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Health & Welfare

Effective antioxidant and antimicrobial feed additives for juvenile Pacific white shrimp farmed at high density

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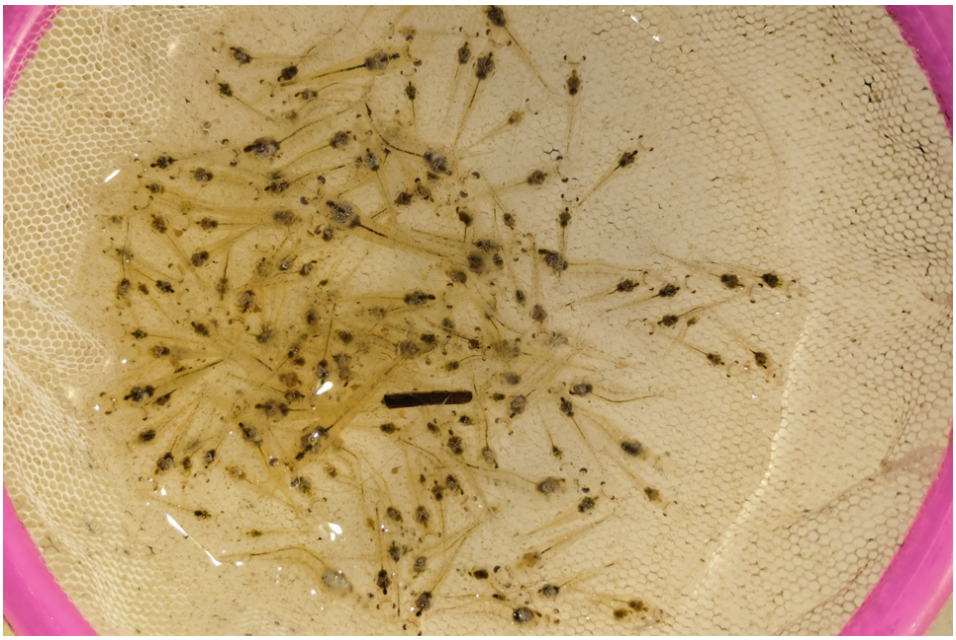
By Shafaq Fatima

The supplementation of N-acetyl-L-cysteine and lauric acid in Pacific white shrimp diets can enhance growth, antioxidant and immune responses



This study evaluated the immune and antioxidant response to dietary lauric acid and N-acetyl-L-cysteine of Pacific white shrimp cultured at high stocking density. The supplementation of NAC and LA at 0.2 percent each in shrimp diets can enhance growth, antioxidant and immune responses. Photo by Francisco Miranda.

The shrimp farming industry globally continues to face significant disease challenges. A highly developed immune system is crucial for preventing disease outbreaks in intensive culture and minimizing the reliance on antibiotics. Several categories of feed additives (amino acids, minerals, probiotics, prebiotics, peptides, organic acids, nucleotides) have been used in aquaculture to improve the innate and adaptive immunity against common pathogens and oxidative stress.

Among a number of innovative immunomodulatory factors, lauric acid (LA) – a saturated, medium-chain fatty acid, naturally found in high concentrations in oils (coconut, palm, black soldier fly larvae) – exhibits strong antibacterial and antiviral properties, is particularly **effective against gram-positive bacteria** (https://doi.org/10.1007/s10529-006-9127-2), inhibits biofilm formation, membrane biosynthesis and virulence factors, may eliminate vegetative cells and spores, and **improves antioxidative capacity** (https://doi.org/10.1128/jb.00743-12). LA benefits have been reported Above mentioned role of purified lauric acid as feed additive has been reported in broilers, mammals and in black sea bream. However, to the best of our knowledge, no study has investigated its role in improving innate immunity in shrimp or other crustaceans.

Hypoxia is the key challenge under high stocking densities and intensive culture which compromises the immune response of animals and increases their **susceptibility to diseases and reduced growth** (https://doi.org/10.1079/NRR200369). Stressors like hypoxia can lead to increased generation of reactive oxygen species (ROS) involved in oxidative stress and ageing. Artificial antioxidants such as N-acetyl-L-cysteine (NAC) are used as agonists of natural ROS scavengers. There is growing evidence that NAC as a feed additive improves antioxidant activity in common carp, tilapia, rainbow trout, large yellow croaker and other aquacultured species.

This article – **summarized** (https://doi.org/10.1371/journal.pone.0315819) from the **original publication** (https://creativecommons.org/licenses/by/4.0/) [Fatima, S. 2025. N-acetyl-L-cysteine and lauric acid; effective antioxidant and antimicrobial feed additives for juvenile Pacific white shrimp (*Litopenaeus vannamei*) cultured at high stocking density. *PLoS ONE 20(1): e0315819*] – discusses the results of a study that investigated if dietary LA and NAC could improve the immune and antioxidant responses in Pacific white shrimp cultured at high stocking density. The main objective was to evaluate their potential use as feed additives in intensive shrimp farming at commercial scale.

Study setup

This study was conducted at Purdue University Fort Wayne (Indiana, USA). Shrimp (initial average weight = 0.65 grams), procured from Auburn University (Alabama, USA) were acclimatized for two weeks, and then 270 animals were randomly distributed in fifteen aquaria (Area = 4.50 cubic feet). The shrimp were grown at low stocking density (LSD) (n = 10/0.80 cubic feet per replicate) and high stocking density (HSD) (n = 20/0.80 cubic feet per replicate). Five shrimp groups were used: T1: negative control at LSD, T2: positive control at HSD, T3: at HSD and fed with LA supplement diet, T4: at HSD and fed with NAC supplemented diet, T5: at HSD and fed with combination of LA and NAC).

The five dietary treatments (used in triplicate) were:

- Treatment T1: low stocking density; fed with diet D0.
- Treatment T2: High stocking density; fed with diet D0.
- Treatment T3: High stocking density; fed with diet D1 (LA).
- Treatment T4: High stocking density; fed with diet D2 (NAC).
- Treatment T5: High stocking density; fed with diet D3 (LA+NAC).

Four different diets were prepared with supplementation of commercially-sourced lauric acid (LA) and N-Acetyl-L-cysteine (NAC) at 0.2 percent each and sprayed on a commercial feed (Zeigler, USA). Shrimp were fed four times a day. An expected growth rate of 1 gram per week was assumed for feed calculation, and the daily feed ration was adjusted according to observed mortality and feed consumption. The trial lasted for 8 weeks, and then the total length and weight of each shrimp in all replicates of each treatment were measured to calculate the mean final weight, weight gain, condition factor and feed conversion ratio (FCR).

For detailed information on the experimental design, diet preparation, animal husbandry; hemolymph and tissue sampling; RNA isolation, cDNA synthesis and qPCR; and data analyses, refer to the original publication.

Researchers examine antimicrobial resistance potential in aquaculture

The influence of antibiotics in fish and their effects on the gut microbiome aren't well understood, says research team examining antimicrobial resistance.

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Results and discussion

Study results show higher weight gains in treatments T3, T4 and T5 while maximum value was noted in T4. Condition values showed a significant difference and were less than 1 in all treatments. FCR was also calculated to be less than 1 in all treatments. The levels of total protein, glucose, total hemocyte count and phagocytic index were noted to be significantly different among all five treatments but quantitatively higher in T3, T4 and T5 as compared to others. The highest values of these parameters were observed in T4.

The levels of malondialdehyde, MDA – a widely used convenient biomarker for oxidative stress to determine lipid peroxidation of polyunsaturated fatty acids, PUFAs, especially omega-3 and omega-6 fatty acids – were significantly different between all treatments (Fig 1A). However, T4 and T5 were found to be in same subset. Lower concentrations of MDA were noted in treatments fed with NAC (T4 and T5) supplementation as compared to the high stocking density (HSD) control. The profile of all antioxidant and immune response genes showed significant difference between all five treatments (Fig 1B–1F). The levels of the enzyme superoxide dismutase (SOD; a very important antioxidant defense against oxidative stress in the body) were significantly lower in treatments fed with NAC and their combination (T4 and T5) (Fig 1B) as compared to T2. Similar results were observed in the profile of the enzyme glutathione peroxidase (GPX; a very important antioxidant enzyme) genes when shrimp fed with NAC supplemented diet showed the significantly lowest levels (Fig 1C).

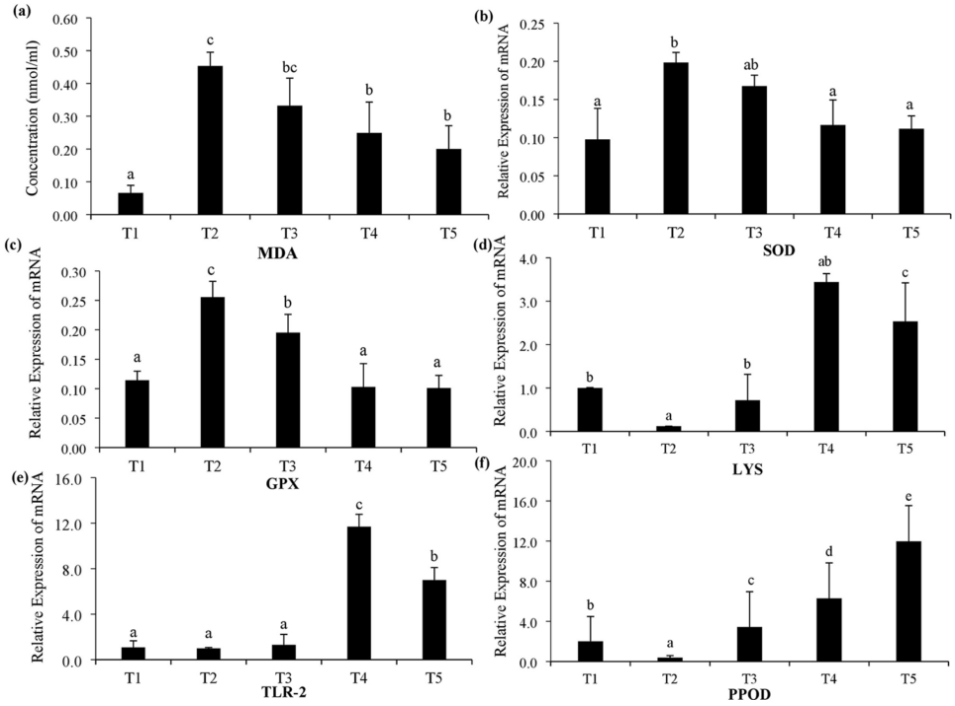

Fig. 1: Concentration of malondialdehyde (MDA) in hemolymph measured by ELISA (a). Relative expression of mRNA of superoxide dismutase (SOD) (b), lysozyme (LYS) (c), glutathione peroxidase (GPX) (d), toll like receptor-2 (TLR-2) (e) and Prophenoloxidase (PPOD) (f) in four different treatments (T1, lows stocking density, T2, high stocking density, T3: lauric acid, T4: N-acetylcysteine, T5: lauric acid+N-acetylcysteine.

The Prophenoloxidase (PPOD) activation system plays a crucial role in pathogen recognition and defense in crustaceans. Lysozyme (LYS) is an enzyme that exerts its activity by hydrolyzing the gram-positive bacterial cell wall. Given its ability to disrupt the bacterial cell wall, lysozyme is recognized as an endogenous antibiotic, playing a vital role in the innate defense against microbes. Our findings reveal an upregulation of LYS and PPOD expression, number of hemocytes and phagocytic index in shrimp fed with NAC and LA supplemented diets, indicating an improved immune function enhanced by these two compounds when compared with controls. A gradual increase in expression of PPOD was noted in T3, T4 and T5 (Fig 1F). The highest expression of this gene was shown in shrimp fed with combination of NAC and LA. In LSD and HSD treatments which were not given any supplement showed lower expression of all genes as compared to other dietary treatments.

Results of the study demonstrated that dietary supplementation of NAC (0.2 percent) and LA (0.2 percent) can improve growth, antioxidant and antimicrobial capacity in Pacific white shrimp. Research on the impact of NAC or LA supplementation on the growth and innate immunity of shrimp is currently limited. Consequently, there is a scarcity of published data specifically addressing and discussing these effects in relation to this species. However, studies reported on humans, broiler, pig and fish strongly support that **NAC plays a critical role** (https://doi.org/10.1042/BJ20130282) as ROS scavenger/antioxidant and protects from damage caused by lipid peroxidation, DNA damage, oxidation of proteins and apoptosis.

Lauric acid is a naturally occurring fatty acid with **significant antibacterial activity** (https://doi.org/10.1016/j.aquaculture.2019.734504) against a wide range of bacteria. It induces microbial membrane disruption, activates reactive oxygen species production in pathogens, and other beneficial effects. Results of the present study confirm the potential use of LA as an additive in shrimp feed to **enhance immunity** (https://doi.org/10.3389/fmicb.2017.02635) against bacteria and viruses. However, further studies are required to investigate its role in signaling pathways against common and virulent pathogens in aquaculture.

Similar to improved antioxidant and antimicrobial activity, the better growth in NAC and LA fed treatments may also be attributed to the multifaceted mechanisms of these nutraceuticals, to activate anti-inflammatory and immunomodulatory functions as observed in several aquaculture species like common carps, Chinese mitten crabs and Nile tilapia. The higher levels of total protein and glucose in T3 and T5 also indicate the improvement in nutrient supply and their transport via globulin and albumin, which may be due to inclusion of LA in diets as observed in poultry and black sea bream.

Similar effects of NAC and LA on growth have been previously indicated by better gut microbiota, longer intestinal villi, and improved regulation of microbiome, thereby **enhancing gut health** (https://doi.org/10.1016/j.fsi.2006.06.004). Particularly, LA maintains its stability as it traverses the gastrointestinal tract, ensuring its eventual absorption. This characteristic brings LA into direct interaction with gut microbiota, contributing to the enhancement of host health and physiology through improved metabolism and immunity. Higher levels of glucose observed in T1 as compared to that in T2 could be attributed to the **fast metabolism of available glucose** (https://doi.org/10.1016/j.fsi.2017.11.016) to cope with high energy needs.

Perspectives

The findings of the present study confirm that the supplementation of NAC and LA in the diet of Pacific white shrimp can enhance growth, antioxidant, and immune responses. Further investigation is required to determine the optimal doses at higher stocking densities that can be more effective against common aquaculture pathogens. Unfortunately, due to restrictions on the use of pathogenic bacteria in laboratory, the present study could not subject the treatments to challenge with virulent bacteria. Future studies can explore these aspects, which will be beneficial in enhancing the antioxidant and immune responses of Pacific white shrimp in intensive culture.

Author

SHAFAQ FATIMA

Department of Biological Sciences, Purdue University Fort Wayne, Fort Wayne, Indiana, United States of America

shaff01@pfw.edu (mailto:shaff01@pfw.edu)