# Effects of Vitamin E on Growth Performance, Antioxidant Capacity, and Resistance to Ammonia Nitrogen Stress in Macrobrachium nipponense (Postprint)

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## Abstract

This experiment aimed to investigate the effects of dietary vitamin E levels on growth performance, antioxidant capacity, and resistance to ammonia nitrogen stress in juvenile Macrobrachium nipponense. Nine hundred healthy juvenile M. nipponense with an average initial body weight of (0.119$±$0.004) g were randomly divided into 6 groups with 3 replicates per group and 50 individuals per replicate. Six semi-purified diets with actual vitamin E contents of 18.31, 37.94, 66.07, 120.25, 212.68, and 388.96 mg/kg were formulated using vitamin E acetate as the supplement (designated as VE1, VE2, VE3, VE4, VE5, and VE6 groups, respectively) and fed for 8 weeks, followed by a 24-h ammonia nitrogen stress test. The results showed: 1) No significant differences were observed in survival rate (SR) among all groups (P>0.05); with increasing dietary vitamin E level, weight gain rate (WGR) of M. nipponense exhibited a trend of initial increase followed by decrease, reaching the maximum in the VE4 group, which was significantly higher than that in the VE1 group (P<0.05); feed conversion ratio (FCR) showed the opposite trend to WGR, with the minimum value in the VE4 group, which was significantly lower than that in the VE1 group (P<0.05). 2) Before ammonia nitrogen stress, hepatic malondialdehyde (MDA) content in M. nipponense decreased initially and then increased with rising dietary vitamin E levels, reaching the lowest value in the VE3 group, which was significantly lower than those in the VE1, VE5, and VE6 groups (P<0.05); total superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities, and total antioxidant capacity (T-AOC) in hepatopancreas of M. nipponense increased initially and then decreased with increasing dietary vitamin E levels. The VE1 and VE6 groups showed higher thioredoxin (Trx) mRNA expression levels and lower thioredoxin reductase (TrxR) mRNA expression levels. Heat shock protein 60 (HSP60) mRNA expression level in the VE3 group was signifi-

cantly lower than those in other groups (P<0.05), heat shock cognate protein 70 (HSC70) mRNA expression level in the VE1 group was significantly lower than those in other groups (P<0.05), and heat shock protein 90 (HSP90) mRNA expression levels in the VE1 and VE2 groups were significantly higher than those in other groups (P<0.05). 3) After ammonia nitrogen stress, the changing trends of SOD, CAT, GSH-Px activities, MDA content, and Trx, TrxR, and HSC70 mRNA expression levels were similar to those before stress, while HSP60 and HSP90 mRNA expression levels showed opposite trends compared with pre-stress conditions. Stress decreased hepatopancreatic GSH-Px activity, T-AOC, and Trx, TrxR mRNA expression levels, while increasing MDA content and SOD activities in the VE4, VE5, and VE6 groups. These results indicate that dietary supplementation with 120.25 mg/kg vitamin E positively promoted growth in M. nipponense, while dietary vitamin E at 66.07, 120.25, and 212.68 mg/kg enhanced antioxidant capacity and resistance to ammonia nitrogen stress.

## Full Text

# Effects of Dietary Vitamin E on Growth Performance, Antioxidant Capacity, and Resistance to Ammonia Nitrogen Stress in Macrobrachium nipponense

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### Abstract

This experiment was conducted to investigate the effects of dietary vitamin E levels on growth performance, antioxidant capacity, and resistance to ammonia nitrogen stress in juvenile Macrobrachium nipponense. A total of 900 healthy juvenile prawns with an average initial body weight of (0.119$±$0.004) g were selected and randomly divided into six groups with three replicates per group and 50 prawns per replicate. Vitamin E acetate was used as the supplement to formulate six semi-purified diets with actual vitamin E contents of 18.31, 37.94, 66.07, 120.25, 212.68, and 388.96 mg/kg (designated as VE1, VE2, VE3, VE4, VE5, and VE6 groups, respectively). The feeding trial lasted for eight weeks, followed by a 24-hour ammonia nitrogen stress test.

The results showed: 1) No significant differences were observed in survival rate (SR) among all groups (P>0.05). The weight gain rate (WGR) exhibited a

trend of initially increasing and then decreasing with increasing dietary vitamin E levels, reaching its maximum in the VE4 group, which was significantly higher than that in the VE1 group ($P<0.05$). The feed conversion rate (FCR) showed the opposite trend to WGR, with the lowest value observed in the VE4 group, significantly lower than that in the VE1 group ($P<0.05$). 2) Before ammonia nitrogen stress, the malondialdehyde (MDA) content in hepatopancreas decreased initially and then increased with rising dietary vitamin E levels, reaching its minimum in the VE3 group, which was significantly lower than those in the VE1, VE5, and VE6 groups ($P<0.05$). The activities of total superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and total antioxidant capacity (T-AOC) in hepatopancreas showed a trend of initially increasing and then decreasing with increasing dietary vitamin E levels. The VE1 and VE6 groups exhibited higher thioredoxin (Trx) mRNA expression but lower thioredoxin reductase (TrxR) mRNA expression. The heat shock protein 60 (HSP60) mRNA expression in the VE3 group was significantly lower than those in other groups ($P<0.05$), while the heat shock cognate protein 70 (HSC70) mRNA expression in the VE1 group was significantly lower than those in other groups ($P<0.05$). The HSP90 mRNA expression in the VE1 and VE2 groups was significantly higher than those in other groups ($P<0.05$). 3) After ammonia nitrogen stress, the activities of SOD, CAT, and GSH-Px, MDA content, and mRNA expressions of Trx, TrxR, and HSC70 showed similar trends to those before stress, whereas the mRNA expressions of HSP60 and HSP90 showed opposite trends compared with pre-stress values. Stress decreased GSH-Px activity, T-AOC, and mRNA expressions of Trx and TrxR, while increasing MDA content and SOD activity in the VE4, VE5, and VE6 groups. In conclusion, dietary supplementation with 120.25 mg/kg vitamin E positively promoted the growth of Macrobrachium nipponense, while dietary vitamin E at 66.07, 120.25, and 212.68 mg/kg enhanced antioxidant capacity and resistance to ammonia nitrogen stress.

**Keywords:** Macrobrachium nipponense; vitamin E; growth; antioxidant capacity; ammonia nitrogen

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## Introduction

Vitamin E is an essential micronutrient required for normal growth and physiological functions in animals. Physiologically, vitamin E participates in various processes including antioxidation, cell signal transduction, reproductive development, immune regulation, and stress resistance. In nutritional physiology research on aquatic animals, the antioxidant and anti-stress effects of vitamin E have consistently attracted researchers' attention. For instance, dietary supplementation with appropriate vitamin E can significantly increase superoxide dismutase (SOD) activity and decrease malondialdehyde (MDA) content in the hepatopancreas of Penaeus monodon, and significantly enhance SOD, catalase (CAT), and glutathione peroxidase (GSH-Px) activities in Litopenaeus

vannamei. Meanwhile, vitamin E effectively protects against stresses induced by pesticides, heavy metal pollution, crowding, and high-fat diets.

With intensifying water pollution and rapid development of intensive aquaculture, ammonia nitrogen has become one of the most common stressors in aquatic animal culture environments. Ammonia nitrogen stress can severely affect physiological functions including growth, respiration, antioxidant capacity, and immunity, with excessive ammonia considered a major environmental factor causing diseases in shrimp and crabs. Nutritional regulation has been identified as a simple and effective technique to alleviate stress. For example, vitamin E can effectively mitigate the adverse effects of ammonia nitrogen stress on Mylopharyngodon piceus and Epinehelus moara.

Macrobrachium nipponense, commonly known as river shrimp or freshwater shrimp, belongs to the phylum Arthropoda, class Crustacea, order Decapoda, family Palaemonidae, and genus Macrobrachium. Due to its delicious taste and high nutritional value, it has become a major freshwater aquaculture species in China, Japan, and other Southeast Asian countries, with primary culture areas concentrated in the middle and lower reaches of the Yangtze River, including Zhejiang, Jiangsu, and Anhui provinces. According to the latest statistics, the national annual production of Macrobrachium nipponense reached 265,000 tons in 2015, representing a 2.88% increase from 2014. The rapid development of its aquaculture industry has driven research demand for its biological characteristics and nutritional physiology. However, current research on the nutritional physiology of Macrobrachium nipponense remains limited, with most studies focusing on protein, lipids, and some trace elements, while nutritional physiology research on vitamin E is scarce. This study aims to investigate the effects of dietary vitamin E on growth performance, antioxidant capacity, and resistance to ammonia nitrogen stress in juvenile Macrobrachium nipponense, providing theoretical reference for the development of formulated feeds and green healthy aquaculture.

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## Materials and Methods

**1.1 Experimental Design** Experimental prawns were purchased from a farm in Nanxun, Huzhou, Zhejiang Province, and acclimated for one week before the formal experiment to adapt to the culture environment. Subsequently, 900 healthy and active juvenile prawns with an average initial body weight of (0.119$±$0.004) g were selected and randomly divided into six groups with three replicates per group and 50 prawns per replicate. The juvenile prawns were randomly stocked into 18 tanks with a volume of 300 L each (50 prawns per tank) for the culture experiment, which lasted for eight weeks.

**1.2 Experimental Diets** Casein and fish meal were used as protein sources, fish oil and soybean oil as lipid sources, and corn starch as carbohydrate source

to formulate the basal diet. The composition and nutrient levels of the basal diet are shown in Table 1 . According to the experimental design, vitamin E acetate (Sigma, USA) was added to the basal diet at different levels to prepare six experimental diets (designated as VE1, VE2, VE3, VE4, VE5, and VE6 groups) with vitamin E levels of 0, 25, 50, 100, 200, and 400 mg/kg, respectively. The experimental diets were prepared as follows: raw materials were ground and passed through an 80-mesh sieve, then accurately weighed and mixed uniformly in stages. Fish oil and soybean oil were added and mixed again, followed by addition of appropriate water for uniform mixing. Finally, the mixture was processed into 1.5 mm diameter pellets using a small feed pelletizer, dried in a 40°C oven to approximately 10% moisture content, sealed in self-sealing bags, and stored at -20°C.

The actual vitamin E contents in the experimental diets were determined to be 18.31, 37.94, 66.07, 120.25, 212.68, and 388.96 mg/kg using high-performance liquid chromatography according to the National Standard of the People' s Republic of China GB/T 17812−2008. Conventional dietary components were analyzed according to AOAC standards with three replicates per sample. Crude protein content was determined by the Kjeldahl method, crude lipid by Soxhlet extraction, crude ash by carbonizing samples at 200°C until no smoke was produced followed by ignition at 550°C in a muffle furnace to constant weight, and moisture content by drying at 105°C to constant weight.

**1.3 Culture Management** The culture experiment was conducted at Zhuangda Aquaculture Farm in Huzhou, Zhejiang Province from July to September 2015. During the experimental period, continuous aeration was maintained to keep dissolved oxygen >6.5 mg/L. Water quality conditions were as follows: temperature 25-30°C, total ammonia nitrogen <0.1 mg/L. Prawns were fed twice daily at 08:00 and 16:00 under natural light conditions using natural river water. To maintain water quality, prawn excrement was siphoned out daily and one-third of the culture water was replaced. Mesh sheets were placed in each tank as shelters to reduce cannibalism during feeding competition.

**1.4 Sample Collection** At the end of the feeding trial, prawns were fasted for 24 h before counting and weighing for growth analysis. Hepatopancreas samples were collected from each group and stored at -80°C for subsequent antioxidant enzyme activity assays and gene expression analysis.

**1.5 Ammonia Nitrogen Stress Test** After the feeding experiment, 60 prawns from each group (20 prawns per tank) were subjected to ammonia nitrogen stress testing. The stress test consisted of three parallels per group with 20 prawns per parallel. Based on the results of Wang et al., the total ammonia nitrogen concentration for the stress test was set at 37 mg/L. Ammonium chloride was used as the stress reagent, with a 10 g/L stock solution prepared and added to tanks according to volume to achieve the target concentration (37

mg/L). Ammonia concentration was measured every 6 h using the Nessler' s reagent method and adjusted with ammonium chloride stock solution as needed. After 24 h of stress, hepatopancreas samples were collected and stored at -80°C for subsequent enzyme activity assays and gene expression analysis.

**1.6.1 Growth Performance Indices** Growth performance indices were calculated using the following formulas: Survival rate (SR, %) = 100 × (number of surviving prawns at experiment end) / (number of prawns stocked at experiment start). Weight gain rate (WGR, %) = 100 × (mean final weight - mean initial weight) / mean initial weight. Feed conversion rate (FCR) = total feed intake / (final total weight - initial total weight). Feeding rate (FR, %) = 100 × total feed intake / [experimental days × (final total weight + initial total weight) / 2].

**1.6.2 Hepatopancreas Antioxidant Enzyme Activities and MDA Content Determination** Approximately 0.50-0.70 g of hepatopancreas was accurately weighed in a 2 mL sterile centrifuge tube, mixed with nine volumes of pre-cooled physiological saline, and homogenized for 10-20 s to prepare a 10% homogenate. The homogenate was centrifuged at 4°C and 4000 r/min for 10 min, and the supernatant was collected for subsequent enzyme activity and MDA content assays. Each group had three replicates. Protein concentration in the supernatant was determined by the Coomassie brilliant blue method. Enzyme activities and MDA content were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute).

SOD activity was determined by the xanthine oxidase method of Elstner et al. The xanthine and xanthine oxidase reaction system generates superoxide anion ($O_2^{-}\cdot$), which oxidizes hydroxylamine to nitrite that appears purple-red in the presence of a color reagent. When the sample contains SOD, it specifically inhibits $O_2^{-}$ generation, thereby reducing nitrite production. One SOD activity unit (U) was defined as the amount of SOD in 1 mg of tissue protein that produces 50% inhibition in a 1 mL reaction system.

CAT activity was determined by measuring the decrease in hydrogen peroxide ($H_2O_2$) content according to Aebi. One enzyme activity unit was defined as the amount of CAT required to reduce 1 μmol of $H_2O_2$ per minute.

GSH-Px activity was determined by analyzing the decrease in glutathione (GSH) during the reaction catalyzed by GSH-Px with $H_2O_2$. One GSH-Px activity unit was defined as the amount that decreases GSH concentration by 1 μmol/L per minute in the reaction system per mg of protein, excluding non-enzymatic reactions.

T-AOC was determined based on the principle that ferrous ions reduced by antioxidant substances under catalysis form stable complexes with phenanthroline compounds, with antioxidant capacity measured through absorbance changes at 520 nm. One T-AOC unit was defined as the amount that increases the

absorbance of the reaction system by 0.01 per minute at 37°C per mg of tissue protein.

MDA content in hepatopancreas was determined by the thiobarbituric acid (TBA) method to assess lipid peroxidation. Thiobarbituric acid condenses with MDA, a product of lipid peroxidation, to form a red product measured colorimetrically at 532 nm.

**1.6.3 mRNA Expression Analysis** Total RNA was extracted from hepatopancreas samples using a tissue/cell RNA rapid extraction kit (Aidlab, Beijing). RNA integrity was detected by 1% agarose gel electrophoresis, while RNA purity and concentration were measured using a Thermo NanoDrop 2000 spectrophotometer. Total RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent Kit (Takara, Dalian) and stored at -20°C.

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to quantify the expression levels of genes encoding heat shock proteins (HSP60 and HSP90), heat shock cognate protein 70 (HSC70), thioredoxin (Trx), and thioredoxin reductase (TrxR) in hepatopancreas before and after stress, with $\beta$-actin serving as the internal reference gene. Each group had three replicates. Primer sequences for qRT-PCR are listed in Table 2 .

PCR reactions were performed using the UltraSYBR Mixture kit (CWBIO, Beijing) in a 20 µL volume containing 10 µL 2× UltraSYBR Mixture, 0.5 µL each of forward and reverse primers (10 µmol/L), 1 µL cDNA, and 8 µL dd$H_2O$. qRT-PCR conditions were: 95°C for 10 min, followed by 40 cycles of 94°C for 15 s, 58°C for 20 s, and 72°C for 20 s. A melting curve was generated after PCR to verify amplification specificity, with temperature increasing from 60°C to 95°C at 0.5°C/5 s. Gene expression levels were analyzed using the $2^{-}\Delta\Delta$Ct method.

**1.7 Statistical Analysis** Experimental data are expressed as mean ± standard error. Data among different vitamin E level groups before or after stress were analyzed by one-way ANOVA, followed by Turkey' s test for multiple comparisons when significant differences were detected. Paired t-tests were used to compare data before and after stress within the same group. The significance level was set at 0.05, and all data were processed using SPSS 19.0 software. Survival rate data were arcsine-transformed before analysis.

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## Results

**2.1 Effects of Vitamin E on Growth Performance of Macrobrachium nipponense** As shown in Table 3 , dietary vitamin E level did not affect the survival rate of Macrobrachium nipponense. Although the VE4 group achieved the highest survival rate (86%), no significant differences were observed among groups (P>0.05). The weight gain rate initially increased and then decreased with increasing dietary vitamin E levels, reaching its maximum in the VE4

group, which was significantly higher than that in the VE1 group ($P<0.05$) but not significantly different from the other four groups ($P>0.05$). The feed conversion rate showed the opposite trend to weight gain rate, with the VE4 group having the lowest value, significantly lower than that in the VE1 group ($P<0.05$). Dietary vitamin E level had no significant effect on feeding rate ($P>0.05$).

**2.2 Effects of Vitamin E on Hepatopancreas Antioxidant Enzyme Activities and MDA Content** As shown in Figure 1 [Figure 1: see original paper], dietary vitamin E level significantly affected hepatopancreas antioxidant enzyme activities and MDA content. Before stress, SOD activity increased with increasing dietary vitamin E levels, reaching its maximum in the VE4 group (120.25 mg/kg), then declined. The VE4 group showed significantly higher SOD activity than the VE1 and VE2 groups ($P<0.05$) but no significant differences compared with the VE3, VE5, and VE6 groups ($P>0.05$). After stress, hepatopancreas SOD activity showed a similar trend, peaking in the VE5 group (212.68 mg/kg) and significantly higher than other groups ($P<0.05$). The VE1 and VE2 groups had significantly lower SOD activities than other groups ($P<0.05$). Post-stress SOD activities in the VE3, VE4, VE5, and VE6 groups were significantly higher than pre-stress values ($P<0.05$), while no significant differences were observed between pre- and post-stress in the VE1 and VE2 groups ($P>0.05$).

Hepatopancreas GSH-Px, CAT activities, and T-AOC showed similar trends to pre-stress SOD activity. Both before and after stress, the VE4 and VE5 groups had significantly higher GSH-Px activities than the VE1, VE2, and VE6 groups ($P<0.05$). Except for the VE2 group, which showed no significant difference in GSH-Px activity before and after stress ($P>0.05$), all other groups exhibited significantly lower post-stress GSH-Px activities compared with pre-stress values ($P<0.05$). Both before and after stress, the VE3, VE4, and VE5 groups had relatively high CAT activities, while the VE1 group had the lowest. Compared with pre-stress values, post-stress CAT activity in the VE3 group decreased significantly ($P<0.05$), while no significant differences were observed in other groups ($P>0.05$). The VE5 group consistently showed the highest T-AOC, followed by the VE3 and VE4 groups, both before and after stress. The VE1, VE2, and VE6 groups had relatively low T-AOC, significantly lower than the VE5 group ($P<0.05$). Post-stress T-AOC decreased significantly in all groups compared with pre-stress values ($P<0.05$).

Both before and after stress, hepatopancreas MDA content showed a trend of initially decreasing and then increasing with rising dietary vitamin E levels, with lower contents observed in the VE2, VE3, and VE4 groups (37.94-120.25 mg/kg), significantly lower than that in the VE6 group ($P<0.05$). Post-stress MDA content increased in all groups compared with pre-stress values, with significant increases observed in the VE1, VE3, VE4, and VE6 groups ($P<0.05$).

**2.3 Effects of Vitamin E on Hepatopancreas mRNA Expression** As shown in Figure 2 [Figure 2: see original paper], pre-stress HSP60 mRNA expression decreased initially and then increased with rising dietary vitamin E levels, reaching its minimum in the VE3 group, which was significantly lower than other groups ($P<0.05$). The VE6 group showed the highest HSP60 mRNA expression, significantly higher than other groups ($P<0.05$). Post-stress HSP60 mRNA expression showed the opposite trend to pre-stress values, with the VE3 group having the highest expression, significantly higher than the VE1 and VE2 groups ($P<0.05$). Post-stress HSP60 mRNA expression in the VE3 group was significantly higher than its pre-stress value ($P<0.05$), while expression levels in other groups were lower than pre-stress values, with significant decreases observed in the VE1, VE4, and VE6 groups ($P<0.05$).

Both before and after stress, the VE3, VE4, VE5, and VE6 groups showed significantly higher HSC70 mRNA expression than the VE1 group ($P<0.05$). Post-stress HSC70 mRNA expression decreased in all groups compared with pre-stress values, with a significant decrease observed in the VE1 group ($P<0.05$).

Pre-stress HSP90 mRNA expression decreased with increasing dietary vitamin E levels, with the VE1 and VE2 groups showing significantly higher expression than other groups ($P<0.05$). Post-stress HSP90 mRNA expression showed a trend of initially increasing and then decreasing with rising dietary vitamin E levels, with the VE3 group significantly higher than other groups ($P<0.05$) and the VE1 group significantly lower than other groups ($P<0.05$). Ammonia nitrogen stress significantly decreased HSP90 mRNA expression in all groups ($P<0.05$).

As shown in Figure 3 [Figure 3: see original paper], both before and after stress, Trx mRNA expression decreased initially and then increased with rising dietary vitamin E levels, with lower expression observed in the VE2, VE3, VE4, and VE5 groups, significantly lower than the VE6 group before stress ($P<0.05$). Post-stress Trx mRNA expression was lower than pre-stress values, with significant decreases observed in the VE1, VE2, and VE6 groups ($P<0.05$), while no significant differences were found in the VE3, VE4, and VE5 groups ($P>0.05$).

Hepatopancreas TrxR mRNA expression increased initially and then decreased with rising dietary vitamin E levels, with the VE2 and VE3 groups showing significantly higher expression than other groups ($P<0.05$). Post-stress TrxR mRNA expression decreased significantly in the VE2, VE3, VE4, VE5, and VE6 groups compared with pre-stress values ($P<0.05$).

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## Discussion

**3.1 Effects of Vitamin E on Growth Performance of Macrobrachium nipponense** Previous studies have shown that dietary vitamin E supplementation can significantly improve weight gain rate or specific growth rate and

reduce feed conversion ratio in Penaeus monodon, Litopenaeus vannamei, and Scophthalmus maximus. This study also found that dietary vitamin E improved the growth performance of juvenile Macrobrachium nipponense, with optimal performance achieved at 120.25 mg/kg vitamin E (VE4 group) and the lowest feed conversion ratio. Low vitamin E level (18.31 mg/kg) inhibited growth performance, while high vitamin E level (388.87 mg/kg) showed a decreasing trend, though not significantly different from the VE4 group. We speculate that even higher dietary vitamin E levels might significantly inhibit growth. Previous reports indicate that high vitamin E levels tend to reduce growth performance in turbot and Penaeus monodon but have no effect on weight gain rate or specific growth rate in Litopenaeus vannamei and Mylopharyngodon piceus. These differential effects of excessive vitamin E among species may be attributed to species specificity, developmental stages, and different vitamin E supplementation forms. The weight gain rate (278%-426%) observed in this study was lower than commercial feed trials, with relatively high feed conversion ratios, possibly because Macrobrachium nipponense is omnivorous with a preference for animal-based diets and is sensitive to fish meal content changes. The low fish meal content in semi-purified diets reduced feed palatability and consequently growth performance.

**3.2 Effects of Vitamin E on Hepatopancreas Antioxidant Capacity of Macrobrachium nipponense** MDA, a product of lipid peroxidation, is commonly used to measure endogenous oxidative damage. This study found that both vitamin E deficiency and excess increased hepatopancreas MDA content, while appropriate vitamin E supplementation (66.07-120.25 mg/kg) significantly decreased MDA content. Similarly, dietary vitamin E supplementation significantly reduced MDA accumulation in tissues of Ctenopharyngodon idella and Rachycentron canadum. These findings indicate that dietary vitamin E deficiency or excess can induce oxidative stress in Macrobrachium nipponense. To reduce oxidative stress and maintain dynamic balance of free radicals, organisms have evolved multiple antioxidant defense responses, including specialized antioxidant enzymes such as SOD, CAT, and GSH-Px. This study demonstrated that dietary vitamin E level significantly affected hepatopancreas antioxidant enzyme activities, with vitamin E at 66.07-212.68 mg/kg (VE3, VE4, and VE5 groups) significantly increasing SOD, CAT, and GSH-Px activities. Additionally, T-AOC showed similar trends to antioxidant enzyme activities with changing dietary vitamin E levels. Similar results have been reported in Litopenaeus vannamei, Ctenopharyngodon idella, Scophthalmus maximus, and tilapia.

Meanwhile, redox systems such as the thioredoxin system play crucial roles in antioxidation. The Trx system consists of reduced nicotinamide adenine dinucleotide phosphate (NADPH), TrxR, and Trx, providing electrons for various enzymes and participating in free radical scavenging, redox state maintenance, apoptosis, and DNA and protein repair. Current research has focused on Trx and TrxR mRNA expression changes under various stresses. The relationship between nutritional status and Trx and TrxR expression has also been sporadically

reported, providing basis for alleviating stress through nutritional approaches. This study showed that dietary vitamin E level significantly affected hepatopancreas Trx and TrxR mRNA expression. Too low or too high vitamin E levels induced Trx mRNA expression, though An Qingcong et al. found that vitamin E deficiency inhibited Trx expression in porcine fetal skin fibroblasts. This discrepancy may be due to species-specific effects of vitamin E on Trx gene expression, but the specific mechanisms require further investigation. External factors such as high temperature, infection, and $H_2O_2$ can induce Trx expression. As an antioxidant, vitamin E deficiency can cause oxidative stress, while excess may exert pro-oxidant effects. Our results indicate that vitamin E deficiency or excess increased oxygen free radicals, causing oxidative stress that induced Trx expression. However, TrxR mRNA expression showed opposite trends to Trx expression, with appropriate vitamin E levels positively promoting TrxR expression. Selenium has been reported to induce TrxR expression, suggesting that vitamin E level affects TrxR expression, with too low or too high levels causing oxidative stress that inhibits gene expression.

These results demonstrate that vitamin E deficiency reduces hepatopancreas antioxidant capacity, increases MDA content, causes lipid peroxidation, and decreases antioxidant enzyme activities or gene expression. Excessive vitamin E exerts pro-oxidant effects, disrupting the oxidation-antioxidation balance, increasing free radicals, causing oxidative damage, and inhibiting antioxidant enzyme expression, while oxidative stress induces Trx expression to cope with the stress.

**3.3 Effects of Vitamin E on Hepatopancreas HSP60, HSC70, and HSP90 mRNA Expression** Heat shock proteins are highly conserved proteins induced in cells by stressors (biological, physical, and chemical factors) that play positive roles in maintaining protein conformation, anti-apoptosis, and cell protection. This study found that dietary vitamin E deficiency or excess significantly induced HSP60 transcription, and HSP90 mRNA expression was also significantly higher in the vitamin E-deficient group than in supplemented groups. These results are similar to findings that appropriate vitamin E reduces HSP expression in goats, further indicating that insufficient or excessive vitamin E causes oxidative stress that induces high HSP60 and HSP90 expression for protective effects. HSC70, a member of the HSP70 family, is a heat shock cognate protein with relatively high constitutive expression in normal cells, participating in cell division, proliferation, and immunity. HSC70 expression can be affected by nutritional status, such as induction by dietary vitamin C in Megalobrama amblycephala. In this study, vitamin E deficiency significantly reduced hepatopancreas HSC70 mRNA expression, indicating that vitamin E supplementation can increase HSC70 expression and enhance protective effects.

**3.4 Protective Effects of Vitamin E Against Ammonia Nitrogen Stress in Macrobrachium nipponense** This study found that post-stress hepatopancreas SOD activity in groups with dietary vitamin E $ $66.07

mg/kg (VE3, VE4, VE5, and VE6) was significantly higher than pre-stress values, indicating that short-term ammonia nitrogen stress induced SOD expression in vitamin E-supplemented groups. Similarly, short-term ammonia nitrogen stress significantly increased SOD activity in Procambarus clarkii and Eriocheir sinensis. This may be because short-term stress has a temporary stimulatory effect on SOD expression to enhance stress resistance, but vitamin E deficiency prevents this stimulatory effect. Meanwhile, ammonia nitrogen stress significantly decreased T-AOC and GSH-Px activity while increasing MDA content, similar to findings in Fenneropenaeus chinensis.

Stress can affect TrxR and Trx expression, such as methylmercury exposure decreasing Trx and TrxR activities in fish liver, and high-concentration quartz dust significantly downregulating Trx and TrxR expression at gene and protein levels in human embryonic lung fibroblasts. Similarly, we found that ammonia nitrogen stress decreased hepatopancreas TrxR and Trx mRNA expression, though no significant differences were observed in the VE3, VE4, and VE5 groups. These results indicate that ammonia nitrogen stress aggravates oxidative damage by downregulating antioxidant enzyme activities and Trx system gene expression, leading to MDA accumulation. However, post-stress T-AOC, GSH-Px, CAT activities, and Trx and TrxR expression were higher in appropriate vitamin E groups than in deficient or excess groups, suggesting protective effects of appropriate vitamin E levels.

Ammonia nitrogen stress differentially affected hepatopancreas HSP60, HSC70, and HSP90 expression. HSP90 has relatively high constitutive expression in normal cells and participates in signal transduction, cell degradation, and immunity. This study found that ammonia nitrogen stress significantly inhibited HSP90 expression, but post-stress HSP90 expression was significantly higher in appropriate vitamin E groups than in deficient and excess groups. Similarly, ammonia nitrogen stress for 48 h decreased HSP90 expression in Fenneropenaeus chinensis. Post-stress HSC70 expression in the VE1 group was significantly lower than pre-stress values. Post-stress HSP60 expression in vitamin E-deficient and excess groups was significantly lower than pre-stress values, while the VE3 group showed significantly higher post-stress HSP60 expression. These findings suggest that decreased HSP expression after stress may be related to tissue damage, but appropriate vitamin E supplementation can increase HSP expression and enhance stress resistance.

In summary: 1) Dietary vitamin E level significantly affected growth performance and antioxidant capacity of juvenile Macrobrachium nipponense, with appropriate levels (66.07-212.68 mg/kg) positively promoting growth and antioxidant capacity. 2) Vitamin E deficiency or excess induced HSP60 and HSP90 mRNA expression but inhibited HSC70 mRNA expression. 3) Ammonia nitrogen stress decreased antioxidant capacity, but appropriate vitamin E supplementation (66.07-212.68 mg/kg) enhanced stress resistance.

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