluble pigments that accumulate in several tissues of aquatic organisms—including muscles and exoskeleton—and play critical roles in growth, immunity, and pigmentation (Shahidi and Brown, 1998; de Carvalho and Caramujo, 2017). In aquaculture, carotenoids are widely applied not only as color enhancers but also as bioactive compounds with multiple physiological functions. They act as precursors for certain vitamins, protect against ultraviolet radiation, prevent the oxidation of essential fatty acids, and contribute to immune development, fertility, and cellular defense mechanisms. Lycopene, in particular, is a natural antioxidant that neutralizes free radicals generated during normal cellular metabolism and under environmental stress, thereby promoting better growth, immunity, and feed utilization in aquatic species (Wade *et al.*, 2017; Nakano and Wiegertjes, 2020).

Increasing global demand for freshwater prawns and the need to improve production efficiency have driven researchers and aquaculturists to develop innovative strategies, including the introduction of new and non-native species with desirable aquaculture traits (Cocke *et al.*, 2017). The oriental river prawn (*Macrobrachium nipponense*), along with other members of the *Macrobrachium* genus, represents an ecologically and economically important group inhabiting freshwater ecosystems such as wetlands, rivers, and estuaries across temperate regions (De Grave and Ghane, 2006). This species exhibits several favorable characteristics for aquaculture, including efficient reproduction under both natural and controlled conditions, adaptability to cage, pond, intensive, and semi-intensive culture systems, and compatibility with polyculture operations. These attributes, coupled with its increasing aquaculture trend since the 1990s, highlight its potential as a valuable candidate for freshwater prawn farming (Lavajoo *et al.*, 2019; FAO, 2020).

Previous studies have shown that *M. nipponense* readily adapts to formulated feeds and displays rapid growth within short rearing periods. Its nutritional and culture requirements, however, demand particular attention, as optimized dietary composition can substantially accelerate growth performance under culture conditions (Ding *et al.*, 2017; Ettefaghdoost *et al.*, 2018; Lavajoo *et al.*, 2019). Moreover, lycopene can be efficiently extracted from tomato processing by-products—such as pulp, seeds, and skins—making it an affordable and sustainable

natural pigment source for aquafeed formulation (Mahfuzur *et al.*, 2018).

Several studies have investigated the effects of carotenoid supplementation in crustaceans, including those of Zhang *et al.* (2013) on whiteleg shrimp (*Litopenaeus vannamei*), Jin *et al.* (2014) on black tiger shrimp (*Penaeus monodon*), and Cheng and Wu (2019) on red swamp crayfish (*Procambarus clarkii*). Building upon these findings, the present study aimed to evaluate the effects of dietary lycopene supplementation on growth performance, antioxidant status, and digestive enzyme activity in the oriental river prawn—a species with high economic potential and adaptability to diverse freshwater environments.

## Materials and methods

### Prawn culture conditions

This study was conducted at Fishland Aquarium (Rasht, Guilan, Iran) for a period of 56 days from September to October 2019. Wild oriental river prawns were collected from the Siah Darvishan River (49°30' E, 25°37' N; -15 m above sea level, Sowmeh Sara, Guilan Province, Iran) using nets and traps. Individuals with a body weight of 1.0–1.5 g and an average total length of approximately 5 cm were selected and transferred to the experimental facility. The prawns were acclimated for 14 days in 700 L polyethylene tanks under controlled physicochemical water conditions. During acclimation, they were fed ad libitum with a basal diet formulated for *M. nipponense* containing 45% protein, 5% lipid, 14% ash, 9–10% moisture, and 18 kJ g<sup>-1</sup> gross energy (1 mm crumble) (Ettefaghdoost *et al.*, 2018). After acclimation, prawns with a

mean body weight of 1.40±0.07 g and length of 5.0±0.32 cm were randomly distributed into 15 glass aquaria (60 L each) with 15 prawns per aquarium. Continuous aeration was provided using air stones connected to a Danner central aerator (AP-100, New York, USA). Approximately one-third of the water volume in each tank was replaced daily before feeding, and full water exchange was performed during biometric measurements. A 12:12 h light–dark photoperiod was maintained using fluorescent lamps throughout the experimental period.

### Diet preparation and experimental design

Experimental diets were formulated based on the basal diet of *M. nipponense* using Lindo™ software (Version 10, Illinois, USA). All feed ingredients were ground using a Moulinex grinder (AR1044, Paris, France) and sieved through a 100 µm mesh to obtain a homogeneous mixture. The ingredients were weighed precisely and mixed thoroughly for 10–15 minutes. Approximately 30% distilled water (based on dry matter) was added to form a cohesive mixture, which was then processed through a Muyang extruder (HSH10, Jiangsu, China) to produce 1 mm pellets. Lycopene powder (10%; Adonis Daru Co., Tehran, Iran) was dissolved in distilled water using an INTLLAB™ magnetic stirrer (MS-500, Kuala Lumpur, Malaysia) and uniformly sprayed onto the diets. The feed was air-dried and stored at -16 °C until use. Because lycopene is sensitive to heat and light, daily rations were kept at 4°C in opaque containers. Five experimental diets were prepared with lycopene levels of 0 (control), 50, 100, 150,

and 200 mg kg<sup>-1</sup>. Each treatment was conducted in triplicate. Prawns were hand-fed four times daily (08:00, 12:00, 16:00, and 20:00 h) at 3% of the average biomass per aquarium (Ettefaghdoost *et al.*, 2015; Ding *et al.*, 2017; Ettefaghdoost and Alaf-

Noveirian, 2017). The composition and proximate analysis of the experimental diets are presented in Table 1 (AOAC, 2016).

**Table 1: Composition and proximate analysis of experimental diets for the oriental river prawn.**

Experimental diets	Lycopene (mg kg <sup>-1</sup> )			
	Control	50	100	150
<b>Ingredients (%)</b>				
Fishmeal <sup>1</sup>	30	30	30	30
Soy meal	30	30	30	30
Wheat meal	7	7	7	7
Corn meal	7	7	7	7
Casein <sup>2</sup>	16	16	16	16
Vitamin premix <sup>3</sup>	2	2	2	2
Mineral premix <sup>4</sup>	2	2	2	2
Cholesterol <sup>5</sup>	0.2	0.2	0.2	0.2
Vitamin C <sup>6</sup>	0.1	0.1	0.1	0.1
Dicalcium phosphate <sup>7</sup>	0.5	0.5	0.5	0.5
Filler (CMC) premix <sup>8</sup>	5.2	5.195	5.19	5.185
Carotenoid pigment (Lycopene) <sup>9</sup>	0	0.005	0.01	0.015
<b>Proximate composition (dry matter basis)</b>				
Moisture (%)	9.43	9.62	9.59	9.32
Crude protein (%)	44.86	44.71	44.57	44.61
Crude lipid (%)	4.88	5.19	4.63	4.49
Fiber (%)	2.79	2.91	2.84	2.78
Ash (%)	14.66	14.83	14.36	14.29
Nitrogen-free extract (%)	32.81	32.36	33.60	33.83
Gross energy (KJ g <sup>-1</sup> ) <sup>10</sup> (%)	18.23	18.38	18.19	18.12
Total carotenoids (mg kg <sup>-1</sup> )	4.52	51.89	108.06	155.77
				198.41

<sup>1</sup> Mazandaran Animal and Aquatic Feed (Sari, Mazandaran, Iran).

<sup>2</sup> Quelab Laboratories Inc. (Montreal, Quebec, Canada)

<sup>3</sup> The Science Laboratories (Qazvin, Qazvin, Iran) – Each 1000 g premix contained; 1600000 IU retinol, 1 IU 400000 calciferol, 40 g alpha tocopherol, 2 g menadione, 6 g thiamine, 8 g riboflavin, vitamin g 12 niacin, 40 g pantothenic acid, 4 g pyridoxine, 2 g f 60 g ascorbic acid, 240 mg biotin, 20 g inositol, 20 g butyl hydroxytoluene

<sup>4</sup> The Science Laboratories (Qazvin, Qazvin, Iran) – Each 1000 g mineral premix contained; 20 iron, 60 g zinc, 4000 mg selenium, 2000 mg cobalt, 5000 mg copper, 4000 mg manganese, 80 mg iodine, 80,000 mg choline chloride

<sup>5</sup> Sigma-Aldrich (St. Louis, Missouri, USA)

<sup>6</sup> The Science Laboratories (Qazvin, Qazvin, Iran) – Each 1000 g vitamin C contained; 500 g Stay-C

<sup>7</sup> The Arastaban Company (Amol, Mazandaran, Iran)

<sup>8</sup> The Kimia Tehran Acid Company (Tehran, Tehran, Iran)

<sup>9</sup> The Adonis Daru Company (Tehran, Tehran, Iran)

<sup>10</sup> Gross energy calculation based on; Protein (16.7 KJ g<sup>-1</sup>), Lipid (37.6 KJ g<sup>-1</sup>), Carbohydrate (16.7 KJ g<sup>-1</sup>)

#### Determination of water quality parameters

Water quality parameters, including temperature and dissolved oxygen, were measured daily, while pH, ammonium, nitrite, nitrate, phosphate, and total

hardness were recorded during biometric sampling (APHA, 2012). Temperature was measured using a TFA digital thermometer (TH-30.1054, Wertheim, Germany), pH with a Milwaukee meter (Mi411, Rocky

Mount, USA), and dissolved oxygen with a Hanna analyzer (HI-9147, Woonsocket, USA). Ammonium, nitrite, nitrate, and phosphate concentrations were determined using Milwaukee photometers (Mi407, Rocky Mount, USA), and total hardness was measured using an HM hardness tester (T-3, Redondo Beach, USA).

#### *Determination of growth indices*

At the end of the 56-day trial, feeding was

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Specific growth rate (\%/day)} = [(\ln. \text{Final weight (g)} - \ln. \text{Initial weight (g)}) / \text{Culture period}] \times 100$$

$$\text{Survival rate (\%)} = [\text{Final number of prawns} / \text{Initial number of prawns}] \times 100$$

**Determination of antioxidant parameters**  
Hepatopancreas samples were collected from each replicate, washed with cold distilled water, and homogenized (1:9 ratio, buffer solution: Tris-HCl 100 mM, EDTA 0.1 mM, Triton X-100 0.1%, pH 7.8) using an IKA® ULTRA-TURRAX® homogenizer (t18, Baden-Württemberg, Germany) at 4°C. The homogenates were centrifuged at 15,000 rpm for 15 min, and supernatants were collected and stored at -80°C. Antioxidant parameters—including total antioxidant capacity, superoxide dismutase, glutathione peroxidase, catalase, and malondialdehyde—were assayed colorimetrically using ZellBio® GmbH kits (Ulm, Baden-Württemberg, Germany) according to the manufacturer's instructions. Optical density was recorded with a BioTek® microplate reader (ELx800™, Vermont, USA) (Zhang *et al.*, 2013; Jin *et al.*, 2014; Han *et al.*, 2018; Cheng and Wu, 2019).

withheld for 24 hours before sampling. The prawns from each replicate were weighed, and growth performance indices and survival rate were calculated using the following equations (Ding *et al.*, 2017):

#### *Determination of digestive enzymes*

To determine digestive enzyme activity, feeding was stopped 48 hours before sampling to ensure the complete evacuation of gut contents. Intestinal tissues were collected on ice and washed with double-distilled water at low temperature. After weighing with an AND digital scale (GF-1000, Tokyo, Japan;  $\pm 0.001$  g), samples were mixed with buffer solution (Tris-HCl 100 mM, EDTA 0.1 mM, Triton X-100 0.1%, pH 7.8) at a 1:9 ratio. Homogenization was performed on ice for 30 seconds, followed by centrifugation at 10,000 rpm for 10 min at 4°C. Supernatants were collected using a Sartorius micropipette (Biohit Proline®, Göttingen, Germany) and stored at -80°C until analysis. Protease (450 nm),  $\alpha$ -amylase (540 nm), and lipase (405 nm) activities were determined spectrophotometrically and expressed as international units per milligram of protein (Castro *et al.*, 2016; Wang *et al.*, 2018; Weilong *et al.*, 2019).

### Statistical data analysis

Normality and homogeneity of variance were tested using the Kolmogorov–Smirnov and Levene's tests, respectively. Data were analyzed by one-way ANOVA using IBM SPSS Statistics (Version 22, North Castle, USA), and mean comparisons were performed using Duncan's multiple range test at a 95% confidence level. Results are presented as mean  $\pm$  standard deviation. Graphs and tables were prepared using Microsoft Excel 2013 (Redmond, USA).

## Results

### Water quality parameters

Throughout the experimental period, no significant differences ( $p>0.05$ ) were observed among treatments in terms of temperature, pH, ammonium, nitrite, nitrate, phosphate, or total hardness (Table 2). However, dissolved oxygen levels were significantly lower in the control group compared with the lycopene-supplemented treatments ( $p<0.05$ ). The highest dissolved oxygen concentrations were recorded in prawns fed diets containing 150 and 200 mg kg<sup>-1</sup> lycopene.

**Table 2: Comparison of mean ( $\pm$ standard deviation) water quality parameters of the oriental river prawn fed with different lycopene levels (mg kg<sup>-1</sup> diet) for 56 days.**

Parameters	Lycopene (mg kg <sup>-1</sup> )					One-way ANOVA			
	Control	50	100	150	200	Mean	Min	Max	P-value
Temperature (°C)	25.57 $\pm$ 0.62	25.72 $\pm$ 0.56	24.85 $\pm$ 0.40	25.04 $\pm$ 0.31	24.70 $\pm$ 0.73	25.18	24.07	26.87	0.364
pH	6.85 $\pm$ 0.04	6.93 $\pm$ 0.13	6.91 $\pm$ 0.28	7.03 $\pm$ 0.25	7.04 $\pm$ 0.10	6.95	6.72	7.32	0.701
Dissolved oxygen (mg L <sup>-1</sup> )	6.52 $\pm$ 0.13 <sup>c</sup>	6.95 $\pm$ 0.16 <sup>b</sup>	6.90 $\pm$ 0.04 <sup>b</sup>	7.14 $\pm$ 0.05 <sup>a</sup>	7.14 $\pm$ 0.07 <sup>a</sup>	6.93	6.35	7.19	0.000
Ammonium (mg L <sup>-1</sup> )	0.83 $\pm$ 0.01	0.78 $\pm$ 0.06	0.82 $\pm$ 0.05	0.75 $\pm$ 0.06	0.76 $\pm$ 0.01	0.79	0.70	0.87	0.240
Nitrite (mg L <sup>-1</sup> )	0.14 $\pm$ 0.01	0.12 $\pm$ 0.01	0.13 $\pm$ 0.02	0.12 $\pm$ 0.03	0.12 $\pm$ 0.01	0.13	0.10	0.15	0.461
Nitrate (mg L <sup>-1</sup> )	0.18 $\pm$ 0.03	0.18 $\pm$ 0.01	0.16 $\pm$ 0.01	0.17 $\pm$ 0.01	0.17 $\pm$ 0.01	0.17	0.15	0.21	0.557
Phosphate (mg L <sup>-1</sup> )	0.02 $\pm$ 0.00	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.00	0.01 $\pm$ 0.01	0.02	0.01	0.03	0.580
Total hardness (mg L <sup>-1</sup> )	135.30 $\pm$ 2.66	129.83 $\pm$ 2.89	131.07 $\pm$ 2.96	132.98 $\pm$ 2.76	132.53 $\pm$ 3.17	132.25	127.8	137.9	0.272

Means with different letters indicate significant differences between various rows ( $p<0.05$ ).

### Growth indices

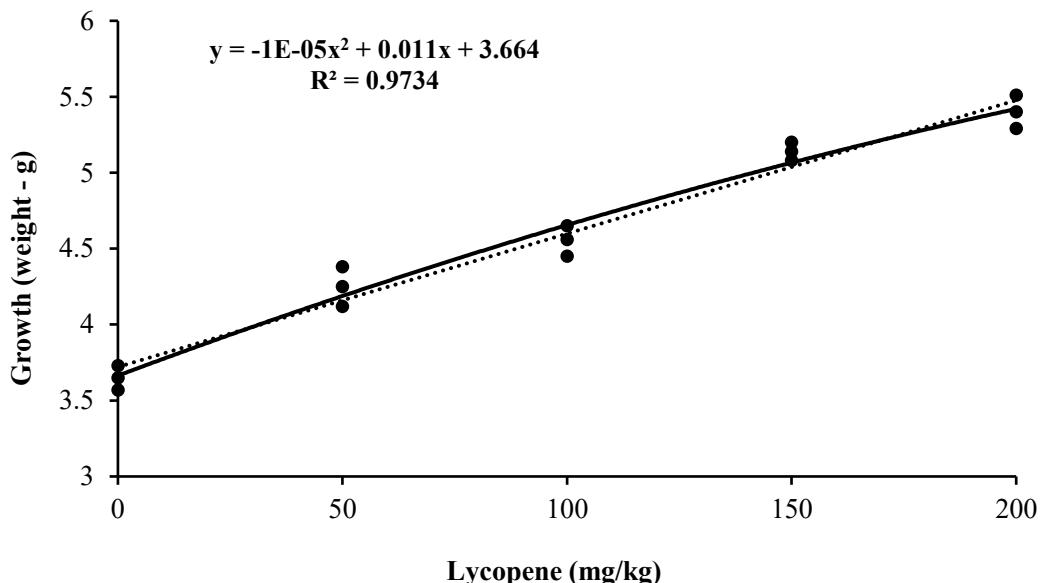
Growth performance and survival rate data for prawns fed diets containing different levels of lycopene are presented in Table 3 and Figure 1. The inclusion of lycopene in the diet significantly enhanced both growth performance and survival rate compared with the control group ( $p<0.05$ ). Final

weight, weight gain, and specific growth rate all showed a positive correlation with increasing lycopene levels. The best results were obtained in prawns fed the 200 mg kg<sup>-1</sup> lycopene diet, whereas the control group exhibited the lowest performance values.

**Table 3: Comparison of mean ( $\pm$ standard deviation) growth indices of oriental river prawn fed with different lycopene levels ( $\text{mg kg}^{-1}$  diet) for 56 days (n=3).**

Parameters	Lycopene ( $\text{mg kg}^{-1}$ )					One-way ANOVA		
	Control	50	100	150	200	F	d.f.	P-value
Initial weight (g)	1.40 $\pm$ 0.07	1.40 $\pm$ 0.07	1.40 $\pm$ 0.07	1.40 $\pm$ 0.07	1.40 $\pm$ 0.07	-	-	-
Final weight (g)	3.65 $\pm$ 0.08 <sup>c</sup>	4.25 $\pm$ 0.13 <sup>d</sup>	4.56 $\pm$ 0.09 <sup>c</sup>	5.14 $\pm$ 0.06 <sup>b</sup>	5.40 $\pm$ 0.11 <sup>a</sup>	384.138	4	0.000
Weight gain (g)	2.25 $\pm$ 0.06 <sup>c</sup>	2.85 $\pm$ 0.08 <sup>d</sup>	3.16 $\pm$ 0.05 <sup>c</sup>	3.74 $\pm$ 0.06 <sup>b</sup>	3.99 $\pm$ 0.04 <sup>a</sup>	296.705	4	0.090
Specific growth rate (% initial weight/day)	0.73 $\pm$ 0.02 <sup>c</sup>	0.85 $\pm$ 0.03 <sup>d</sup>	0.91 $\pm$ 0.01 <sup>c</sup>	0.99 $\pm$ 0.02 <sup>b</sup>	1.04 $\pm$ 0.02 <sup>a</sup>	230.379	4	0.000
Survival rate (%)	75.44 $\pm$ 3.95 <sup>c</sup>	86.67 $\pm$ 6.66 <sup>b</sup>	95.55 $\pm$ 3.85 <sup>ab</sup>	93.33 $\pm$ 6.66 <sup>ab</sup>	97.78 $\pm$ 3.85 <sup>a</sup>	9.092	4	0.002

Means with different letters indicate significant differences between various rows ( $p<0.05$ ).

**Figure 1: The relationship between lycopene concentration in the diet and growth (weight) of the oriental river prawn.**

#### Antioxidant parameters

The effects of dietary lycopene on antioxidant indices are shown in Table 4. Total antioxidant capacity increased significantly ( $p<0.05$ ) with rising lycopene inclusion levels, while superoxide dismutase, catalase, and malondialdehyde activities decreased significantly compared

with the control. In contrast, glutathione peroxidase activity was not significantly affected ( $p>0.05$ ). The highest total antioxidant capacity was observed in prawns fed the 200  $\text{mg kg}^{-1}$  lycopene diet, indicating a strong enhancement of antioxidant status in these treatments.

**Table 4: Comparison of mean ( $\pm$ standard deviation) antioxidant parameters of the oriental river prawn fed with different lycopene levels ( $\text{mg kg}^{-1}$  diet) for 56 days (n=3).**

Parameters	Lycopene ( $\text{mg kg}^{-1}$ )					One-way ANOVA		
	Control	50	100	150	200	F	d.f.	P-value
Total antioxidant capacity (U/mg protein)	2.26 $\pm$ 0.24 <sup>d</sup>	2.63 $\pm$ 0.20 <sup>c</sup>	3.24 $\pm$ 0.11 <sup>b</sup>	3.40 $\pm$ 0.31 <sup>ab</sup>	3.62 $\pm$ 0.18 <sup>a</sup>	30.725	4	0.000
Superoxide dismutase (U/mg protein)	7.09 $\pm$ 0.30 <sup>a</sup>	5.89 $\pm$ 0.48 <sup>b</sup>	5.58 $\pm$ 0.37 <sup>bc</sup>	5.03 $\pm$ 0.29 <sup>c</sup>	5.17 $\pm$ 0.44 <sup>c</sup>	17.893	4	0.000
Glutathione peroxidase (U/mg protein)	29.43 $\pm$ 1.21	29.27 $\pm$ 2.05	26.29 $\pm$ 1.59	26.17 $\pm$ 1.77	26.91 $\pm$ 1.72	2.737	4	0.090
Catalase (U/mg protein)	12.87 $\pm$ 0.62 <sup>a</sup>	8.87 $\pm$ 0.97 <sup>b</sup>	7.43 $\pm$ 0.60 <sup>c</sup>	8.07 $\pm$ 0.51 <sup>bc</sup>	6.90 $\pm$ 0.21 <sup>c</sup>	42.504	4	0.000
Malondialdehyde (nmol/mg protein)	8.41 $\pm$ 0.22 <sup>a</sup>	6.65 $\pm$ 0.47 <sup>b</sup>	6.21 $\pm$ 0.65 <sup>bc</sup>	5.27 $\pm$ 0.58 <sup>d</sup>	5.35 $\pm$ 0.40 <sup>cd</sup>	21.000	4	0.000

Means with different letters indicate significant differences between various rows ( $p<0.05$ ).

### Digestive enzymes

Results of digestive enzyme activity are summarized in Table 5. Dietary lycopene supplementation significantly influenced digestive enzyme activities ( $p<0.05$ ). Protease,  $\alpha$ -amylase, and lipase activities increased progressively with higher lycopene concentrations, reaching their

highest levels in the 200 mg kg<sup>-1</sup> treatment. The control group exhibited the lowest activity across all measured enzymes. These findings demonstrate that dietary lycopene supplementation can markedly improve digestive efficiency in *M. nipponense*.

**Table 5: Comparison of mean (±standard deviation) digestive enzymes of the oriental river prawn fed with different lycopene levels (mg kg<sup>-1</sup> diet) for 56 days (n=3).**

Parameters	Lycopene (mg kg <sup>-1</sup> )					One-way ANOVA		
	Control	50	100	150	200	F	d.f.	P-value
Protease (U/mg protein)	1.25±0.03 <sup>d</sup>	1.38±0.01 <sup>c</sup>	1.45±0.04 <sup>bc</sup>	1.51±0.02 <sup>ab</sup>	1.57±0.05 <sup>a</sup>	30.004	4	0.000
$\alpha$ -amylase (U/mg protein)	1.79±0.13 <sup>d</sup>	2.21±0.11 <sup>c</sup>	2.40±0.05 <sup>b</sup>	2.69±0.08 <sup>a</sup>	2.58±0.06 <sup>ab</sup>	41.719	4	0.000
Lipase (U/mg protein)	0.73±0.05 <sup>c</sup>	0.81±0.02 <sup>b</sup>	0.87±0.03 <sup>b</sup>	0.92±0.03 <sup>a</sup>	0.97±0.02 <sup>a</sup>	30.145	4	0.000

Means with different letters indicate significant differences between various rows ( $p<0.05$ ).

### Discussion

Lycopene is one of the most potent carotenoid pigments, widely recognized for its strong antioxidant capacity and its role as a vital micronutrient in aquatic nutrition. Structurally, it contains the longest hydrocarbon chain among carotenoids, with eleven conjugated double bonds and numerous geometric isomers (Lee and Chen, 2002; Sahin *et al.*, 2014). Like other carotenoids, lycopene cannot be synthesized de novo by prawns and must therefore be supplied through the diet. Nevertheless, crustaceans possess the metabolic ability to interconvert carotenoid compounds (Mao *et al.*, 2017).

In the present study, the measured water quality parameters remained within optimal ranges and did not differ significantly among treatments, except for dissolved oxygen. These findings align with Niu *et al.* (2009), who observed a similar pattern in *Litopenaeus vannamei* fed diets supplemented with astaxanthin. The higher

dissolved oxygen observed in lycopene-fed treatments may indicate reduced oxidative stress and improved physiological efficiency, consistent with previous reports on carotenoid-supplemented diets (Zhi *et al.*, 2018; Abdel-Daim *et al.*, 2019).

The current findings also demonstrated that dietary lycopene supplementation significantly enhanced growth performance and survival rate in *M. nipponense*. These results corroborate the observations of Cheng and Wu (2019), who reported similar improvements in *Procambarus clarkii* fed diets enriched with astaxanthin. Lycopene may contribute to these effects by improving feed utilization, promoting gut health, and reducing oxidative damage in metabolic tissues.

Antioxidant responses were markedly influenced by lycopene levels. The total antioxidant capacity increased significantly, while superoxide dismutase, catalase, and malondialdehyde levels decreased with higher lycopene inclusion.

Comparable patterns were reported by Han *et al.* (2018) in *Portunus trituberculatus* and by Zhang *et al.* (2013) in *L. vannamei* fed diets containing astaxanthin. In both studies, carotenoid supplementation led to elevated total antioxidant capacity, indicating enhanced resistance to oxidative stress. Similar outcomes have been observed in *Penaeus monodon* (Chien *et al.*, 2003; Pan *et al.*, 2003; Jin *et al.*, 2014). Total antioxidant capacity reflects the collective function of enzymatic and non-enzymatic antioxidants, serving as a reliable indicator of the organism's defense balance against reactive oxygen species (Sahin *et al.*, 2014; Zhi *et al.*, 2018). The significant increase in this parameter observed in the present study suggests that lycopene effectively strengthens the antioxidant defense network, improving the prawn's resilience to environmental and metabolic stressors.

The observed reductions in superoxide dismutase and catalase activities indicate a decreased need for enzymatic scavenging of reactive oxygen species, likely due to the direct radical-quenching properties of lycopene (Girao *et al.*, 2012; Zhang *et al.*, 2013). Lycopene can directly neutralize superoxide anion radicals, thereby reducing the demand for superoxide dismutase and subsequent hydrogen peroxide detoxification by catalase (Sahin *et al.*, 2014). Likewise, the marked decrease in malondialdehyde levels suggests that lycopene effectively inhibits lipid peroxidation, consistent with previous findings in crustaceans and fish (Da Silva *et al.*, 2015). Reduced lipid peroxidation contributes to membrane stability and

improved cellular function under oxidative stress conditions.

Dietary lycopene also significantly enhanced digestive enzyme activities, including protease,  $\alpha$ -amylase, and lipase. Similar improvements have been reported by Wang *et al.* (2018) and Weilong *et al.* (2019) in *Marsupenaeus japonicus* fed astaxanthin-supplemented diets. Enhanced digestive enzyme activity is generally associated with improved nutrient absorption and overall feed conversion efficiency. These findings collectively indicate that carotenoid pigments such as lycopene play an important role in supporting digestive physiology and metabolic regulation in aquatic invertebrates (Wade *et al.*, 2017).

## Conclusion

The present study provides compelling evidence that dietary lycopene supplementation exerts a significant positive influence on the growth performance, antioxidant defense system, and digestive physiology of the oriental river prawn (*Macrobrachium nipponense*). Prawns fed lycopene-enriched diets exhibited higher survival rates, improved feed efficiency, and enhanced enzymatic and non-enzymatic antioxidant capacities compared with the control group. Concurrently, the reduced activities of superoxide dismutase, catalase, and malondialdehyde indicate that lycopene effectively mitigates oxidative stress by directly scavenging free radicals and minimizing lipid peroxidation. Furthermore, the upregulation of digestive enzymes, including protease,  $\alpha$ -amylase, and lipase, suggests that lycopene promotes

better nutrient digestion and assimilation, contributing to superior growth outcomes. These findings highlight lycopene's multifunctional role as both an antioxidant and a metabolic enhancer in crustacean nutrition. Overall, the inclusion of 200 mg kg<sup>-1</sup> lycopene in the diet is recommended as the optimal supplementation level for *M. nipponense* juveniles to improve physiological resilience, digestive efficiency, and growth performance under aquaculture conditions. Future research should focus on elucidating the molecular mechanisms underlying lycopene's regulatory effects on antioxidant-related gene expression and metabolic pathways to further optimize its application in aquafeed formulations.

### Conflicts of interest

The Authors declare that there is no conflict of interest.

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