天然由来アスタキサンチンのシロアシエビ Penaeus vannameiのストレスおよび免疫関連遺伝子への影響

[課程博士・論文博士共通]

博士学位論文要約
Summary of Dissertation

専　攻 Major	Applied Marine Biosciences	氏　名 Name	THANOMCHAISANIT PHANTIWA
論文題目 Title	Effects of naturally derived astaxanthin on stress- and immune-related genes in Whiteleg shrimp *Penaeus vannamei*		

Astaxanthin is the pigment in xanthophyll the major group of carotenoids that appears in red, or orange-reddish colors and plays a role in circulating lipoproteins and cell membranes, anti-inflammatory activity and has significantly high antioxidant. The natural source of astaxanthin is produced naturally in many creatures including *Haematococcus pluvialis* (Green algae), *Phaffia rhodozyma* (Red yeast) *Mycobacterium lacticola* (Gram-positive bacteria), and *Euphausia superba* (Antarctic krill). Astaxanthin is a high-value carotenoid produced by microalgae, and it is widely used as a feed supplement for the growth of salmon, shrimp, and ornamental fish. Research on astaxanthin supplements for *P. vannamei* natural astaxanthin supplements have shown potential benefits in enhancing tolerance to environmental pressure in *P. vannamei*. Astaxanthin, a powerful antioxidant, is found in marine ecosystems and is known for its various health-promoting. Natural astaxanthin supplements offer promising benefits for enhancing tolerance to environmental pressure in *P. vannamei*. Further research and application of these supplements could prove beneficial for aquaculture practices involving *P. vannamei*. The immune system of *P. vannamei* plays a crucial role in protecting the organism from pathogens and maintaining its health, various factors, such as hemocyte count, phenoloxidase activity, respiratory burst, and the use of probiotics, can significantly impact the immune response and resistance to pathogens in white shrimp. Heat shock proteins (HSPs) are indeed involved in the *P. vannamei*. Research has shown that HSP70 and HSP90 are involved in the shrimp's tolerance to certain strains of bacteria and viruses, such as *Vibrio parahaemolyticus* and white spot syndrome virus (WSSV).

Research has demonstrated that dietary supplementation of astaxanthin can lead to better growth performance and coloration in *P. vannamei*. Astaxanthin is also reported to enhance the immune response and resistance to Vibrio infection in white shrimp, in addition to improving red coloration. Astaxanthin can improve the color of exoskeletons. The results show the impact of astaxanthin as a feed additive on color intensity after a month feeding trial, astaxanthin can improve the color of shrimp. Growth performance, there was no significant difference in shrimp weight. However, after a one-month feeding trial, there were slight differences between the dietary supplementation of astaxanthin and the commercial shrimp diet. This study has shown that dietary supplementation with astaxanthin leads to a significant increase in crustin gene expression in gills along with the upregulation of other immune-related genes and antioxidant enzymes including lysozyme, proPO, trypsin, hemocyanin C chain-like, and hepatic lectin-like. This indicates that astaxanthin enhances the immune response and antioxidant status of *P. vannamei*, potentially contributing to improved resistance to bacterial infections and overall health. Dietary supplementation with astaxanthin has been shown to enhance the growth performance including increased phenoloxidase activity, phagocytic activity, and immune response of *P. vannamei*. The growth indices, such as final weight, weight gain, and specific growth rate, were significantly higher in shrimp fed with astaxanthin-enriched diets compared to the control group. Heat shock proteins are important markers in the cellular stress response. Studies have shown that astaxanthin supplementation can lead to an enhanced elevation in HSPs, indicating a potential augmentation of the cellular stress protective response to heat stress. This suggests a relationship between astaxanthin and the expression of HSPs, highlighting the potential of astaxanthin to influence the cellular stress response.

Salinity is one of the most important environmental factors in shrimp farming, as it can significantly impact the growth, food intake, and survival rate of shrimp. Variations in salinity can lead to stress, slow growth, and low survival rates, causing high economic loss in shrimp culture. Dietary astaxanthin has several benefits for *P. vannamei* in salinity stress. Astaxanthin, a powerful antioxidant, can improve growth performance, enhance stress tolerance, and increase survival in shrimp under different environmental stresses, including salinity stress. It has been shown to increase the total antioxidant capacity, decrease oxidative stress, and enhance immune response and resistance to salinity stress. Low salinity stress led to the significant upregulation of HSP gene throughout the test period under normal feed. Astaxanthin supplementation can alleviate stress in shrimp and enhance immune-related genes. Astaxanthin-supplemented feed lowered the expression of HSP indicating enhanced capacity to tolerate low salinity. HSP are rapidly induced after stresses that the result showed in normal feed. This suggests that the shrimp fed with astaxanthin may already have sufficient proteins necessary to protect them against stress. Therefore, it is evident that salinity stress can significantly impact the immunity of *P. vannamei*, and measures such as maintaining high salinity levels and dietary supplementation can help mitigate these effects.

The aquaculture industry faces a significant hurdle in managing these ever-changing environmental parameters, particularly in outdoor settings where nature's unpredictability significantly impacts shrimp farming success. Temperature fluctuations impact crucial chemical and biological processes, including dissolved oxygen levels, chemical reactions, photosynthesis, aerobic respiration, and organism metabolism. Our findings showed significantly higher expressions of CAT and SOD in the hepatopancreas of shrimp fed with astaxanthin-supplemented feed before the stress test, highlighting the potential of astaxanthin to bolster antioxidant capacity. Although not all gene expressions significantly increased, astaxanthin supplementation notably improved shrimp survival under acute low-temperature stress. The hepatopancreas, crucial for nutrient storage and immune function, exhibited the most pronounced response in terms of gene upregulation, suggesting the positive impact of astaxanthin on immune-related and antioxidant genes, potentially enhancing stress tolerance in shrimp. In conclusion, the study underscores the potential benefits of astaxanthin supplementation in mitigating low-temperature stress effects on *P. vannamei*. Astaxanthin enhances immune responses and antioxidant capacity, contributing to increased survival rates. While the FDA approves astaxanthin for fish feed, further research should explore optimal dosages and application methods tailored to shrimp farming. Long-term effects and cost-effectiveness in commercial shrimp farming warrant additional investigation for sustainable and profitable aquaculture practices.

Vibrio parahaemolyticus is a gram-negative bacterium that can cause various illnesses, including gastroenteritis and wound infections. It was first discovered in the 1950s in Japan. Acute hepatopancreatic necrosis disease (AHPND) caused by toxin from *V. parahaemolyticus* the disease that has devastated the global shrimp industry, can spread quickly in the shrimp's tissues, particularly targeting the hepatopancreas, and cause damage even after the bacteria are eliminated by the shrimp's immune system The disease has been associated with substantial mortality and economic losses in shrimp farming, making it a critical area of research for understanding its virulence, pathogenesis, and mitigation strategies. Astaxanthin contributes to increased survival rates. Therefore, the result suggests that astaxanthin has a positive effect on the resistance of white shrimp to *V. parahaemolyticus*.