Effects of High-Salt Stress on Digestive and Immune-Related Enzyme Activities in Litopenaeus vannamei (Postprint)

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Abstract

To investigate the effects of high salinity on the activities of digestive and immune-related enzymes in the Pacific white shrimp (Litopenaeus vannamei), four salinity gradients of 30, 40, 50, and 60 were established. The shrimp had a body length of (7.84±0.68) cm and were stocked at a density of 333 individuals/m^3; each gradient included three replicates, and the experimental duration was 30 days. Hemolymph, muscle, hepatopancreas, and other tissues were sampled to assay the activities of superoxide dismutase (SOD), catalase (CAT), alkaline phosphatase (AKP), acid phosphatase (ACP), protease, lipase, and amylase. The results demonstrated that salinity significantly influenced the activities of pepsin, lipase, and amylase in the hepatopancreas of L. vannamei ($P<0.05$); the activities of all digestive enzymes decreased progressively with increasing salinity, with significant differences among treatments ($P<0.05$). Salinity also affected immune parameters in different tissues of L. vannamei: in hemolymph, AKP activity increased gradually, whereas ACP, CAT, and SOD activities initially increased then decreased; in muscle, AKP, ACP, and SOD activities showed a similar pattern of initial increase followed by decline; in hepatopancreas, AKP activity exhibited a trend of decrease-increase-decrease, ACP activity showed no significant differences among high-salinity treatments ($P>0.05$), CAT activity decreased initially then increased, and SOD activity declined gradually after salinity 40. These findings preliminarily indicate that high salinity significantly impacts the activities of digestive and immune-related enzymes in L. vannamei, with tissue-specific effects of salinity on immune enzyme activities across different tissues, and that salinity stress above 50 exerts particularly pronounced effects on digestive and immune enzyme activities.

Full Text

Preamble

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Effects of High Salinity Stress on Digestive and Immunity-Related Enzyme Activities in *Litopenaeus vannamei*

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Abstract

Litopenaeus vannamei is native to the Pacific coastal waters of Central and South America and is considered one of the most important euryhaline aquaculture shrimp species worldwide. Due to its high disease resistance, rapid growth, suitability for high-density culture, and other excellent biological characteristics, it has become a major aquaculture species with significant development potential. Salinity is one of the most important and variable water quality factors affecting the physiology of aquatic organisms. Salinity variation can trigger various physiological responses, including enhanced stress-related hormones in plasma, stimulated energy metabolism, and disrupted electrolyte equilibrium. Consequently, marine organisms have developed various survival mechanisms to cope with salinity fluctuations. For example, crustaceans adjust their osmolarity and maintain stable internal conditions by altering related enzyme activities. The immune enzyme activities of crustaceans play crucial roles in disease resistance and health maintenance. Additionally, digestive enzyme activities directly reflect the ability of the organism to digest and absorb nutrients, which affects survival and growth. However, few studies have investigated the influence of high salinity on the immune and digestive indices of *L. vannamei*. There are extensive high-salinity water resources in China' s coastal and northwestern regions that could be developed for aquaculture. Analyzing the effects of high-salt stress on digestive and immune-related enzyme activities in *L. vannamei* can enhance our understanding of shrimp stress biology and provide a theoretical reference for future shrimp cultivation in high-salt environments.

Digestive and immunity-related enzyme activities were investigated in *L. vannamei* (body length: 7.84±0.68 cm) exposed to salinity levels of 30, 40, 50, and 60 psu. The stocking density was 333 individuals/m³ with three replicates per treatment, and the experiment lasted 30 days. The activities of immunity-related enzymes, including superoxide dismutase (SOD), catalase (CAT), alkaline phosphatase (AKP), and acid phosphatase (ACP), as well as digestive enzymes (pepsin, lipase, and amylase), were measured in different tissues (hemolymph, muscle, and hepatopancreas). The results showed that salinity significantly influenced pepsin, lipase, and amylase activities in the hepatopancreas, with enzyme activities decreasing as salinity increased. In hemolymph, ACP, CAT, and SOD activities showed an "increase-decrease" trend, whereas AKP activity increased with salinity. In muscle tissue, AKP, CAT, and SOD activities also showed an "increase-decrease" trend. In the hepatopancreas, no obvious changes were observed in AKP and ACP activities, while SOD activity decreased significantly when salinity exceeded 40 psu. These results indicate that high salinity can affect digestive and immune-related enzyme activities in different tissues, particularly under high-salinity conditions (above 50 psu).

Keywords: *Litopenaeus vannamei*; high salinity; digestive enzymes; immunity-related enzymes

Introduction

Litopenaeus vannamei is native to the tropical and temperate waters of the Pacific coast of Central and South America and exhibits broad salinity tolerance. Since its introduction to China, it has been widely cultured in waters of varying salinities [1]. Salinity is one of the most variable water quality factors that directly affects osmoregulation, metabolism, and energy budget in aquatic organisms, thereby influencing their growth and survival. Previous studies on salinity effects in *L. vannamei* have primarily focused on low-salinity conditions [2-6], while research on shrimp physiology and immunity has concentrated on salinity shock [7-8] and long-term salinity effects [9-10].

China' s coastal and northwestern regions contain extensive high-salinity water resources, such as salt fields in Shandong, which could be developed for *L. vannamei* aquaculture. Although some high-salinity waters have been used for shrimp culture, yields are generally not higher than 400 kg/hm^2—substantially lower than those in other salinity environments. Achieving high yields in high-salinity shrimp culture requires fundamental research, yet studies on the effects of high salinity on shrimp growth, physiology, and molecular indicators remain scarce. This experiment investigates the effects of high-salinity stress on digestive and immune-related enzyme activities in hemolymph, hepatopancreas, and other tissues of *L. vannamei* to enrich stress biology theory and provide references for utilizing high-salinity waters.

Materials and Methods

1. Source of Experimental Animals and Acclimation

Experimental *L. vannamei* were purchased from Changyi Haifeng Aquaculture Co., Ltd. Shrimp were transported to the laboratory at Qingdao Agricultural University and temporarily reared in aquaria (30 cm × 40 cm × 30 cm) with aeration. Individuals of similar size (body length: 7.84±0.68 cm; weight: 3.98±0.71

g) were selected. During acclimation, salinity was gradually increased from ambient levels to experimental gradients at a rate of 1 psu per day. Temperature was maintained at 27±0.5°C using heating rods. After reaching target salinities, shrimp were held for an additional 24 h before experiments began. High-salinity seawater was prepared by adding crude salt to natural seawater.

2. Experimental Design and Management

Four salinity treatments (30, 40, 50, and 60 psu) were established, with 30 shrimp per treatment and three replicates each. Shrimp were fed commercial formulated feed at 3-5% of body weight at 19:00 daily. Uneaten feed and feces were siphoned after 60 minutes, with 30% water exchange using pre-heated, pre-adjusted salinity water. Other conditions remained the same as during acclimation.

3. Sample Collection

After the experiment, five shrimp were randomly selected from each treatment. Hemolymph (1.0-1.5 mL) was withdrawn from the heart using a sterile syringe and centrifuged at 4,000 rpm for 10 minutes; serum was collected and stored at -80°C. Hepatopancreas and muscle tissues were excised, weighed accurately, and placed in glass homogenization tubes with 9 volumes of physiological saline. Tissues were homogenized and centrifuged at 3,000 rpm for 15 minutes; supernatants were collected and stored at -80°C. All procedures were performed on ice.

4. Enzyme Activity Assays

Activities of pepsin, lipase, and amylase were measured in hepatopancreas. Activities of superoxide dismutase (SOD), catalase (CAT), alkaline phosphatase (AKP), and acid phosphatase (ACP) were measured in hepatopancreas, muscle, and hemolymph. Protein content was determined using the Coomassie brilliant blue method. All enzyme activity kits were purchased from Nanjing Jiancheng Bioengineering Institute. Data were analyzed using SPSS 19.0 software with one-way ANOVA and Duncan' s multiple range tests. Differences were considered significant at $P<0.05$.

Results

1. Effects of High Salinity on Digestive Enzyme Activities

Digestive enzyme activities in the hepatopancreas decreased significantly with increasing salinity. Amylase and lipase activities were significantly lower at 50 and 60 psu compared to 30 and 40 psu ($P<0.05$), though no significant differences were observed between 30 and 40 psu treatments ($P>0.05$). Pepsin activity showed a similar declining trend, with significant differences among all treatment groups ($P<0.05$) [Figure 1: see original paper].

2. Effects of High Salinity on AKP Activity

AKP activity in hemolymph increased significantly with salinity, with the 60 psu treatment showing markedly higher activity than other groups (P<0.05). In muscle, AKP activity first increased then decreased, peaking at 40 psu and declining thereafter, with significant differences between treatments (P<0.05). In hepatopancreas, AKP activity showed no clear trend and no significant differences among treatments (P>0.05) [Figure 2: see original paper].

3. Effects of High Salinity on ACP Activity

ACP activity in hemolymph increased then decreased with salinity, peaking at 40 psu (P<0.05). In muscle, ACP activity showed a similar pattern, with the 40 psu group significantly higher than others (P<0.05). In hepatopancreas, no significant differences were observed among treatments (P>0.05) [Figure 3: see original paper].

4. Effects of High Salinity on CAT Activity

CAT activity in hemolymph first decreased, then increased, then decreased again, with the 40 psu group showing significantly higher activity than the 30 psu group (P<0.05). In muscle, CAT activity increased then decreased, peaking at 40 psu. In hepatopancreas, CAT activity decreased then increased, with no significant differences among treatments (P>0.05) [Figure 4: see original paper].

5. Effects of High Salinity on SOD Activity

SOD activity in hemolymph increased then decreased, peaking at 40 psu (P<0.05). In muscle, SOD activity showed the same trend, with the 40 psu group significantly higher than others (P<0.05). In hepatopancreas, SOD activity decreased gradually with increasing salinity, showing significant differences among treatments (P<0.05) [Figure 5: see original paper].

Discussion

1. Effects of High Salinity on Digestive Enzyme Activities

Previous studies have extensively investigated digestive enzyme activities in *L. vannamei*, but few have examined salinities exceeding normal seawater levels. Dalla Via [11] found that when environmental salinity decreases, shrimp expend substantial energy on osmoregulation. Liu Guoxing [12] analyzed salinity effects on digestive enzymes in *Procambarus clarkii* and found that enzyme activities decreased with increasing salinity, consistent with our results. Babkin [13] reported that digestive enzymes exhibit an overall effect—when one enzyme' s activity increases, others tend to increase as well, and vice versa. Our findings align with this concept, showing that all digestive enzyme activities declined with increasing salinity, indicating reduced digestive and absorptive capacity at high salinities, which may affect growth. This corresponds with slower growth observed in high-salinity production environments.

2. Effects of High Salinity on Immune Enzyme Activities

Salinity stress significantly affects antioxidant system function in aquatic animals [16]. SOD and CAT are important antioxidant enzymes that work synergistically to scavenge excess oxygen free radicals, playing crucial protective roles. Their activities are related to immune performance and environmental stress [17-18]. In this study, different tissues showed varying responses: SOD and CAT activities were higher in hepatopancreas and muscle, while SOD was more active in hemolymph. Both enzymes generally showed an "increase-decrease" trend across salinity treatments, peaking at 40 psu, suggesting that moderate salinity stress activates antioxidant defenses. However, at higher salinities, antioxidant capacity was suppressed, significantly reducing the shrimp' s stress resistance. Deng Pingping et al. [19] reported similar adaptive changes in antioxidant enzymes of turbot (*Scophthalmus maximus*) in response to salinity variation.

AKP and ACP are important for calcium absorption and chitin formation during molting. ACP is a lysosomal component released during hemocyte phagocytosis and encapsulation reactions [20-21]. Wang Gengshen et al. [23] found that salinity significantly affects AKP and ACP activities. Liu Cunqi et al. [22] reported that environmental factors and Ca^2 concentration influence phosphatase activity, which can indicate immune capacity. In our study, both enzymes showed higher activities in hepatopancreas than in muscle, with the highest activities in hemolymph and hepatopancreas, consistent with Wang Gengshen' s findings. The activity patterns likely reflect adaptive responses to osmoregulatory demands–synthesis of AKP and ACP requires inorganic phosphate, and increased enzyme production at moderate salinities (40 psu) may support osmoregulation. However, at extreme salinities, enzyme activities were suppressed.

This study demonstrates that amylase, lipase, pepsin, CAT, SOD, ACP, and AKP activities are closely related to salinity, with optimal activities around 40 psu. Excessively high salinity reduces enzyme activities, suggesting that salinity should be maintained below 50 psu in *L. vannamei* culture to support optimal digestive and immune function.

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Note: Figure translations are in progress. See original paper for figures.