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

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[課程博士・論文博士共通]

博士学位論文内容要旨
Abstract of Dissertation

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論文題目 Title	Effects of naturally derived astaxanthin on stress- and immune-related genes in Whiteleg shrimp *Penaeus vannamei*		

Whiteleg shrimp (*Penaeus vannamei*) is a commercially important aquaculture species farmed worldwide, however, it is susceptible to stress caused by fluctuations in environmental conditions, particularly salinity and temperature. These variations can disrupt the delicate balance necessary for optimal shrimp growth and health. 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. Studying the potential application of natural feed additives in aquaculture is an important and growing area of research. Thus, this study investigated the efficacy of dietary astaxanthin supplementation on the survival, behavior, and gene expression of *P. vannamei* subjected to environmental fluctuation stress, namely: low salinity and low-temperature stress.

Astaxanthin, a member of the Xanthophyll carotenoid group, includes β-cryptoxanthin, β-carotene, lycopene, and zeaxanthin. This compound is red or orange-reddish in color and occurs naturally in various organisms such as *Haematococcus pluvialis* (green algae), *Phaffia rhodozyma* (red yeast), and *Mycobacterium lacticola* (a Gram-positive bacteria) *Euphausia superba* (Antarctic krill) which have large yields and serve as main sources of astaxanthin. Furthermore, natural astaxanthin is accumulated in animals that consume microalgae such as in shrimp, crab, and the flesh of salmonids, and these serve as secondary sources. In fact, astaxanthin was originally isolated from lobsters in 1937, and predominantly used in the feed industry to enhance growth in many species of aquatic animals. Additionally, it exhibits significant antioxidants, immunomodulatory, anti-inflammatory, anti-proliferative, anti-apoptotic, and anti-cancer properties in humans.

After one month of the feeding period, the dietary supplementation with astaxanthin influenced the growth of shrimp since the overall body weight was higher compared to normal-fed shrimps, although the difference was not statistically significant. Moreover, the shrimp showed intense color change after feeding with astaxanthin. Lastly, through Quantitative PCR (qPCR) analysis, it was discovered that astaxanthin supplementation upregulated the expression of immune-related genes such as crustin, lysozyme, and

prophenoloxidase (proPO) were upregulated after astaxanthin feeding – indicating an increase in immune capacity.

Effects of astaxanthin supplementation on the adaptability to acute low salinity stress were analyzed in the expression of stress-, immunity-, and antioxidant-related genes. Briefly, shrimp were acclimated and divided into two groups: one fed with a normal commercial diet (N) and the other with astaxanthin-supplemented feed (Ax). Shrimp were subjected to low salinity stress at 10 ppt for 24 hours after continuous feeding for 1, 2, 3, and 4 weeks.

During the low salinity stress all shrimp were alive, and the gene expression of stress-induced genes (heat shock proteins: HSP60, HSP70, HSP90), immune-related genes (crustin, lysozyme, proPO), hepatic lectin-like, hemocyanin C chain-like), antioxidant genes (superoxide dismutase (SOD)), and digestive gene (trypsin) were analyzed. Results showed that astaxanthin supplementation can reduce stress in shrimp, improve the expression of genes involved in the immune system, and lower the expression of HSPs suggesting enhanced capacity to tolerate low salinity.

The defensive potential of dietary supplementation against low-temperature stress was first screened by analyzing the survival rates, and the expression of stress-, immunity-, and antioxidant-related genes. After a feeding period of 2 and 4 weeks, shrimp were exposed to low-temperature stress at 10°C.

Results showed that astaxanthin supplementation significantly improved the survival rates of shrimp after low-temperature stress, indicative of its potential to enhance tolerance against low temperature. Furthermore, qPCR analyses also revealed that astaxanthin supplementation induced the expression of stress-induced genes, possibly priming the shrimp from stress. After being subjected to low-temperature stress, astaxanthin supplementation reduced the expression of stress-induced genes in shrimp. This suggests that the shrimp may already have sufficient proteins necessary to protect them against stress. Additionally, the expression of immune-related and antioxidant genes was positively influenced by astaxanthin supplementation-indicating improved immune response and antioxidant capacity.

In summary, although not all gene expressions significantly increased, astaxanthin supplementation notably improved shrimp survival under acute low-temperature stress. Immune-related genes and antioxidant genes showed upregulation in shrimp fed with astaxanthin-supplemented feed. Antioxidant enzymes play critical roles in combating oxidative stress induced by temperature fluctuation.

The study underscores the potential benefits of astaxanthin supplementation in mitigating low salinity and low-temperature stress effects on Whiteleg shrimp. Astaxanthin enhanced 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.

Keywords: Whiteleg shrimp, astaxanthin supplementation, low-temperature stress, survival, gene expression, immune response, antioxidant capacity.