Effects of Dietary Linolenic Acid Content on Growth, Antioxidant Capacity, Non-Specific Immunity, and Resistance to Ammonia-Nitrogen Stress in Macrobrachium nipponense (Postprint)

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Abstract

Linolenic acid (C18:3n-3, LNA) plays an important regulatory role in growth, immune protection, and environmental stress resistance in crustaceans. This study investigated the effects of dietary LNA content on antioxidant capacity, non-specific immune performance, and ammonia-N stress resistance in the Oriental river prawn (Macrobrachium nipponense), and determined the optimal dietary LNA requirement. Six iso-nitrogenous and iso-lipidic semi-purified diets with LNA levels of 0 (L0, control), 0.5% (L0.5), 1.0% (L1.0), 1.5% (L1.5), 2.0% (L2.0), and 2.5% (L2.5) were formulated and fed to juvenile Oriental river prawns (initial body weight: (0.12$±$0.01) g) for 8 weeks. Each diet was assigned to 5 aquaria (replicates) with 50 experimental prawns per aquarium. Following the feeding trial, 10 prawns from each aquarium were subjected to a 24-h ammonia-N stress challenge (total ammonia-N concentration: 7.922 mg/L). The results showed that specific growth rate, weight gain rate, and survival rate of prawns exhibited a quadratic response to increasing dietary LNA, though differences among groups were not significant ($P>0.05$). Tissue LNA content in both hepatopancreas and muscle increased with dietary LNA supplementation. Hepatopancreatic superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and total antioxidant capacity (T-AOC) generally increased initially then decreased with dietary LNA elevation, peaking in the L1.0 group. Hepatopancreatic malondialdehyde (MDA) content in groups L0.5, L1.0, L1.5, L2.0, and L2.5 was significantly lower than in the L0 group ($P<0.05$). Alkaline phosphatase (ACP) activity was highest in the L1.0 group, though not significantly different from L1.5 ($P>0.05$). Lysozyme (LYZ) activity displayed a quadratic response, reaching maximum activity in the L1.5 group, which was significantly higher than all other groups ($P<0.05$). Following 24-h ammonia-N stress, hepatopancreatic MDA content in groups L0.5, L1.5, L2.0, and L2.5 was significantly lower than in L0 ($P<0.05$), with the L1.5 group showing the lowest value, significantly below all other groups ($P<0.05$). Hepatopancreatic SOD activity and T-AOC exhibited quadratic responses to dietary LNA, with SOD peaking in L1.5 and T-AOC in L1.0. Hepatopancreatic GSH-Px activity was highest in L0, but not significantly different from L1.0 ($P>0.05$). Quadratic regression analysis based on hepatopancreatic SOD activity indicated the LNA requirement of Oriental river prawns to be 1.19%. In conclusion, appropriate dietary LNA levels (1.0%~1.5%) can improve growth performance, enhance antioxidant capacity and non-specific immunity, and mitigate the negative impacts of ammonia-N stress in Oriental river prawns.

Full Text

Effects of Dietary Linolenic Acid Content on Growth, Antioxidant Capacity, Non-Specific Immunity, and Anti-Ammonia-Nitrite Stress Ability in Oriental River Prawn (Macrobrachium nipponense)

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Abstract: Linolenic acid (C18:3n-3, LNA) is an important polyunsaturated fatty acid (PUFA) that regulates growth, immune protection, and environmental stress resistance in crustaceans. This study investigated the effects of dietary LNA content on antioxidant capacity, non-specific immune performance, and anti-ammonia-nitrite stress ability in oriental river prawn (Macrobrachium nipponense) to determine the optimal dietary LNA level. Six isonitrogenous and isolipidic semi-purified diets were formulated with LNA contents of 0 (L0, control), 0.5% (L0.5), 1.0% (L1.0), 1.5% (L1.5), 2.0% (L2.0), and 2.5% (L2.5). Juvenile prawns with initial body weight of (0.12$±$0.01) g were fed for 8 weeks, with five replicate tanks per diet and 50 prawns per tank. After the feeding trial, 10 prawns from each tank were subjected to 24 h ammonia-nitrite stress (total ammonia-nitrite concentration of 7.922 mg/L). The results showed that specific growth rate, weight gain rate, and survival rate of prawns increased initially and then decreased with increasing dietary LNA content, though differences among groups were not significant ($P>0.05$). LNA content in both hepatopancreas and muscle increased with dietary LNA levels. Hepatopancreas superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, and total antioxidant capacity (T-AOC) generally showed a similar trend, peaking in the L1.0 group. Hepatopancreas malondialdehyde (MDA) content in L0.5, L1.0, L1.5, L2.0, and L2.5 groups was significantly lower than in the L0 group ($P<0.05$). Acid phosphatase (ACP) activity was highest in the L1.0 group, though not significantly different from L1.5 ($P>0.05$). Lysozyme (LYZ) activity increased initially then decreased, reaching maximum activity in the L1.5 group, which was significantly higher than other groups ($P<0.05$). After 24 h ammonia-nitrite stress, hepatopancreas MDA content in L0.5, L1.5, L2.0, and L2.5 groups was significantly lower than in L0 ($P<0.05$), with L1.5 showing the lowest value ($P<0.05$). SOD activity and T-AOC followed a similar pattern, peaking in L1.5 and L1.0 groups, respectively. GSH-Px activity was highest in L0 but not significantly different from L1.0 ($P>0.05$). Quadratic regression analysis based on hepatopancreas SOD activity indicated that the optimal dietary LNA requirement was 1.19%. In conclusion, appropriate dietary LNA content (1.0%~1.5%) can improve growth, enhance antioxidant capacity and non-specific immunity, and mitigate the negative effects of ammonia-nitrite stress in oriental river prawn.

Keywords: oriental river prawn (Macrobrachium nipponense); linolenic acid; growth; antioxidant; non-specific immunity; anti-ammonia-nitrite stress

Introduction

Fatty acids play crucial roles in immune and inflammatory responses in aquatic organisms [1-2]. Studies on fish have demonstrated that dietary polyunsaturated fatty acids (PUFA) possess immunomodulatory effects, including enhanced phagocytosis, respiratory burst activity, antigen presentation, and humoral immunity [3-5]. Linolenic acid (C18:3n-3, LNA), an 18-carbon PUFA, serves as a precursor for eicosanoid synthesis (including prostaglandins and leukotrienes) and can enhance immune function by activating prostaglandins and improving antioxidant stress capacity [6]. For instance, Wu et al. [7] found that in grouper (Epinephelus malabaricus), a dietary LNA to linoleic acid (C18:2n-6, LOA) ratio of 3.3 (28.8% linseed oil, 1.3% LOA) significantly enhanced head kidney leukocyte phagocytosis and respiratory burst activity. Conversely, complete replacement of fish oil with linseed oil (48.1% LNA in linseed oil group vs. 3.3% in fish oil group) did not affect non-specific immune performance or pathogen resistance in Eurasian perch (Perca fluviatilis) [2].

Crustaceans generally have limited capacity to convert 18-carbon PUFA to highly unsaturated fatty acids (HUFA), which is insufficient to meet their growth and developmental needs, necessitating dietary supplementation. Different species exhibit varying requirements for LNA and LOA. According to NRC (2011), shrimp have higher LNA requirements than LOA requirements [8]. Researchers have established LNA requirements for several shrimp species, including Chinese shrimp (Penaeus chinensis) [9], brown shrimp (Penaeus aztecus) [10], and Indian white shrimp (Penaeus indicus) [11], using growth performance as the primary evaluation criterion.

Oriental river prawn (Macrobrachium nipponense), belonging to Arthropoda, Crustacea, Decapoda, Palaemonidae, and Macrobrachium, is widely distributed in alkaline and freshwater habitats. Valued for its delicious taste and nutritional quality, it has become a major economic freshwater aquaculture species in China [12]. Over the past decade, production of oriental river prawn has remained relatively stable [13-14]. However, this species exhibits relatively weak stress resistance, and high-density culture often triggers stress responses that severely constrain aquaculture efficiency and sustainable industry development. Stress responses and disease outbreaks in cultured shrimp are closely related to their immune function [15], and dietary fatty acid composition has been shown to affect animal immunity [16]. For example, in Pacific white shrimp (Litopenaeus vannamei), dietary arachidonic acid (C20:4n-6, ARA) content affected expression of immune-related genes including Toll-like receptor (TLR), immune deficiency (IMD), and lysozyme (LYZ), with ARA' s immunomodulatory effects influenced by dietary eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) levels [17]. However, the effects of dietary unsaturated fatty acids on antioxidant capacity and immune enzyme activity in oriental river prawn remain unreported. Therefore, this study investigated changes in growth performance, hepatopancreas antioxidant and immune enzyme activities, and antioxidant indices under ammonia-nitrite stress in oriental river prawn fed diets with varying LNA content, aiming to determine optimal dietary LNA supplementation levels and provide theoretical foundations for research on resistance to environmental stress in shrimp.

Materials and Methods

1.1 Experimental Diets Peruvian white fish meal (crude protein 66.69%, crude lipid 9.10%) was provided by Zhejiang Jingbao Feed Co., Ltd. Linseed oil (50% LNA) was supplied by Lino (Shanghai) International Trade Co., Ltd. Six isonitrogenous and isolipidic semi-purified diets were formulated with fish meal and casein as protein sources, containing 0 (L0, control), 0.5% (L0.5), 1.0% (L1.0), 1.5% (L1.5), 2.0% (L2.0), and 2.5% (L2.5) LNA. Diet preparation involved grinding ingredients through an 80-mesh sieve, accurately weighing, and thoroughly mixing micronutrients (vitamins, minerals, attractants) using a stepwise expansion method. Linseed oil and soybean lecithin were then added and kneaded evenly, followed by water addition (400 mL/kg) and mixing to form a dough. The mixture was pelleted into 1.0 mm diameter particles using a small feed pelletizer, dried at 40°C to approximately 10% moisture content, sealed, and stored at -20°C. Diet composition and nutrient levels are shown in Table 1 , and fatty acid composition is presented in Table 2 .

1.2 Experimental Prawns and Culture Conditions Juvenile oriental river prawns were obtained from Huzhou Bangda Ecological Agriculture Co., Ltd. After one week of acclimation, 1,500 healthy prawns with uniform body weight [(0.12$±$0.01) g] were randomly distributed into six groups with five replicates each (50 prawns per replicate). Each replicate was stocked in a 300 L aquarium containing mesh shelters to reduce cannibalism. The experiment was conducted from July to September 2015 at the Zhejiang Provincial Key Laboratory of Aquatic Resources Conservation and Development. Daily maintenance included siphoning waste and water exchange (approximately 1/3 volume) at 07:30. Aerated tap water was used with the following parameters: temperature 25-29°C, pH 7.6-8.1, dissolved oxygen >6.5 mg/L, and total ammonia-nitrite <0.01 mg/L. Prawns were fed twice daily at 08:30 and 16:00 at 4-5% of body weight for eight weeks.

Following the 8-week feeding trial, 10 prawns per tank were randomly selected for a 24 h ammonia-nitrite stress test, following the method of Zou et al. [18]. Ammonium chloride was used to adjust water total ammonia-nitrite concentration to 7.922 mg/L. During stress testing, continuous aeration maintained dissolved oxygen $ $5.0 mg/L, pH 7.6-8.1, and temperature 25-29°C.

1.4 Sample Collection After the feeding trial, prawns were fasted for one day before final weighing and survival counting. For both feeding and stress tests, hepatopancreas was dissected from the cephalothorax of sampled prawns, and both prawn bodies and hepatopancreas were stored at -80°C for subsequent analyses.

1.5 Analytical Methods

1.5.1 Growth Performance Calculations Growth parameters were calculated as follows:
Survival rate (SR, %) = 100 × (final prawn number / initial prawn number)
Weight gain rate (WGR, %) = 100 × (final weight - initial weight) / initial weight
Specific growth rate (SGR, %/d) = 100 × (ln final weight - ln initial weight) / experimental days
Feed conversion ratio (FCR) = feed intake / (final mean weight - initial mean weight)
Feeding rate (FR, %) = 100 × total feed / [experimental days × (final mean weight + initial mean weight) / 2]

1.5.2 Proximate Composition Analysis Dietary crude protein was determined by Kjeldahl method (Kjeltec 2200, FOSS, Denmark), crude lipid by Soxhlet extraction (Soxtec™ 2043, FOSS, Denmark), and crude ash by muffle furnace combustion at 550°C for 14 h. Hepatopancreas lipid content was measured using the method of Folch et al. [19].

1.5.3 Fatty Acid Composition Analysis Fatty acid composition was analyzed following Chang et al. [20] using an HP-6890 gas chromatograph equipped with an Agilent 19091J-413 capillary column (30.0 mm × 0.25 mm). Injection port temperature was 200°C, detector temperature 260°C, and initial column temperature 140°C, gradually increased to 240°C until all components eluted. Quantification was performed by area percentage method.

1.5.4 Hepatopancreas Antioxidant Indices Approximately 0.5 g of hepatopancreas was homogenized in ice-cold 0.86% saline at a 1:9 mass/volume ratio to prepare 10% homogenate, centrifuged at 3,500 r/min for 15 min, and the supernatant collected. Protein content in supernatant was determined by Coomassie brilliant blue method. All indices were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute). Lipid peroxidation was assessed by malondialdehyde (MDA) content using the thiobarbituric acid (TBA) method, where MDA reacts with TBA to form a red product measured at 532 nm. Superoxide dismutase (SOD) activity was determined by xanthine oxidase method, with one unit defined as the amount causing 50% inhibition of the reaction per mg protein. Glutathione peroxidase (GSH-Px) activity was measured based on its catalysis of H_2O_2 with reduced glutathione (GSH), with activity calculated from GSH consumption. Total antioxidant capacity (T-AOC) was determined by the reduction of Fe^{3+} to Fe^{2+}, which forms a stable complex with phenanthroline; one unit was defined as the amount causing a 0.01 absorbance increase per minute per mg protein at 37°C.

1.5.5 Hepatopancreas Non-Specific Immune Indices Acid phosphatase (ACP) and lysozyme (LYZ) activities were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute). ACP activity was determined by its ability to decompose disodium phenyl phosphate, producing free phenol that forms a red quinone derivative with 4-aminoantipyrine and potassium ferricyanide, with activity proportional to color intensity. LYZ activity was based on its ability to hydrolyze peptidoglycan in bacterial cell walls, causing bacterial lysis and increased light transmittance.

1.6 Statistical Analysis Data are presented as mean ± standard deviation (SD). One-way ANOVA was performed using SPSS 19.0, followed by Tukey’ s multiple comparison test when significant differences were detected ($P < 0.05$).

Results

2.1 Effects of Dietary LNA Content on Growth Performance As shown in Table 3 , weight gain rate and specific growth rate of oriental river prawn increased initially then decreased with rising dietary LNA content, reaching maximum values in the L1.5 group, though differences among groups were not significant ($P>0.05$). Survival rates did not differ significantly among groups ($P>0.05$). Dietary LNA content had no significant effect on feed conversion ratio or feeding rate ($P>0.05$).

2.2 Effects of Dietary LNA Content on Hepatopancreas Lipid Content Figure 1 [Figure 1: see original paper] shows that hepatopancreas crude lipid content decreased with increasing dietary LNA content, reaching the lowest value in the L2.5 group, which was significantly lower than other groups ($P<0.05$). However, no significant differences were observed among L0, L0.5, L1.0, and L1.5 groups ($P>0.05$).

2.3 Effects of Dietary LNA Content on Hepatopancreas and Muscle Fatty Acid Composition Tables 4 and 5 show that saturated fatty acids were most abundant in both hepatopancreas and muscle, followed by monounsaturated fatty acids. In hepatopancreas, saturated fatty acid content in L0.5 group was significantly higher than all groups except L0 ($P<0.05$), while monounsaturated fatty acid content in L0 group was significantly higher than other groups ($P<0.05$). In muscle, saturated fatty acid content in L1.0 group was significantly higher than other groups ($P<0.05$), and monounsaturated fatty acid content in L0 group was significantly higher than other groups ($P<0.05$). C18:3n-3 (LNA) content in both tissues increased with dietary LNA content, while C20:5n-3 (EPA) and C22:6n-3 (DHA) contents showed an initial increase followed by a decrease.

2.4 Effects of Dietary LNA Content on Hepatopancreas Antioxidant Indices Table 6 shows that hepatopancreas SOD, GSH-Px activities, and T-AOC generally increased initially then decreased with rising dietary LNA content, all peaking in the L1.0 group. SOD activity in L1.0 was significantly higher than other groups ($P<0.05$), GSH-Px activity was significantly higher than all groups except L0 ($P<0.05$), and T-AOC was significantly higher than L1.5, L2.0, and L2.5 groups ($P<0.05$). Hepatopancreas MDA content was highest in L0 group, significantly exceeding L0.5, L1.0, L1.5, L2.0, and L2.5 groups ($P<0.05$). Within the 1.5-2.5% range, hepatopancreas MDA content showed an increasing trend with dietary LNA content.

2.5 Effects of Dietary LNA Content on Hepatopancreas Non-Specific Immune Indices As shown in Figure 2 [Figure 2: see original paper], hepatopancreas ACP activities in L0.5, L1.0, and L1.5 groups were significantly higher than in L0 ($P<0.05$), with L1.0 showing the highest activity, though not significantly different from L1.5 ($P>0.05$). Hepatopancreas LYZ activity increased initially then decreased, reaching maximum activity in L1.5 group, which was significantly higher than other groups ($P<0.05$).

2.6 Effects of Dietary LNA Content on Anti-Ammonia-Nitrite Stress Ability Table 7 shows that after 24 h ammonia-nitrite stress, hepatopancreas MDA content in L0.5, L1.5, L2.0, and L2.5 groups was significantly lower than in L0 ($P<0.05$), with L1.5 showing the lowest value, significantly lower than all other groups ($P<0.05$). Hepatopancreas SOD activity and T-AOC increased initially then decreased, peaking in L1.5 and L1.0 groups, respectively. GSH-Px activity was highest in L0 group but not significantly different from L1.0 (P>0.05).

Based on these results, quadratic regression analysis of hepatopancreas SOD activity (y) against dietary LNA content (x) yielded the equation $y = -0.4021x^2 + 0.9539x + 0.5125$, $R^2 = 0.7184$ (Figure 3 [Figure 3: see original paper]), indicating the optimal dietary LNA content for maximum SOD activity was 1.19%.

Discussion

3.1 Effects of Dietary LNA Content on Growth Performance Previous studies have shown that dietary LNA supplementation promotes fish growth, while excessive LNA inhibits rather than enhances growth [1]. Similar results were observed in this study, where specific growth rate and weight gain rate of oriental river prawn increased when dietary LNA content ranged from 0-1.5%, but declined when LNA exceeded 1.5%. Comparable findings have been reported in fish; for example, grass carp (Ctenopharyngodon idellus) showed improved growth with dietary n-3 HUFA up to 0.25%, but significantly reduced performance at 0.83% or 1.13% [21]. Transcriptomic analysis of grass carp hepatopancreas after three months of n-3 HUFA feeding revealed that n-3 HUFA affected expression of 36 protein metabolism-related genes, upregulating protein digestion genes (trypsin, chymotrypsin), protein translation genes (RNA polymerase II core promoter sequence, eIF-4A), and downregulating protein catabolism genes (ubiquitin ligase, ubiquitin) [22], suggesting that dietary PUFA may promote growth through protein-sparing mechanisms.

Quadratic regression analysis indicated that the dietary LNA requirement for maximum hepatopancreas SOD activity in oriental river prawn was 1.19%, which is higher than requirements reported for Chinese shrimp (0.7-1.0%) [9] and kuruma shrimp (Penaeus japonicus) (1.0%) [23], but lower than that for Indian white shrimp (2.0%) [11]. These differences may reflect species-specific LNA metabolism rates and are also influenced by diet composition, prawn size, feeding frequency, and experimental conditions.

Previous research has shown that dietary LNA reduces body lipid content [24], consistent with our findings that hepatopancreas lipid content decreased with increasing dietary LNA. This aligns with results in darkbarbel catfish (Pelteobagrus vachelli) [25] but contrasts with grass carp studies showing increased body lipid with n-3 PUFA [21], possibly due to grass carp’ s lower lipid requirement.

Body fatty acid composition generally reflects dietary fatty acid composition [25-26]. In abalone (Haliotis discus hannai Ino), high dietary LNA increased muscle LNA content [27], consistent with our observation that hepatopancreas and muscle LNA content increased with dietary LNA. The initial increase then decrease in EPA and DHA contents may be attributed to enhanced fatty acid desaturase and elongase activities at optimal LNA levels, promoting conversion of 18-carbon PUFA to HUFA [27-28].

3.2 Effects of Dietary LNA Content on Antioxidant Capacity and Non-Specific Immunity Studies have shown that hepatopancreas non-specific immune enzyme activities in darkbarbel catfish increased with dietary LNA but were significantly downregulated when LNA exceeded 1% [29]. Similar results were observed in this study, where hepatopancreas SOD, GSH-Px activities, and T-AOC increased initially then decreased, peaking in the L1.0 group. Excessive LNA may induce lipid peroxidation, generating toxic reactive oxygen species (ROS) such as superoxide anion radicals ($O_2^{-}\cdot$), H_2O_2, and ozone. SOD catalyzes ROS to H_2O_2, mitigating cellular damage [30-32]. In this study, hepatopancreas SOD activity increased then decreased, with L1.0 significantly higher than other groups, though no significant differences existed among L0, L0.5, L1.5, L2.0, and L2.5 groups, indicating that 1.0% dietary LNA enhanced SOD activity, but PUFA peroxidation products beyond this threshold did not further increase SOD activity.

T-AOC reflects overall antioxidant capacity, representing the combined effects of enzymatic (SOD, CAT, GST) and non-enzymatic (vitamins, amino acids, metalloproteins) factors. Our results showed maximum hepatopancreas T-AOC at 1.0% dietary LNA, consistent with Pan et al. [33], who reported maximum T-AOC in common carp (Cyprinus carpio) when 25% of fish oil was replaced by linseed oil, indicating that optimal LNA levels can improve antioxidant capacity.

MDA, a product of lipid peroxidation induced by oxygen radical attack on PUFA in biological membranes, reflects the degree of lipid peroxidation and indirectly indicates cellular damage from free radicals. The significantly higher MDA content in L0 group suggests that LNA deficiency caused cellular damage, likely due to essential fatty acid deficiency [34]. However, the increasing trend in MDA content with dietary LNA levels from 1.5-2.5% resembles findings in marbled rockfish (Sebastiscus marmoratus), where high n-3 HUFA increased MDA accumulation [35]. Based on hepatopancreas antioxidant capacity, the optimal dietary LNA content appears to be 1.0%.

Moderate HUFA supply can enhance immunity in freshwater fish, while excessive levels may cause negative effects [36-37]. We therefore measured hepatopancreas ACP and LYZ activities as non-specific immune indices. ACP participates in degradation of foreign macromolecules and functions as a lysosomal hydrolase in macrophages, with increased activity enhancing pathogen clearance [38]. LYZ mediates non-specific immune responses, resisting parasitic, bacterial, and viral infections [17,40]. Our results showed highest ACP activity in L1.0 group and maximum LYZ activity in L1.5 group. Reports on n-3 PUFA effects on fish immunity vary. Yang et al. [41] found that serum LYZ activity and blood cell phagocytosis in rice field eel (Monopterus albus) increased with dietary LNA, peaking at 1.55% LNA. Li et al. [5] reported significantly higher antibody titers in darkbarbel catfish fed 6% linseed oil compared to 4% and 2% groups. These findings demonstrate that appropriate dietary LNA levels can promote immunity.

3.3 Effects of Dietary LNA Content on Anti-Ammonia-Nitrite Stress Ability After 24 h ammonia-nitrite stress, L0 group showed higher hepatopancreas MDA content and lower SOD activity and T-AOC than other groups, likely due to LNA deficiency. Both SOD activity and T-AOC increased initially then decreased with dietary LNA content. Zhao et al. [42] reported that appropriate dietary DHA significantly increased SOD activity and decreased MDA content in Chinese mitten crab (Eriocheir sinensis) under hypoxia stress, similar to our results. However, excessive DHA significantly increased MDA content, possibly due to DHA oxidation producing more MDA than could be protected against by SOD and other antioxidant enzymes. Conversely, Ji et al. [21] found that high dietary HUFA significantly increased both SOD activity and MDA content in juvenile grass carp, suggesting excessive HUFA induced oxidative stress, with similar results reported in Nile tilapia (Oreochromis niloticus) [43]. These findings suggest that within an appropriate range, increased dietary PUFA enhances antioxidant capacity. Our results showed that after 24 h ammonia-nitrite stress, 1.5% dietary LNA resulted in lowest MDA content and highest SOD activity and T-AOC, indicating that 1.5% LNA enhances antioxidant capacity and improves stress response to ammonia-nitrite.

Conclusions

1. Considering the comprehensive effects on growth, antioxidant capacity, non-specific immunity, and anti-ammonia-nitrite stress ability, the suitable dietary LNA content for oriental river prawn is 1.0%~1.5%.
2. Under the conditions of this experiment, quadratic regression analysis indicates that a dietary LNA content of 1.19% yields maximum hepatopancreas SOD activity in oriental river prawn.

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