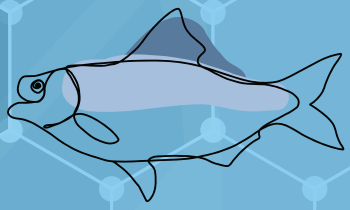
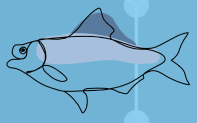
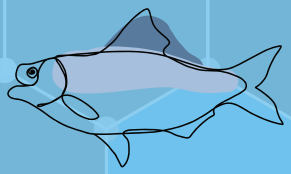
Book of Abstracts





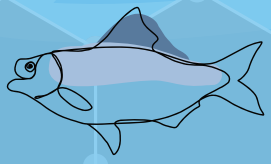


AQUA-THAILAND

The 2nd INTERNATIONAL CONFERENCE ON SUSTAINABLE AQUACULTURE

HEALTH AND DISEASE MANAGEMENT

17 - 18 MARCH 2022



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AQUA-THAILAND

The 2nd International Conference on Sustainable Aquaculture: Health and Disease Management

(Virtual Conference)

17 – 18 March 2022

Organized by

National Center for Genetic Engineering and Biotechnology
National Science and Technology Development Agency
Ministry of Higher Education, Science, Research and Innovation

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The 2nd International Conference on Sustainable Aquaculture: Health and Disease Management

17 – 18 March 2022

First Edition, 17 March 2022

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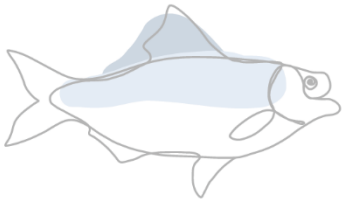
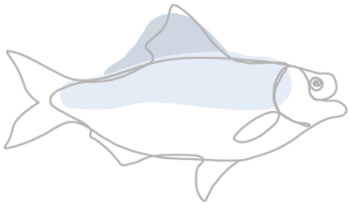
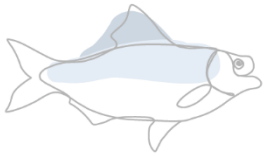






ABOUT THE CONFERENCE

The 2nd International Conference on Sustainable Aquaculture: Health and Disease Management

DATE: 17 – 18 March 2022

ORGANIZED BY:
National Center for Genetic Engineering and Biotechnology
National Science and Technology Development Agency
Ministry of Higher Education, Science, Research and Innovation

EVENT CONCEPT:
This virtual conference on aquatic animal health and disease management is part of a series of international conferences on aquaculture technology in the early and late stages of development of the production process. This goal of this conference is to provide a platform for the exchange of knowledge, and technologies in the field of health and disease management both domestically and internationally. It also aims to highlight trends that are critical for the aquaculture industry to meet global sustainability standards in the future and provide a platform for collaboration among researchers or organizations working on aquaculture health worldwide.

RATIONALE:
Aquaculture is the farming of aquatic animals. Global aquaculture production set a new record in 2018 at 114.5 million metric tons live weight, with a total commercial value of $263.6 billion (the price of product available at the farm, excluding separately invoiced transportation or delivery costs) (*FAO,2020*). The total production was composed of 82.1 million tons of aquatic animals, 32.4 million tons of aquatic algae, and 26,000 tons of shells and pearls for ornamental purposes.

Aquaculture will continue to be the driving force behind growth in global fish production, continuing a decades-long trend. Aquaculture production is projected to reach 109 million metric tons in 2030, but the average annual growth rate of aquaculture is expected to slow from 4.6 percent in 2007-2018 to 2.3 percent in 2019-2030.

Despite the important role that aquaculture plays in the global economy, the success and sustainability of the sector depends heavily on the issue of disease management. Often, the success or loss of aquaculture enterprises depends on how effectively farmers and researchers are able to manage diseases in the farmed environment. Therefore, successful disease management is one of the critical keys to achieving high profits for farmers and ensuring food security for consumers.

The 2nd International Conference on Sustainable Aquaculture aims to advance global understanding of disease control strategies and research trends to improve aquatic animal

health. The conference theme, **Health and Disease Management**, recalls approaches to mitigate and prevent disease outbreaks while striving for sustainable aquaculture production. The conference theme encourages dialog and collaboration to share advances in aquaculture research, knowledge, and technology. Recent understandings of the shrimp immune system, pathogenesis, and microbiome management will be covered, and the developments of feed additive, vaccine, and other health management strategies will also be discussed.

We invite interested professionals and researchers to present original papers and participate in the conference to respond to the theme of **Health and Disease Management** and link all these issues to sustainability and excellence in aquaculture production.

CONFERENCE SESSIONS:

- Research trend to improve shrimp health and well-being
- Managing the microbiome and other strategies for better aquatic animal health and disease mitigation

SESSION CHAIRS:

- Dr. Kallaya Dangtip — Aquatic Animal Health Research Team, Integrative Aquaculture Biotechnology Research Group, National Center for Genetic Engineering and Biotechnology
- Dr. Wanilada Rungrassamee — Biosensing and Bioprospecting Technology Research Group, National Center for Genetic Engineering and Biotechnology

SESSION COORDINATORS:

- Dr. Sage Chaiyapechara — Aquaculture Service Development Research Team, Integrative Aquaculture Biotechnology Research Group, National Center for Genetic Engineering and Biotechnology
- Dr. Wananit Wimuttisuk — BioAssay Research Team, Biosensing and Bioprospecting Technology Research Group, National Center for Genetic Engineering and Biotechnology

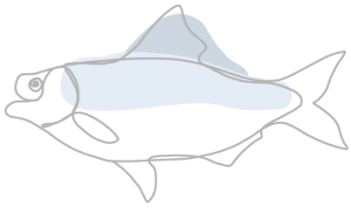
ABSTRACT LIST

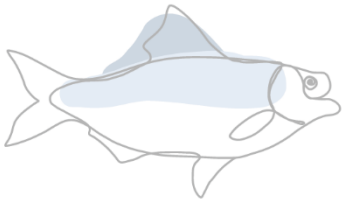
CONFERENCE SESSION

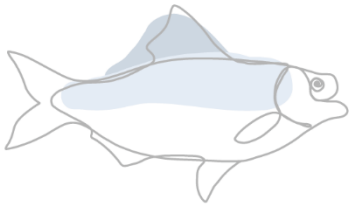
E-POSTER SESSION

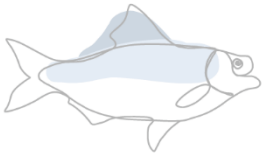
ABSTRACT

CONFERENCE SESSION



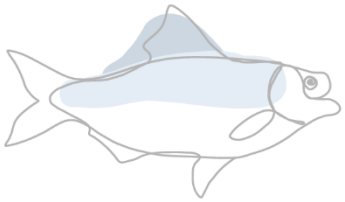


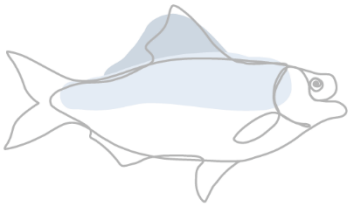


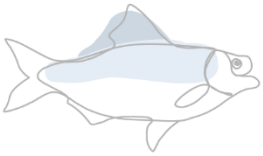


SESSION 1

RESEARCH TREND TO IMPROVE SHRIMP HEALTH AND WELL-BEING







A comprehensive and collaborative approach is critical to promote successful shrimp aquaculture

Han-Ching Wang[1, 2,*] and Chu-Fang Lo[2]

[1]Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, Tainan, Taiwan
[2]International Center for Scientific Development of Shrimp Aquaculture, National Cheng Kung University, Tainan, Taiwan

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Abstract:

Aquaculture development aligns with the United Nations Sustainable Development Goals (SDGs), including SDG 2 [Zero hunger], SDG13 [Climate action] and SDG 14 [Life below water]. Asia is the world's largest producer of shrimp aquaculture, with >70% of total global production. For decades, the biggest challenge to this industry has been infectious diseases, especially white spot disease (WSD) and acute hepatopancreas necrosis disease (AHPND), as listed by the OIE [World Organization for Animal Health]. Therefore, reducing the threat of infectious disease outbreaks has always been important in shrimp aquaculture management. In this talk, we will reflect on the journey to date and look forward to the future for research to evidence and shape shrimp health management. Various omics technologies and approaches and integrated systems-biology have been critical to generate new knowledge and elucidate WSSV and AHPND pathogenesis, physiological constraints, and metabolism in shrimp. In addition, many complex molecular mechanisms involved in shrimp immunity, host-pathogen interactions, and the crucial roles of environment and microbiota in disease pathogenesis have been discovered. Furthermore, new knowledge from these holistic omics approaches has been used for evidence-based approaches to breeding, disease management and biosecurity in shrimp aquaculture. Taken together, to combat such multi-factorial diseases, it is essential to use a comprehensive strategy involving a collaborative effort from the global shrimp research community.

The holistic approaches for sustainable aquaculture and modernized shrimp health management practices 2022

Prakan Chiarahkhongman

Aquatics Animal Health Care Products Specialists and Solutions provider (AAHCPS), The Representative of Charoen Pokphand Food Public Company (CPF), Charoen Pokphand Group Global (CPG), Thailand

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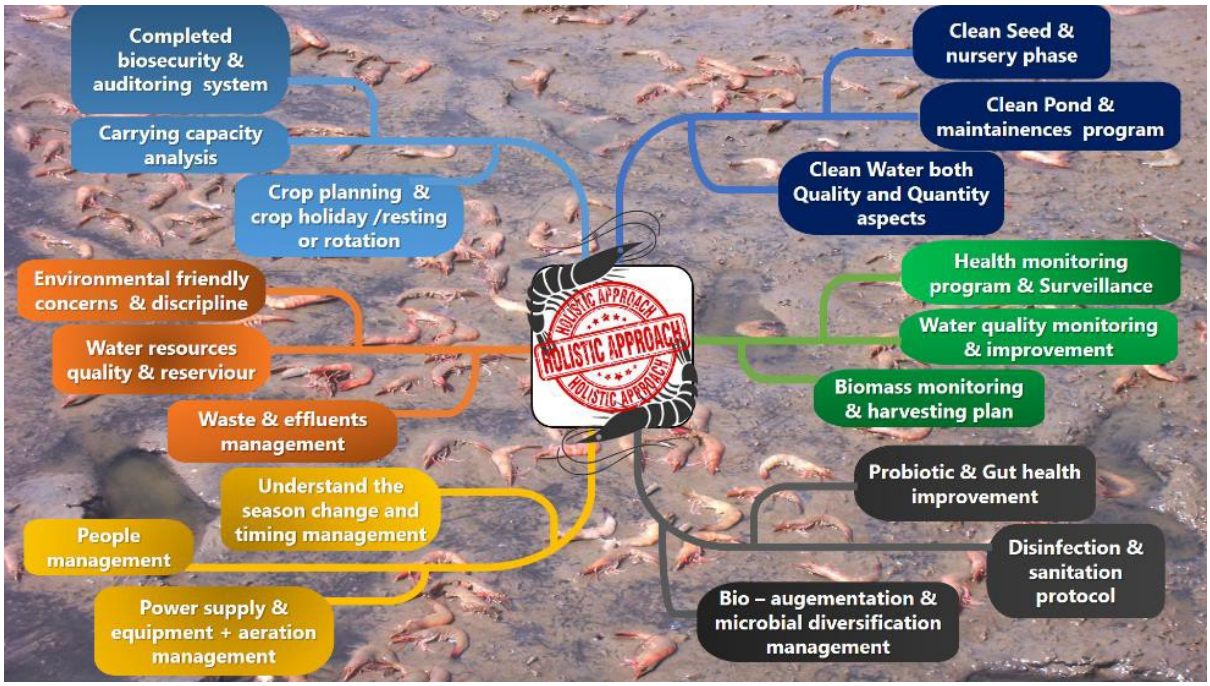
Abstract:

The holistic approaches for sustainable aquaculture to help the farmers to cope with an increasing number of important shrimp diseases i.e. **WSSV, IHHNV, IMNV, DIV1, AHPND, EHP, BACTERIA, WFS** etc. by integrating the 360 degree of the best aquaculture practices with the understanding of environmental deterioration impact which including of the eutrophication, high organic loaded & pathogens accumulation, invasive of virus & microsporidian EHP into bio – carrier or mechanical vector found accumulating in the natural resources water including water system inside the farms. Dramatically increasing environmental impact and overloaded of pathogens shown by active pathogens surveillance program have been done in Thailand and many countries 2022. The holistic approach with the overall understanding of the impact and interaction between all the shrimp farming KPI, diseases challenges & threat factors and environmental impact have been found to be crucial & very important for shrimp farmers to be survived with the diseased environment which definitely cause poor performance, low survival, economically losses & high production cost.

The holistic approach by adopting and combine a range of different culture management strategies can be started with the concept of avoiding the contamination within the ponds and water by using only ***Clean seed*** from ***Clean SPF brood stock*** with the extension of ***Nursery phase*** to promote the bigger and stronger juvenile finally will be benefited by the compensatory growth resulting in able to finish the crop faster and lower the risk during the pond periods. ***Clean water***, in term of both quality and quantity aspects have to be well prepared. Reservoir, Settling, Treatment ponds are needed to be designed. Aging water or resting water until the quality are good for water exchanged are always safe for shrimp. ***Clean pond*** bottom with routinely maintenance program which mean earthen pond and HDPE lining pond must be well examined and cleaned, repeated uses and bad bottom quality must be avoided, leaving sun drying after harvest, burnt liming or disinfecting to eliminate the pathogens and EHP spore have to be considered. ***Completed biosecurity concerns*** comprised with physical, chemical and biological precaution measures with audit system have to be implemented to exclude all of the pathogens. ***Routinely water quality parameter examination & improvement*** and ***Shrimp health monitoring program, Surveillance program, Carrying capacity and Crop planning analysis*** also needed to be considered. ***Pond aids, labor, Equipment and Power supply*** also needed to have a backup plan. ***Season change and Timing management*** which mean understanding of seasonal factors and decreasing the contact time between pathogens and shrimp to be as less as possible are always safe for shrimp, many of modern shrimp farms have been adapted for ***Smaller size culture unit*** such as using HDPE round tanks or Concrete tanks for easily managing the pathogens & EHP spore by ***Exchanging more water*** or ***Transferring (partial harvesting)*** shrimp to new cleaner tanks in case of

overcapacity, deteriorated or contaminated. Biological control by ***Bio– augmentation*** to increase ***MMW (Microbially mature water)*** and microbial diversification including ***Probiotic and Gut health improvement*** have been beneficial and chemical control by ***Disinfecting and Sanitation protocol*** have to be introduced. ***Sludge ponds and ETP*** (Effluent treatment plant) to keep all solid waste and effluents in place to be well treated before discharging also play an important role for diseases mitigation and sustainability environmental-friendly aquaculture system in 2022.



The exploitation of the viral accommodation mechanism to control viral diseases in shrimp

Suparat Taengchaiyaphum

Aquatic Animal Health Research Team (AQHT), Integrative Aquaculture Biotechnology Research Group, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand

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Abstract:

Shrimp cultivation is threatened by viral diseases that have caused serious losses of economy and society to the shrimp farming industry worldwide. The most effective strategies to overcome this problem remain unclear. Recent studies on penaeid shrimp genome have revealed that endogenous viral elements (EVEs) of major shrimp viruses, including white spot syndrome virus (WSSV) and Infectious hypodermal and haematopoietic necrosis virus (IHHNV) occur in the shrimp genome. It was hypothesized that EVE with high sequence identity to extant viruses in shrimp and insects arise via host recognition of viral messenger RNA followed by formation of variable cDNA fragments, here called viral copy DNA or vcDNA from it by host reverse transcriptase (RT). Integration of those vcDNA fragments into the host genome is via host integrase (IN). Some of these EVE could be protective if they produced negative sense-RNA transcripts that result in degradation of viral RNA by the RNA interference (RNAi) pathway. If protective EVE occurred in germ cells, they could be passed on to the next generation and constitute heritable, adaptive immunity. It was proposed that this is the underlying natural mechanism that leads to balanced persistent infections in which one or more viruses are tolerated by shrimp and insects, sometimes for a lifetime, without signs of disease. This phenomenon of tolerance to persistent viral infections had been called viral accommodation hypothesis proposed for the first time in 2009 (VAH-2009). Until today, there are several publications from our team supported VAH-2009 including identification of protective EVEs and their cognate transcripts in the *P. monodon* domesticated broodstock, the Mendelian inheritance of EVEs in *P. monodon* and establishment of a protocol for cvcDNA (circular form of vcDNA) preparation for extracting IHHNV-cvcDNA that matched the sequence of infective IHHNV in shrimp. The extracted IHHNV-cvcDNA was shown to inhibit IHHNV replication when it was injected into *P. vannamei* challenged with IHHNV. Our interesting results supported the VAH-2009 and opens potential application of cvcDNA for shrimp vaccination and for improvement of viral tolerance in shrimp breeding stocks. In order to do that, the detailed mechanisms related to the production of cvcDNA from infecting viruses and from EVE in shrimp remain to be revealed.

Keywords:

Viral accommodation; Persistent infection; Endogenous Viral Elements (EVEs); viral copy DNA (vcDNA); RNA interference (RNAi); shrimp; viral diseases

Exposing the secrets of the nephrocomplex 1 ("antennal gland"): A major portal of entry for pathogens in *Penaeus vannamei* shrimp

G.M.A. De Gryse, V.K. Thuong, B. Descamps, W. Van Den Broeck, C. Vanhove, P. Cornillie, P. Sorgeloos, P. Bossier, H.J. Nauwynck*

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Abstract:

Viruses, such as white spot syndrome virus, and bacteria, such as Vibrio species, wreak havoc in shrimp aquaculture. As the main portal of entry for various pathogens in shrimp remain unclear, infectious diseases are difficult to prevent and control. Because the cuticle is a strong pathogen barrier, regions that lack cuticular lining, such as the shrimp's excretory organ, "the antennal gland", are major candidate entry-portals. The antennal gland, up till now morphologically underexplored, was studied using several imaging techniques. Using histology-based 3D-technology, we demonstrated that the antennal gland resembles a kidney, connected to a urinary bladder with a nephropore (exit opening) and a complex of diverticula, spread throughout the cephalothorax. Micro Magnetic Resonance Imaging of live shrimp not only confirmed the histology-based model, but also indicated that the filling of the diverticula is linked to the molting cycle and possibly involved therein. Based on the hemolymph filtration function and attached diverticle complex, we propose to rename the antennal gland as the "nephrocomplex". By an intrabladder inoculation, we showed high susceptibility of this nephrocomplex to both white spot syndrome virus and Vibrio infection compared to peroral inoculation. An induced drop in salinity allowed the virus to enter the nephrocomplex in a natural way and caused a general infection followed by death; fluorescent beads were used to demonstrate that particles may indeed enter through the nephropore. These findings pave the way for an oriented disease control in shrimp.

Updated research on hormonal regulation on crustacean health: Bursicons and their role in immune modulation

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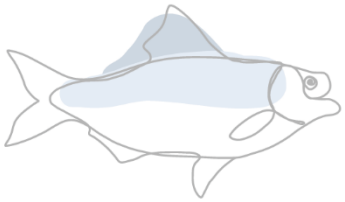
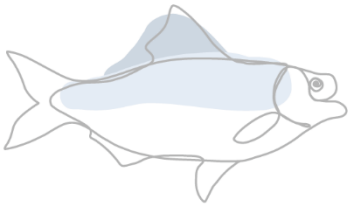
Abstract:

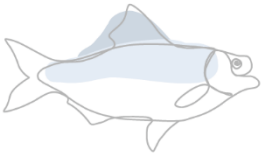
Neuropeptides are small proteins or polypeptides generally synthesized and secreted by neurons or neuroendocrine cells to regulate the physiological functions of animals through activation of specific receptors. In crustaceans, eyestalk and the central nervous system (CNS) are the primary sites from which most crustacean neuropeptides are synthesized and released into the hemolymph. So far, more than one hundred neuropeptides have been identified in crustaceans, which play central roles in the control of various physiological processes, including reproduction, growth, molting, metabolism, and immunity. Transcriptome analysis and in silico mining of neuropeptides in the freshwater prawn *Macrobrachium rosenbergii* eyestalk and central nervous system could predict various preproneuropeptide transcripts, including bursicons (Burs), crustacean cardioactive peptide (CCAP), crustacean hyperglycemic hormones (CHH), eclosion hormone (EH), pigment-dispersing hormones (PDH), diuretic hormones (DH), neuropeptide F (NPF), neuroparsins (NPs), SIFamide, and sulfakinin (SK). These transcripts are most prominent within the eyestalk and CNS. Bursicons, α and β Burs, are initially identified to be responsible for cuticle hardening during the molting cycle and vitellogenesis stimulation in crustaceans. In prawn, Burs are expressed primarily in ventral nerve ganglia. Recently, they have been shown to involve in the crustacean immune system. We show that the significant increase of Burs expression is related to the increase of ammonia-N and salinity stress and the decrease of phenoloxidase (PO) activity and immune-related genes. The homodimeric form of Burs is proposed to regulate hemocyte granulation. We show significant differences in the proportions of agranulocytic and granulocytic hemocytes that corresponded with the upregulation of Burs in acute stress response. However, during chronic stress, the proportions of the two types of hemocytes returned to normal levels, which coincided with the downregulation of Burs. Therefore, changing hemocyte type is apparently under the influence of the Burs neurohormones. In addition, we show that Burs are likely under the control of ecdysteroid hormone, which controls molting. Although molting is essential to animal growth, animals at pre-and post-molt stages are more sensitive to stress and less resistant to bacterial infection. We found that ecdysteroid hormone could activate Burs expression and modulate immune gene expressions. Moreover, increases in hematopoietic cell proliferation and circulating hemocyte numbers, and increased crustacean hematopoietic factor (CHF) and anti-lipopolysaccharide factor (ALF) gene expression were demonstrated. Understanding hormonal effects on animal health may potentially provide a way to manipulate hormones to improve the animal's health in the future.

SESSION 2

MANAGING THE MICROBIOME AND OTHER STRATEGIES FOR BETTER AQUATIC ANIMAL HEALTH AND DISEASE MITIGATION







Gut microbiota of fish and shrimp: A key endpoint for immunity

Tufan Eroldogan

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Corresponding author, e-mail: mtufan@cu.edu.tr

Abstract:

Aquaculture is among the fastest growing food-producing industries in the world presently. One of the foremost challenges encountered by the aquaculture sector is infectious diseases problems. In last decade, the gut health became one of the vital research areas in the aquaculture field to overcome these problems. The gut microbiota is known to play crucial roles in development of aquatic animals' immune system and help in optimal nutrient absorption as gut microbiome produce a range of enzymes which contribute to digestion. Thus, the diet is an important environmental factor in shaping aquatic animal's microbiota and thereby gut health. The facts and evidence of various researches on the effects of microbiota on aquatic animal's health, one of the key issues is to understand the dynamics of the gut microbiota in shrimp and fish. Therefore, in-depth understanding the microbiota in shrimp and fish gut can help to enhance both the aquaculture management for higher productivity and the safety of animal as food. In doing so, we should interpret the strong link between gut microbiota communities and immunity of the animal.

Understanding fish and microbe interactions for aquaculture production and health: Tilapia as case study

Fotini Kokou

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Abstract:

Fish, like other animals, host a complex set of microbes in their gut, which play a crucial role in nutrition and health through host-microbe interactions. These microbes contribute to nutrient digestion, development of the immune system and defense from pathogenic microbes; therefore, the establishment of a healthy gut microbiome is important for host fitness. Over the past decade, we have gained knowledge on how the environment, the diet or the fish health status can shape the gut microbiome and fish performance. However, we still have to understand what is a healthy microbiome and how we can manage it through dietary, genetic or rearing interventions. While microbes can have profound effects on their hosts, we are only beginning to understand how microbial colonization either through feed or the rearing environment may shape host performance. When looking at larval stages, the rearing environment and the feed are important factors shaping the initial gut colonization, while their long-term effects on fish performance, health or disease resilience are not well-understood. Looking at microbial dynamics and microbe-microbe interactions can possibly indicate new targets for probiotic development or microbiome manipulation. In this presentation, I will discuss our latest research on host-microbe and microbe-microbe interactions in the fish gut, and how we can utilize this information to promote improved fish performance and health.

Effects of polyunsaturated fatty acid supplementation on the eicosanoid biosynthesis pathway in penaeid shrimp

Wananit Wimuttisuk*, Pacharawan Deenarn, Punsa Tobwor, Pisut Yotbuntueng, Sage Chaiyapechara, Waraporn Jangsutthivorawat, Metavee Phromson, Atikorn Panya, Natthaporn Phonsatta, Siriporn Tala, and Vanicha Vichai

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ABSTRACT:

Dietary supplementation of polyunsaturated fatty acids (PUFAs) has been shown to improve survival, growth, and reproductive maturation in crustaceans. PUFAs not only provide energy source for these biological processes, but also serve as precursors of eicosanoids, which are a group of pro- and anti-inflammatory signalling molecules that include prostaglandins and hydroxy fatty acids. In mammals, the consumption of n-3 PUFAs has been shown to reduce inflammation in the gastrointestinal tract. However, their effects on crustacean inflammatory pathway remain unknown. In this study, the juvenile Pacific white shrimp *Penaeus vannamei* were fed with feed pellets with similar proximate compositions but varying levels of PUFAs, including minimal PUFA requirement (control feed), high level of arachidonic acid (ARA feed), and high level of docosahexaenoic acid (DHA feed). After four weeks of feeding, shrimp were challenged with white spot syndrome virus (WSSV) and collected at 24 h post-infection. Fatty acid analysis of shrimp muscles and hepatopancreases revealed that muscles contained similar fatty acid profiles to feed pellets whereas the fatty acid profile of hepatopancreases differed. Moreover, WSSV infection increased the levels of PUFAs in muscles of shrimp in DHA feed group. The effects of dietary PUFAs on gastrointestinal tract were also examined by monitoring levels of two eicosanoids, namely prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in shrimp intestines. In shrimp fed with control feed and ARA feed, WSSV infection resulted in higher levels of PGE_2 and $PGF_{2\alpha}$ in intestines than uninfected shrimp. However, levels of both prostaglandins were comparable in the DHA feed group. As both PGE_2 and $PGF_{2\alpha}$ are proinflammatory signaling molecules whereas DHA are known for its strong inflammatory effects, we believe that DHA supplementation in shrimp feed counteracts the inflammation caused by WSSV infection in shrimp intestines. Further study is required to determine whether the reduction of prostaglandins in shrimp intestines due to DHA supplementation are beneficial shrimp health and survival.

KEYWORDS:

Shrimp; eicosanoid; feed; polyunsaturated fatty acids; intestine; white spot syndrome virus.

The impact of host-genetics on the microbiota, health and development of *L. vannamei*

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Abstract:

There is growing evidence on the importance of the gut microbiota to improve and maintain the host's health. However, the influence of the host genome on the microbiota composition is still unexplored.

To study this association on shrimp under aquaculture conditions, we used 16S rRNA amplicon sequencing to characterize the hepatopancreas and gut microbiota of two genetically different populations of *L. vannamei* shrimps collected from three ponds of a shrimp hatchery in Mexico.

We observed that the organ was the first variable that impacts the microbiota composition, followed by the host genetics. Further, the genetic line also influenced the functions associated with the microbiota. Interestingly, the first genetic line showed a functional profile similar to wild-type shrimps, while the second genetic line showed functions related to xenobiotics degradation.

These observations can help better understand the shrimp's development and provide clues for better management of shrimp under aquaculture conditions.

Understanding microbiome and transcriptome in the early developmental stages of the black tiger shrimp

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Abstract:

Shrimp aquaculture is an important to aquatic animal industry and human nutritional. However, the industry faces numerous challenges, especially disease infections. A high quality of shrimp larva is a crucial key that could help to improve the shrimp production. Here, we examined the bacterial community structures and transcriptomic profiling in four life stages (nauplius, zoea, mysis and postlarva) of black tiger shrimp (*Penaeus monodon*) using 16S rDNA amplicon sequencing and RNA sequencing, respectively. The dominant bacterial phyla were Proteobacteria, Bacteroidetes and Planctomycetes, and these phyla were also found as common bacterial phyla associated though developmental stages. Moreover, bacterial communities associated with black tiger shrimp were different from their rearing water, and bacterial profiles had more similar pattern when shrimp developed to postlarva, suggesting that the differences of shrimp physiology, morphology, diets and immune level could influence on bacterial profiles. Gene enrichment analysis showed that most of transcripts were mainly related to metabolic processes, cell and growth development, and immune system. In particular, several innate immune genes were found in early larval developmental including Toll signaling pathway, pathogen pattern recognition proteins, prophenoloxidase system, antimicrobial peptides, blood clotting system and heat shock protein. Our finding revealed that these immune responses are important for defense mechanisms against invading pathogens in early larval stages.

Phytobiotic health additive improves shrimp farm operation harvest results and sustainability

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ABSTRACT:

For the past 20 years, one of the key strategies of the world aquaculture industry has been to reduce use of fish meal and fish oil in feed formulations, to improve its sustainability. The growing world conscience and concern with climate change means that many industries, including aquaculture, are embarking in Life Cycle Analysis of their products and services, which consumers and seafood importers in developed countries are increasingly demanding. Shrimp farming still faces the major challenge to overcome widespread and severely damaging diseases that threaten sustainability of farm operations. This study follows the successful application of a phytobiotic health additive, in significantly improving survival, harvest performance, and profitability of a large Indonesian farm. A Life Cycle Analysis was conducted on these results, proving how improved survival and performance also mitigated the shrimp farm contribution to Green House Gases and other environmental impact parameters.

KEYWORDS:

Shrimp farming, diseases, white feces syndrome, Life Cycle Analysis (LCA), sustainability, phytobiotic

The application of chenodeoxycholic acid in the low fishmeal diet of shrimp

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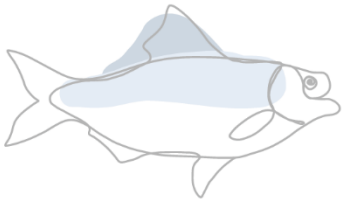
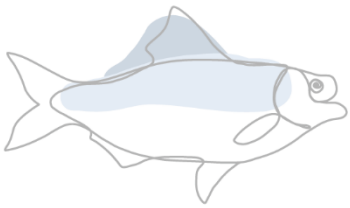
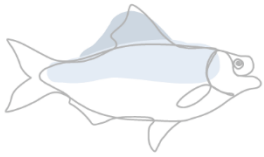
Abstract:

Fish meal (FM) replacement is one of the critical issues exists in the shrimp feed industry. Dietary FM alternatives have been reported disturbing bile acids (BA) status of aquatic animals, especially when FM is replaced by plant proteins. An eight-weeks feeding experiment was conducted to evaluate the effects of dietary chenodeoxycholic acid (CDCA) supplementation on growth, immune response, and intestinal health of *Penaeus monodon* fed a low FM diet. Three diets were formulated: high FM (25%) diet (HF), low FM (15%) diet (LF), and low FM diet supplemented with 0.1% CDCA (LFC). Shrimp fed Diet LF showed lower weight gain and feed efficiency than those fed Diet HF. Low dietary FM up-regulated the mRNA level of *xbp1*, and down-regulated the mRNA levels of *sod*, *iap* and NF-κB signaling pathway-related genes (*traf6*, *tube*). Dietary supplementation of CDCA up-regulated the expression of *relish* and anti-oxidative-related genes (*sod* and *cat*) in the hepatopancreas and midgut. Low dietary FM impaired the intestinal fold and induced the endoplasmic reticulum swollen of intestinal epithelial cell, dietary supplementation of CDCA alleviated this problem. The intestinal microbiota diversity was lower in shrimp fed Diet LF than those fed HF. Dietary supplementation of CDCA in a low FM diet increased the intestinal microbiota diversity by decreasing the relative abundance of dominant phylum Proteobacteria. Low dietary FM increased the relative abundance of a dominant pathogenic bacterium (*Vibrio*), which was decreased by the supplementation of CDCA. These results indicated that dietary supplementation of CDCA in a low FM diet improved the growth performance, immune response and intestinal health of shrimp.







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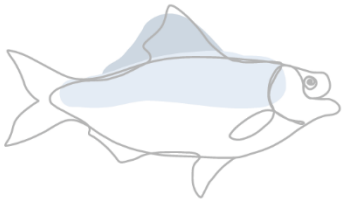

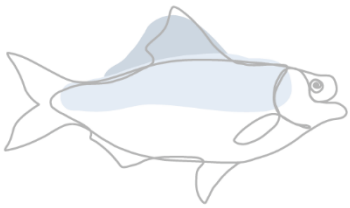

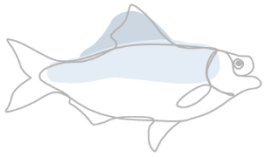

White spot syndrome virus infection triggered the activation of prostaglandin biosynthesis pathway in the Pacific white shrimp *Penaeus vannamei*

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ABSTRACT:

Prostaglandins, including prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), are known proinflammatory signaling molecules that regulate immunity and inflammation in insects and mammals. Although the roles of prostaglandins in crustacean ovarian development have been well-established, whether prostaglandins function as signaling molecules during pathogenic infection has yet to be examined. In this study, the juvenile Pacific white shrimp *Penaeus vannamei* were injected intramuscularly with either white spot syndrome virus (WSSV) or phosphate buffered saline (control). Shrimp were collected at 0, 6, 12, 24, 48, and 72 h post-infection (psi) and the effects of WSSV infection on shrimp prostaglandin biosynthesis pathway were examined. Enzyme immunoassays revealed that WSSV infection resulted in increasing levels of prostaglandin E_2 (PGE_2) in shrimp hemolymph at 6 h psi compared to controls. Similarly, levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in WSSV-infected shrimp were also higher than those of the control shrimp at 6 and 24 h psi. To determine whether changes also occurred transcriptionally, shrimp hemocytes were subjected to quantitative real-time PCR analysis, revealing *prostaglandin E synthases* and *prostaglandin F synthase*, which are responsible for the production of PGE_2 and $PGF_{2\alpha}$, respectively, were upregulated from 6 to 72 h psi. Additionally, injection of ibuprofen, which is an inhibitor for the prostaglandin biosynthesis pathway, in WSSV-infected shrimp also reduced levels of PGE_2, but not $PGF_{2\alpha}$, in shrimp hemolymph. Our data not only suggests a conserved function of the prostaglandin biosynthesis pathway in shrimp immunity, but also suggests that the rapid upregulation of prostaglandin biosynthesis genes during WSSV infection indicates that they may be used as early response markers to monitor shrimp health.

KEYWORDS:

Prostaglandins; white spot syndrome virus; shrimp; cyclooxygenase; eicosanoids

Extraction optimization and analysis of eicosanoids and polyunsaturated fatty acids in the black tiger shrimp *Penaeus monodon*

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ABSTRACT:
Eicosanoids, which are oxygenated derivatives of polyunsaturated fatty acids (PUFAs), serve as signaling molecules to modulate growth, sexual maturation, and immune response in shrimp. However, the analysis of eicosanoids in shrimp tissues were challenging due to small sample size, strong matrix effects from high fat or high protein content in shrimp tissues, and wide polarity range of the eicosanoid compounds. In this study, liquid-liquid extractions were optimized to obtain maximum recovery yields of PUFAs and eicosanoids from shrimp hemolymph, hepatopancreas, and intestines. Hemolymph were subjected to ethyl acetate extraction at a 1:7 ratio (v/v) of hemolymph: ethyl acetate, which provided the highest recovery yields of deuterated standards, namely prostaglandin E_2-d_4 and eicosapentaenoic acid-d_5 at 96.5 and 93.1%, respectively. Hepatopancreas and intestine homogenates were first adjusted to pH 4 and subjected to ethyl acetate extraction at a 1:1 (v/v) ratio tissue homogenate: ethyl acetate. The organ extracts were then analyzed using ultra-high performance liquid chromatography coupled with Orbitrap high resolution mass spectrometry, revealing that two, nine, and nine eicosanoids were detected in shrimp hemolymph, hepatopancreas, and intestines, respectively. High levels of arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were also detected in all three organs. Comparative analysis of PUFAs and eicosanoids in wild-caught and domesticated black tiger shrimp *Penaues mondon* revealed that hemolymph of wild-caught shrimp contained higher levels of (±)8-HETE, (±)8-HEPE, ARA, EPA, and DHA than those of domesticated shrimp. On the other hand, hepatopancreas of wild-caught shrimp contained higher levels of ARA and EPA, but lower levels of (±)11-HETE, (±)5-HEPE, (±)9-HEPE, and (±)15-HEPE than domesticated shrimp. Lastly, intestines of wild-caught shrimp contained higher levels of prostaglandin D_2 (PGD_2) prostaglandin E_2 (PGE_2), 15-deoxy-prostaglandin J_2 (15d-PGJ_2), (±)8-HETE and (±)8-HEPE, but lower levels of ARA, EPA, and DHA than domesticated shrimp. Our analysis not only reveals the presence of PGD_2, 15d-PGJ_2, and (±)15-HEPE, which are new in crustaceans, but also indicates that eicosanoid profiles are unique to different shrimp organ and rearing environment.

KEYWORDS:
Hemolymph; hepatopancreas; hydroxy fatty acids; intestines; prostaglandins; shrimp.

Effects of dietary supplementation with *Pichia jadinii*, *Bacillus subtilis* and probiotics on antimicrobial peptides gene expression of Pacific white shrimp (*Litopeneaus vannamei*) and its resistance against *Vibrio parahaemolyticus* infection

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ABSTRACT:

Use of probiotics and immunostimulants has been widely accepted in shrimp aquaculture for prevent and control diseases. The objective of this study is to determine the effect of whole cell marine yeast *Pichia jadinii*, bacteria *Bacillus subtilis* and probiotics prepared from fermentation on antimicrobial peptides gene expression in hemocyte of shrimps cultured in ponds and investigated resistance against *Vibrio parahaemolyticus* infection. The expression of AMPs gene; Crustin, Penaeidin3 (LitvanPEN3) and Antilypopolysaccharide factor (LvALF1) were observed by quantitative real time PCR at 40, 60 and 90 days after cultured in pond. The result showed that shrimps fed diet supplemented with whole cell marine yeast, bacteria and probiotics prepared from fermentation had up-regulation of Crustin and LitvanPEN3 expression whereas LvALF1 had down-regulation. Although, immune related gene expression of treated shrimps was no statistically significant difference with control group, higher bacterial resistance were recorded in treated group than in the control group. Therefore, it is considered that whole cell marine yeast *Pichia jadinii*, *Bacillus subtilis* and probiotics prepared from fermentation could be used as a natural alternative in shrimp farm to reduce the risk of infectious disease caused by pathogenic bacteria.

KEYWORDS:

Probiotics; marine yeast; gene expression; antimicrobial peptide; *Litopeneaus vannamei*

Minimum inhibitory concentration of a bronopol solution against common threats in freshwater and brackish aquaculture

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ABSTRACT:

Intensive culture systems have introduced stressful environments to animals, which may cause an increase in susceptibility to disease threats and opportunistic pathogens that are normally present in water sources used for aquaculture. Many farmers try to disinfect these potential factors, for example by applying antibiotics and other highly toxic chemicals, as preventative and/or remedial measures. Bronopol has been widely known as a safe alternative for the purpose of controlling many aquatic disease threats including fungi, parasites, bacteria and myxobacteria. This study was to determine minimum inhibitory concentration (MIC) and/or minimum bactericidal concentration (MBC) of Antizol (with 50% bronopol) against common aquatic threats such as bacteria (*Edwardsiella ictaluri*, *Aeromonas hydrophila*, *Streptococcus agalactiae*, *Vibrio parahaemolyticus* and *Vibrio harveyi*), harmful algae (*Oscillatoria* and *Microcystis*) and *Enterocytozoon hepatopenaei* microsporidian parasite (EHP). The results showed that Antizol could inhibit and kill all shrimp pathogens tested in different salinity conditions (5, 10 and 20 ppt) at the dose of 0.08 ppm, and various fish pathogens could be inhibited at 0.2 ppm or killed at 0.4 ppm. For harmful algae, Antizol controlled almost all cell division of *Microcystis* and *Oscillatoria* (>90 µm) in lab conditions at the doses of 50 ppm and 3.13 ppm, respectively. In addition, inhibition of spore polar tube extrusion of two different batches of EHP had the MIC effect on ~90% spores and above at the testing range of 10-40 ppm Antizol. Further investigations such as toxicity to various animals, preventive/remedial approaches and application routes are ongoing and recommended.

KEYWORDS:

Antizol; aquatic disease; bronopol; control; minimum inhibitory concentration.

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