Effects of Chitosan on Oxidative Stress Biomarkers and Histopathological Changes in Whiteleg Shrimp (*Litopenaeus vannamei*)

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Abstract

Whiteleg shrimp (*Litopenaeus vannamei*) is a crustacean species that is extensively cultivated and exported across the Americas, Europe, and Asia, including Thailand. As a result, whiteleg shrimp has become one of the most important aquaculture species. According to viral, bacterial, parasitic, and fungal infections, as well as physiological stress arising from an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms, can lead to abnormal clinical manifestations in shrimp and, in severe cases, mortality. In the present study, chitosan, a biopolymer widely utilized in pharmaceutical and nutraceutical applications, was incorporated into shrimp feed and divided into 5 groups, including the control group, the non-irradiated chitosan group, the irradiated chitosan at different concentrations of 0.1%, 0.2% and 0.4% of feed, respectively. Shrimp samples were collected and analyzed through biochemical and histopathological examinations to assess the effects on various organs in comparison with a control group. The results indicated that shrimp receiving irradiated chitosan at 0.2% of feed demonstrated effective antioxidant activity and a reduction in oxidative damage across several organs. These findings were consistent with the histopathological observations, which revealed normal organ structures. In contrast, shrimp fed non-irradiated chitosan, and those receiving irradiated chitosan at 0.1% of feed did not exhibit sufficient efficacy in reducing reactive oxygen species. For supplementation with irradiated chitosan at 0.4% of feed significantly increased glutathione (GSH) levels, it may have induced tissue stress in certain organs, such as the hepatopancreas and intestine.

Introduction

Whiteleg shrimp (*Litopenaeus vannamei*) is an economically important aquatic species that is widely consumed. As a result, the farming of whiteleg shrimp has increased significantly in recent years. However, one of the major challenges in shrimp aquaculture is disease, which can arise from several factors such as viruses, bacteria, parasites, fungi, and stress conditions caused by an imbalance between free radicals and antioxidants in the body, known as oxidative stress. These conditions can lead to abnormal symptoms in shrimp and, in severe cases, may result in mortality. Currently, there has been increasing research on the development of biopolymers to enhance immunity and improve resistance to diseases. In this study, chitosan was used to evaluate its effectiveness in antioxidant activity and its ability to protect cellular integrity. The findings obtained from this research may provide guidance and contribute to further development in shrimp aquaculture.

Materials and Methods

Experimental treatments



- Control group
- Normal chitosan
- Irradiated chitosan 0.1% 0f feed
- Irradiated chitosan 0.2% 0f feed
- Irradiated chitosan 0.4% 0f feed

Sample extraction

Hemolymph, Hepatopancreas, Intestine, Muscle, Gill



The samples were mixed with 0.1 M PBS, homogenized and centrifuged at 3,500 rpm at 4 °C for 10 min to collect supernatant



Stored supernatant at -20 °C

Biochemical assays

Biochemical assays in hemolymph and vital organs

MDA, GSH, GPx, GR, SOD, CAT

Tissue collection and preparation



Hepatopancreas, Gill, Intestine, Muscle



fix in 10% formalin

Histopathological examination

Tissue processing → Embedding → Sectioning → Staining → Microscopic observation



Data analysis

RStudio

One-way ANOVA test using Rstudio program

Results

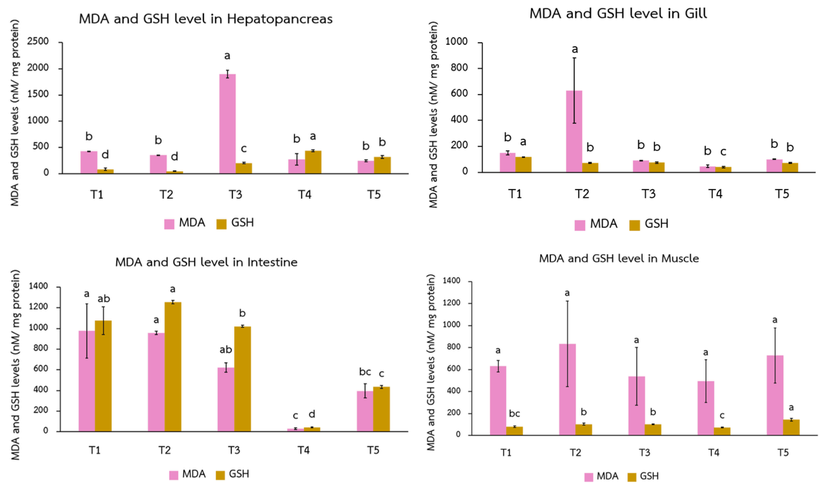


Fig 1 Oxidative stress markers (MDA and GSH) in each organ under different dietary chitosan concentrations for 4 weeks. Data are presented as mean ± standard error (S.E.). Superscript letters denote significant differences ($p < 0.05$) between experimental treatments.

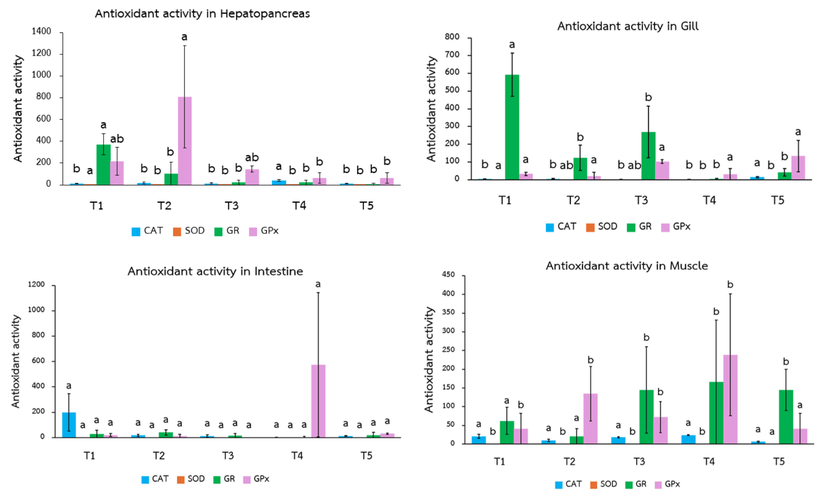


Fig 2 Antioxidant enzyme activity (CAT, SOD, GR and GPx) in each organ under different dietary chitosan concentrations for 4 weeks. Data are presented as mean ± standard error (S.E.). Superscript letters denote significant differences ($p < 0.05$) between experimental treatments.

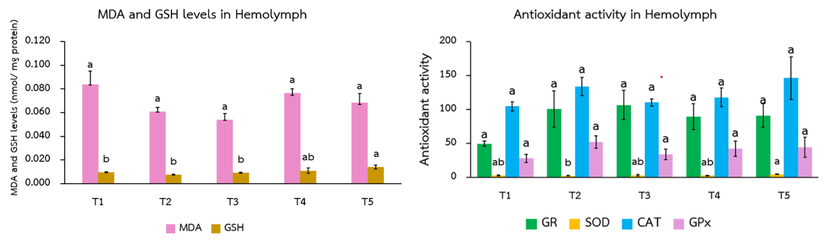


Fig 3 Oxidative stress markers (MDA and GSH) and Antioxidant enzyme activity (CAT, SOD, GR and GPx) in hemolymph under different dietary chitosan concentrations for 4 weeks. Data are presented as mean ± standard error (S.E.). Superscript letters denote significant differences ($p < 0.05$) between experimental treatments.

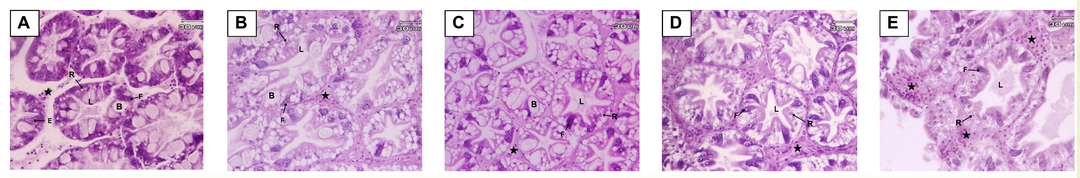


Fig 4 Hepatopancreatic tissues of shrimp fed with different concentrations of chitosan and control group. Control (A), normal chitosan group (B), irradiated chitosan 0.1% of feed (C), irradiated chitosan 0.2% of feed (D) and irradiated chitosan 0.4% of feed (E). H&E, 20x magnification. B; B cell, E; E cell, F; F cell, R; R cell, L; lumen, black star; hemocytic infiltration.



Fig 5 Gill tissues of shrimp fed with different concentrations of chitosan and control group. Control (A), normal chitosan group (B), irradiated chitosan 0.1% of feed (C), irradiated chitosan 0.2% of feed (D) and irradiated chitosan 0.4% of feed (E). H&E, 20x magnification. C; cuticle, E; epithelial cell, H; hemocyte, IS; interlamaellar space.

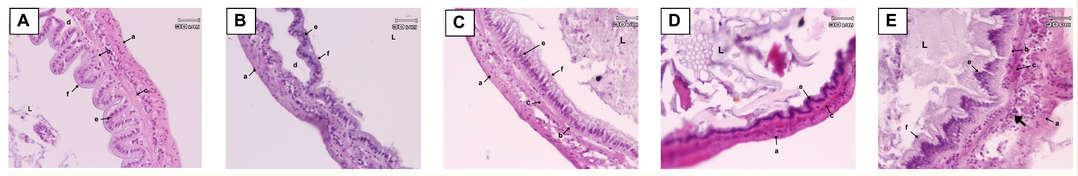


Fig 6 Intestinal tissues of shrimp fed with different concentrations of chitosan and control group. Control (A), normal chitosan group (B), irradiated chitosan 0.1% of feed (C), irradiated chitosan 0.2% of feed (D) and irradiated chitosan 0.4% of feed (E). H&E, 20x magnification. a; external muscle layer, b; basement membrane, c; circular muscle layer, d; epithelial cell peel, e; epithelial cell, f; brush border, L; lumen, black arrow; hemocytic infiltration.

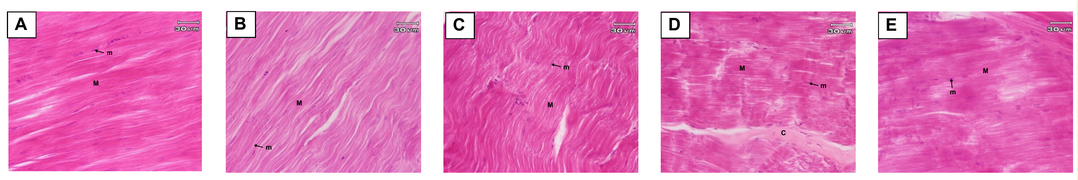


Fig 7 Muscular tissues of shrimp fed with different concentrations of chitosan and control group. Control (A), normal chitosan group (B), irradiated chitosan 0.1% of feed (C), irradiated chitosan 0.2% of feed (D) and irradiated chitosan 0.4% of feed (E). H&E, 20x magnification. m; myocyte, C; connective tissue, M; muscle fiber.

Conclusion

The supplementation of chitosan, both in its normal and irradiated forms, in shrimp diets showed a tendency to enhance the antioxidant defense system. In particular, irradiated chitosan at concentrations of 0.2% and 0.4% of the feed demonstrated promising potential as a dietary supplement to promote shrimp health. However, in terms of histological observations, irradiated chitosan at 0.4% of the feed caused abnormalities in the tissues of the hepatopancreas and intestine. These findings suggest that chitosan supplementation at appropriate levels can be applied in the aquaculture industry, especially in the culture of whiteleg shrimp and other economically important aquatic species.

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