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Marketing fresh hybrid groupers in Langkawi, p55

Editor/Publisher

Zuridah Merican, PhD
Tel: +60122053130
Email: zuridah@aquaaasiapac.com

Editorial Coordination

Corporate Media Services P L
Tel: +65 6327 8825/6327 8824
Fax: +65 6223 7314
Email: irene@corpmediapl.com
Web: www.corpmediapl.com

Design and Layout

Words Worth Media
Management Pte Ltd
Email: sales@wordsworth.com.sg
Web: www.wordsworth.com.sg

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Aqua Research Pte Ltd

3 Pickering Street,
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Singapore 048660
Web: www.aquaasiapac.com
Tel: +65 9151 2420
Fax: +65 6223 7314

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Zuridah Merican

What does sustainability mean for aquafeeds?

The sustainable development goals set by the United Nations in 2015 were designed to be a "blueprint to achieve a better and more sustainable future for all" to be attained by 2030. Climate change and sustainability are back on everyone's agenda. It is no longer a philosophical discussion but how does this affect the aquafeed industry or more importantly, how can the aquafeed industry contribute to this goal?

Although the underlying principles impact all aquafeeds, the key drivers for the various feeds in Asia differ greatly. The driver for sustainable shrimp feeds is the augmentation of fishmeal but total fishmeal replacement may not be the initial goal. Shrimp production increased by 350% during the period 2000-2019 and high-quality fishmeal has surpassed the USD 2,000 per tonne mark. To date, shrimp nutritionists have not been able to totally replace fishmeal at the same cost and at the same performance. Cost has been the major driver of fishmeal substitution over the past 20 years and has now added sustainability as another driver. Recent increases in the price of soybean meal together with an impending commodity

super cycle have already forced feed companies in some countries to increase feed prices. With the increase in shrimp production and demand for feeds, additional supply of quality protein ingredients is required to maintain stability in the supply chain. Novel ingredients such as single cell proteins attributing to its small footprint and insect meals attributing to its circular economy are already here. However, push factors alone can only do so much. To accelerate the process, there must be a demand from consumers willing to pay a slight premium for sustainable shrimp, at least for the initial period.

Marine fish feed faces a different key driver. Despite the large volume of marine fish produced in Asia, feed consumption is relatively low. This mismatch is due to a preference for trash fish which leads to poor water quality, conditions for disease outbreaks and low survival rates. In this issue, we learn that ectoparasites infestations, often propagated by poor culture environments, result in poor market value due to undesirable appearances. It is not that farmers do not realise this but this fetish for trash fish must be stopped. Markets do not place enough emphasis on environmental sustainability in marine fish production. Despite faster growth rates and a shorter cycle due to conducive temperatures and no over wintering, here in Asia, we are not taking the opportunity to progress. Let us look at how salmon aquaculture has developed in Europe and South America. This is due to the use of quality compound feed which does not deteriorate water quality, allowing for higher carrying capacity and better survival rates. Many feed companies are ready with nutrient rich diets and could do with some help from government legislation to ban the use of trash fish.

Freshwater will become a limiting commodity with increasing population

and pollution. Cage culture of freshwater species such as tilapia has advantages over ponds such as a lack of off-flavour in the fish but the culture system is perceived to damage the water quality of the freshwater body and compete with tourism for its pristine nature. All freshwater bodies have a carrying capacity for sustainable aquaculture which can be increased if pollution is reduced from faeces and uneaten feed. One of the major polluting factors is high unavailable phosphorus in the feed and ultimately in the faeces which acts as a fertiliser. While efficient feeding can be managed by automated feeders and real time monitoring of feed consumption, phytase enzymes can improve the availability of phosphorus, utilisation by the fish and consequently reduced amounts in the faeces. This is where freshwater fish feeds can contribute to the UN's SDGs and still provide food and employment to the community and economic development to the country.

This Covid-19 pandemic highlighted the importance of food security when many nations were forced to close its borders in 2020. However, food security is not only confined to poultry, meat and fish production. Moving upstream along the supply chain, it covers feed and protein ingredients as well. This concern does not only affect smaller nations but larger countries as well, with populations to match, such as China.

Is the UN deadline of 2030 too ambitious or is the aquafeed industry moving too slowly?

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New research in microbial management reveals potential for improvements in farming efficiency

The development of effective microbial management strategies to reduce disease outbreaks in aquaculture is often hindered by limited knowledge of the microbiology in fish and shrimp farms. New research by the Centre for Microbial Ecology and Technology (CMET, Ghent University) in collaboration with INVE Aquaculture, the Advanced Nutrition business area of Benchmark (BAN) has revealed opportunities to drive farming efficiencies through a comprehensive understanding of the microbiome.

“In aquaculture, the presence of bacteria in farming water influences nutrient cycles, metabolic waste degradation, digestion and health of the animals so managing these microbes is an important tool in steering the overall health of the system”, says Peter De Schryver, Group Leader Health and Environment at INVE (BAN) and co-author of the publication.

A good performing microbial community in the water is vital, as well as the need to avoid the microbiome becomes suddenly disrupted (a process called “dysbiosis”). By accurately assessing the bacteria, it allows companies to begin to track changes and link this with the health status of their stock. In the future, this may also allow producers to predict when animal health may be compromised.

First of its kind technology

The researchers focussed on the rearing water in the culture of the white leg shrimp (*Litopenaeus vannamei*). They assessed microbiome composition and dynamics using a combination of established molecular and novel flow cytometry analysis methods, which allows for the measurement and quantification of all bacteria, but also

algae and sometimes even viruses. This is a differential to conventional diagnostics which focuses on a single organism at a single moment.

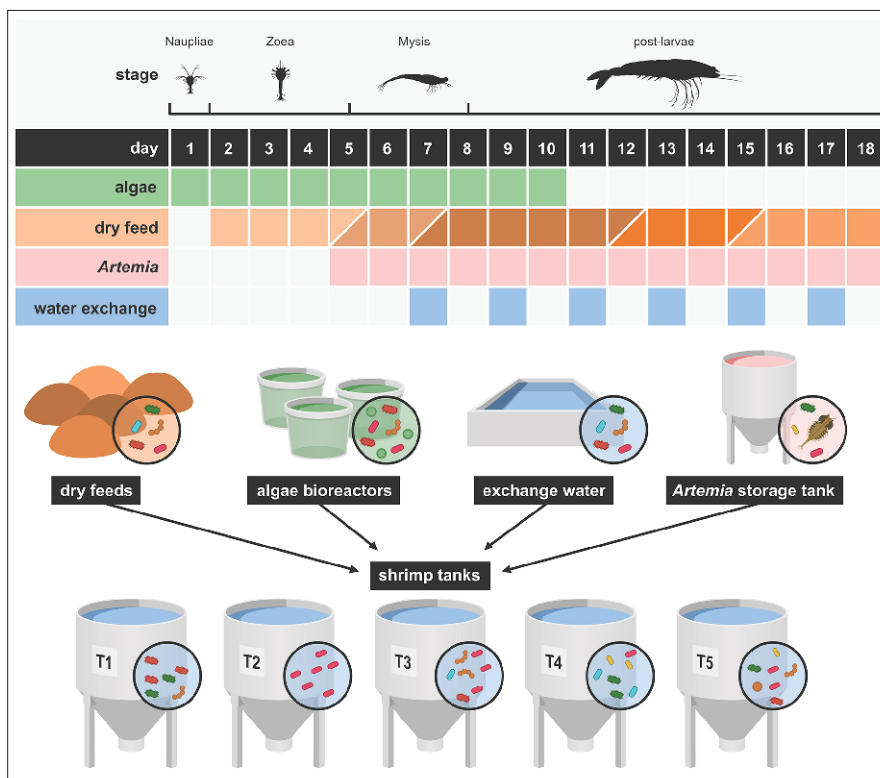
Lead author, Jasmine Heyse of Ghent University said, “We tracked contributions of microbes from external sources, including live or dry feed products, to the rearing water. It is the first time that these contributions have ever been quantified.”

Improving efficiency and efficacy of products

Ruben Props, also of Ghent University said, “The application of this new flow cytometry methodology means that we can accurately assess changes in microbiomes that are linked to the use of products. I believe this opens up new opportunities for product suppliers such as INVE but also fish and shrimp producers to know that the products they are using are having the desired effect.”

“Via CMET’s academic research but also our upcoming UGent spin-off company KYTOS we are continuing to create larger datasets which will allow us to pinpoint the microbiological markers that determine the survival and health of animals. This will be a step change in farm management,” added Ruben.

“The concept behind the paper* sets out the way forward. It was a great partnership between industry and academics and shows with the correct tools and knowledge we can make great steps forward in improving the health and efficiency of aquaculture systems,” concluded Peter.



* Full publication

Heyse, J., Props, R., Kongnuan, P., De Schryver, P., Rombaut, G., Defoirdt, T. and Boon, N. (2021), Rearing water microbiomes in white leg shrimp (*Litopenaeus vannamei*) larviculture assemble stochastically and are influenced by the microbiomes of live feed products. *Environ Microbiol*, 23: 281-298. <https://doi.org/10.1111/1462-2920.15310>

ASC launches consultations on proposed improvements to the programme

The Aquaculture Stewardship Council (ASC) has launched one of its largest ever public consultations. It is asking stakeholders to have their say on major proposed improvements to the programme. One set of consultations is seeking feedback on proposed environmental requirements for the aligned Farm Standard. This will cover all ASC certified species bringing efficiencies and improvements without compromising on quality. The second consultation covers the expansion of ASC's supply chain assurance with the development of the ASC Chain of Custody (CoC) module introducing important requirements specifically for farmed fish.

Chris Nannes, ASC CEO, said, "Both of these consultations concern important innovations in the ASC programme, and as always we want to use the knowledge and experience of our stakeholders to ensure these improvements are as effective as they can be. Multi-stakeholder working is one of ASC's biggest strengths and I'd like to thank in advance everyone who can contribute to this next chapter in the ASC programme."

Anyone can take part in these public consultations. ASC seeks feedback of certificate holders, auditors, NGOs and supply chain partners. The expertise, experience, and opinions of all ASC's stakeholders will once again help to shape the future of a programme that is continuously improving.

Stakeholders can view webinars setting out what the new proposals are for each topic, read additional information, and can feedback on any or all of these topics using a survey, on the ASC website. All of this information is available on the ASC Farm Standard consultation page and the ASC Chain of Custody Module consultation page. Both consultations will run for two months, from 8 March to 7 May 2021.

Farm standard

The project on the aligned Farm Standard will cover all ASC certified species and will bring major improvements to the programme. It will provide greater consistency across species and culture systems, delivering efficiency and simplifying the introduction of updates across the programme.

Currently, ASC manages several separate farm standards covering different species. They all provide an industry-leading set of robust environmental and social requirements. However, because they have been developed at different times, they can vary on how some of the impacts that are common across aquaculture are addressed. The aligned Farm Standard will cover all these common impacts in one robust and thorough standard, which includes species-specific requirements which must also be met by the relevant farms.

The current consultation is looking at P2 in the Farm Standard –the environmental indicators. Because of the large number of environmental issues covered by ASC standards, these will be divided into two rounds of public consultation. The second round of consultation will take place in September 2021.

Chain of Custody module

Since 2012, ASC has used MSC's CoC certification to cover ASC certified product supply chains. This robust system is the leading international standard for seafood segregation and traceability and it covers the entire supply chain, from farm to final sale. The new requirements will be in addition to the existing MSC CoC requirements and will apply only to ASC CoC certificate holders. The new module will have implications for stakeholders, particularly CoC certificate holders and Certification Assessment Bodies (CABs). However, ASC will work to ensure that impacts are minimised.

Next step

Feedback from both consultations will be used to adapt the new proposals. The Farm Standard's P1 and P3 requirements, covering legal and social indicators respectively, are currently being finalised following feedback from stakeholders and are due to be released this year. The next round of public consultation for the remaining P2 indicators will take place in September this year. After this feedback has been collated, a final round of public consultation on the complete proposed P2 will take place next year, followed by its release. The ASC CoC module will be adapted based on this round of public consultation. All final releases will be followed by an effective period of 6-12 months to allow auditors and certificate holders to adjust.



Postponement

With advice from the Singapore government and Singapore tourism board, the World Aquaculture Society in partnership with Singapore Food Agency has agreed to postpone the **World Aquaculture 2020 to Dec 5 to 8, 2021.**
www.was.org



Cancellation

Following local regulations and advisories from Indonesia, the APA21 Steering Committee, **Asian Pacific Aquaculture (APA 21)** organised by the World Aquaculture Society – Asian Pacific Chapter and hosted by the Ministry of Marine Affairs & Fisheries (MMAF) in Indonesia originally planned from September 7 to 10, 2021 will be cancelled.
www.was.org

Data sharing for disease management in shrimp farming: More to gain

Sharing data on disease outbreaks could add real value to the industry if all segments of the supply chain work together and if regulators use systems that provide real-time data to support swift action.

By Pau Badia Grimalt and Anton Immink

Diseases and poor health management in shrimp farming are still key obstacles to sustainable growth of the shrimp industry and have a huge economic impact. Shinn et al. (2018) estimated economic losses in Thailand due to episodes of acute hepatopancreatic necrosis disease (AHPND) throughout 2009 to 2016 at around USD7.38 billion. Data collection is not performed regularly by farmers or regulators and disease reporting is not obligatory in many countries. Diseases differ in how they infect and spread but early knowledge of the risks through data sharing amongst producers and regulators can help in ensuring early prevention measures and in reducing the impacts. Relevant data sharing for health management purposes has helped to reduce the negative impact of disease in salmon farming, for example with sea lice, and in livestock commodities such as the swine or cattle industry, for example with foot and mouth disease (FMD).

The global shrimp industry has been impacted by a succession of diseases such as white spot syndrome virus (WSSV), Taura syndrome virus (TSV), AHPND and more recently by the microsporidian parasite *Enterocytozoon hepatopenaei* (EHP). In Thailand, shrimp is the most important aquaculture species in terms of volume and revenue. In 2010, shrimp production in Thailand fell from 600,000 tonnes to 189,000 tonnes due to disease outbreaks. In 2020, the production was 275,000 tonnes.

Recording disease

Most shrimp farmers do not collect data in a systematic and digital way and therefore it is difficult to move towards an evidence-based approach to improve their businesses. In addition, in Thailand there is no mandatory reporting or record keeping of farmed shrimp disease outbreaks by farmers, although the Department of Fisheries reports to the OIE- World Organisation for Animal Health, notifiable diseases such as WSSV or AHPND.

Nevertheless, numerous technology companies currently offer aquaculture farmers and other industry stakeholders a range of mobile applications, sensors, and other internet of things (IoT) devices to monitor farm performance and to conduct data analysis. Uptake of these technologies in the shrimp industry appears to be slow. Engaging shrimp farmers to use these technologies appears to be more challenging than in other commodity sectors such as salmon, poultry or swine.

Disease control in shrimp farming (like most farming sectors) is complex, as the routes and mechanisms of infection are multifarious. Environment, culture conditions and management practices, all play a role in disease control. To understand disease outbreaks, it is important



Understanding disease management through data sharing with the MyShrimp app in Thailand.

to be aware of the dynamics within the culture systems, such as the potential source of each infection, their routes of infection, how they might spread or propagate, the conditions required or associated with infection events, and the relative susceptibility or resilience of groups within the population (age, developmental state, strain, etc.). The subsequent development of control strategies can then be based on this baseline information. Shrimp culture systems are not standardised, further adding to the complexities of developing management and control strategies. If the routes of infection can be identified and control measures implemented quickly, then it is possible to reduce the probability of a disease event occurring or, depending on the mechanism involved and pathogenicity, reduce the severity of the event.

Coping with disease

Farmers and the industry have adopted different strategies to cope with pathogens and reduce disease outbreaks. Biosecurity measures and prevention such as key control points at the hatchery are still among the most effective ways to minimise the probability of infection or reduce the spread and magnitude of an infection. Crab fences are common in shrimp farms and are one of the main effective barriers to avoid WSSV infections. Crabs are carriers and act as entry points of this virus into the ponds.

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Training on farm biosecurity measures and disease data sharing.

Once a pond is infected, in most situations, there is little a farmer can do to eradicate the infection, but through quick interventions, it may be possible to manage the infection during the remaining culture period. In many cases of a WSSV outbreak within a pond, there is no other option open to farmers but to harvest early, if there are interested buyers, or to cull the pond if some of the shrimp are still below market size. This, however, has economic implications as larger shrimp are both more valuable and subject to less price variation than smaller ones. If the shrimp are too small to be sold, farmers typically destroy the whole stock and disinfect the pond. Some farmers may choose to live with the disease where subsequent crops may have lower production such as the case of EHP infections.

Data sharing

Data sharing can help in different ways, but each disease or health management issue needs to be identified and understood to know where, how and when it needs to be solved. The kind of data, when and where to be shared, to and from whom, and why sharing it are some of the key questions and approaches that need to be considered when sharing disease monitoring data. Increased and early knowledge of a disease outbreak provides time to react and introduce or expand biosecurity measures. It can also mean lower intervention costs as opposed to addressing more advanced infections or even worse, when there are no options left other than harvest or destroy.

A good recent example of health management and data sharing is the sharing of COVID-19 information across countries and regions, including daily reports on the number of new infections, deaths, control measures and recoveries. Identification and understanding of the disease spread routes is very important to control and reduce risks of disease outbreaks. Therefore, sharing data is beneficial to farmers if those routes are controlled. Both in terrestrial

and aquatic systems, the mechanisms and routes of transmission of diseases are similar. They are associated with the movement of live animals, water or inert objects where the disease can live, and these constitute the main routes that need to be controlled.

Even though there is no structured or digitalised data sharing on shrimp farming and disease outbreaks, regular informal data sharing is happening among farmers. Personal or group communications in online chat groups and in farmers' organisations such as clubs and cooperatives play an important role in information dissemination. Seminars, monthly meetings and sharing experiences are common practices among farmers within a cluster, where they learn from each other through the sharing of past experiences and from new knowledge. Examples of farming practices, news on regulation updates, new technology, updates on requirements from processing plants, difficulties in each step of the supply chain, etc. are shared yearly in shrimp fairs or seminars.

There are of course, negative outcomes to reporting on diseases or losses. Shrimp farming has been hit before by international news focussing on the environmental issues of shrimp farming and decontextualising some of the information related to disease. Processing plants and shrimp buyers may take advantage when they know that a pond needs to be harvested due to diseases by offering prices lower than the market price. A farmer who is known to have disease on their farm may face inconvenience or alienation. But shrimp farming is not alone; similar issues also happen in the farming of other commodities such as swine and cattle.

“There is the social responsibility to share for the good of the industry and working towards more sustainable practices.”



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Sharing the common challenges faced in a farm due to diseases and farm assessment.

Alerts are needed

Shrimp health management programmes need to be upgraded in their objectives, scope and approach to reduce the impact of diseases. Recent alerts from international experts about the diseases DIV1 (decapod iridescent virus 1) and HPTV (hepatopancreatic translucence virus) reported from hatcheries present an immediate and urgent concern to the industry, on top of the already numerous existing diseases. Globally, hatchery sources present significant concerns because diseased post larvae movement results in the vertical transmission and spreading of diseases to grow-out ponds. Farmers need to be reminded to source from reputable hatcheries, perform effective sanitation of ponds and test post larvae before stocking to ensure that their farms remain disease-free.

It is strongly recommended that regulators for aquatic animal health follow recommendations such as strengthening hatchery biosecurity, with a focus on the control and monitoring of imported broodstock and post larvae as advised by the OIE. They should support more informed policy development and more responsive disease management strategies by increasing post larvae and grow-out testing and publishing real-time disease outbreak and control data. All tiers of the supply chain have data that help in disease management. There are tools available for reporting this data in real-time that could help to control diseases. All farmers should be socially responsible to share data for the good of the industry and working towards more sustainable practices.

Balancing the benefits

It is challenging for individuals to adopt approaches that may 'point the finger' in the short term. However, if the majority of farmers were to use data more effectively and if regulators required it and acted on it in real time, there is a tangible opportunity to overcome the on-going and repeated disease cycles that adversely impact the Asian shrimp industry. Acting fast and sharing data will help prevent a repeat of the chronic economic impacts associated with established diseases such as AHPND, WSSV and EHP amongst others – and ultimately lead to a more resilient and robust shrimp industry.

Real-time data about disease issues and farm management could also benefit the whole industry through increased transparency and greater market value of products. Who knows, in the near future it may turn out to be an alternative to certification. We leave you here to ponder: should the industry miss this opportunity to improve governance and aim for a more sustainable industry with less risks from disease outbreaks?



Pau Badia Grimalt is a biologist from Barcelona with an international master's degree in Thailand in aquaculture and fisheries. Since 2018, he is pursuing a PhD degree in aquaculture from the University of Stirling (UK). Pau is also the manager of ThaiTIP, a multi-stakeholder innovation platform between European and Thai aquaculture stakeholders.

Anton Immink is the CEO of ThinkAqua – a new NGO promoting innovation in aquaculture to help the industry move to greater sustainability. He was formerly Global Aquaculture Director at Sustainable Fisheries Partnership. Email: anton.immink@thinkaqua.org

Pau and Anton are colleagues on the IDH and Walmart Foundation supported SHRImp project (Shrimp Health Resources Improvement project) that aims to improve health management in shrimp farming in Thailand through data sharing. The work referenced here is part of that project and has involved a wide team of partners all contributing to greater and improved learning.

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Artemia decapsulation: Transitioning from tradition

A more efficient and sustainable method enables farmers to increase the quality and survival of harvested nauplii.

Artemia is the most widely used live larval feed in fish and shrimp hatcheries. As prey they have a suitable size, are highly nutritious and stimulate the feeding response of marine fish and shrimp. Besides, *Artemia* nauplii can be enriched with nutrients to improve their nutritional profile. Before hatching, *Artemia* are, however, enclosed in a rigid shell (chorion). On the one hand, this means that *Artemia* cysts can be stored indefinitely and hatched into live nauplii on demand. On the other hand, the chorion of the cyst is non-digestible and must be removed from live nauplii before offering the live prey to larval aquaculture species. Removal is a challenge even to the modern aquaculture sector and current practices can compromise human welfare and raise environmental concerns.

A commonly used approach to decapsulate *Artemia* cysts is a chemical process using hypochlorite. This method frees the embryos to allow them to develop and hatch into free swimming nauplii. However, this process carries many risks, not only for the *Artemia* but also for the operators and the environment. The chemical oxidation process catalysed by hypochlorite and the heat produced in this exothermic reaction can damage the embryos, requiring a well-managed, closely timed and complex decapsulation protocol to avoid significant losses or minimise reduced hatchability of *Artemia* embryos. The complexity of decapsulation calls for highly skilled and trained operators, making it a costly process. Moreover, these workers are at risk of exposing themselves to hazardous chemicals and inhaling the toxic gases and fumes produced in the reaction. The waste products, such as adsorbable organic halogen compounds (AOX), are harmful to the environment and difficult to degrade; some are toxic to humans and other organisms through which they can accumulate in

the food chain. These concerns regarding the disposal of the by-products make it unsustainable and costly to carry decapsulation at a large-scale.

A more sustainable method

It is therefore essential to switch to more efficient and sustainable methods to produce live *Artemia* nauplii and promote further growth of the aquaculture industry. Answering to these needs INVE (Benchmark Advanced Nutrition) developed the SEP-Art technology. This technology separates nauplii from their cysts using magnetism (Figure 1). SEP-Art cysts are coated with a non-toxic layer of magnetic material that does not affect the overall hatching characteristics of the cysts. The SEP-Art separation tool uses magnets that solely attract the cysts and empty shells, freeing the *Artemia* nauplii during the process. Once the cysts attach to the magnets, they can be removed easily from the *Artemia* nauplii suspension.

This allows the harvest of more and better quality *Artemia* nauplii in less time. As opposed to the traditional methods, SEP-Art does not compromise the vitality of the nauplii as it does not employ physical force or a chemical reaction. Furthermore, it maximises the recovery of the hatching output and speeds up the harvest and collection of the nauplii, making it more efficient. Compared to decapsulation, SEP-Art tools are intuitive and easy to use, and, hence, accessible to untrained workers. The process does not produce chemical waste, making it more sustainable to the environment even at large scale. Overall, a more efficient approach that enables farmers to increase the quality and survival of the harvested nauplii and support the healthy growth of fish and crustaceans.

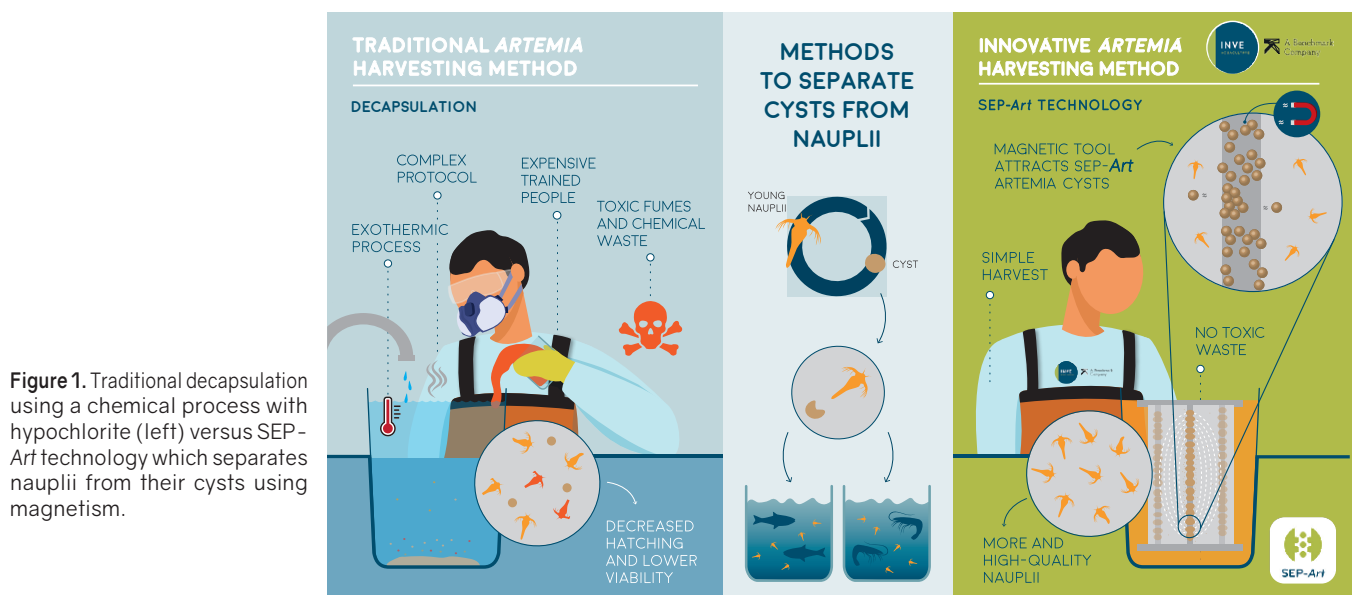


Figure 1. Traditional decapsulation using a chemical process with hypochlorite (left) versus SEP-Art technology which separates nauplii from their cysts using magnetism.



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Summarized results from the top three shrimp producing countries

Thailand

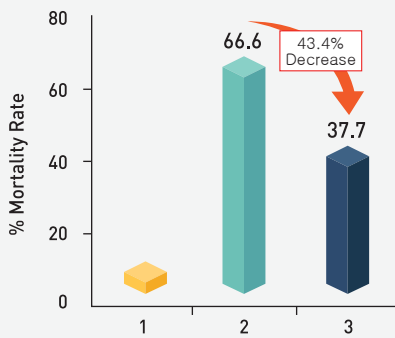


Fig1: Cumulative mortality rate after EMS challenge

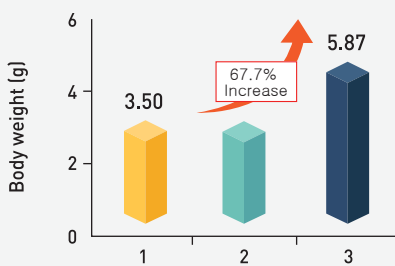


Fig4: Body weight after 42 days

Vietnam

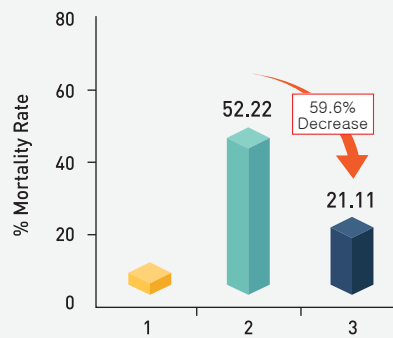


Fig2: Cumulative mortality rate after EMS challenge

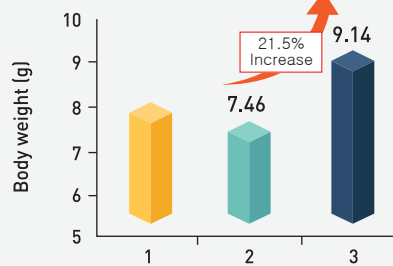


Fig5: Body weight after 42 days

China

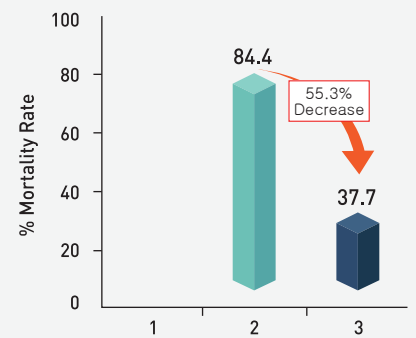


Fig3: Cumulative mortality rate after EMS challenge

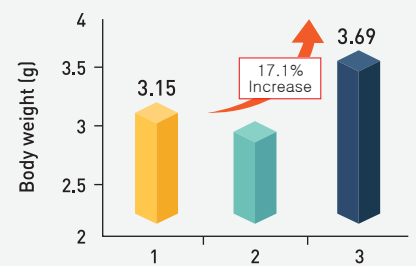


Fig6: Body weight after 42 days

1. Positive control 2. Negative control (challenge) 3. Ig-Guard A

Study confirms that a broad spectrum aquatic disinfectant is highly effective against tilapia lake virus

With no therapy or vaccine in sight to reduce the impact of TiLV, biosecurity and disinfectants to reduce occurrence and severity of the virus are critical. In this study, virucidal inactivation at 28°C occurred within 10 minutes of exposure at a low 50ppm concentration.

By Win Surachetpong and Tanja Gerharz

Tilapia is currently produced in over 140 countries from a wide range of culture conditions (Figure 1). Tilapia lake virus (TiLV) is an important emerging disease, causing massive mortality in tilapia farms. Some countries are reporting mortalities up to 90%. TiLV outbreaks in several countries affect fry and fingerling production to affect the price of tilapia products. Many outbreaks are associated with TiLV infections occurring during the summer months (May to October), when water temperatures range from 22°C to 32°C (Eyngor et al., 2014).

Under laboratory settings, exposing susceptible fish to moribund fish via cohabitation has led to disease progression within ten days (Eyngor et al., 2014; Liamnimitr et al., 2018). This suggests that controlling the release of virus from infected fish or removing contaminated equipment could reduce the occurrence and severity of the disease.

There is no therapy or vaccine available to reduce the impact of this emerging virus; therefore, the application of biosecurity and disinfectants to control the spread of the virus is critical. Various factors have been shown to affect disinfectant efficacy, including disinfectant concentration, contact time, temperature, and soiling (organic matter) conditions. However, there has been little research into the effects of common disinfectants on TiLV.

The present study investigated the efficacy of a wide spectrum disinfectant (Virkon™ Aquatic, Lanxess, UK) for inactivating TiLV under laboratory conditions such as different contact time, temperature, and soiling condition. To enable data comparisons with other viruses, the tests used a standard temperature of 4°C and a temperature of 28°C, which was chosen to represent natural conditions. TiLV has been reported in water temperatures above 25°C.

Experimental set-up

In this study, a tilapia lake virus (TiLV) strain VETKU-TV01 was isolated from the liver of diseased tilapia collected from Pathum Thani province, Thailand in 2016. To prepare a virus stock, confluent E-11 cells, originating from snakehead fish (*Ophicephalus striatus*) were inoculated with TiLV. Aliquots of the viral stock were prepared and stored at -80°C for further experiments.

The cytopathic effect (CPE) was monitored daily following the protocol of Reed and Muench (Reed & Muench, 1938). The experiments were performed in triplicates and interpretations of these results were by assessing the reduction of virus >4 log₁₀ reductions of TCID₅₀ titre compared to the standard virus control at 4°C or 28°C. The average log reduction of virus concentration analysis was presented as a graph with standard error. The TCID₅₀ for viral reduction assay was calculated according to viral titre reduction.

The effects of low soiling (LS), or high soiling (HS) as interfering substances were tested against this aquatic disinfectant. The low soiling condition was prepared by dissolving 3g/L of bovine serum albumin (BSA), while high soiling consisted of 10g/L BSA and 10g/L yeast extract (British Standards Institution, 2015). The side effects of soiling substances, neutralisers, and Virkon Aquatic on cell lines were verified to confirm that no cytotoxicity would be falsely concluded to be a CPE caused by the viruses. The toxicity tests of disinfectant and neutralisers in E-11 cells were evaluated as well, with dilutions according to the method described in W.H.O. standard hard water (total hardness of 342ppm).



Figure 1. Tilapia farming in Thailand: A. Tilapia hatchery, B. Earthen pond farming and C. Grow-out cages. Close positioning of tilapia grow-out cages is a recipe for the spread of TiLV.

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Experiments	L15+TiLV	L15+TiL +Amicon	Treatments with Virkon™ Aquatic	
			500ppm	5,000ppm
Exp 1	3.56E+05	2.00E+05	1.12E+05	0.00E+00
Exp 2	3.56E+05	2.00E+05	1.12E+05	0.00E+00
Exp 3	6.32E+05	2.00E+05	2.00E+05	0.00E+00
Average	4.48E+05	2.00E+05	1.41E+05	0.00E+00

Table 1. Log values of TiLV titre (TCID₅₀/mL) load after treatment with Virkon™ Aquatic at 500 and 5,000ppm at 4°C; 10 minutes of contact time).

Efficacy test against TiLV at 4°C

The efficacy of this aquatic disinfectant was tested at 4°C against other fish viruses. In this study, the evaluation was based on the CPE formation in E-11 cells. First, we tested the concentration of Virkon Aquatic at 500 to 5,000ppm for the inactivation of TiLV. While 500ppm had less efficacy against TiLV, a concentration at 5,000 ppm completely inhibited TiLV at >5 log₁₀ TCID₅₀/mL reductions at 4°C (Table 1).

“These findings suggested that temperature plays an important role in the virucidal properties of disinfectant.”

Notably, efficacy improved dramatically at 28°C and in particular, 50–5,000ppm showed 5 log₁₀ TCID₅₀/mL reductions of TiLV concentration at 28°C with 10 min of exposure (Table 2). These findings suggested that temperature plays an important role in the virucidal properties of disinfectant.

Subsequently, we did a series of experiments to investigate the virucidal effect at 5,000ppm at 1 min exposure and the result showed that Virkon Aquatic achieved >5 log₁₀ TCID₅₀/mL reduction at 4°C for 1 min exposure (Figure 2 and Table 3). Similarly, its efficacy to inactivate TiLV reached >5 log₁₀ TCID₅₀/mL reduction when the duration of exposure was extended to 10 min (Figure 2).

Virus concentration	TR	Concentrations of Virkon™ Aquatic		
		50ppm	500ppm	5,000ppm
Mean titre		<0.00	<0.00	<0.00
6.32E+05		>5.80	>5.80	>5.80

Table 2. Log values of TiLV titre (TCID₅₀/mL) and titre reduction (TR) of TiLV incubated with Virkon™ Aquatic at concentrations of 50–5,000ppm at 28°C for 10 minutes.

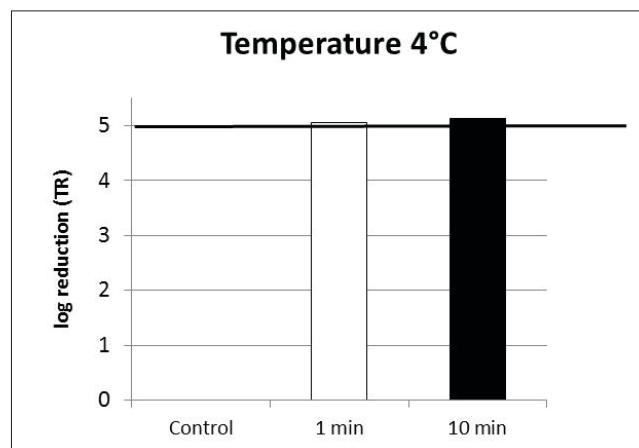


Figure 2. Log reduction value of TiLV titre (TCID₅₀/mL- exposed to 5,000ppm Virkon™ Aquatic at 4°C for 1 and 10 min in hard water. The log₁₀ titre reduction was calculated by comparing with the control group. The efficacy was tested in three independent replicates. A horizontal line indicates the detection limit of approximately 5 log₁₀ reductions.

Contact time [min]	Log titre of virus control	Log titre after treatment	Log reduction (TR) (mean±SD)
1	5.05, 5.05, 5.05	0.00, 0.00, 0.00	≥ 5.05
10	5.30, 5.05, 5.05	0.00, 0.00, 0.00	≥ 5.13

Table 3. Log values of TiLV titre (TCID₅₀/mL- before and after treatment with Virkon™ Aquatic 5,000ppm at 4°C; 1, and 10 minutes of contact time).



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Interfering Substance	Contact time [min]	Log titre of virus control	Log titre after treatment	Log reduction (TR) (mean±SD)
Hard water	1	5.05, 5.05, 5.05	0.00, 0.00, 0.00	≥ 5.05
High soiling	1	5.05, 5.30, 5.30	0.00, 0.00, 0.00	≥ 5.22
Hard water	10	5.05, 5.05, 5.05	0.00, 0.00, 0.00	≥ 5.05
Low soiling	10	5.80, 5.80, 5.30	0.00, 0.00, 0.00	≥ 5.63
High soiling	10	5.05, 5.30, 5.30	0.00, 0.00, 0.00	≥ 5.22

Table 4. Log values of TiLV titre (TCID₅₀/mL) before and after treatment with 5,000ppm Virkon™ Aquatic at 28°C and 1, 10 min of contact time under different soiling conditions (low-soiling and high-soiling).

Efficacy against TiLV at 28°C

As TiLV is regularly reported in water temperatures above 25°C, the effect of this aquatic disinfectant was validated at a temperature that mimicked natural exposure. Accordingly, we tested the efficacy against TiLV at 28°C (Figure 3). As shown in Figure 3, Table 2 and Table 4, a concentration at 5,000ppm Virkon Aquatic inactivated the virus at a titre reduction of >5 log₁₀ within 1 min incubation. For natural water conditions, the efficacy was tested in the condition containing interfering substances by mimicking the low and high soiling conditions at 28°C. In hard water, 5,000ppm concentration completely demonstrated results of >5 log₁₀ reductions for inactivated TiLV at 28°C and 1 and 10 min exposure. At low-soiling conditions, Virkon Aquatic showed 5 log₁₀ titre reduction (Figure 3 and Table 4). Additional tests under high-soiling conditions revealed a log reduction of >5 log₁₀ of TiLV, respectively. Interestingly, the efficacy of this aquatic disinfectant against TiLV under high-soiling conditions at 1 min of contact time also verified a reduction of >5 log₁₀ viral loads (Figure 3 and Table 4). Furthermore, the application of soiling conditions did not affect the recovery of the virus by Amicon® filtration device (data not shown).

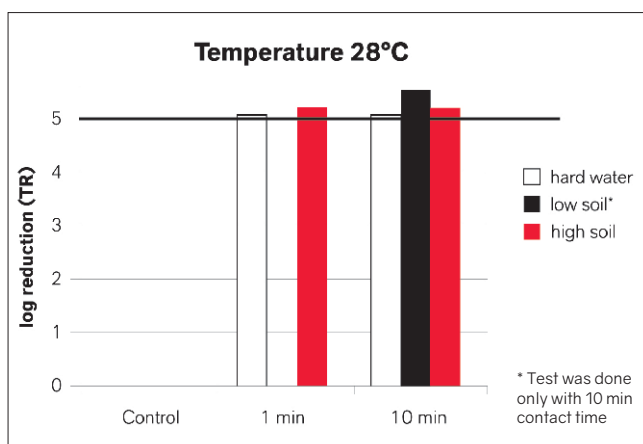


Figure 3. Log reduction value of TiLV titre (TCID₅₀/mL) exposed to 5,000ppm Virkon™ Aquatic at 28°C. Virkon™ Aquatic was incubated with TiLV for 1 and 10 min in hard water (HW), low-level soiling (LS), and high-level soiling (HS) conditions. Each treatment was performed in three independent replicates. A horizontal line indicates the detection limit of approximately 5 log₁₀ reductions.

Conclusion and recommendations

In this study, we tested the efficacy of Virkon Aquatic at 50-5,000ppm against TiLV. The results demonstrated the high virucidal property of this aquatic disinfectant in inhibiting TiLV. Certainly, the factors that contributed to the efficacy of disinfectants, such as contact time, water temperature and soiling interference, should be taken into account when using such substances to eliminate the aquatic virus. Our results revealed that high soiling substances affected the efficacy of other disinfectants (data not shown), but not Virkon Aquatic. Generally, the efficacy against an emerging virus in tilapia under various physical conditions was demonstrated and this aquatic disinfectant can be applied to reduce or control the spread of this virus. We recommend the use of 5,000ppm (correspond to a 1:200 dilution) Virkon™ Aquatic to completely inactivate TiLV at 28°C in 1 min under high soiling conditions.

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Win Surachetpong, PhD is an Associate Professor in the Department of Veterinary Microbiology and Immunology, Kasetsart University, Bangkok, Thailand.

Tanja Gerharz, PhD is Head of Global R&D Disinfection at LANXESS Deutschland GmbH Material Protection Products, Germany.
Email: tanja.gerharz@lanxess.com

Infection of chalimus stages of caligid copepods in the buccal cavity of cultured hybrid grouper

In a cage farm in Malaysia, a large number of leech infections prompted a survey on other ectoparasites in crimson snappers and hybrid groupers.

By Leong Tak Seng

Large numbers of hatchery-produced hybrid groupers (*Epinephelus polyphelcadion* x *Epinephelus fuscoguttatus*), known as the cantik grouper in Indonesia and pearl grouper in Malaysia, were imported from Sabah and Indonesia for culture in floating cages in Peninsular Malaysia in the latter part of 2020. The change to culture hybrids from the original grouper species was an attempt to reduce the problem of leech infections. However, a greater number of leeches were found infecting hybrid groupers as compared to the other grouper species. There is no solution to this leech problem except to constantly treat the groupers regularly with freshwater to reduce the leech population in the cage.

It all started when a fish farmer in Pulau Pangkor (an island off Perak state), indicated problems with his hybrid grouper culture. The groupers in the cages were very thin and not feeding well (Figure 1).

Within the buccal cavity were large numbers of reddish nodule-like outgrowths affecting all immature groupers cultured in the farm. For some unknown reasons, hybrid groupers have large numbers of these reddish nodule-like outgrowths in the buccal cavity (Figure 2).



Figure 1. Skinny infected hybrid grouper.



Figure 2. Reddish nodule-like outgrowths in buccal cavity.

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On closer examination, large numbers of encapsulated chalimus stages of caligid copepods *Caligus minimus* were found (Figure 3) and when teased apart large numbers of chalimus were set free (Figures 4 and 5).

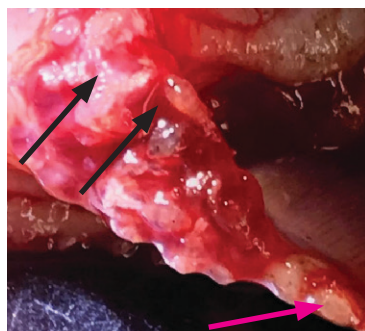


Figure 3. Encapsulated chalimus stages of caligid copepods *Caligus minimus*.

Figure 4. Free chalimus stages teased from buccal cavity capsules.

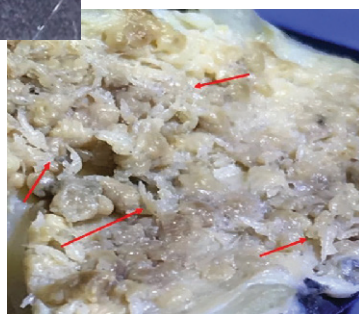


In the preserved mouth of an infected grouper, numerous chalimus stages of caligid copepods were seen attached to the buccal cavity (Figure 6).



Figure 5. Chalimus stages removed from nodule-like outgrowths in buccal cavity.

Figure 6. Attachment of chalimus stages of caligid copepods *Caligus minimus* at roof of buccal cavity.



Spread of ectoparasites

In this farm, only crimson snappers *Lutjanus erythropterus* and hybrid groupers were cultured. When the hybrid groupers were reported to have chalimus stages infection, monitoring studies on the ectoparasites in two cages of crimson snappers and a cage of groupers were undertaken. The whole cage of snappers or groupers was treated with freshwater for 10 minutes. Ectoparasites were collected from the container, preserved in 100% alcohol, identified and counted under a dissecting microscope in the laboratory.

A total of 17,330 ectoparasites were removed after the freshwater treatment from the crimson snappers in the first cage. The ectoparasites consisted of the numerically dominant *Benedenia/Neobenedenia* spp.

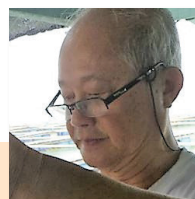
(17,114; 98.8% of total ectoparasites), 14 leech and 202 *Caligus* spp. Of the 17,114 *Benedenia/Neobenedenia* spp., 14,333 were *Benedenia lutjani* (83.7% of total *Benedenia/Neobenedenia* spp.). The caligid copepods were identified and counted and comprised *Caligus rotundigenitalis* (67; 33.2% of total *Caligus* spp.) and *C. minimus* (135; 66.8%).

During the freshwater treatment of crimson snappers in the second cage, a total of 133,941 ectoparasites were removed from the snappers. The *Benedenia/Neobenedenia* spp. was again the most numerically dominant ectoparasite (133,468) comprising 99.6% of total ectoparasites. There were 372 *Caligus* spp. and 101 *Zeylanicobdella arugamensis* leech recovered from the sampling. Of the 372 caligid copepods recovered, 359 were *C. rotundigenitalis* and only 13 were *C. minimus*. During this second sampling, most groupers in the farm had died.

A whole cage containing the hybrid grouper was freshwater-treated and ectoparasites were collected, identified and counted. A total of 1,060 ectoparasites were recovered, of which the leech, *Z. arugamensis* was dominant amounting to 637 (60.1% of total ectoparasites). Of the ectoparasites, there were 355 *Benedenia/Neobenedenia* spp. at 33.5% and caligid copepods at 6.4% (68). All 68 caligid copepods recovered were identified as *C. rotundigenitalis* and there were no *C. minimus*. No chalimus stages infections were found in the buccal cavity of the hybrid groupers.

Seabass (*Lates calcarifer*) cultured in Penang, Malaysia were found infected with parasitic copepods *C. minimus* and its life cycle was reported by Khoa et al (2017). In Pulau Pangkor, cultured crimson snappers were also infected with parasitic copepods *C. minimus* and chalimus stages of this parasitic copepods were found attached in the buccal cavity of hybrid groupers.

We have seen large numbers of infected hybrid groupers dying and presently there is no effective treatment for their infection. In Egypt and Turkey, farmed European seabass (*Dicentrarchus labrax*) and mullet (*Mugil cephalus*) in marine fish farms were infected with *C. minimus*, resulting in serious economic losses (Özak, et al. 2006, Jithendran, et al. 2008, Noor El-Deen, et al. 2012; Noor El-Deen, et al. 2013). Infected fish can be treated with freshwater or hydrogen peroxide to remove the parasitic copepods on the external surface but not in the buccal cavity.



Dr. Leong Tak Seng was Professor of Parasitology at Universiti Sains Malaysia. He was a founder and former Chairperson (1986 - 1989) of the Fish Health Section of the Asian Fisheries Society. He spent more than 40 years on the study of parasites and diseases in marine fish cultured in floating cages.
Email: leongtakseng@gmail.com



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Tilapia lake virus: Clinical signs, disease diagnosis and prevention

By Raanan Ariav and Natan Wajsbrot

Tilapia is the second most important finfish cultured worldwide. Among the secrets for tilapia's success are its low production costs while maintaining high product quality, particularly its nutritional content. Additionally, tilapia tolerates high stocking densities and is highly resistant to diseases. Indeed, tilapia farmers were able to effectively deal with bacterial disease agents, such as *Streptococcus* spp. and *Aeromonas* spp., through different approaches, including the use of feed additives and antimicrobials, coupled with improved management and strict biosecurity strategies. However, the tilapia industry has recently witnessed the emergence of highly virulent infectious diseases such as the tilapia lake virus (TiLV) that has been affecting wild and farmed tilapia for over a decade.

TiLV was first officially identified and reported in 2014 at the Sea of Galilee, Israel. After learning more about the disease's origin and progression, the sharp decline of wild-caught tilapia in the Sea of Galilee since 2009 was linked to an increasing presence of TiLV in the wild fish populations. Awareness on this novel virus spreads rapidly and by May 2017 the Food and Agriculture Organization of the United Nations (FAO) triggered a global alarm. The FAO warned that the virus can adversely impact global food security and nutrition and advised countries importing tilapia to take appropriate risk management strategies such as diagnostic testing, health certification, and imposition of quarantine measures and contingency plans to contain the outbreaks.

Transmission and risk factors

Tilapia is the main host of TiLV but other important warm water aquaculture species can also be infected. Some fish species such as snakeskin gourami (*Trichogaster pectoralis*), iridescent shark (*Pangasianodon hypophthalmus*), walking catfish (*Clarias macrocephalus*), striped snakehead fish (*Channa striata*), climbing perch (*Anabas testudineus*), common carp (*Cyprinus carpio*), silver barb (*Barbodes gonionotus*), Asian sea bass (*Lates calcarifer*) and Indian major carp (*Labeo rohita*) have been found to be resistant to this emerging aquatic disease. This apparent resistance to TiLV might be due to the lack of viral receptors or other mechanisms that enable replication in the hosts. Nevertheless, other factors, such as stress, co-infection and environmental conditions also play important roles in the vulnerability to TiLV.

TiLV is transmitted both horizontally (among and between individuals of the same generation) and vertically from broodstock to offspring. Under experimental conditions, the virus was detected in faeces and contaminated water after a successful intragastric infection, suggesting an oral–faecal route of transmission. The virus can thus spread horizontally amongst conspecifics inhabiting the same water body.

The vertical transmission of TiLV was detected in infected broodstock and their offspring (early 2-day

old larvae). This also means that it is theoretically possible to test larvae for the presence of TiLV before shipping them to farms; this represents a significant opportunity to create TiLV pathogen-free larvae – a major biosecurity step. It is also hypothesised that molluscs, aquatic insects and invertebrates are potential carriers of TiLV and thus the virus could also be transmitted through these organisms. However, more research needs to be performed to confirm transmission pathways via these other vectors. Overall, these multiple transmission pathways make the containment of outbreaks particularly challenging.

Despite TiLV being a recently identified virus, some direct effects have already been identified as significant risk factors for its propagation, namely the presence and proximity of infected, wild or farmed populations, and water temperatures ranging from 25°C to 31°C. Other risk factors include any change that might affect the immune status of the fish and make them more vulnerable to the presence of the virus, either directly altering the fish immunological competence or disrupting their homeostasis. The latter, which forces the fish to energetically rebalance its physiological conditions, are referred to as immunosuppression drivers and include sub-optimal environmental parameters, increased stocking densities and presence of secondary bacterial and/or parasitic infection in the background.

Clinical signs and diagnosis

Clinical signs of TiLV include lethargic movements and changes in fish behaviour, such as swimming near the water surface, swirling motion, reduced schooling, imbalanced movements and loss of appetite (Figure 1). Ocular and skin lesions, discolouration and abdominal swelling can also be present. However, TiLV infection is not necessarily limited to these symptoms and behavioural changes. Since these clinical signs are similar to those associated with various other tilapia diseases, the identification of the disease often relies on more specific diagnostic methods, such as histological observations or more recent molecular tools, involving polymerase chain reaction (PCR).

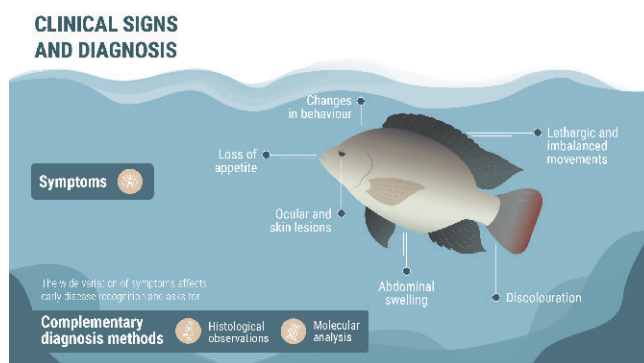


Figure 1. Clinical signs of TiLV and additional methods to complement the diagnosis.



Unilateral or bilateral opacity of the lens and cataract due to TiLV

Histological observations

Histopathological changes associated with TiLV are observed in the brain, liver, spleen, gills, eyes and kidney. The most common histopathological feature in infected tilapia is syncytial hepatitis, characterised by the evident development of multiple nuclei in a single hepatic cell. In addition, recurrent histopathological lesions found in TiLV infected fish were driven by the obstruction of the kidney and the brain. Ocular infections can also be recorded in fish over the course of the disease, which lead, in extreme cases, to endophthalmitis and cataracts.

Molecular analysis

The development of molecular detection techniques for TiLV, such as *in situ* hybridisation (ISH) and PCR, allow early detection of the disease. In addition, these molecular techniques provide a

greater understanding of the multiple factors involved in the disease process, fostering the knowledge of TiLV interaction with tilapia and the development of effective methods for controlling the virus.

TiLV control and prevention

Currently, biosecurity measures and good management practices are the best prevention and control measures for TiLV (Figure 2). All live tilapia shipments (including eggs) must be highly regulated and monitored for possible presence of TiLV. Since TiLV can be transmitted vertically, the establishment of a specific pathogen-free (SPF) stocking broodstock is essential to avoid the spread of the disease. Although this requires rigorously screening broodstock for this disease, it will ensure TiLV-free fingerlings, which is key to controlling the disease during early production.

Aquaculture facilities should apply strict biosecurity measures and guidelines to prevent the virus from spreading in the farm. The starting point must be a biosecurity plan, which outlines disease monitoring routines, strict regulations for quarantine of new fishes and specific protocols for disinfecting materials, water



Superficial ulceration and secondary *Aeromonas* infection due to TiLV



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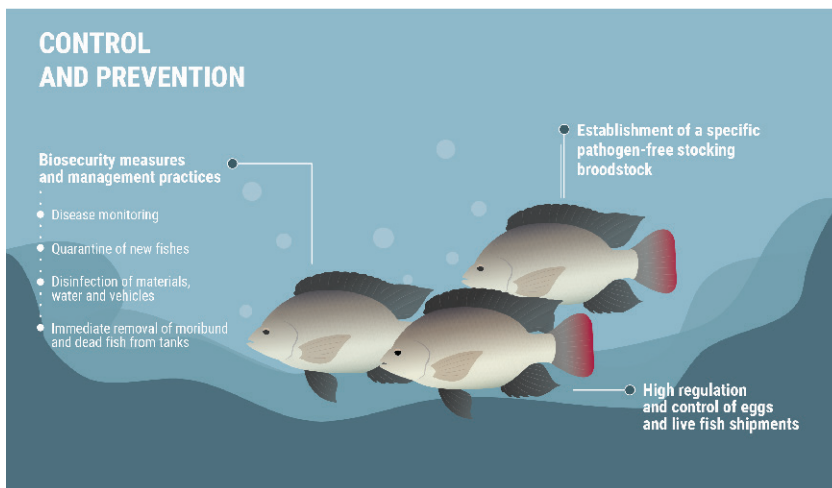


Figure 2. Control and prevention measures for TiLV.

and vehicles. A vital routine task is also the immediate removal of moribund and dead fish from tanks to avoid the transmission of TiLV. The use of disinfectants should also be promoted, following several studies showing that common disinfectants, such as sodium hypochlorite (NaOCl), hydrogen peroxide (H₂O₂) or formalin, can reduce TiLV load to a minimum level.

Vaccination may also be an effective tool for preventing TiLV outbreaks. A vaccine for TiLV management is not yet available, but studies have shown that fish that survive the infection present effective protective immunity to TiLV. These findings suggest that tilapia can develop immunity to the virus and thus a TiLV vaccine may control the spread of the disease. TiLV vaccines (both attenuated and inactivated) based on cell culture preparations are currently being developed and their efficacy is being tested in laboratory conditions. Vaccination seems a promising solution, but further research is required to determine the best vaccine type (live-attenuated or inactivated virus) and the most effective vaccination method (immersion, injection or oral).

The way forward

The spread of TiLV to Latin America, Africa and Asia may cause a significant economic loss to tilapia producers. This will consequently lead to social and environmental challenges. Therefore, the FAO emphasises the need for an international monitoring program aimed at identifying populations of tilapia that are infected and implementing strict preventive measures among TiLV-free tilapia populations.

While the global trade of live fish is nowadays a common reality, the transport of live tilapia, from broodstock to eggs or juveniles,

must be properly regulated to avoid the spread of TiLV. However, and since it is not clear yet if other aquatic animals can spread the virus or act as a reservoir, it is imperative that monitoring and research efforts are promoted to better understand the role of different transmission vectors and how to regulate them.

References are available on request



Dr Raanan Ariav, DVM, is VP Aquaculture and Executive Manager at Phibro Animal Health.
Email: Raanan.ariav@pahc.com



Natan Wajsbrot, MSc is Fish pathologist and Microbiologist in the R&D team at Phibro Aqua.



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Effects of dose variation of an exogenous enzyme complex in diets for hybrid red tilapia

A higher dose of an enzyme complex supplemented into diets enhanced health and growth performance of hybrid red tilapia.

By Phuong Do, Liem T. Pham, Tu L.C. Tran, Hien T.T. Tran, Philippe Mahl and Hoang Phan

The hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*) is an important species and able to adapt itself to different production systems and diets, as well as feed on available plankton in the culture system. This high market value tilapia has flesh with excellent nutritional qualities. However, feed represents a major cost for intensive tilapia production and it is one of the most important factors influencing the ability of fish to attain its genetic potential for growth and maintain fish health. Research on nutrition and feeding of tilapia has been expanding steadily over the past three decades including the potential use of new functional ingredients, probiotics and enzymes to improve growth, feed utilisation and fish health (Verschuere et al., 2000).

Enzymes can be considered as promising feed additives since they enhance the availability of nutrients and energy, resulting in better growth performance and reduced waste production (Jegannathan & Nielsen 2013). Similar to other monogastric animals, fish are not able to metabolise some nutrients due to insufficient production or absence of specific enzymes (Taniguchi & Takano, 2004). This justifies the need for an exogenous supply of specific enzymes in diets such as an enzyme complex.

We conducted a study on the effects of a combination of exogenous enzymes (protease, phytase, amylase, xylanase, cellulase) and vitamin D3 at six different levels (0, 1, 2, 4, 6 and 8g/kg feed) in tilapia diets on growth parameters, feed conversion ratio (FCR), nutrient digestibility and body composition.

Fish and diet preparation

A total of 4,000 hybrid tilapia were sourced from a local hatchery and transported to the test facilities. Fish were acclimated for several weeks in 2m³ tanks to adapt to environmental conditions. During this acclimation period, fish were fed with formulated commercial feed. Then, 1,440 fish of ~8.7g from the same cohort were selected for the experimental study, based on similarity in size (<10%). Fish were starved of feed for 1 day prior to the experiment.

Commercial feed pellets were used in this research to meet the nutritional requirements for tilapia (30% crude protein, 3.5% crude lipid, 54.5% carbohydrate, 11.8% crude ash and gross energy of 18.1 KJ/g). For this growth trial, we used the enzyme mixture of protease, phytase, amylase, xylanase, cellulase, vitamin D3 (Zymgrow, Virbac Vietnam).

This enzyme complex was diluted with 150 mL at various doses; 0, 1, 2, 4, 6, 8g of enzyme complex/kg of feed to be added homogeneously onto the feed, then dried at 37°C for 2 days. For the digestibility trial, the commercial feed was grounded and 1% chromic oxide Cr₂O₃ and water (37%) were added. The mixture was then pelleted to form 3mm diameter pellets and dried at 60°C for one day. The enzyme complex was added homogeneously at the above doses onto the prepared pellets as described above.

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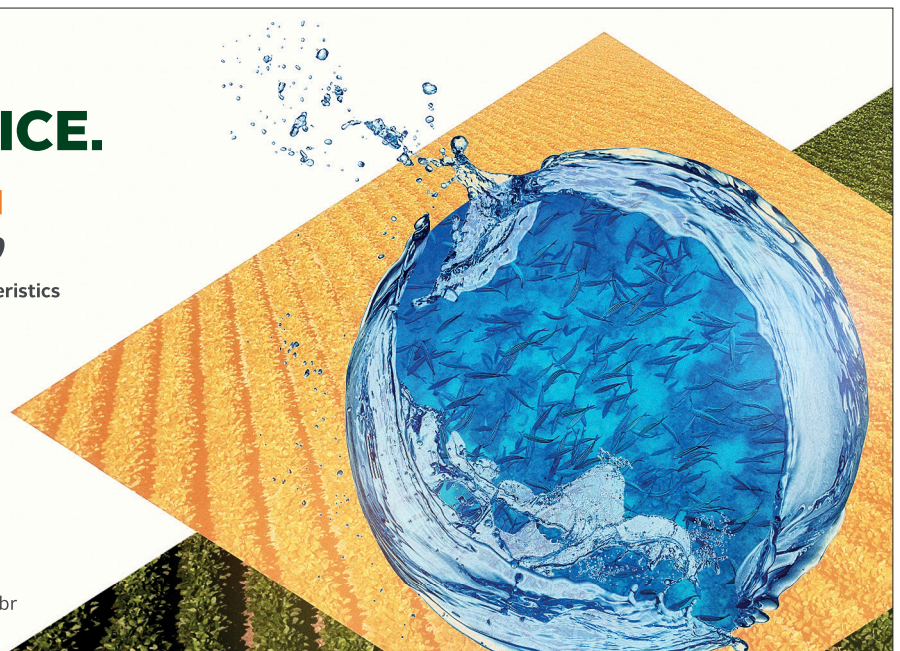
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Growth trial

Experimental fish (~8.7g) were stocked into composite 500-L tanks (350L of water) at a density of 80 fish/tank. There were six experimental treatments, conducted in triplicates and they were randomly assigned to 18 tanks. Experimental tanks were aerated continuously and water was exchanged at around 20% per day. Fish were fed with the experimental diets for 12 weeks, twice a day at 9am and 3pm. Fish were fed manually to satiation and the amounts of eaten/uneaten feed were recorded daily. Fish mortality was recorded daily, and dead fish were removed from the experimental tanks and weighed for calculations of final survival rate (SR) and feed conversion ratio (FCR).

All the fish in each tank were weighed at the beginning, on day 28, day 56 and at the end of the experiment (day 84) as a group. Growth performances were calculated for weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), SR, FCR, protein efficiency ratio (PER) and apparent net protein utilisation (ANPU). Fish were randomly collected from all tanks and analysed for proximate composition at the beginning (10 fish/tank) and the end of the experiment (3 fish/tank). The proximate composition analyses were done following standard methods (AOAC, 2016) including moisture, ash, crude protein and crude lipid.

Digestibility trial

Following the growth stage, fish in each tank were transported to one of 18 cylindrical-conical sloped bottom tanks. Fish were stocked at a density of 50 fish/tank and fed with prepared diets with the digestibility marker Cr_2O_3 for two weeks. This was then followed by a period of faecal collection to reach a total of ~10g of dry faecal material per replicated tank.

Feeding to satiation was carried out carefully in the morning with uneaten feed removed. Water was exchanged 2 hours after feeding (20% daily) and faecal collection was done using a small net. Faeces were dried at 60°C, and then kept frozen at -20°C for analyses of crude protein, crude lipid, moisture and ash following AOAC (2016). Chromic oxide (Cr_2O_3) was analysed following the method of Furukawa & Tsukahara (1966).

The calculations were:

(1) Apparent Digestibility Coefficient of the diet (ADC_{diet})

$$\text{ADC}_{\text{diet}} = 1 - \frac{\%A}{\%B}$$

(2) Apparent Digestibility Coefficient of a nutrient in diet ($\text{ADC}_{\text{Nu-Diet}}$)

$$\text{ADC}_{\text{Nu-Diet}} = 1 - \frac{\%A}{\%B} \times \frac{\%B'}{\%A'}$$

where A is % Cr_2O_3 in feed, B is % Cr_2O_3 in faeces, A' is % nutrient in feed, and B' is % nutrient in faeces.

Throughout these trials, water quality parameters were maintained in ranges for normal development of fish. Dissolved oxygen and water temperature were 5.1–6.0 mg/L and 25.8–32.8°C, respectively. pH ranged between 8.1–8.3, NO_2 was below 0.1 mg/L and TAN was below 1 mg/L.

All data were analysed for significant differences using one-way ANOVA and Duncan post-hoc test via SPSS 16.0.

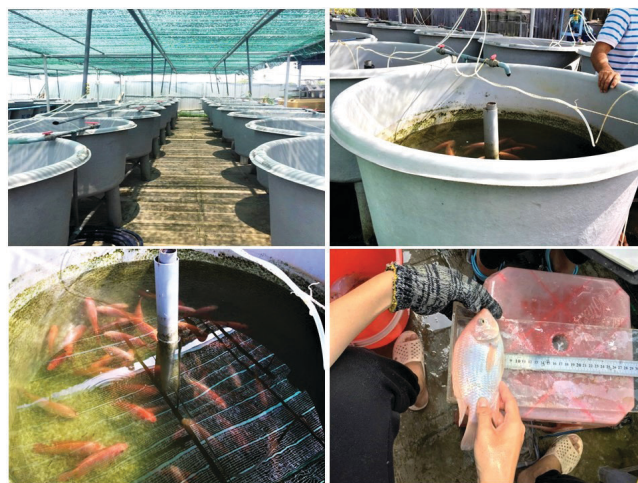
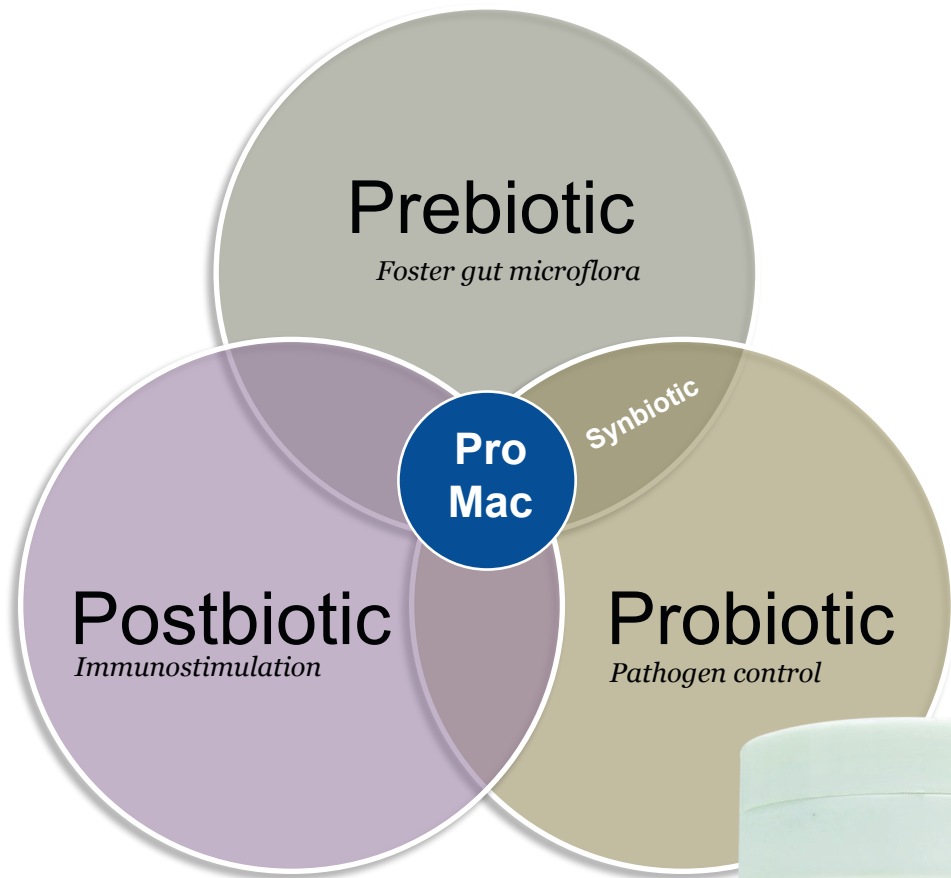


Figure 1. Experimental setup and fish sampling at the end of the growth stage. Fibreglass tanks of 500L with 350L of water were stocked with 80 fish/tank.

Enzyme (g/kg feed)	W_i (g)	W_f (g)	WG (g)	DWG (g/day)	SGR (%/day)	SR (%)
0	8.88±0.38 ^a	136.2±2.12 ^a	127.3±1.81 ^a	1.52±0.02 ^a	3.25±0.04 ^{ab}	72.9±5.05 ^a
1	9.08±0.23 ^a	132.7±5.57 ^a	123.6±5.71 ^a	1.47±0.07 ^a	3.19±0.07 ^a	76.3±10.2 ^a
2	9.15±0.17 ^a	134.7±10.3 ^a	125.6±10.3 ^a	1.50±0.12 ^a	3.20±0.09 ^a	81.7±9.04 ^a
4	9.04±0.26 ^a	152.5±3.37 ^b	143.5±3.60 ^b	1.71±0.04 ^b	3.36±0.06 ^{bc}	69.2±6.88 ^a
6	9.16±0.09 ^a	155.1±10.0 ^b	145.9±10.0 ^b	1.74±0.12 ^b	3.37±0.08 ^{bc}	74.2±12.6 ^a
8	8.80±0.32 ^a	158.7±3.11 ^b	149.9±2.85 ^b	1.78±0.03 ^b	3.44±0.03 ^c	75.8±5.05 ^a

Different superscripts in the same column indicated a significant difference ($P < 0.05$)

Table 1. Growth performance of tilapia fed with diets supplemented with different enzyme levels



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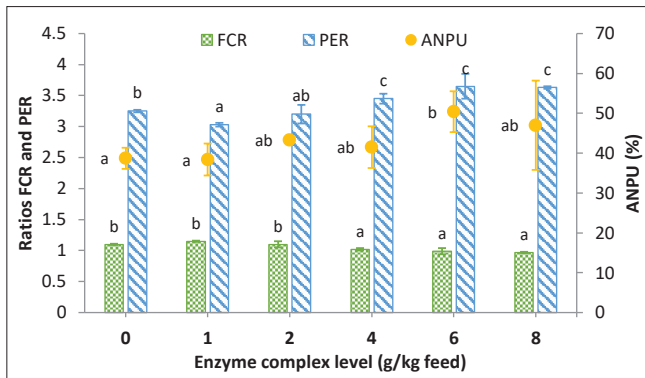


Figure 2. Ratios of FCR and PER, and percentage of ANPU of red tilapia fed with various supplemented levels of enzyme complex. Different superscripts in the same parameter indicated a significant difference ($P < 0.05$)

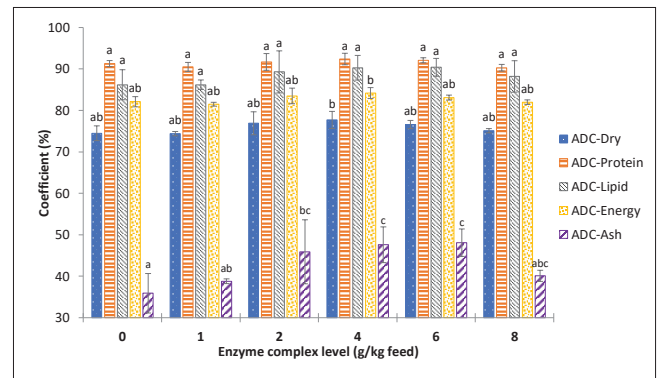


Figure 3. Apparent digestibility coefficients of experimental feed for dry matter, protein, lipid and energy shown in red tilapia fed with different levels of the enzyme complex. Different superscripts in the same parameter indicated a significant difference ($P < 0.05$)

Growth performance

At the end of the trial, the final weight (Wf), WG, DWG and SGR were recorded. The sampling conducted at the end of the growth trial is shown in Figure 1. Fish growth performance is presented in the Table 1. A significantly higher ($P < 0.05$) growth rate was shown in the treatment groups with higher levels of enzyme supplementation (4, 6, 8 g/kg feed) in comparison to the diets with lower enzyme supplementation (0, 1, 2 g/kg) as final weight (Wf), WG, DWG and SGR expressed this tendency. Feeding tilapia with different supplementation levels of the enzyme complex did not affect their survival rate which averaged ~75% for the experimental conditions.

Feed utilisation

Feed utilisation efficiency is presented in the Figure 2. A better FCR was obtained in the diet treatments supplemented with 4g enzyme complex per kg feed and above (≤ 1), in comparison to other treatments with lower enzyme supplementations (≥ 1.1 ; $P < 0.05$). This indicates a better growth performance of fish when they were fed with higher doses of the enzyme complex. A similar trend was observed with protein utilisation represented by PER which was above ~3.5 for the higher

dose treatments ($P < 0.05$). Better ANPU was obtained in groups of 2, 4, 6 and 8g enzyme complex per kg feed with the highest value at the supplementation of 6g ($> 50\%$) compared with the control (no enzyme addition; $P < 0.05$).

Composition of whole-body fish

Proximate compositions of whole-body fish before and after the growth trials are presented in Table 2. There were no significant differences in proximate compositions of experimental fish, except for total ash. Results showed that additions of enzyme in the diet increased the total ash of whole body of red tilapia significantly ($P < 0.05$) compared to fish from the treatment group with no enzyme added.

Digestibility trial

Apparent digestibility coefficients of dry matter (ADC-Dry), protein (ADC-Protein), lipid (ADC-Lipid) and energy (ADC-Energy) are presented in Figure 3. All parameters were found to be highest in the treatment with 4g enzyme complex/kg feed, particularly in ash, energy and dry matter. However, there were no

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Enzyme (g/kg feed)	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
0	77.9±1.60 ^a	11.6±0.73 ^a	7.01±1.01 ^a	2.67±0.19 ^a
1	77.1±2.14 ^a	12.3±1.09 ^a	6.98±1.00 ^a	3.40±0.53 ^b
2	76.3±1.09 ^a	13.1±0.63 ^a	6.15±0.17 ^a	3.41±0.28 ^b
4	76.4±1.32 ^a	11.8±1.58 ^a	7.64±0.62 ^a	3.29±0.48 ^b
6	75.4±2.26 ^a	13.5±1.72 ^a	6.63±1.04 ^a	3.53±0.16 ^b
8	76.1±2.95 ^a	12.7±2.96 ^a	6.62±0.57 ^a	3.49±0.14 ^b
Initial fish	87.1±0.83 [*]	7.60±0.04 [*]	1.08±0.05 [*]	2.50±0.22 [*]

*Values were not statistically compared; Different superscripts in the same column indicated a significant difference (P < 0.05)

Table 2. Proximate compositions (% as is) of whole body of red tilapia before and after feeding with diets containing different levels of the enzyme complex.

significant differences in ADC-Protein and ADC-Lipid among the treatments, and there were no significant differences in $ADC_{Nu-Diet}$ among the treatments with 4, 6 and 8g enzyme complex/kg feed. The increase in dosage of enzyme complex in the diet (up to 6g/kg feed) for red tilapia increased ADC of feed.

Discussion

Supplementation of diets with exogenous enzymes in a complex preparation is considered effective to eliminate the anti-nutritional factors and improve feed utilisation in terms of energy and amino acids, resulting in improved fish performance (Lin et al., 2007). Exogenous protease can compensate for the deficiency of endogenous enzymes especially for young animals and assist in the

breakdown of macromolecular proteins, thus improving their digestibility (Shi et al., 2016).

For tilapia (*Oreochromis niloticus* and *O. aureus*), the digestibility of dry matter and crude protein increased with supplementation of protease (Li et al., 2016). In a previous study, *O. mosambicus* fed with a kikuyu-based diet supplemented with a multi-enzyme complex comprising cellulase, xylanase and phytase improved growth, lowered feed conversion ratio, increased protein efficiency/digestibility and increased fish activities (Hlope-Ginindza et al., 2016). Hybrid red tilapia supplemented with a combination of protease, cellulase, glucanase, pectinase and pure mannanase in the diet significantly improved dry matter and energy digestibility coefficients (Ng & Chong, 2002).

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In this study, the supplementation of the enzyme complex in the diet for red tilapia significantly enhanced digestibility of ash to increase the ash content in the fish body. Additionally, fish growth and feed utilisation improved with dietary enzyme supplementation. The appropriate amount of dietary enzyme supplementation is ≥ 4 g/kg. Positive effects of the enzyme complex were shown in the results on performance and digestibility of fish fed with the treatment diets. However, some previous studies found limited effects of enzymes (Ogunkoya et al., 2006; Yigit and Olmez, 2011), whereas others found improved nutrient digestibility and growth of fish (Ghomi et al., 2012; Lin et al., 2007); Therefore, it is difficult to make comparisons across studies.

Conclusion

Overall, the supplementation of the Zymgrow enzyme complex used in this study for hybrid red tilapia did not show any toxicity effect. The addition of the enzyme complex improved fish body proximate composition and enhanced their digestibility for better growth performances. We observed better WG, DWG, SGR, FCR, PER and ANPU, particularly at the dosages of 4g/kg of feed or more as compared to the control diet.

Lastly, in our opinion, these findings are also important for the whiteleg shrimp and therefore, investigations merit for this important farmed species.

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Phuong Do is Clinical Executive with a Master of Science in aquaculture disease. **Philippe Mahl** is Manager of Corporate Aquaculture Development and Technical Support.



Hoang Phan, PhD is Manager of Asia Aqua Development and Technical Support.

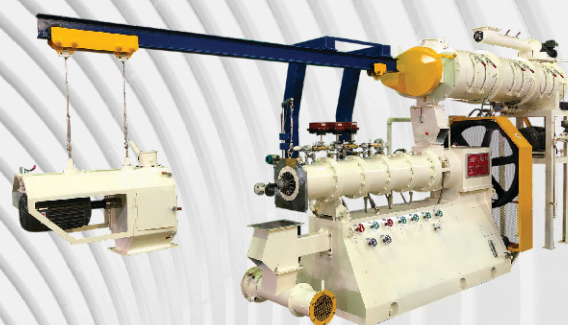
All are with Virbac, Aquaculture Division, France and Vietnam. Email: hoang.phan@virbac.vn



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Inhibitory capacity of a microbial enhanced protein against *Vibrio* spp. in Pacific white shrimp

A series of trials demonstrated that diet supplementation confers a protective effect against the *Vibrio* species associated with AHPND.

By Darwin Zambrano and Sergio F. Nates

Acute hepatopancreatic necrosis disease (AHPND) or early mortality syndrome (EMS) has been associated with *Vibrio parahaemolyticus*. AHPND has spread to the Western Hemisphere and emerged in Mexico in early 2013. Since the first reported case in southern China in 2009, AHPND has also been observed in shrimp farms in other parts of Asia and the Americas. AHPND outbreaks have inflicted severe damage to the global shrimp farming industry with annual losses of more than USD 1 billion.

In 2017, the first cases of AHPND were reported in Ecuador with levels of *V. parahaemolyticus* and *V. vulnificus* of 3×10^4 CFU/g and mortalities up to 100% in larval culture tanks. The disease affects larvae in early stages with mortalities up to 100% while in juvenile shrimp, mortalities of 30–40% have been reported during the first 20–30 days after stocking.

To offset high mortalities from AHPND, supplements have been incorporated into diets. These include a wide variety of probiotics and products such as organic acids, essential oils, biomolecules and in some cases, antibiotics. On the other hand, molecular diagnostics developed in recent years have made it possible to determine the presence and quantification of *Vibrio* loads by PCR techniques, using specific primers to detect the AP4 toxin. In addition, a more specific media (ChromAgar) was also developed to differentiate the growth of various strains of *Vibrio*.

A promising mitigation step

Recent studies on the development of practical diets for shrimp production systems using a microbial enhanced protein, ME-PRO® (Prairie Aquatech, South Dakota, USA), have shown to be a promising solution to produce eco-friendly aquafeeds. The protein is processed at a state-of-the-art plant using non-GMO (non-genetically modified) soybean meal and a naturally occurring, non-toxicogenic fungi, *Aureobasidium pullulans*. The fermented co-product also offers significant amounts of short-chain peptides and free amino acids that confer excellent attractability and palatability properties. Results from numerous feeding trials have demonstrated that this product can sustain shrimp health, high-performance growth, and feed efficiency with inclusion levels as high as 50% of the total amount of ingredients in the diet.

The objective of this study was to evaluate the inhibitory capacity of ME-PRO at different doses (0.5%, 1% and 2%) on *Vibrio* spp. extracted from the midgut of Pacific white shrimp *Litopenaeus vannamei*. This study was carried out at the Microbiology and Molecular Biology Laboratory, QSBIOTECH, located in La Libertad, Santa Elena province, Ecuador.

Agar medium for analysis

For the analysis of total *Vibrio*, thiosulfate citrate bile sucrose agar (TCBS, Difco™) was prepared, supplemented with 2% sodium chloride and incubated at 35°C for 24 hours. The pH of a litre solution was adjusted to 8.6 and heated to boiling point with frequent stirring for 2 minutes. The solution was then placed on sterile plates and stored at 2°C–8°C until use.

ChromAgar *vibrio* medium was used for *V. parahaemolyticus* and *V. vulnificus*. Powdered agar (74.7g) was dissolved in 1L of distilled water, homogenised and heated to boiling at 100°C.

For isolation, purification and growth tests with probiotic strains, tryptic soy agar medium or TSA (Difco) was used. Powdered agar (40g) was dissolved in 1L distilled water. The solution was homogenised for 2 minutes and subsequently plated and autoclaved at 121°C for 15 minutes.

TSB (trypticase soy broth, Difco) was used for the isolation, purification and growth tests of strains of *V. parahaemolyticus*, *V. vulnificus* and several commercial probiotics. Powdered agar (30g) was dissolved in 1L distilled water. The solution was homogenised for 2 minutes and subsequently was plated and autoclaved at 121°C for 15 minutes.

Test doses

Sub-samples ME-PRO were resuspended in distilled water at the test doses (0.5%, 1% and 2%) and were added to all the agar and liquid media at three concentrations. All inclusions into the culture media at the indicated concentrations were used to measure the effect against *V. parahaemolyticus* and commercial probiotics from three companies.

Agar inoculations

Shrimp post larvae (0.1g) were weighed, macerated and dissolved in 200uL sterile distilled water. A 50uL sample was inoculated in TCBS agar and ChromAgar. All *Vibrio* and probiotic bacteria were resuspended in TSB medium. A 50uL sample was inoculated in TSA agar plates in the case of probiotics and on ChromAgar for the *Vibrio* species.

The results of bacterial growth count in agar were expressed as CFU/g for larvae macerates and mL for resuspensions. Growth curves for *Vibrio* species and probiotics were generated from readings of their optical density at 600nm using a Photometer 9300 and expressed as cell/mL.

PCR analysis

DNA extraction and evaluation protocols were completed using a lysis buffer and PCR techniques. The amplification conditions in the MiniAmp Plus thermal cycler for denaturation were 94°Cx0:30 sec; hybridisation 55°Cx0:30 sec; and polymerisation 72°Cx1:45 min. There were 25 cycles for both the first and second PCR tests.

Inhibitory capacity

Results showed that inhibitory capacity of ME-PRO was present at inclusion levels of 0.5%, 1% and 2% against *Vibrio* bacteria in macerates of shrimp larvae and inclusion in culture media ChromAgar *Vibrio* and TCBS.

Samples of shrimp larvae were evaluated to determine the presence of *V. parahaemolyticus* and *V. vulnificus*, as a routine protocol established to evaluate the health of the animals when shrimp larvae are being purchased from a hatchery. Daily, over seven consecutive days, five samples of live larvae from commercial hatcheries (suspected to be infected with AHPND) were collected and processed. When the microbial enhanced protein was incorporated in TCBS and ChromAgar culture media, results indicated inhibitory activity for *Vibrio* type 1 (yellow colonies) between 38%, 48% and 57% respectively. For type 2 *Vibrio*, the inhibition rate was 78% and 100% for the 1 and 2% doses and for *V. parahaemolyticus*, 38%, 45% and 62% respectively (Figure 1). The evaluation of shrimp larvae macerates using TCBS agar indicated an inhibitory activity of *Vibrios* in the presence of the protein (Figure 2). Similarly, in ChromAgar a reduction in *V. parahaemolyticus* occurred when the 0.5% dose was used (Figure 3).

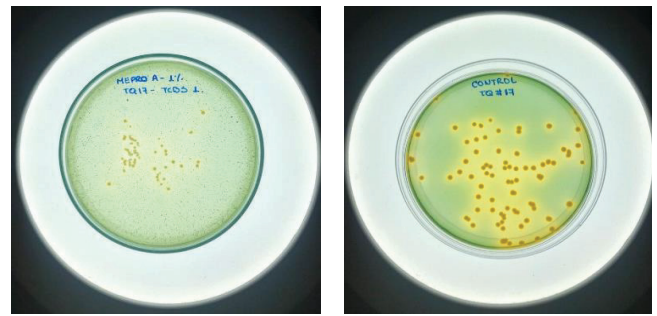


Figure 2. Photographs of ME-PRO® activity at 1% (left) versus control (right) using TCBS and shrimp larvae macerates.

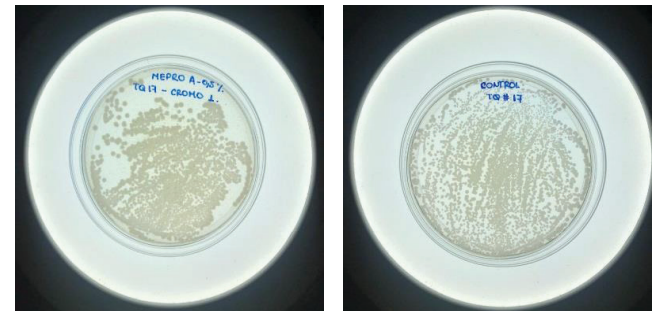


Figure 3. Photographs of ME-PRO® activity at 0.5% (left) versus control (right) using ChromAgar and shrimp larvae macerates.

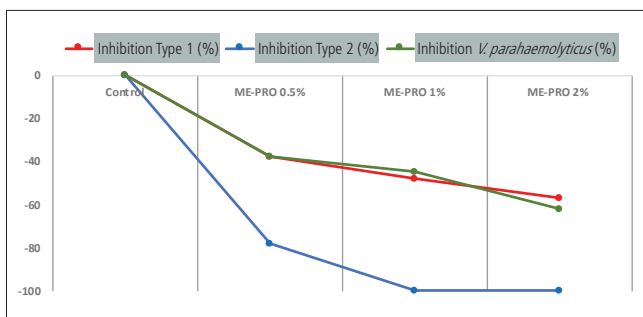


Figure 1. Inhibitory activity of ME-PRO® against *Vibrio parahaemolyticus* at three doses, 0.5%, 1% and 2%.

VP AHPND inhibition dynamics with time

Three EMS-associated strains were isolated from larval samples, grown on ChromAgar and TCBS and tested using PCR. In addition, a strain of *V. parahaemolyticus* (VP AHPND) was cultured in liquid TSB medium with the inclusion of ME-PRO at 1 and 2%. Every 6 hours petri dishes were inoculated to verify the bacteria dynamics and the effect of the enhanced microbial protein on bacteria growth. The results showed that at 1 and 2% at time zero an inhibition effect of 27% was obtained. The growth of *V. parahaemolyticus* was compromised after the first 6 hours of culture for both dosages with a 40% reduction in growth. At 12 hours there was a slight recovery, and at 18 hours, the reactivation bacteria growth was back to normal (Figure 4).

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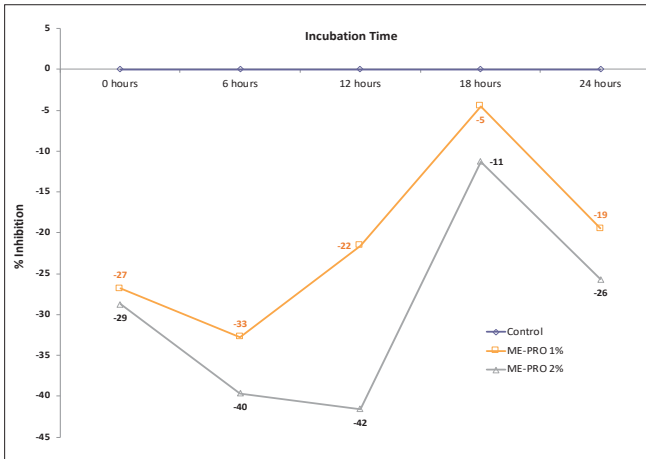


Figure 4. Inhibition (%) of *Vibrio parahaemolyticus* in the presence of ME-PRO® at 1 and 2% inclusion levels; incubation of 0hrs, 6hrs, 12hrs, 18hrs and 24hrs in ChromAgar Vibrio.

Inhibition of *V. vulnificus*

Strains of *V. vulnificus* were isolated from larval samples with disease signs and used in this test. In Ecuador, the association of *V. parahaemolyticus* and *V. vulnificus* at levels of 10⁴ CFU/g has been determined to cause high mortalities.

Strains of *V. parahaemolyticus* and *V. vulnificus* were resuspended and incubated to reactivate for 1 hour at 32°C in TSB culture medium with the inclusion of ME-PRO® at 5%, 10% and 15%. Incubations were carried out immediately in ChromAgar Vibrio to determine the effect on bacteria growth. The results showed that ME-PRO at 10% could strongly affect *V. vulnificus* growth rate with a 72% reduction in bacteria populations at 18 hrs. At the 15% inclusion level the growth rate of *V. parahaemolyticus* was reduced significantly up to 63% (Figure 5).

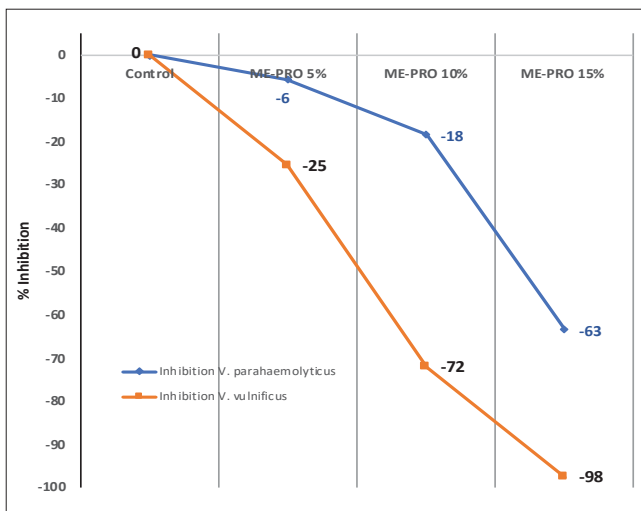


Figure 5. Inhibition (%) of *Vibrio parahaemolyticus* in the presence of ME-PRO® at 5%, 10% and 15% inclusion levels; incubation in ChromAgar Vibrio.



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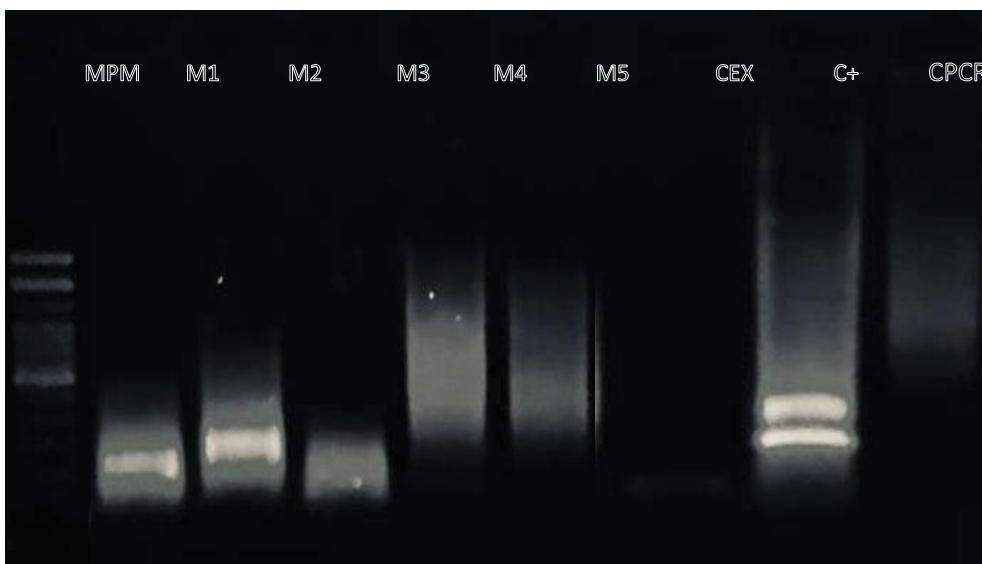


Figure 6. PCR amplification of *Vibrio parahaemolyticus*. Line 1: MPM; Line: 2,3 (PCR positive); line: 4-6 (PCR negative); line 7: Cex; line 8: C+ PCR. Line 9: CPCR.

Detection of VPQB020 with PCR

For this test, the strain VPQB020 (*V. parahaemolyticus*) previously identified by PCR was used. Bacteria were grown in ChromAgar *Vibrio* with ME-PRO inclusion levels of 1% and 2%. CFU were counted in plates and PCR of each of the bacteria that grew in the culture medium was carried out at the different inclusion levels.

The results of PCR amplification using the VP4 toxin primers indicated the detection of AHNPD in samples 1 and 2; samples 3-5 were negative. The extraction controls, PCR and positive PCR control were excellent (Figure 6).

The results of the bacterial CFU counts showed that a 2% inclusion level of ME-PRO inhibited the growth of *V. parahaemolyticus* load by up to 50% resulting also on a negative PCR for the AP4 toxin (Table 1).

Treatment	<i>Vibrio parahaemolyticus</i> (UFC)	% Inhibition	PCR EMS
Control	95,200	0	Positive
ME-PRO® inclusion			
1%	61,600	-35	Positive
2%	48,800	-49	Negative

Table 1. Growth of *Vibrio parahaemolyticus* in the presence of ME-PRO® at inclusion levels of 1%, 2%. Bacteria presence was detected using PCR.

Inhibitory activity with probiotics

An evaluation of the inhibitory activity of ME-PRO at inclusion levels of 1% and 2% against three strains of commercial probiotics was carried out. The strains were reactivated in TSB culture medium at these two doses and incubated at 30°C for 24 hrs. Subsequently, samples were incubated every 6 hours in TSA medium without salt. The results were expressed as CFU readings and bacteria levels counted in the agar plates versus CFU counts obtained in the controls. The ratio was used to find a relationship; commercial probiotics showed an inhibition of 71%, 38% and 6% with ME-PRO at 1%, while at 2%, it was 72%, 44% and 8%. (Figure 7).

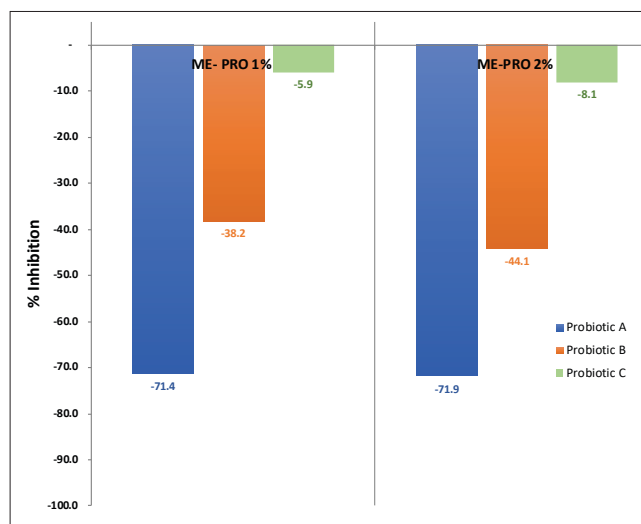


Figure 7. Inhibition (%) of three commercial probiotics in the presence of ME-PRO® at 1% and 2% inclusion levels; incubation in TSB.

Conclusion

The assessment in this study indicated that an inclusion level as low as 1% ME-PRO improved resistance to *V. vulnificus* and *V. parahaemolyticus* associated with high mortalities in shrimp aquaculture. Similar to previous studies, the results confirmed that supplementation into the diet of white shrimp, *L. vannamei*, will confer a protective effect against the *Vibrio* species associated with EMS.



Darwin Zambrano is President, Q'Earth Solution S.A.

Sergio F. Nates, PhD is Executive Vice President, Prairie Aquatech, USA.
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2020 – Watershed year for alternatives to fishmeal and fish oil

A turning point of incorporating more of the most novel alternative ingredients to effectively displace the high cost fishmeal and fish oil in aquafeeds

By Kevin Fitzsimmons



A variety of fish-free ingredients used in aquafeeds. Credit: F3 Challenge/Rick Barrows

In many ways, 2020 marked a turning point in the aquafeed industry. The COVID-19 pandemic led to global interruptions in marine fishing with many viral outbreaks occurring on fishing boats and in processing plants, causing fishmeal and fish oil prices to steadily increase.

It also seems to have been the year when the industry made the mental shift that fishmeal and fish oil were no longer considered commodity ingredients but specialty feed additives due in part to rising costs.

At the same time, a plethora of alternative products continue to flood the market. Algae, insect meals, single-cell proteins, bio-tech treatments of ingredients and by-products have become widely available at competitive prices. Each of these new ingredient supplies are replacing larger fractions of fishmeal and fish oil in aquafeeds.

Algal based oils

Algae-based products, including those produced by Corbion and Veramaris, now account for 25% of the supply of the omega-3 fatty acids used in Norwegian salmon feeds and are making rapid inroads to the rest of the global salmon feed supply. Retailers in Europe and in the US are marketing premium salmon products as fed with algal oils and not wild-sourced fish oil.

Nutrition research has shown that other farmed seafood are equally able to utilise oils derived from algae, which is the original source of omega 3s that bioaccumulate up the food chain. In addition, a recent study in the *British Journal of Nutrition* found that seed oil from genetically modified plants are just as effective as

fish oil in providing healthy docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) benefits to humans, further confirming that it is nutrients not ingredients that are critical to produce healthy farmed seafood (cambridge.org). The lack of any heavy metal pollutants such as those found in fish oil also make these fish-free oils more appealing to consumers. Safeguarding our food supply from these toxins that negatively affect brain development is evident in the fact that the EU is considering regulating fish oils to enhance consumer safety.

The algae producers' rapid expansion plans, partnered with feed companies that have expressed their intentions to shift to algal-derived oils, confirm the expectation that algae will produce most of these essential ingredients in the near future and that oil from fish will be used exclusively as a special additive for high-value products. Another algae-based ingredient of interest is the algal meal after processing to remove the polyunsaturated fatty acids. These meals, available post-extraction from the oil for aquaculture, human use and biofuels still have high protein, vitamin and antioxidant concentrations that are valued in aquafeeds. Seaweeds that have had carrageenan and agar extracted are also recognised as an increasingly important ingredient.

Insect meals

The mass production of insects for meal and oil was equally exciting in 2020. Virtually every month saw news of the “world’s biggest insect farm to date” coming on-line, or multi-million dollar or euro investments made to build even bigger farms. Black soldier fly and other insect meals were bought in large quantities for

inclusion in aquafeeds by major producers including Skretting and Mowi. Protix, Ynsect, Agri-Protein, Entobel, Chapul Farms, and others are dropping their cost of production and improving the quality of their final products by adjusting the feeds for the insects. The environmental benefits of utilising food processing wastes as feed sources have provided extra impetus to the insect producers.

Single-cell proteins

Single-cell protein sources including bacteria, yeast, and other microbes are providing some of the most exciting sectors of new ingredients. Bacteria produced using methane (Calysta), ethanol (KnipBio) or even carbon dioxide (NovoNutrients), have seen large investments, including from multinational petroleum giants. Feeding trials have been successful with a variety of aquatic species and as the production rate of bacteria is so fast, construction of the bioreactors (basically similar to fermentation tanks used for alcoholic beverage production) is the time constraint. Yeast products used in aquaculture are not new, but the multiplicity of new products and product forms is astounding. Many of these products that started as specialty additives continue to edge more into conventional ingredients.

Ag-tech products

Related to the microbes as ingredients, are the ag-tech products that have taken increasing fractions of the ingredient mix. Brewery and distillery by-products have been joined by dried distillers grains with solubles (DDGS) and now companies including Montana Microbials, Jiangsu Fuhai, Scoular, and Menon Renewables are using fermentation, enzyme treatments, lysates, and other microbially mediated methods to treat various ag products and by-products to provide a variety of up-cycled products including high protein meals, polyunsaturated oils, and complex carbohydrates. One of the most interesting side stories of the microbial and microbial mediated ingredients is the ability of so many of the ingredients to also serve as probiotics with several functional abilities. There have been a number of peer-reviewed articles documenting improved growth and disease resistance for diets incorporating many of these ingredients.

The steady increase in plant-based proteins and oils used in aquafeeds has been occurring for 30 years, with soy products making up a large fraction. Strong development efforts breeding soy varieties with fewer anti-nutritional factors and better amino acid and fatty acid profiles have encouraged more use. Both GMOs and conventional selective breeding are contributing to the rapid advances in soy product capabilities. Other crops including pulses, nuts, and various grains have also introduced new products including protein concentrates from barley, corn, faba bean, pea, pistachio, and potato. More oilseed meals including camelina, canola, cottonseed, palm, peanut, sunflower, and sesame are appearing in high quality and quantity.

By-products

Animal processing by-products have also taken another important fraction of the ingredient mix. Poultry and pork by-products have been prominent, but so have meat, bone and blood meal products. The quality and

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functional properties of many of these ingredients in aquafeeds are still being documented. Regulations restricting the use of many of these by-products derived from warm-blooded vertebrates for use back into other warm-blooded animals do not apply when fed to cold-blooded animals that are not known to be able to sustain the pathogens of concern, even if the pathogen survived the rendering and feed manufacturing processing steps.

Looking forward

2020 was an unprecedented year, not only because of the global COVID-19 pandemic, but also because the aquafeed industry saw a turn towards incorporating more of the most novel alternative ingredients with new and improved versions of plant and animal products that effectively displace more of the high cost fishmeal and fish oil. Aquaculture farms also appreciate the ability to stabilise feed costs and benefit from the highly nutritious feeds that are formulated from the expanding menu available to aquafeed producers.

Looking forward, we expect exponentially more alternatives, as the rapid advances in synthetic biology propelled by the search for the COVID-19 vaccine make their way into the feed industry, reducing the cycle time from nutrient identification into feed incorporation. As alternatives proliferate, they will continue to diminish the need for fishmeal and fish oil in aquafeeds, creating more certainty and freeing aquaculture to continue to grow independently of fluctuations and decreasing availability of wild-caught resources. We anticipate that this window of opportunity for new nutrient and

feed startups due to advances in synthetic biology will continue to progress and flourish. Then, at some point, there will be consolidation in line with the product cycles in other industries. But we are optimistic of the greater sustainability of new feeds, and that they will also become more economically sustainable as the prices of fishmeal and fish oil increase, and the prices of alternatives drop as they scale up production.

The F3 Future of Fish Feed will convene a series of webinars in 2021 to discuss the growing opportunities for alternative ingredients in aquafeed. In addition, registration for the F3 Challenge - Carnivore Edition is open to companies and teams developing and selling a fish-free feed in three categories: Salmonid, Shrimp, or Other Carnivorous Species. The total prize has increased to USD300,000.



Kevin Fitzsimmons is Professor at the University of Arizona, USA and is Future of Fish Feed (F3) Challenge chair and judge.
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Hepatic health—A key challenge in aquaculture

With current demands for high productivity in fish farming, optimal fish liver conditions assure growth performance and farm profitability.

By Álvaro Rodríguez

Optimal liver status is essential for farmed fish throughout the production cycle. The liver is the organ responsible for all the metabolic processes where waste substances are produced for subsequent elimination through urine and faeces. At the same time, the liver is involved in many other important organic activities. Its proper functionality has a close relationship with other important organs such as the heart and kidneys.

In fish farming, liver health problems have different origins ranging from infectious, toxic, pharmacological or nutritional. Those of nutritional origin are usually due to an imbalance between energy and protein in feed. Feed contamination can also cause liver problems. For example, the presence of mycotoxins in feed ingredients or finished feed for the trout can induce hepatomas, caused by contamination with aflatoxins, which are toxic metabolites produced by the fungus *Aspergillus* sp. The most common aflatoxins include B1, B2, G1, G2, M1 and M2, with B1 being the most implicated in cancer risk (Barrios-Cisneros, 1995).

Liver diseases may evolve with loss of liver functions, but in some cases but in some cases do not cause specific symptoms.

Hepatic lipidosis

Steatosis or fat accumulation in the liver affects production yield by causing growth reduction and spoilage of zootechnical parameters. Sometimes, when the etiology is toxic in origin, it can lead to high mortality rate. The existence of hepatic steatosis associated with dietary deficiencies of vitamin E or dietary lipid rancidity has also been demonstrated (Caballero et al., 2002; Iregui, 2004), although in this case necrotic lesions can be seen in other tissues.

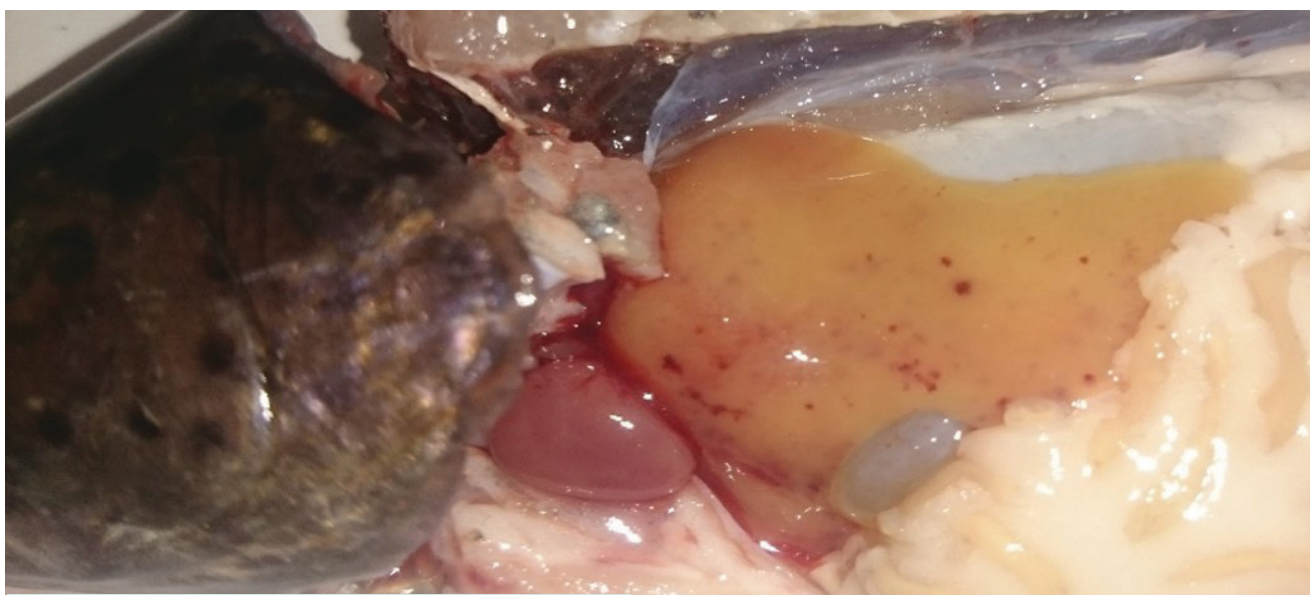
Hepatic lipidosis or steatosis is produced by an excessive accumulation of fat, mainly triglycerides, in the cytoplasm of hepatocytes (Hibiya, 1982; Carlyle and Duncan, 1990; Trigo, 1998). This happens frequently in some fish species such as the salmonids and tilapia, which are prone to store fat in liver tissues.

Hepatic functionality and productivity

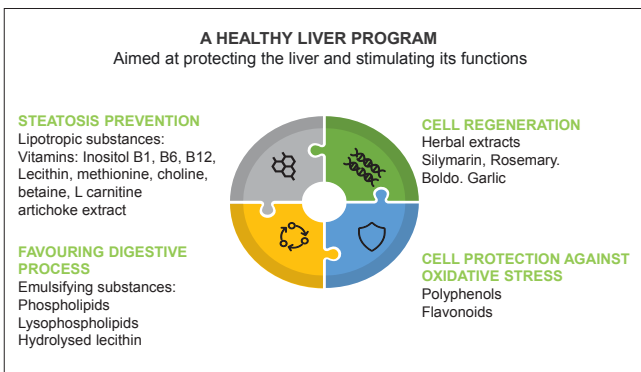
It has been reported that marine fish are particularly susceptible to hepatic steatosis caused by physiological malfunction (Spin et al., 1998). Some fish can support a high fat accumulation in liver without symptoms. They, however, may show some abnormal behaviour, such as manifesting lower than expected growth, reduced feed intake or even a marked lack of appetite to eliminate excess fat accumulated both in the liver and perivisceral space.

The high productivity demanded by current fish farming requires that fish sustain an optimal liver condition. Otherwise farm profitability will be compromised at some point of the production cycle and the expected results will not be achieved. The liver of farmed fish is subjected to constant detoxification challenges with a very high metabolic demand to successfully metabolise the high energy level of aquafeed. That is why it is necessary to adopt a set of measures aimed at protecting the liver.

In the first place, it is essential to estimate the nutritional needs of the fish so that its full genetic growth potential can be expressed to achieve farm profitability. A good liver condition will not only influence growth rate but will also act as an efficient barrier against opportunistic pathogens.



Hepatic steatosis caused by raw material rancidity in the rainbow trout.



Occasionally, to avoid stressing the liver, dietary changes are made by incorporating lower energy aquafeed into the feeding program. This strategy, which may give a good initial result by reducing liver lipid overload in the liver, always leads to a decrease in growth rate.

The hepatic protection

A liver protection program starts with selecting the best raw materials for the feed and allowing a balanced formulation with the right fatty acid profile for each fish species. The incorporation of lipotropic substances with hepato-protective function together with nutraceutical substances with hepato-regenerative and fat digestibility properties (emulsifiers) will complete the strategy for efficient liver protection.

The culture duration will also influence the possibility of steatosis development, with the risk increasing when fish are subjected to longer culture periods.

It is remarkable that due to the high feeding rates that fish species are subjected to, there is a clear tendency to accumulate fat in the liver, even when feeds are of high nutritional quality. As the hepatocytes are infiltrated with more fat, liver performance will decrease, and therefore nutrient absorption will be affected. This is specifically so, with the absorption of fat from the feed.

Steatosis can be prevented through a set of nutritional measures which, if applied continuously, will make it possible to optimise the use of fat, mobilise fatty deposits of hepatocytes and regenerate damaged liver cells. Optimal liver condition has many advantages that will be manifested in better health and fish will be able to utilise food more efficiently, resulting in a shorter production cycle time.



A healthy liver program.

Hepatoprotectors are nutritional complexes designed to improve the functionality of hepatocytes. Liptosa has an attractive liver health program called "Healthy Liver" aimed at optimal fish production. It is made up of three nutraceutical solutions that can be applied continuously and either singly or together, depending on the type of farm or liver condition to be resolved. These are:

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Álvaro Rodríguez is Aquaculture Manager at LiptoAqua, part of Liptosa, Spain.
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Liptosa ... the green way

A new feed prophylactic to improve resistance of white shrimp post larvae against EHP

Laboratory trials in Vietnam indicate a promising prophylactic application during EHP outbreaks in white shrimp.

By Stephane Frouel, Julie Castier, Maxime Hugonin and Thomas Pierrot

The shrimp aquaculture industry is consistently confronted with disease outbreaks, including the introduction and dispersion of novel pathogens such as parasites. This pathogenic pressure significantly impacts on the profitability of shrimp farming. Chemicals are often used to treat diseases. However, the massive use of chemicals in aquaculture is nowadays a high public health concern and has detrimental effects on the environment. Our current research work is aimed at developing a specific solution against parasites in aquaculture, in particular against endoparasites.

Enterocytozoon hepatopenaei (EHP) is a microsporidian parasite that was first characterised in the black tiger shrimp *Penaeus monodon* in 2009 in Thailand. EHP is restricted to the shrimp hepatopancreas and its occurrence was usually given scant attention because of the overwhelming focus on some other diseases, such as early mortality syndrome (EMS) and white faeces syndrome. However, although EHP does not appear to cause high mortality, it is associated with severe growth retardation and depressed feed conversion ratio (FCR) in shrimp, leading to economic losses for the farmers who may need to resort to an early harvest.

Experimental design

A 64-day trial was conducted in a laboratory in Vietnam to evaluate the potential of a new feed solution called A-Coverost supplied by MiXscience (Avril Group, France). This prophylactic was tested to gauge the response of white shrimp *Litopenaeus vannamei* post larvae (~PL30 with average initial weight 0.5g) challenged by EHP.

The experimental shrimp were initially checked for important pathogens (WSSV, EHP, IMNV, TSV, and AHPND) to confirm their disease-free status before starting the trial. In parallel, a stock shrimp specifically and experimentally infested with EHP were also used for the cohabitation challenge.

The challenge trial comprised four phases: an adaptation period of 1 day, 14 days of prophylactic treatment (pre-challenge), 7 days of EHP cohabitation challenge, followed by a post challenge of 42 days to evaluate the growth performance of the challenged shrimp (Figure 1).

Fifteen tanks (350L) were used for the trial. The stocking density during the challenge was 40 post larvae/tank which was equivalent to 160 PL/m³. The tanks were filled with 250L of brackishwater, and were continuously aerated. Temperature, salinity, and dissolved oxygen (DO) were kept within the following range: temperature 27–29°C; salinity 20ppt and DO 6–7ppm.

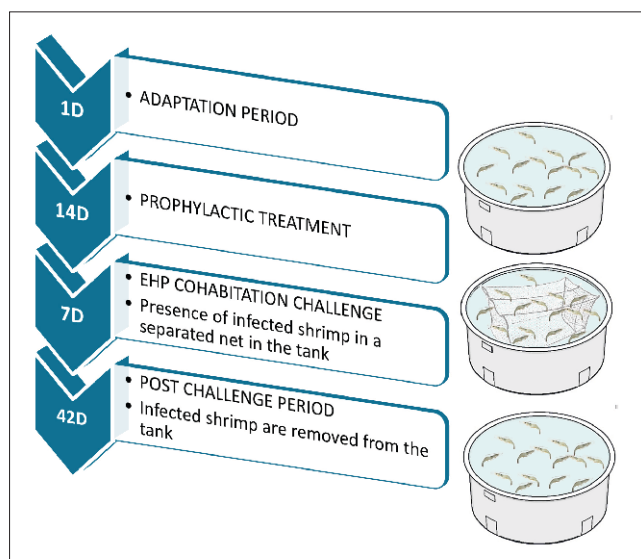


Figure 1. Chronology and layout of the trial phases

Treatment	Test product	Dosage of test product (g/kg of feed)	EHP challenge
Non-challenged Control	No	-	Cohabitation SPF shrimp
Challenged control	No	-	Cohabitation with EHP-infected shrimp/inoculum
Experimental product	yes	4	

Table 1. Description of experimental treatments

The completely randomised trial comprised three treatment groups (Table 1). Each group had five replicates.

The test product was mixed and incorporated into a basal pelleted feed adapted for shrimp post larvae. All ingredients were mixed together including the experimental product before the pelletising process. Experimental shrimp were kept on the test diet throughout the challenge. Shrimp were fed with their respective diet to satiation with four meals per day during the trial. The feeding rate ranged from 5–10% of the estimated body weight.

Challenge method

Shrimp were challenged under a standardised cohabitation method. An inoculated stock of EHP-

infected shrimp with an average EHP load/density of 2.5×10^8 CFU/g was used for the challenge. They were cultured in a separate tank.

Five tanks for the treatment and five tanks for the challenged control were co-cultured by co-habitation method with 20 EHP infected shrimp and 20 specific pathogen free (SPF) shrimp for each tank. Non challenged control tanks were co-cultured with 40 SPF shrimp.

The SPF and infected shrimp were separated by a net divider that allowed the free exchange of water. The cohabitation lasted 7 days, then, the inoculated shrimp and SPF shrimp were removed from the tanks.

Study parameters

During the trial, shrimp were analysed for their EHP load by quantification of the hepatopancreas using a qPCR technique at two time-points of the post-challenge (10 days post-challenge, day 32 of the trial, and 42 days post-challenge, day 64 of the trial).

Feed efficiency (FCR) and growth performance parameters (specific growth rate-SGR, body weight gain), final survival and EHP load of the shrimp hepatopancreas were monitored during the trial. Dead shrimp were removed from the tanks daily.

Water quality parameters such as dissolved oxygen, pH and temperature were measured daily. Total ammonia-nitrogen, nitrite, and alkalinity were measured twice a week.

Efficacy of the additive to reduce the EHP impact

The trial results indicated that the experimental product (applied at 4g/kg of feed) demonstrated some positive effects on growth performance (Table 2). A significant reduction of EHP load in the hepatopancreas of the infected shrimp was also observed (Figure 2).

Growth and feed efficiency

The test product restored the feed efficiency and the associated growth performance altered by the presence of the parasite. The results with the product were, however, statistically non-significant to the non-challenged control for FCR and final body weight gain (Table 2). This showed that the impact of EHP was neutralised. In contrast, the growth performance parameters of the shrimp of the challenged control were extremely poor.

Infected shrimp showed a slight reduction in survival rate. Compared to the challenged control, survival rate of shrimp fed with the test product was slightly enhanced but not significantly ($p > 0.05$). These results confirmed the fact that EHP mainly affected the growth rather than the survivability of shrimp.

Parasitic load

The EHP load density of shrimp in non-challenged group was zero at two different sampling time-points of the trial. This indicated that the experimental design was acceptable and no cross-contamination happened between the non-challenged group and the other treatment groups throughout the trial.

Treatment	Non-challenged control	Challenged control	Experimental product
iIBW (g)	0.54 ± 0.03 ^a	0.54 ± 0.02 ^a	0.51 ± 0.07 ^a
fIBWG (g)	8.83 ± 0.90 ^a	7.52 ± 0.91 ^b	8.85 ± 0.64 ^a
FCR	1.16 ± 0.08 ^a	1.60 ± 0.26 ^b	1.38 ± 0.24 ^{ab}
Survival rate (%)	90.50 ± 7.98 ^a	80.00 ± 6.85 ^b	81.46 ± 6.68 ^{ab}

iIBW - initial Individual body weight (g); fIBWG - final individual bodyweight gain (g); FCR - feed conversion ratio. Values with different letters in the same rows are significantly different at $p > 0.05$

Table 2. Performance of *Litopenaeus vannamei* after EHP exposure (a, b: ANOVA $p < 0.05$)

Based on the qPCR results, EHP load increased in all the challenged groups but in two different and significant ways between challenged control and shrimp fed with the experimental product. Shrimp in all replicates of the challenged control group were infected with high density of EHP parasites at the end of the trial. Meanwhile, a rapid response was observed in the treatment group. The product tended to quickly induce a positive effect in terms of EHP multiplication, recording the smallest value of EHP load 10 days after challenge. This low value persisted even after 32 days post challenge whereas it significantly increased (more than 1 log) in the challenged control.

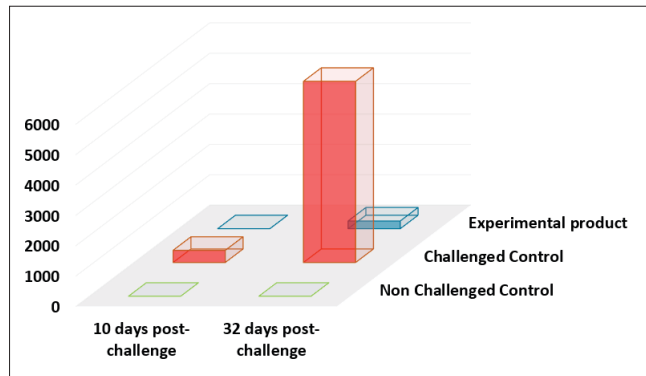


Figure 2. Dynamics of *Enterocytozoon hepatopenaei* (EHP) in shrimp hepatopancreas after the cohabitation challenge. The EHP load is expressed at 10^5 CFU/g of shrimp hepatopancreas.

Mode of action of the experimental product

A-Coverost is an innovative blend of oleochemicals associated to a specific active matrix. The combined positive effects of the product that explain the observed bio-efficacy on parasites are described in figure 3.

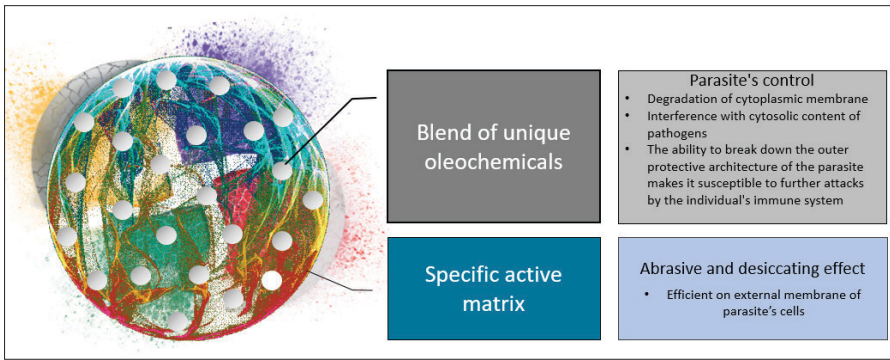


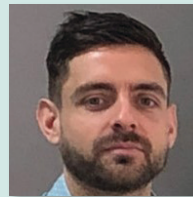
Figure 3. An illustration of the mode of action of the experimental product

Conclusion

Strict biosecurity and disease surveillance are key criteria in controlling EHP in shrimp farm environments. However, potential treatments are emerging and these preliminary results demonstrated that the blend of oleochemicals developed by MiXscience seemed to counteract the adverse effects of EHP on infected shrimp. The test product shows promise as a preventive product for shrimp farms that are heavily impacted by microsporidians.



Dr Stephane Frouel is an aquaculture specialist, in charge of aquaculture projects management including feed additive assays and worldwide trials.
Email: stephane.frouel@mixscience.eu



Maxime Hugonin is an aquaculture specialist, in charge of product development of the current solution against EHP disease.
Email: maxime.hugonin@mixscience.eu



Julie Castier is a technical specialist, in charge of research dedicated to providing an innovative solution against EHP disease.
Email: julie.castier@mixscience.eu



Thomas Pierrot is an aquaculture specialist, in charge of business development of the aquaculture department.
Email: thomas.pierrot@mixscience.eu

All authors are with MiXscience, part of the Avril Group, France.

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Evaluating retail consumer behaviour and shrimp purchases in China

A survey reveals that consumers from six major cities in China prefer shrimp live and darker in colour, raw or cooked, relating these traits to freshness, better quality and health. Such preferences are relevant to the global shrimp supply chain, particularly producers and exporters to a growing China market, as well as other markets with similar quality expectations.

By Thiago Soligo, Chiow Yen Liew, Daranee Seguin, Eduardo Yamashita, Darryl Jory and Zuoyong Luo



Chinese consumers associate red colour with freshness and quality and are willing to pay for these attributes.

According to the latest estimate of the Food and Agriculture Organisation, FAO (2020), the annual Chinese seafood consumption is around 41kg per capita, or about double FAO's estimated world average. A recent publication (Crona et al., 2020) stated "China is a key player in the global seafood trade, and represents one of the largest producers, consumers, importers and exporters of seafood in the world.... consumption is steadily growing and shifting toward an increasing amount of high-value marine species". Given China's dominance in the sector, Chinese choices regarding what to eat, and how and where to source this seafood, are increasingly important, not just for China, but for the rest of the world.

Globally, China is one of the largest shrimp importers – while its exports continue to decrease – and there are estimates that, on average, each Chinese consumes 1 kg of shrimp/year leading to an annual consumption of 1.4 million tonnes. Since it has a strong economy, China is already rebounding well from the Covid-19 pandemic. A growing number of Chinese consumers continue to shift from a diet based mostly on carbohydrates to one with more protein. This is helped by a growing expansion of e-commerce and online sales of seafood, including shrimp. The Chinese market for shrimp is expected to continue growing steadily.

Chinese consumers are increasingly aware of and concerned about the quality, safety, characteristics and origin of the food products they eat. Proper evaluation of their preferences and purchasing criteria is important for shrimp producers and exporters, as well as for support industries in the shrimp value chain – from aquafeed producers to processors. They need to consider and incorporate new information on consumer preferences and factors affecting purchase decisions, adjust production procedures and inputs

and tailor them to better meet consumer demands and expectations.

Through a contracted survey, we evaluated purchasing behaviour for shrimp at the retail level by consumers in six major cities in China, to better understand factors that buyers consider when buying shrimp, and consumer perceptions of shrimp quality. The survey results support industry players, especially those who are producing and exporting shrimp to China, to have a better understanding of consumer behaviour.

Study design

In this study, an online survey commissioned to a top, specialised commercial company (Kantar; www.kantar.com) was carried out in November 2019 with 3,661 initially screened consumers, from which an effective sample of 1,800 consumers finished a complete survey. The gender, age and monthly household income distributions were closely monitored during the screening stage to ensure that the sample size is representative.

Survey data was analysed and applied to evaluate the shrimp purchasing behaviour of Chinese consumers in six different, major cities. These cities were Beijing, Shanghai, Guangzhou, Hangzhou, Wuhan and Chengdu; each city had an effective sample of 300 consumers.

The selection criteria for survey respondents included the following:

- Male and female consumers, 22-50 years old.
- Local residents for more than two years.
- Decision makers of fresh, food-related household purchases; and
- Had purchased shrimp within the last three months prior to the survey.



Figure 1. Survey data on shrimp purchase incidence and purchase frequency.

During the <20 min long online survey, survey respondents were asked various questions related to their shrimp purchasing criteria, frequency and behaviour; and their perception of shrimp quality and possible influences on purchases.

The shrimp consumer market in China

Data collected during the survey shows that shrimp is an important delicacy in the Chinese family cuisine. Shrimp purchasing within the past three months was very high at 89%, and an average consumer had a purchasing frequency of 1.5 times per week, with 41% of respondents buying shrimp twice a week or more, and 24% reporting purchases of 2-3 times per month. Only 3% of consumers reported purchasing shrimp once a month or less (Figure 1).

The overall purchase incidence in different regions was around 90%, with the eastern region cities (Shanghai and Hangzhou) having the highest purchase frequency of 1.7 and 1.6 times per week, compared to 1.5 or fewer times per week in other regions. Shrimp consumption was quite common in major cities across the country, with the eastern coastal area having higher purchase frequency (Figure 2).

Results show that purchase incidence in the three months prior to the survey in different respondent groups ranged from 83% to 93%, where females and young consumers between the ages of 22 and 35 years old frequently bought shrimp (1.6 – 1.7 times per week). This was more often than for males and middle-aged consumers above 35 years old (1.5 or fewer times per week), echoing the trend of young women today actively adopting and searching for healthier diets with low-fat, high-protein ingredients (Figure 3).

Our survey data also shows that purchase channels are getting diversified. Conventional places like supermarkets and food markets were the key channels (adding up to 55% of the total purchase channel distribution), but new retailing and e-commerce channels continue to develop and expand. Online purchase was popular due to the ease of transaction and delivery convenience. The speed of delivery has also opened the accessibility to purchasing from inland areas. There were differences in purchase channels among regions. Consumers from the eastern or southern coastal cities bought more from food markets. In the north there were more purchases from supermarkets and fresh food stores, while online purchases were more popular in the middle and western regions (Figure 4).

Species awareness

The survey also addressed consumer awareness of the different species of shrimp available in the market. Results

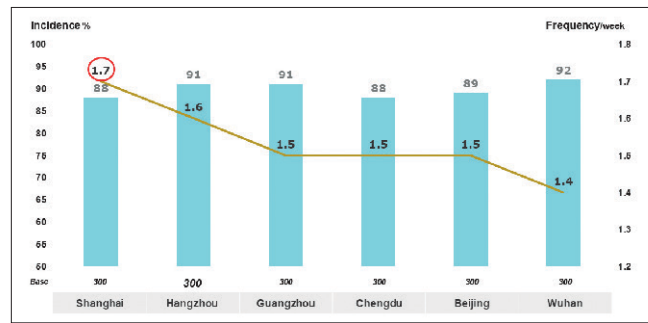


Figure 2. Survey data on shrimp purchase incidence (%) and frequency per week, by cities.

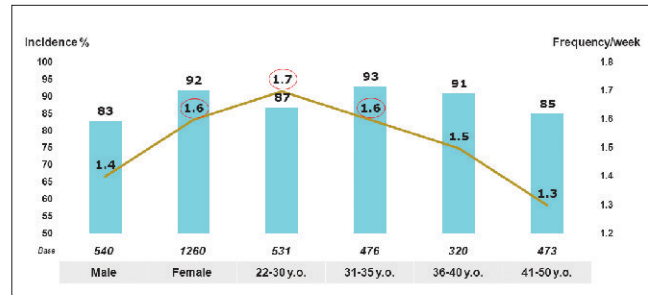


Figure 3. Demographic survey data on shrimp purchase incidence (%) and frequency per week, by group.

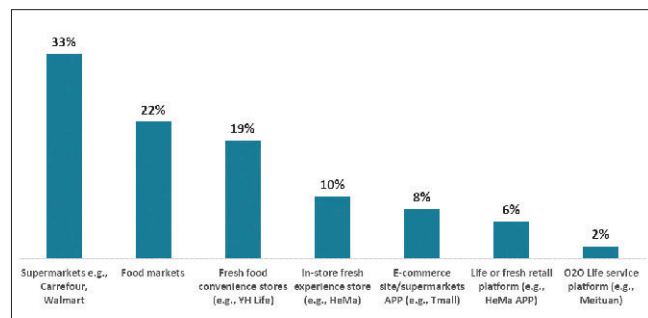


Figure 4. Results on percent distribution of shrimp purchase channels.

show consumers were more aware of local species; Asian black tiger prawn (*Penaeus monodon*), greasyback shrimp (*Metapenaeus ensis*) and Chinese white shrimp (*Fenneropenaeus chinensis*) rather than imported species, such as the kuruma shrimp (*Marsupenaeus japonicus*), Pacific white shrimp (*Litopenaeus vannamei*) and the black tiger prawn (also *Penaeus monodon*). This was particularly marked in the eastern and southern coastal areas.

However, imported shrimp also enjoyed significant consumer recognition and showed competitiveness in local markets, particularly in Tier 1 cities (the largest and wealthiest), where imported species attracted many consumers. It should be noted that the Asian tiger prawn and the black tiger prawn are both the same species (*Penaeus monodon*), and that different names are probably commercial ones used by local vendors to possibly differentiate between locally sourced and imported *P. monodon* (Figure 5).

Survey data for purchase rates show a similar tendency regarding awareness: consumers from coastal areas – such as Shanghai and Guangzhou – purchased significantly more local species as indicated by significantly higher

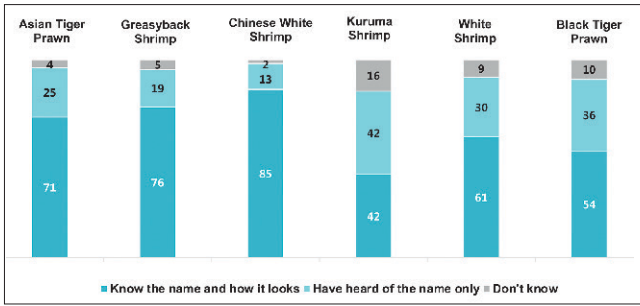


Figure 5. Survey data showing consumer awareness (%) of the different shrimp species available.

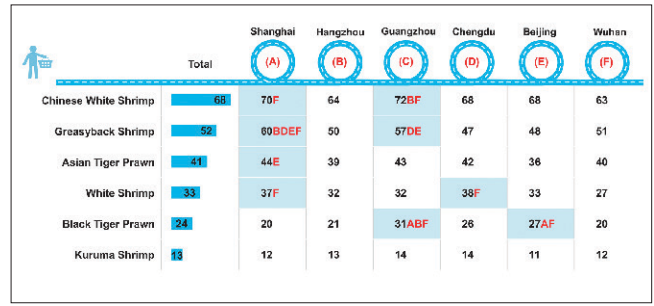


Figure 6. Survey data showing consumer purchase rates (%) by shrimp species, by cities surveyed. Letters ABDEF indicate significantly higher values at 95% confidence interval.

purchase rates highlighted in blue colour. Likewise, those from Tier 1 cities such as Shanghai, Guangzhou and Beijing, preferred more imported species (white shrimp, black tiger prawn and Kuruma shrimp).

Also, greasyback and Chinese white shrimp had the highest conversion rates (the conversion rate from awareness of the product/species to purchase percentage). It is worth mentioning that imported species had fair conversion rates despite lower awareness, which implied good market potential (Figure 7).

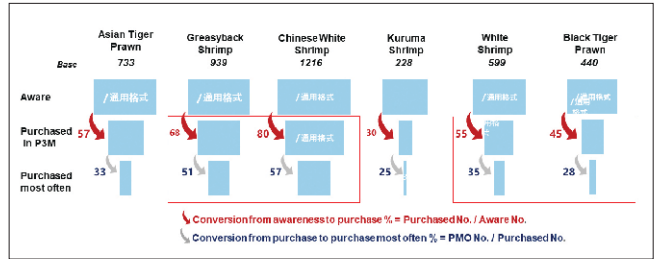


Figure 7. Conversion rate for species (%), P3M – Past three months. Greasyback and Chinese white shrimp had the highest conversion rates. Imported species had fair conversion rates despite lower awareness, which implies good market potential.

Purchasing and consumption behaviour

In this survey section, data show a clear consumer preference for live (fresh), followed by whole (frozen) shrimp. Generally, it is more common for Chinese consumers to have live shrimp or whole shrimp in their meals, as these are better suited to Chinese cooking practices. Compared to other cities, live shrimp had more demand in Guangzhou (south), while frozen whole shrimp had higher consumer demand in Shanghai (east) and Beijing (north).

key drivers for the purchase of live and fresh whole shrimp, while for frozen/cooked shrimp, easy to cook/eat was the major allure (Figure 8).

Quality was first, according to survey data. Consumers paid significant attention to the liveness, colour, overall size and size similarity for live shrimp; for frozen shrimp, important traits were species and colour; and for cooked shrimp, taste and aroma were the most desirable characteristics (Figure 9).

Regarding reasons given by survey respondents for processing preference, freshness and health were the

Purchase rate % by region	Shanghai	Hangzhou	Guangzhou	Chengdu	Beijing	Wuhan
	(A)	(B)	(C)	(D)	(E)	(F)
# Fresh or live	94	92	94	93	92	91
Live	65 F	65 F	77 ABDEF	64	69 F	57
Fresh whole	34 C	31	25	35 C	35 C	29
Fresh peeled	25	21	20	20	20	22
Fresh headless	15	18 E	12	13	11	21 CDE
# Frozen	72	70	71	71	72	66
Frozen whole	47 BCF	33	38	39	47 BCF	36
Frozen peeled	25	29	34 AD	26	27	29
Frozen headless	20	23	18	18	17	18
Frozen mixed seafood	15	11	12	11	11	12
# Cooked	47	41	46	44	40	41
Ready to serve	21	17	20	17	17	22
Cooked whole	24 BE	17	20	19	16	18
Cooked peeled	22 F	17	22 F	19 F	18 F	12

Figure 8. Survey data showing consumer purchase rates (%) by regions, for different shrimp products and presentations. Letters ABC indicate significantly higher values at 95% confidence interval.

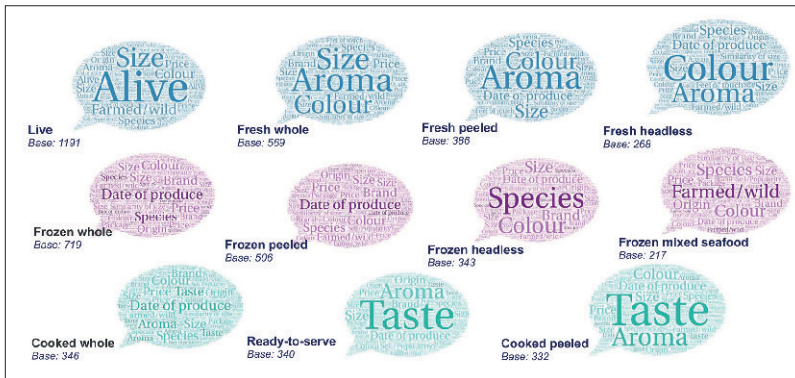


Figure 9. Survey data showing consumer criteria for different shrimp products and presentations.

Health and convenience were the main concerns when purchasing, while freshness was the main advantage reported for live shrimp. Consequently, consumers paid more attention to quality-related factors such as shrimp vitality, colour and size. In addition, frozen and cooked shrimp provided convenience to consumers, so the species, colour, aroma and flavour were more important (Figure 9).

The survey results for consumption patterns showed that consumers tended to eat shrimp simply just after preparing/cooking it. Overall, most consumers in China have shrimp at their normal dinners, and simple Chinese cooking methods (boiling and steaming) are preferred. For consumers in coastal areas (east & south), the survey showed that it was more common to consume shrimp daily, while for those in the mid and western areas, shrimp tended to be served more often on special occasions (Figure 10).

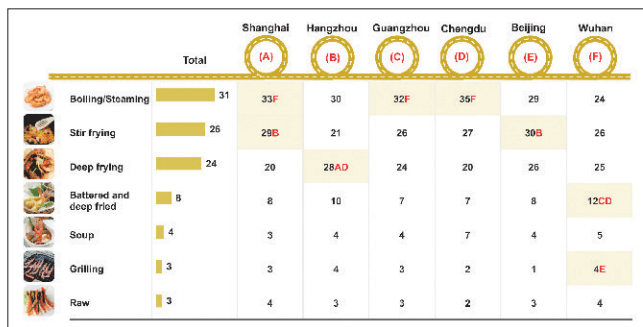


Figure 10. Survey data showing consumer preference (%) for different cooking methods of shrimp, by cities surveyed. Boiling, steaming and frying are the most common cooking methods reported. Letters ABC indicate significantly higher values at 95% confidence interval.

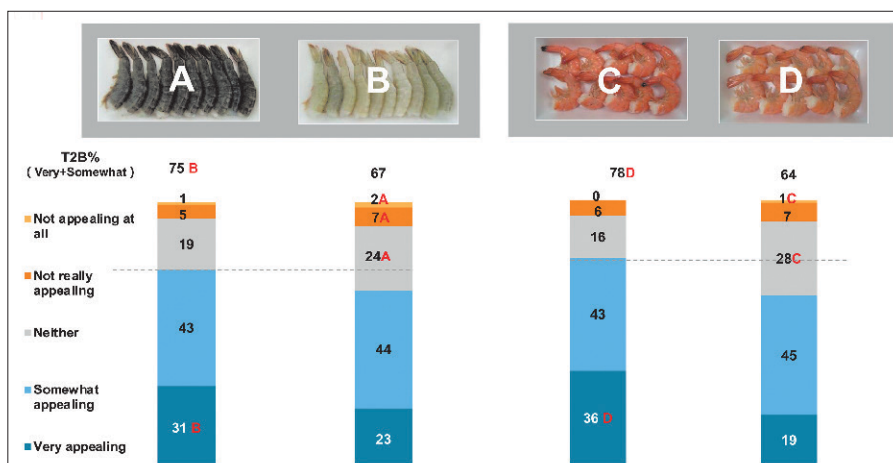


Figure 11. Survey data on attractiveness (%) of shrimp pigmentation show that strong/darker pigmented appearance is more attractive both for fresh and cooked shrimp. Letters ABC indicate significantly higher values at 95% confidence interval.

Quality comparison test

Preferences according to the appearance of fresh and cooked shrimp were evaluated in this section. The results demonstrate consumer preference for darker/strongly pigmented shrimp for both cooked and live shrimp. A darker/stronger pigmented shrimp was deemed attractive. The preference for pigmented shrimp was found to be more prominent in cooked shrimp with consumers preferring red coloured shrimp (Figure 11).

When asked for the reason(s) for their preference, respondents stated that freshness and quality were the reasons why colour is considered. Consumers usually associated strong/darker colour with freshness and good quality, and clearly the redness of cooked shrimp was a key driver in purchasing decisions, because consumers were willing to pay more for shrimp with darker pigmentation. In the case of fresh shrimp, a darker colour was perceived to be from wild-caught species of shrimp (Figure 12).

A strongly pigmented shrimp was often higher priced compared to a paler shrimp. It was found that the price gap was even wider in cooked shrimp.

Perspectives

Overall, the survey results presented here show a higher incidence of purchase in large and coastal cities and consumer preference for live shrimp, darker/stronger in colour in both raw or cooked presentations. These are traits that are related to the perception of freshness, better quality and healthy. These results are relevant to the global supply chain of shrimp, particularly those producing and exporting shrimp to the growing market in China and other export markets with similar quality expectations.

China consumers perceived freshness, quality and health status of the shrimp with colour. The survey found that consumers in China were willing to pay more for shrimp with better quality, appearance and colour. The survey data also revealed a high degree of knowledge on the part of respondents regarding their quality criteria and expectations. These findings should be considered by the entire production value chain for farmed shrimp targeting export markets such as China, as well as other markets with similar quality expectations.

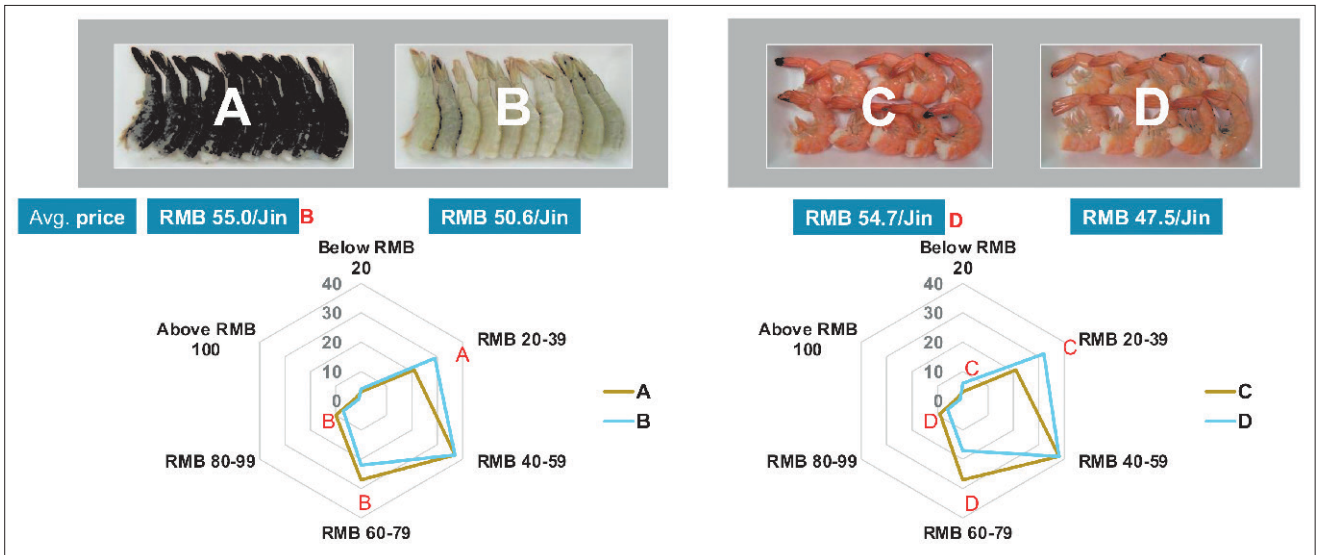


Figure 12. Survey data for pigmentation and price perception (%) for raw and cooked shrimp, showing that darker pigmented shrimp generate a perception for higher prices. The price gap is even larger in cooked shrimp. Note: The Jin is a common unit of weight in China (1 Jin = 0.5kg). Base total N = 1800. Letters ABC indicate significantly higher values at 95% confidence interval. USD = RMB 6.46 on 13 February 2021)

Understanding consumers' preferences for seafood products is crucial to increase their consumption and spending habits. One significant takeaway from the results of this survey is that Chinese consumers prefer certain product presentations and qualities, including live and fresh forms and shrimp with darker pigmentation. The preference for darker/stronger colour is also common in other countries, including major markets such as France, Spain and Australia.

The darker colouration in shrimp is derived from ingestion of certain carotenoid pigments, natural or synthetic, as shrimp cannot synthesize these pigments *de novo*. Carotenoids, while influencing flesh colour also have important, health-related metabolic roles in shrimp. Culture conditions are one of the key factors determining the availability of the pigments in farmed shrimp. Shrimp in semi-intensive culture systems with open ponds obtain adequate levels of carotenoids compared to those in high density production systems. In the former, supply of carotenoids come from consumption of microalgae and natural live feeds.



Under an intensive culture regime, there is little or no dependence on pond natural productivity. Therefore, to achieve a desired colour score (such as 28 on the DSM colour fan), the farmers must supplement the feed with the right level of a carotenoid source. This depends on the feed producer and the farmers' willingness to absorb the extra cost for aquafeed formulas with inclusion levels of these carotenoid pigments that can improve the pigmentation of the shrimp.

The efficacy of the immune system, which is the primary line of defence, is dependent on an adequate supply of nutrients such as carotenoids and vitamins for maintaining biological processes and optimal growth, improving immunity and disease resistance of the animals. Among the vitamins, vitamin A, vitamin D3, and its metabolite 25OH2D3, vitamin E and vitamin C have been shown to strengthen immunity and health. Moreover, antioxidants like vitamins C and E are scientifically proven to slow down oxidation processes, extend shelf life and reduce spoilage and waste in post-harvest phases, including storage, processing and distribution.

One significant takeaway from this survey is that Chinese consumers prefer certain product presentations and qualities, including live and fresh forms, and darker/stronger pigmentation.

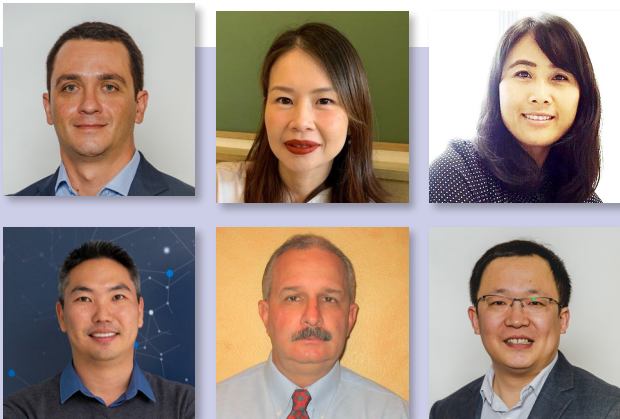
In this article, we show that an accurate and up-to-date understanding of consumer preferences and the drivers influencing their purchasing criteria is important for the entire shrimp value chain. A darker colour trait can provide a significant competitive advantage to differentiate a product from other commodity shrimp in some very important markets. As the results of the survey described here indicate, this would provide producers and exporters with valuable market information as well as a potential price advantage with darker shrimp. Subsequently, their production procedures and inputs could be adjusted to better meet the expectations and preferences of those consumers.

This understanding of consumer behaviour can help shrimp producers and exporters to differentiate their shrimp, so they can gain buyer preference and convey a better selling price through a higher colour score compared to a pale looking shrimp.

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Thiago Soligo, MSc, is Aquaculture Marketing Manager, DSM Nutritional Products Latin America San José, Costa Rica.
Email: Thiago.Soligo@dsm.com

Chiow Yen Liew is Aquaculture Marketing Manager, DSM Nutritional Products Asia Pacific

Daranee Seguin, PhD., is Manager, Nutrition and Health Solution, Aquaculture, DSM Nutritional Products Asia Pacific

Eduardo Yamashita, MBA, is Regional Technical Service Manager, Aquaculture DSM Nutritional Products Latin America

Darryl Jory, PhD., is a Consultant based in Florida, USA.

Zuoyong Luo is Aqua Technical & Marketing Manager, DSM Animal Nutrition and Health Greater China.



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Sustainable aquaculture: What does it mean?

Creating safe products for human nutrition and global food security is crucial for inclusive social, environmental and economic sustainability.

By Benedict Standen

As we enter a second year of COVID-19, it is the collective responsibility of all to work together and do our part to limit the spread of the virus. Individual measures can include wearing masks, social distancing and working from home. Although this can be mentally challenging, it also gives one an opportunity to reflect on the more important things in life— what are our individual and our companies' professional goals. From a corporate view, very often these objectives are a tangible goal, for example a financial target. This approach covers aspects such as 'what', but very often we forget about the 'why'. Whilst 'money talks', a company's motivation to serve an industry is often a lot deeper. This applies strongly in aquaculture.

Social sustainability

Food security

Food security exists when all people, at all times, have access to sufficient, safe and nutritious food sources that meet their dietary requirements. According to the UN, the world population is growing by approximately 81 million people every year. If forecasts are correct, then globally there will be 8.5 billion people in 2030, 9.7 billion in 2050 and over 11 billion by 2100 (Figure 1). On top of this, in part due to advances in medical technology, global life expectancy is also increasing, with the average person living 5.5 years longer in 2016, compared to 2000, based on World Health Organisation (WHO) data. With more mouths to feed, the pressure on our animal production sectors will be greater than ever. Aquaculture products have a crucial role in human nutrition and global food security as they generate a high-quality source of valuable nutrients, and micronutrients for a diversified and healthy diet. This is most important in developing countries, where, already, aquatic protein can contribute to >50% of animal protein. Many fish species are an important source of fatty acids, essential amino acids, minerals such as calcium, phosphorous, zinc, iron, selenium and iodine; as well as vitamins A, D and B, all of which have a vital function in many biological systems.

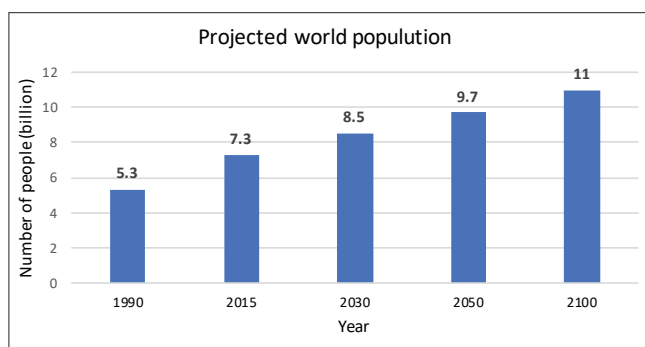


Figure 1. Projected world population until 2100. Source: United Nations Department of Economic and Social Affairs.

Producers can produce more aquatic protein for human consumption by improving growth performance and survival. Additives such as phytochemicals and pigments can improve fillet and flesh quality, in volume terms and in nutritional value.

Feed and food safety

As well as ensuring that we have enough food to eat, of equal importance is that the food is safe for both human and animal consumption. There are a number of contaminants which can enter the food chain, for example, microbiological contaminants, heavy metals, microplastics, antibiotic residues and mycotoxins to name a few. These can negatively affect aquatic animal health and performance, and some (e.g. aflatoxin) can also have carry-over effects into the muscle, implicating human health.

Aquaculture allows us to have better control of production, compared to its fishery counterpart for example. Contaminants entering animals through the diet can be managed at the feed mill. Raw material selection in combination with methodical and routine sampling is important to ensure that only the highest quality ingredients are utilised in aquafeed.

Prophylactic usage of additives can prevent the build-up of aquatic pathogens. This reduces the need for medicines, so our food is less likely to contain such residues. Further, foodborne pathogens such as *E. coli* and *Salmonella* can also be controlled. Mycotoxin risk can be mitigated using adsorption and biotransformation tools (Figure 2).

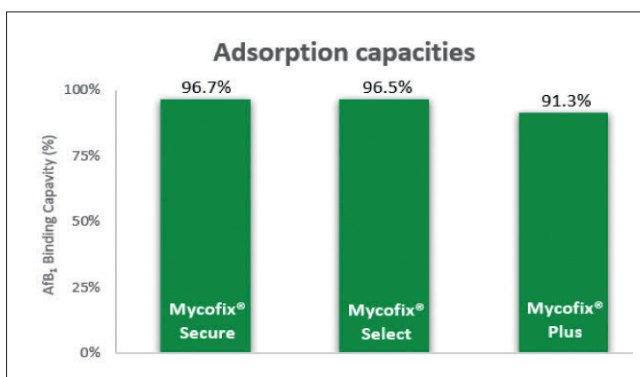


Figure 2. Adsorption capacities of several mycotoxin binders produced by BIOMIN.

Protect antibiotic efficacy for human medicine

The United Nations has stated that antimicrobial resistance (AMR) is "one of the biggest threats to global health, food security, and development today." Although a

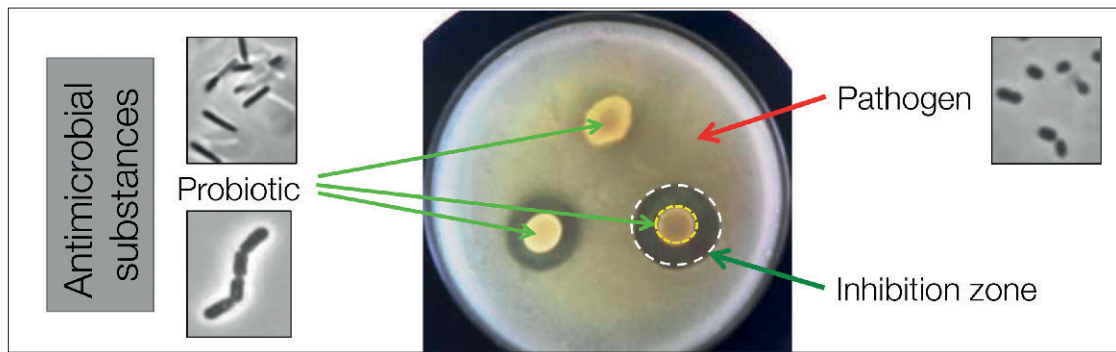


Figure 3. Probiotics to reduce pathogens.

natural process, the spread of such resistance mechanisms is being artificially fast tracked, through our extensive usage of antimicrobials in animal production. This has led to many markets to evaluate antibiotic use. By reducing our dependence on such therapies, we can preserve the efficacy of these medicines for use as a treatment for animal and human pathologies, when they are really critical.

Whilst aquaculture is also a contributor, it is also a pioneer in antibiotic free and antibiotic reduced animal production. For example, Norway has almost eradicated the use of antibiotics and all salmon are completely free from residues from antibiotics or other medicines. In fact, a report in 2015 by the Norwegian Veterinary Institute demonstrated that the domestic salmon industry used 273kg of antibiotics in 2015, compared with 5,850kg for terrestrial livestock species. It should be noted that in the same period, salmon production in Norway was four times higher than terrestrial animal production, which puts antibiotic use at 80 times lower in salmon culture per unit biomass. This success can be attributed to a holistic approach over many years, and from all stakeholders in the aquaculture sector, specifically, through improved nutrition, usage of functional feeds, rigorous biosecurity measures, vaccination, genetic improvements and governmental policy. Other aquaculture markets are also following this model, with variable success.

When used as a prophylactic strategy, additives can improve the disease resistance of aquatic animals, without the need for antibiotic intervention. Areas of focus are direct pathogen control and improvement of the immune response (Figure 3).

Animal welfare

In many markets, especially the consumer driven ones in the Western Hemisphere, awareness around animal welfare is becoming more important in food supply. Many welfare concepts have been developed for warm-blooded terrestrial species, and it is still unclear how these can be applied to aquatic species. A key challenge in developing recommendations and regulations on the welfare of farmed fish, is that both scientific knowledge and practical experience in this area are limited. According to EFSA (2009), "the concept of welfare is the same for all farm animals, i.e. mammals, birds and fish," but goes on to state that fish welfare "has not been studied to the same extent."

Eleven years on, we have not made much progress in assessing fish welfare, in part due to the huge number of species cultured, without even considering different developmental stages and their specific demands. This

means that there are relatively few operational welfare indicators in aquaculture. However, it can be agreed that poor animal welfare can lead to stress, and inevitably, the animals may become immunocompromised, increasing their risk of disease. Thus, maintaining optimum health and performance should be a key target.

Job creation

The aquaculture industry employs millions of people worldwide, many of whom may struggle to maintain reasonable livelihoods without it. According to FAO, 19.3 million people were employed in the aquaculture primary sector in 2016, with >96% of all engagement in Asia. The industry must also strive to provide best practices and welfare for its human resources. This means that as well as creating more jobs, the workers must be treated fairly, with good working conditions, decent salary and a positive impact on all employees and the host community.

Environmental sustainability

Water quality

Whilst nature has 'built-in' measures to correct imbalances in its biological systems (e.g. nitrogen cycle), the addition of extra organic material in aquaculture, primarily through feeding, can overload aquatic systems if left unmanaged. Such practices can often lead to friction between industry and public stakeholders, especially where there is a competition for resources in coastal areas. Poor water quality has a direct negative impact on aquatic animals, as they are continuously ingesting water when they eat and drink, and for respiration and osmoregulation. Excess nutrients can enter local waterways damaging local ecosystems, affecting flora and fauna and causing phytoplankton blooms. Further, inland shrimp culture can lead to the gradual salinisation of underground fresh water reserves. This is one reason why a number of producers are looking at reduced or zero water exchange systems.

A number of actions are being taken to reduce the environmental impact. Feed manufacturers are researching novel technologies to improve the digestibility of aquafeeds and linked to this, it is important that feeding management is optimised at the farm site to reduce waste. Site selection and production type are also important mitigating factors, and there is a greater effort to move away from traditional culture techniques. In order to operate a cleaner production cycle, there is a trend to pursue alternative production systems, for example integrated multi-trophic aquaculture (IMTA), aquaponics, offshore farming, and recirculating aquaculture systems (RAS). Whilst none of these are currently mainstream, they all pave the way for a cleaner production cycle in the future.

We can improve the digestive efficiency of aquafeeds, reducing FCR and ensuring that less nutrients are released into the environment (Figure 4).

Fishery protection

Until the late 1990s, virtually all of our aquatic protein came from capture fisheries; however, due to overfishing, many of the wild stocks crashed and the industry has now plateaued. This means that additional demand must be derived from aquaculture, and for the first time in 2014, more than half of aquatic protein for human consumption came from farming. The successful growth and productivity of aquaculture put less pressure on wild fish stocks.

Capture fisheries are not only sourcing for human consumption, but also other industries, such as fishmeal production. Traditionally, fishmeal has been a staple ingredient in aquafeed. However, pressure on wild stocks, especially for small pelagic fish, and fluctuating prices have forced the industry to explore alternative protein sources. Although the absolute volume of fishmeal is slowly increasing, it cannot keep up with the fast growth of the aquaculture sector. It is anticipated that in 2030, fishmeal production will be 19% greater than it is today, but more than half of this growth will come from a better usage of fish waste derived from processing facilities.

Although there are many alternatives being investigated (e.g. krill, insects, single cell proteins, animal by-products), many currently have biological or commercial disadvantages, so plant proteins are currently most relied upon. Fish oil is also a valuable resource, as it is a key provider of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), amongst others. This is an important requirement for marine

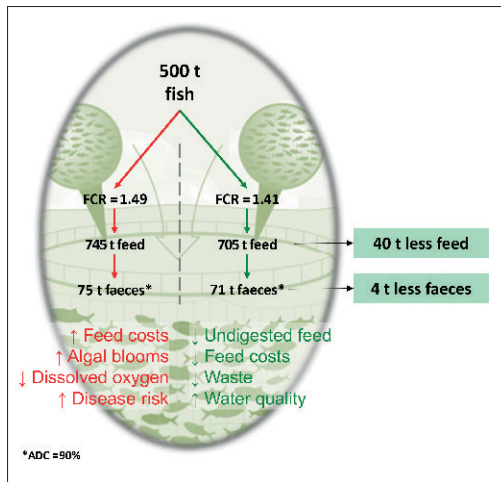


Figure 4. Phylogenics can increase the digestibility of aquafeeds. This means that farmers can produce the same amount of biomass with less inputs (feed), and with a lower environmental footprint (less waste/faeces). Data comes from a trial where Digestarom® PEP MGE, on the right, was compared to control treatment, on the left.

fish, and there is ongoing research into finding other EPA/DHA sources, with the most promising coming from algae and/or GMO crops.

Carbon footprint

Carbon footprint is typically defined as the total greenhouse gas emissions caused by a specific activity, expressed as CO₂ equivalent (CO₂ eq). The Global Salmon Initiative reported that a 40g serving of farmed salmon produces 0.60g CO₂ eq, compared to 0.88g for chicken, 1.30g for pork and 5.92g for beef. This makes it one of the most environmentally sustainable animal proteins to consume. It should be noted, however, that other aquatic species have a greater environmental cost. For example, tilapia and carp have a slightly higher value, followed by shrimp and catfish production which has a similar carbon footprint to beef (Figure 5). Molluscs have the lowest, even smaller than that of farmed salmon.

by shrimp and catfish production which has a similar carbon footprint to beef (Figure 5). Molluscs have the lowest, even smaller than that of farmed salmon.

Economical sustainability

Aquaculture must be a viable business with good long-term prospects. In production terms, the FAO SOFIA 2018 report claims the first-sale value of aquaculture products alone was USD243.5 billion. In recognition of the economic benefits of aquaculture, many agencies (local, national, regional and international) provide incentives for aquaculture development. These can include financial subsidies, tax rebates, public investment in collective infrastructure (e.g. water channels and electricity supply), marketing and investment in job creation, skill development, education, research and training programs. These projects are often targeted at generating income and employment for rural communities, which may otherwise have limited opportunities. By bringing jobs and infrastructure to these areas, aquaculture can also be a driver for secondary industry and services.

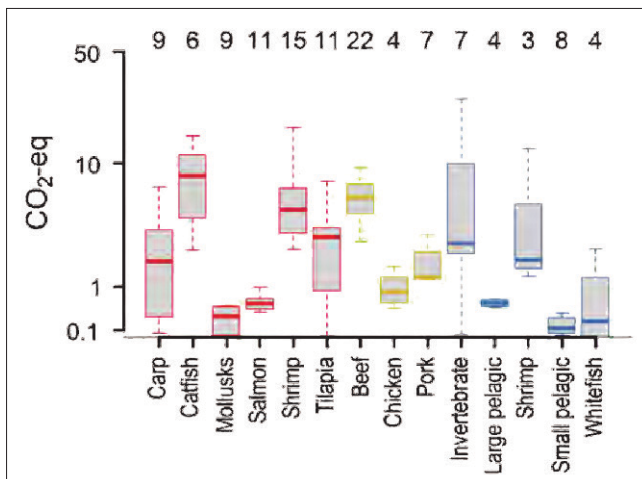


Figure 5. CO₂-eq associated with aquaculture (red), livestock (yellow) and capture fisheries (blue), per 40g protein produced. Numbers above each box represent the number of studies included in each product. Source: Hilborn et al. 2018.

The aquaculture industry has come a long way, in a relatively short period of time. Whilst there are inevitably improvements and bottlenecks to overcome, the sector continues to develop and contribute to food security worldwide. True sustainability must come by engaging all stakeholders and working together towards a holistic solution, for the good of the people, the environment and for the bottom line!



Benedict Standen, PhD. is Product Manager, BIOMIN Holding GmbH, Austria. Email: benedict.standen@biomin.net

Hybrid grouper farming: Manoeuvring colossal lows in demand in 2020

A year-long impact of the Covid-19 pandemic on the demand for live fish required strategic changes at a farm.

Operation Manager, Mohd Addin Aazif Hj Mokhtar oversees operations at Grouper Palace, a group cage farm located off Langkawi Island, in the state of Kedah in northern Peninsular Malaysia. Grouper Palace is part of JK Investments, a Kuwait-based agriculture company. Langkawi is a major destination for local and foreign tourists and it is an ideal market for fresh seafood. Set up in 2018 through the acquisition of several cage farms off Langkawi, the farm began its first harvest in 2019.

The farm focusses solely on the hybrid grouper (tiger grouper, *Epinephelus fuscoguttatus* x giant grouper, *E. lanceolatus* (TGxGG) which Addin, as an MSc student at the Borneo Marine Research Institute, Universiti Malaysia Sabah (UMS), is familiar with. He was part of the team working on the aquaculture development of this grouper with Dr Shigeharu Senoo. Asian table size (1-1.5kg) hybrid and several groupers are usually marketed live or as fresh fish.

The company has a good business plan for 2018-2023. All was going according to plan, with the first sales at the end of 2019. Grouper Palace focusses on innovative and eco-friendly marine fish farming. "In 2019, we stocked 100,000 fingerlings in the cages. We were happy as survival was good at 70-80%," said Addin. "Juveniles of 7 inches (17.5cm) weighing 150g grow to 1-1.5kg in less than 12 months. We had tonnes of fish to sell as live fish to Hong Kong using well-boats, to local seafood restaurants and the domestic market as well as for export. Everything was according to plan," said Addin during a webinar organised by the Malaysian Fisheries Society, held in October 2020 to discuss the impact of Covid-19 on the aquaculture sector in Malaysia.

"We had good targets for 2020 and I was expecting to harvest more fish in early 2020 too. Of course, we did not expect this Covid-19 pandemic. Now even though the farm has fish in the cages, we are not able to sell the fish."



Mohd Addin Aazif injecting fish with hormones for the first sex change prior to breeding.



Some 300g hybrid groupers, two months after stocking at 100g/fish

Marketing live hybrid groupers

The company had already lined up plans for marketing its stock. It had planned to sell in domestic and international markets – the latter being a key market for live groupers. The target export markets were China, Hong Kong, Singapore and other Southeast Asian countries. Marketing included promotions in aquaculture and seafood trade shows. The target buyers were agents, distributors, restaurants and fish markets but the company also planned direct sales. "The Hong Kong well-boats would collect the live groupers and would henceforth distribute them regionally. But way before 2020, there were no well-boats plying the area. For us in Langkawi, we depend fully on these boats; if we were to do our own transportation to other parts of Malaysia by land, it will be costly. Furthermore, these boats take large volumes of fish."

Malaysia imposed several lockdowns throughout 2020. Stricter lockdowns included the closure of all restaurants and hotels throughout the country. The major markets for live groupers are seafood restaurants where the demand is particularly high during the end of year wedding season, which is followed by the Chinese New Year in February 2021. Flights were cancelled and sending air cargo to Hong Kong and China, as well as to Singapore was not possible.

Lower prices

Prices were depressed in international and domestic markets in 2020. Based on data from the fish marketing organisation in Hong Kong, the declining price over the period from May to October 2020 affected the following fish: brown marble, brown spotted, camouflage and green groupers. Prices/kg dropped in October 2020 to the following: brown marble grouper, (HKD237.57; USD30.64) brown spotted grouper (HKD213.00; USD27.5), camouflage grouper (HKD290.33; USD37.4) and green grouper (HKD144.33; USD18.6).

“In 2019, we could sell our fish at MYR33-35/kg (USD8.3-8.8/kg) but in October 2020, the prices were only MYR18/kg (USD4.5/kg). We have stories of farmers throwing away their fish. At the same time, fish were coming in from Indonesia, undercutting with lower offer prices. We only saw the large price drops with the groupers as these are 'luxury fish'. Prices for other marine fish such as pompano and snappers remain relatively stable.” Addin attributed lower prices to changing buying patterns- with incomes affected, consumers opted for cheaper fish.

Changing strategy

Over at Grouper Palace, Addin adopted a strategy of maintaining the groupers in the cages until they reach 5-7kg and target a different market, rather than compete with the smaller farmers. “As a big company, these low prices are at the edge of our production costs. In the meantime, we also changed our species to snappers but it will be 1-1.5 years before we can harvest them.

“At Grouper Palace, we learnt to change our stocking plans, change species in line with demand and also our feeding regime.”

“At the farm, it has been our strategy to only use extruded feeds. With full feeding, our feed consumption is around 20-30 tonnes/month and this is very costly at MYR5/kg. To cut costs, we feed for 2 days, with one day off. Fish will still grow but it will be slower to reach our target of 5-7kg per fish.”

Over the past year, the situation for marine fish farms, not only for Grouper Palace, has been dire. In October, there was a slight reprieve when the government allowed for interstate travel and Langkawi attracted domestic tourists. With hotels and restaurants open, but not at full capacity, Addin said, “We saw prices creeping up, demand was up but towards the end of 2020, it slowed again.”

Lessons learnt

“What we have learnt from this pandemic is that original plans may get derailed. We always need to innovate according to situations and market demand. At Grouper Palace, we learnt to change our stocking plans, species in line with demand and feeding regime. This applies not only in this pandemic year but also for any adverse situation in the future,” said Addin.

Fortunately, the farm produces its fry and fingerlings from its own broodstock. It did not face any fry shortage. In 2020, more than 70% of the hybrid grouper fry supply from Indonesia was disrupted when the Indonesian authorities closed its biosecurity facility for exports. “It was farming as usual for snappers, pompano and seabass farms as fry supply is from local hatcheries. In the future, we should be thinking of self-sufficiency in hybrid grouper fry supply, as its farming is increasing,” said Addin.

“My advice is that we should always have a plan B for the farm, perhaps a diverse set of species to farm. We could plan better to meet market needs, like the higher demand at the end of the year to match with wedding seasons in Malaysia. Situations change and so should plans.”



At Grouper Palace, from left, Abdul Karim, farm team leader holding a 3kg hybrid grouper; a harvest of 3-4kg hybrid groupers and Marvin Loh, Manager of Langbiru Fisheries, selecting fish for the Singapore market.

Managing ectoparasites in farmed marine fish

What is known of the life cycle of major sea lice, skin flukes and leeches and how to manage them.

Disease problems caused by various parasites in farmed fish are often overlooked mainly because they have less adverse impacts on production compared to viral and bacterial diseases. However, some of these parasites, if left uncontrolled, can cause significant production losses and affect the marketability of harvested fish leading to significant economic losses.

The webinar, “Beauty and the Beast: Important parasites of fish” brought insights on how important parasites are in aquaculture, in particular to marine fish with three out of the four presentations focussing on sea lice, skin flukes and leeches. This webinar organised in December 2020 by the Fish Health Section (FHS) of the Asian Fisheries Society (AFS) was attended by 339 participants from at least 20 countries.

“The aims were to raise awareness on the importance of parasites on both farmed and wild fish populations, to understand their mechanisms of infestation (including their life cycle), as well as disease prevention and control,” said Dr Eduardo Leano, Network of Aquaculture Centres in Asia. **Dr Erlinda R. Cruz-Lacierda** of the University of the Philippines Visayas (UPV) also encouraged the younger generation to continue research into fish parasitology, to add to the current information and to help industry better manage them.

Sea lice in the Philippines

In the Philippines, 21 species of sea lice have been identified. Two species, *Caligus epidemicus* and *Lepeophtheirus spinifer* are the most abundant and damaging to wild and farmed marine fish. An updated checklist from 1977–2019 showed infestations of *C. epidemicus* in milkfish *Chanos chanos*, seabass *Lates calcarifer*, mangrove snapper *Lutjanus argentimaculatus*, cobia *Rachycentron canadum*, green grouper *Epinephelus coioides* and siganids *Siganus* spp., among others. Cruz-Lacierda said, “Occurring on the body surface of the fish, the prevalence of sea lice is high, reaching up to 100%. One study indicated that *C. epidemicus* showed a 100% prevalence in siganids *Siganus guttatus* and Asian seabass farmed in tanks and ponds. *Lepeophtheirus spinifer* was 100% prevalent in the pompano *Trachinotus blotchii* farmed in cages. In siganid broodstock, symptoms are extensive erosion and haemorrhagic lesions, particularly on the anterior ventral part of the fish with 10% mortality.

Pompano reared in net cages showed that *L. spinifer* and attached themselves to the body surface, fins and eyes. In severe infestations, there was exophthalmia and haemorrhagic areas on the operculum. Co-infection with the monogenean *Neobenedenia* spp. was also recorded on the eye. Pakingking et al. (2018) isolated *Vibrio harveyi* in *L. spinifer* infected pompano broodstock with exophthalmia. In 2018, Beluso et al. (2019) reported that *L. spinifer* infestation reached 220 individuals/fish when the water temperature was lowest at 27°C and salinity was highest



Dr Erlinda R. Cruz-Lacierda



Pompano *Trachinotus blotchii* broodstock infected with *Lepeophtheirus spinifer* with exophthalmia (top left) and haemorrhagic areas on the operculum (top right). Pictures provided by Dr Erlinda R Cruz-Lacierda.

at 35ppt. Previous studies showed that the development and survival of sea lice were optimal at high salinity. Cruz-Lacierda also discussed the prevalence of the different development stages of the sea lice.

The whole life cycle of *Caligus epidemicus* covers 11 stages over 17 days, at 20ppt and 24–25°C (Lin and Ho, 1993). In the case of *L. spinifer*, the life cycle is completed within 6–7 days at 29°C and 30ppt (G. Erazo-Pagador, SEAFDEC AQD, pers. comm.). Skin lesions on the fish reduce market value of pompano. “There have been various treatment methods but the parasite remains a problem. Trichlorphon (Neguvon) is used to treat *C. patulus* on milkfish broodstock but this treatment has potential risks to both handler and environment,” said Cruz-Lacierda. Freshwater baths are ineffective as reinfection occurs after the fish return to the net cages. At SEAFDEC, trials using emamectin benzoate as treatment of *L. spinifer* on the pompano are ongoing.

Life cycle to control marine leech in Malaysia

In Malaysia, **Dr Kua Beng Chu**, National Fish Health Research Centre, Fisheries Research Institute is closely monitoring and working on control measures for the marine leech *Zeylanicobdella arugamensis*. Kua explained, "We have recorded losses at MYR0.5 million in 2006 to 2007, and in 2016-2017, the losses were estimated at MYR1.5 million. These losses are not only because of fish mortality but also the loss in value of the fish due to the unattractive appearance. In Malaysia, this ectoparasite is prevalent in hybrid groupers, which are usually sold as live fish where appearance is crucial. The value of an infected fish can be a tenth that of an uninfected grouper." The leech uses its proboscis to puncture the fish skin and sucks blood. It then leaves small pores which allow secondary bacterial infections that often lead to fish mortality.

Kua said that a better knowledge of the life cycle is important to develop control measures. As for the marine leech *Z. arugamensis*, it demonstrated a short period between 16 to 22 days to complete their life cycle on different hosts at a different temperature, salinity and locations. Under laboratory conditions (27°C and 28ppt), it takes 16 to 17 days to complete their life cycle. Fortunately, the marine leech is macroscopic and the three stages in the life cycle can be seen with the naked eye.

"It takes 7 days for the new egg inside the cocoon to develop into juvenile under 27°C at 28ppt. The juvenile then looks for a new host. It takes another 9 to 10 days for the juveniles to grow to mature adults. Adult *Z. arugamensis*



Dr Kua Beng Chu



Grouper infected with leech in the eye. Picture provided by Dr Kua Beng Chu

can survive for 12 days without food while the juveniles can do so for 19-21 days. The optimal salinity range for survival is 10-40ppt but the leech may even survive at 0ppt for a few hours. The optimum temperature range for survival is 25-35°C.

Z. arugamensis is not host specific and can infect any marine species. Mean intensity increases with the culture period and can reach up to 250 leeches per fish after 24 months of culture from less than 50 leeches per fish at 7 months of culture in groupers.

The most effective way to remove the leech is via fresh-water or formalin baths, but these baths do not kill all the leeches. Farmers also remove the leech manually with a toothbrush and towel. Kua explained the efficacy in the physical removal of the leeches using several versions of a substrate based on the principle that the leech will be



11TH SYMPOSIUM ON DISEASES IN ASIAN AQUACULTURE

23rd – 26th August 2021

Land of Adventure: Exploring Aquatic Animal Health for Sustainable Aquaculture

Plenary Speakers		Keynote Speakers		Date	Item
 Plenary I : State of Aquaculture Dr Rohana Subasinghe (Sri Lanka)	 Session I : Biosecurity in Aquaculture Dr Edgar Brun (Norway)	 Session III : Detection method/Diagnostic (Parasitic, Bacterial & Viral diseases) Prof Karin Pittman (Norway)	 Session V : Trends in Fish Health Management Dr. Kua Beng Chu (Malaysia)	MARCH 31 ST 2021	Abstract Submission Dateline
 Plenary II : Drivers and pathways of disease emergence in aquaculture Dr Melba B. Reantaso (Philippines/Italy)	 Session II : Epidemiology (Parasitic, Bacterial & Viral diseases) Prof. Dr. Kenton L. Morgan (United Kingdom)	 Session IV : Prevention & Control Measures Dr Huang Jie (China/Thailand)	 Session VI : Trends in Shrimp Health Management Prof Chu-Fang Lo (Taiwan)	JUNE 1 ST 2021	Notification of Abstract Acceptance
				JUNE 30 ST 2021	Early Registration Deadline
				JULY 1 ST 2021	Normal Registration


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attracted to lay cocoons on the substrate. In the hybrid grouper floating cages, 2 to 3 times application of BP2 substrates significantly reduced the prevalence of adult leeches in grouper cages where substrates were installed (40%) as compared with grouper cages without substrate (100%). With the red snapper, the installation of BP2 up to three times in the cages managed to eliminate the leeches. After the BP2 substrate was removed from the cages over a certain fixed period, the prevalence of marine leeches in cages was still lower than those cages where the substrate was not installed. The uniqueness of the BP2 substrate is due to the material which attracts the leech; the size of the device and its placement in the net cages are also important considerations. The BP2 innovation has undergone field trials and is now undergoing pre-commercialisation and will be available for the target group by 2021. An ongoing research is using a functional diet containing commercially available essential oils to reduce marine leech infestations.

Can we innovate to successfully manage skin flukes, based on current knowledge?

The current management of skin flukes in tropical aquaculture is certainly not successful, said **Dr Susan Gibson-Kueh**, Senior Research Fellow Aquaculture, James Cook University in Singapore, as she brainstormed possible innovative ideas to manage these parasites in sea cages. *Neobenedenia* is a skin fluke causing severe production losses, in Asian seabass *Lates calcarifer* (or barramundi) and snappers *Lutjanus* spp. Some of these skin flukes are non-host species specific, and therefore hard to control (Trujillo, 2015). Parasitised fish often have linear abrasions on the skin.

Control of this parasite is difficult as it produces a large number of eggs, attaching steadfastly to net cages. Adult stages of these parasites can be as large as rice grains and are visible as they drop off during a freshwater bath treatment. The miracidium hatched from eggs presumably seeks the host with the aid of 4 eyespots. "Knowledge of the parasite life cycle may help shed some light on innovative ways to manage these parasites. For example, knowing how much the miracidium depends on light to find new hosts may be useful, as shown by research on sealice (Hamoutene et al., 2016). Generally, juveniles of these parasites have limited energy resources to move and stay alive and will perish if a host is not found!", said Gibson-Kueh.

These parasites have a very short generation time. Sexual maturity occurs in 10 days at 24°C and 35ppt after attaching to a host. These parasites lay eggs continuously but egg production increases in the dark. A study showed that 81% of oncomiracidium emerges from the eggs in the first three hours of light (Dinh Hoai & Hutson, 2014).

With regards to control measures, "Immersing fish in a freshwater bath causes the parasite to drop off. But reinfection occurs soon after the freshwater bath. In order to break the life cycle and re-infection, the period between treatment must be shorter than the time it takes the parasite to achieve sexual maturity. Farmers treat fish every few weeks and juveniles, especially snappers, are the most susceptible. Doing this every two weeks is not only laborious but moving fish around is stressful. Furthermore, the eggs are very resistant. So, we need to think of a way to kill or prevent infective stages from infecting fish," added

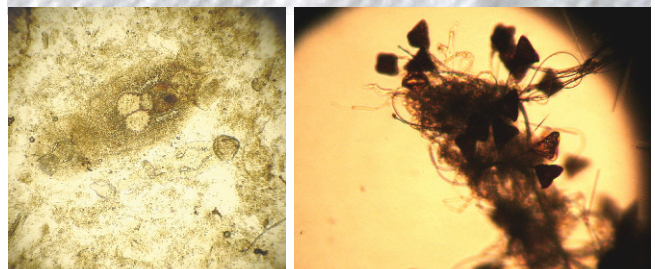
Gibson-Kueh. Synchronised treatment at an aquaculture site will reduce spread between farms.

Gibson-Kueh also said that various studies using oral anti-parasitic drugs have met with variable success due to the re-infection after treatment or low palatability of medicated feed (King et al., 2000; Morales-Serna et al., 2018). Praziquantel (one of two anti-parasitic treatments available in the market) has a bitter flavour (Partridge et al., 2014). Citing examples of vaccines against the cattle tick and recombinant vaccine against Chilean sea louse, she said that genomic sequencing of this parasite may lead to an effective vaccine antigen (Raynard et al., 2002). Plant based remedies such as water-ethanol extracts of ginger, basil and bitter chaparro have been found to be toxic against some life stages of *Neobenedenia* sp (Trasvina-Moreno et al. 2019). An extract of seaweed was found to reduce hatching success to 3% as compared to 99% in a seawater control.

References are available on request.



Dr Susan Gibson-Kueh



Top, snapper infected with *Neobenedenia* sp.; left, *Neobenedenia* sp. on skin and on the right, eggs. Photo credit: Ethan Goh and Jeremy Yeo.

Integrated cage-cum-pond culture of Indian pompano

A high-density multispecies fish culture approach for enhancing marine fish production in India.

By Sekar Megarajan, Ritesh Ranjan, Biji Xavier, Ponnaganti Shiva, Shubhadeep Ghosh and Bobby Ignatius



Pompano are harvested when fish reach 700g after a grow-out duration of 210 days. In this integrated cage -cum-pond system, it is possible to partially harvest fish from the cages. The market price of pompano was INR330/kg in February 2021

Aquaculture continues to play an important role in meeting an ever-increasing demand for fish globally. This is more so in India where the population is projected to rise to 1.65 billion by 2050. It has been estimated that India must produce nearly 10.5 million tonnes of fish annually to meet the requirement of this growing population. Most of this is expected to be from freshwater fish aquaculture. However, a significant contribution from mariculture/coastal aquaculture is essential, to complement the annual supply from capture fisheries which has stagnated at 3.5-4.0 million tonnes.

In recent years, India has become a leader in coastal aquaculture production, but this is solely dominated by the marine shrimp sector. On the other hand, India is still at the initial stage in marine finfish aquaculture production. Today, the contribution of marine aquaculture to national fish production is insignificant. There is scope for mariculture development in India through the efficient use of technologies, resources and species available in the country. To meet the demand for domestic consumption, a sustainable development of the sector is essential.

Farming the pompano in India

Species diversification is one of the major approaches adopted to increase fish supply from aquaculture. A suitable marine finfish candidate is the pompano *Trachinotus* spp, in the family Carangidae. The pompano, with the following

characteristics is the preferred species for mariculture: ease of breeding in controlled condition; quick adaptability to different culture conditions; tolerant to a wide range of salinity; fast growth rate; pleasant appearance; good meat quality and high consumer preference. Unlike some of the other cultured marine fishes, the pompano is quick to readily accept artificial pelleted feed and can complete their entire culture cycle solely with artificial feeds.

At the Central Marine Fisheries Research Institute (CMFRI), under Indian Council of Agriculture Research (ICAR), researchers have succeeded in breeding two species of pompano and produced a consistent seed supply. The breakthrough for the Snubnose pompano (*Trachinotus blochii*) was in 2010, while that for the Indian pompano (*Trachinotus mookalee*) was in 2016.

Culture technology for these species was subsequently standardised in different culture systems such as coastal ponds and sea cages in different locations. Results have been very encouraging. Thereafter, these culture methods were demonstrated in different parts of the country under different research programmes by ICAR – CMFRI, and with financial support from the National Fisheries Development Board (NFDB), Government of India. The demonstration and dissemination of technology have created awareness among different aquaculture farmers and have convinced them to venture into pompano farming.

Integrated culture systems

Several culture methods have been used for marine finfish production, including pond culture, recirculating aquaculture system (RAS), in-pond raceway recirculating (IPRS) culture systems and cage culture (Wang et al., 2020). Pond-based culture has a low stocking density of 1 fish/m², whereas other methods use a high stocking density of 15-30 fish/m³ of water body. The challenges with the high stocking density culture systems include high cost of production and dependency on skilled manpower.

Therefore, we have investigated alternative aquaculture production systems for finfish, to target efficient land utilisation and high productivity. One such alternative is the integrated cage-cum-pond culture system where fish are cultured in cages that are integrated with semi-intensive culture of other or same fish species in open ponds (Sipauba-Tavers et al., 2016). Fish in cages are fed a complete feed, while the same species or other low value fish stocked in the open pond are fed at low feed frequency or depend only on the natural food in the pond. The pond reared fish derive nutrients from uneaten foods from the cages or from autotrophic and heterotrophic food chains.

In this farming method, there is a high stocking density for fish in cages located in ponds and a low stocking density for the fish in the open area of the ponds. The total production efficiency increases 3-4 times, compared to the fish cultured in open pond alone. This culture system has been mainly practised for freshwater fish at a high stocking density of up to 100 fish/m³. A maximum production of 24kg/m³ was achieved by utilising 20% of the pond area for cage culture (Quagraine et al., 2011). However, this culture method has not been adopted well for marine finfish culture, as most of the marine fish species are being cultured in cages in open waters.

Integrated cage-cum-pond system

To evaluate the feasibilities of this culture method for marine finfish, a study was conducted for Indian pompano *T. mookalee* under an integrated culture system in coastal ponds by ICAR-CMFRI, Visakhapatnam Regional Centre in different locations in the Andhra Pradesh state. The result of the study was promising. The Indian pompano juveniles produced at the mariculture hatchery, Visakhapatnam Regional Centre of ICAR-CMFRI was stocked at a high density of 15 fish/m³ in a net cage of 8 X 8 X 1.5m. The stocking density was reduced to 10 fish/ m³ after reaching 250g size. A total of four cages were created in the pond and these cage structures occupied less than 15% of the pond area. At the same time, the same fish species were also stocked in the open pond at a low stocking density of 0.25 fish/ m³ (Sekar et al., 2020). The Indian pompano stocked in the integrated cages and open pond were fed with pelleted feed with 40% of crude protein and 10% of crude fat (Growel Feeds Pvt Ltd, India).

After one year of grow-out culture, the fish in the net cages had reached the size of 880g, whereas the same fish stocked at low stocking density in the open pond had reached the size of 695g during the same period. The result showed that the fish cultured in cages grew 31% faster than the fish stocked in the open pond at low stocking density. Pompano is harvested when fish reach 710g after a grow-out duration of 210 days. In this integrated cage-cum-pond system, it is possible to partially harvest fish from the cages. The market price of pompano was INR330/kg (USD 4.7/kg) in February 2021.



Culture of the Indian pompano (*Trachinotus mookalee*) in integrated cage cum pond system. Cages are placed in 1ha ponds and fish in the cages are fed with 40% crude protein diets.

Thus, this method provides farmers with an opportunity to use their limited pond resources to increase fish yield and generate more income. The information gained from these preliminary studies suggested that the pompano is a potential candidate for culture under integrated cage-cum-pond culture methods.

Prospects and opportunities for integrated marine finfish culture in India

Aquaculture is expanding in India in different ways. There is a variety of production systems but ponds are currently the most common. This integrated cage-cum-pond system allows for the use of an existing pond without further modifications. If water quality is managed well, this method would yield 2-3 times more production than if the same pond is used for fish culture without integration.

In India, marine shrimp culture is one of the fastest-growing farming systems, using a culture area of 93,496ha during 2017-2018. However, at the same time primarily, because of disease related setbacks, shrimp farmers had suffered frequent losses (Kalaimani et al., 2013). Some farmers are opting for alternative farming methods, which produce profits similar to shrimp farming. In this context, we think that introducing high-density culture of pompano and integrating it with other species will play an important role. It can be an alternative for existing shrimp farmers where intercropping with finfish can be the best option to break the chain of disease-causing pathogens.

It has been estimated that about 1.2 million ha of potential brackish water are available for fish culture in India. Only 10% of this area has been utilised. Thus, a large area is still available for pond-based marine fish culture. Moreover, the culture technologies for milkfish (*Chanos chanos*) and grey mullet (*Mugil cephalus*) are well established in India and widely adopted by farmers. Being euryhaline in nature, these species could also be cultured in saline ponds fed with low protein feeds. A high-density culture of pompano in integrated cages fed with high protein feed combined with milkfish or mullet in the open pond fed with low protein feed could effectively utilise the pond space as well as avoid feed wastage.

Conclusion

We envisage that an estimated production of 5 million tonnes of marine fish could be achieved via aquaculture by 2050 to meet India's fish demand. At the same time, the government has been focusing on doubling farmer's income



An integrated cage-cum-pond system experiment conducted by ICAR-CMFRI, Visakhapatnam Regional Centre in coastal ponds at Bhavedevarapalli, Krishna District, Andhra Pradesh, India.

through various means. Thus, introducing new culture methods in addition to the existing cage and pond-based culture systems can help to achieve the government's goal. This integrated cage-cum-pond culture method would be one of the approaches for increasing fish production and doubling farmer's income by effective utilisation of culture area.

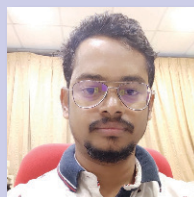
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Dr Sekar Megarajan, Dr Ritesh Ranjan, Dr Biji Xavier, Dr Ponnaganti Shiva and Dr Shubhadeep Ghosh are with ICAR-Central Marine Fisheries Research Institute, Visakhapatnam Regional Centre, Andhra Pradesh, India. Email: sekarrajaqua@gmail.com



Boby Ignatius is with ICAR-Central Marine Fisheries Research Institute, Kochin, Kerala, India.

NEXT ISSUES

May/June 2021

Issue focus: Demand and Supply Equilibrium

Industry review: Aquafeed Production

Feed/Production Technology: Extrusion & Pelleting/Hatchery Technology

Deadlines: Articles – March 12/Adverts – March 19

July/August 2021

Issue focus: Sustainable & Responsible Aquaculture

Industry review: Tilapia

Feed/Production Technology: Sustainable Feeds/ Big Data

Deadlines: Articles – May 14/Adverts – May 21

Email: zuridah@aquasiapac.com; enquiries@aquasiapac.com for details

A strategic partnership to develop the aquafeed business in Vietnam



Viet Uc AquaFeed Company Limited in Ben Tre Province.

In early March, **BioMar** took another important step to expand its global footprint within the shrimp feed business. It has acquired a majority ownership of the Viet-Uc feed business and is ready to pursue a position within high-end shrimp feed in Vietnam. This creates a synergy with its existing shrimp businesses in Latin America.

After a comprehensive due diligence, BioMar and Viet-Uc have signed an agreement for BioMar to acquire the majority of the Viet-Uc feed business. Through this acquisition BioMar establishes a partnership together with one of the leading seafood groups in Vietnam active in shrimp hatcheries, fish hatcheries and shrimp farming. The ambition is to grow the market for high-end feed products focusing on sustainability, traceability, quality, and performance. The acquisition is subject to customary approvals by the authorities.

“With this partnership we open an important door into the Vietnamese market, one of the world’s leading shrimp producing countries with a production close to 500,000 tonnes of shrimp. Considering the performance and agility of the Vietnamese shrimp industry during the last decade and not at least during this last year of pandemic, I am sure the market holds a great potential for growth.” explains Carlos Diaz, CEO, BioMar Group.

Looking at market forecasts and the increasing consumer focus on healthy and sustainable seafood, BioMar expects there will be an increasing market share for high-end feed, ensuring customers can capture value by delivering certified and tailored products towards global retail channels. Building upon its business model of promoting local market agility in combination with global excellence, the company will shortly be able to turn research and knowledge within shrimp feed into new product and service offerings to the Vietnamese market.

“BioMar is already a significant shrimp feed producer in South and Central America and it is our ambition to continue the journey to become a major player within



At a Viet Uc's super-intensive greenhouse shrimp farming facility. From left, Tuan Quoc Tran, Viet Uc; Carlos Diaz and Michael Gammelgaard, Director, Global Business Development, BioMar.

high-end shrimp feed. Within the past 5 years, we have established a significant production of shrimp feed in Ecuador and Costa Rica in partnership with large-scale local farmers. Now, adding Vietnam into the equation will enable us to build a strong relationship both to the market in Vietnam but also to the nearby countries, and at the same time creating benefits from the relation between genetic and nutritional development. Partnering with one of the leading companies within this field in order to develop high performance feeds, concepts and value chain collaborations makes a lot of sense in order to promote a positive development of the shrimp industry “, concludes Carlos Diaz.

Since 2012, BioMar has been engaged in the development of feed and technical services for shrimp utilizing the capabilities of global R&D in combination with trial facilities around the world, most recently through the advanced internal feed trial unit in Ecuador dedicated to shrimp and local trials in Vietnam. Moving into Asia will strengthen the position of BioMar in the global shrimp industry and make it possible to share know how and best practice across the continents.

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Asia's shrimp production continues to be plagued by diseases, bringing down survival rates and increasing costs of production. Conversely, market prices have dropped due to higher supply from new production areas in Asia and from Latin America, placing considerable pressure on margins.

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Breaking ground for a new €24 million aquafeed mill in Vietnam

Skretting has announced its plan to construct a new factory in 2021 as part of its commitment to aquaculture in Vietnam and the broader southeast Asia region. The new factory will supply the growing market with an annual capacity of 100,000 tonnes.



Reflecting an investment of €24 million, the state-of-the-art facility will enable effective feed supply to key farming provinces in the Mekong Delta and beyond. With existing facilities at full capacity, the new factory will alleviate the current demand on supply. The new factory is located next to Skretting Vietnam's existing facilities in Thuan Dao Industrial Zone, Long An.

"With a mission and commitment of bringing good solutions to farmers and contributing to the sustainable development of aquaculture in Vietnam, Skretting Vietnam is dedicated to leveraging our global resources to offer a complete value proposition for our customers," said Bui Thuy Tien, General Manager of Skretting Vietnam.

Vietnam became part of the Skretting family in 2010

through the acquisition of Tomboy Aquafeed JSC, a reputable Vietnamese fish and shrimp feed company. In the ten years since, Skretting Vietnam has fully embedded the Skretting culture into all of its operations – from research and raw material procurement to products and services for aquaculture. The latest shrimp feed factory opened in 2017.

"This new factory in Vietnam is a logical and necessary step towards strengthening Skretting and Nutreco's position as a global competitor in Asia," said Therese Log Bergjord, Skretting CEO. "We have a very solid growth strategy, matched with the huge potential of the sector. We have activated an expert team to make sure we deliver, and I'm excited to see how we continue to grow in the coming years." www.skretting.com

Newest mycotoxin risk management solution in Asia Pacific

Leading animal nutrition and feed additive firm **BIOMIN** has launched its newest mycotoxin risk management solution in selected markets across the Asia Pacific region. Mycofix Plus 5.Z with ZENzyme is an innovative, all-in-one feed additive providing next-generation mycotoxin risk management for breeding animals and their offspring. ZENzyme is the first and only purified enzyme that degrades zearalenone (ZEN) fast, specifically and irreversibly into non-toxic and non-estrogenic metabolites.



Ursula Hofstetter is Head of Global Product Management Mycotoxins at Biomin

"With the introduction of ZENzyme into the Mycofix range, we raise the bar once again in terms of state-of-the-art protection against mycotoxins for our customers," noted Ursula Hofstetter, Head of Global Product Management Mycotoxins at Biomin.

"ZENzyme offers the animal protein sector full and complete protection against zearalenone. With consistent and proper application, farmers will have the confidence that their animals will not face ZEN-induced reproduction or health challenges," added Franz Waxenecker, Managing Director.

Zearalenone, a naturally-occurring toxin produced by fungi, commonly contaminates crops including corn (maize), wheat and soy at potentially harmful levels according to the Biomin Mycotoxin Survey. Binders are not sufficient against zearalenone and other techniques are needed. Therefore, Biomin has pioneered the field

of mycotoxin mitigation using enzymatic biotransformation, beginning with the introduction of FUMzyme – the world's first-ever commercially available mycotoxin-deactivating enzyme that degrades fumonisins in 2013.

Enzymatic biotransformation involves enzymes designed to specifically target a mycotoxin and detoxify the fungal metabolite irreversibly, thus protecting animals from potential harm. The Mycofix range of feed additives is the only mycotoxin solution on the market that includes purified mycotoxin-degrading enzymes, namely: FUMzyme and ZENzyme, as well as a mycotoxin-degrading microorganism, BBSH® 797, for

the degradation of trichothecene mycotoxins.

"Over the years we have invested considerably in technologies to counteract mycotoxins, and many of these novel technologies have achieved EU authorizations for their scientifically proven safety and efficacy," explained Hofstetter. "While we have had some success in counteracting zearalenone in the past, ZENzyme is quite simply faster and better than any other technology available."

In aquaculture, zearalenone is known to have several negative consequences for fish and shrimp, including reduction in broodstock productivity, reduction in offspring survival, increased rate of mutations and malformations, effects on gonads and roe. www.biomin.net

Startup networking in Indonesia



The strategy in Indonesia is to ensure that the economy moves from one which is based on its resources to one based on innovations and digitalisation to drive fisheries and aquaculture production. For breakthroughs in innovations, there should be a good research ecosystem with synergy and participation among academics, government and industry. The first startup in Indonesia's aquaculture, eFishery began in 2013. Today, there are more than 30 startups in the fisheries and aquaculture sectors and more than 700 people involved with startups. Leading startups include Aruna Marketplace (a digital fish auction platform, as well as marketplace for seafood products), eFishery and JALA.

In 2018, Minapoli, which is itself a startup created in 2018 and led by CEO Rully Setya Purnama began organising Digifish. Following this, the Digifish Network of startups was created. Since then, there is this annual gathering of startups in Indonesia. The vision of the Digifish Network is to propel fisheries and aquaculture in Indonesia with innovations and digitalisation. Discussions at this year's Digifish included the impact of these startups on Indonesia's aquaculture and fishery businesses during this very challenging pandemic year, and on its resilience and sustainability.

Digifish 2020 was held virtually on December 15-16, with the theme "Accelerating the impact of innovation ecosystem on the fisheries sector". It was attended by more than 450 participants; among them, were fish and shrimp farmers, startups/innovators, government institutions, fisheries and aquaculture businesses, universities, NGOs and fisheries associations. There was a startup carnival with presentations from eight startups describing their innovations and achievements to date. A new session - a farmers' showcase, presented innovations that farmers have adopted. Rully introduced two German companies with innovations targeted at shrimp aquaculture in Indonesia. Below are excerpts from some presentations and brief profiles of some startups making waves in 2020.



Rully Setya Purnama, CEO Minapoli with presenters, Agus Ciputra (BASF Indonesia) and Aries Fajar Dwiputera (Bosch Aquaculture).

Targets for a digital economy

The Ministry of Marine Affairs and Fisheries (MMAF) has ambitious targets for 2024, and among them is to increase shrimp production by 250%. There is also a target to be one of the leading seafood producers globally. MMAF wants to increase per capita fish consumption in Indonesia from 58.3kg in 2020 to 60.9kg by 2024. In 2020, Indonesia's seafood exports contributed USD6.1 billion and MMAF wants to increase this to USD8.2 billion in 2024, with a focus on the shrimp and tuna sectors. In 2020, (January to September), shrimp exports led at 19.36% in terms of volumes and 39.78% in terms of value at USD1.49 billion.

Demands of export markets

Budhi Wibowo, Head of the Seafood Products Processing and Marketing Association (AP51) discussed collaboration, and upstream and downstream digitalisation to support seafood marketing. Budhi said that food safety, traceability and sustainability are crucial demands on Indonesia's exports. "We need to have certification. Certifications such as BRC, BAP, GlobalGAP, ASC are required at B2B; this is the responsibility of the producer." But he added that this is not all, as there is certification required at the country level before certification at the consumer level. Upstream there is food safety which means that shrimp ponds, suppliers and fishing vessels need to be certified. "As these are requirements at the country level, the government must fulfill them. If we do not, we cannot export to some countries or if we do so, the risks are high".

Traceability requirements by buyers include catch certificates by the EU (European Union); traceability data are required by Chinese buyers and USA buyers ask for Seafood Import Monitoring Program (SIMP) since 2019. This enhanced traceability is a major requirement which Indonesian producers must follow. Traceability requires data and this is where support with digitalisation and firm cooperation among stakeholders are essential.

Sectorial effect of the pandemic

Lelly Hasni from the Ministry of National Development said that the adverse Covid-19 effects include poor demand, market disruptions and poor prices. There was an urgent need to hasten digitalisation to help industry

In April 2020, with the Ministry of Marine Affairs and Fisheries, the Digifish Network initiated a movement "makanikanitusehat" (eating fish is healthy) to provide access to quality seafood by "buying from home".

players market products. Innovation is also essential to pull investments into the sector. In terms of numbers, Budhi said that in 2020, the food service sector (hotels, restaurants, cafes, catering) fell by 80% but today, this has improved to the 60% level. The retail segment including supermarkets and online sales grew by 30%. The demand for ready-to-eat and value-added products rose very quickly. AP5I promoted the switch from food service to retail, especially for ready-to-cook and value-added products.

The discussion was also on upgrading marketing in local markets. According to Budhi, other than increasing the number of cooked and ready-to-eat products, reduction in the number of steps in the supply chain and upgrading in ecommerce are important targets. A major bottleneck is cold chain logistics, especially in cities with small market demand. He wants to see the distribution network shortened: from processing plants to distributors, frozen marts (offline/online) and finally the consumer. Currently distribution starts from processing plants to distributors, wholesalers, small retailers and consumers.

However, the frozen mart will need to have freezer facilities, with promotions online and collaboration with delivery partners such as Gojek and Grab. "We want to have frozen marts in as many areas as possible, with participation of small cooperatives. We need multi-stakeholder collaborations to increase seafood sales in domestic markets via digital platforms (Lazada etc.) for door-to-door distribution from producers to frozen marts, resellers and logistics," said Budhi. "We need to effectively link buyers and producers. If we have better collaboration with producers, marketing is made easier."

The Digifish Network initiated a movement "makanikanitusehat" (eating fish is healthy) to provide access to quality seafood by "buying from home". It opened centres in 10 major cities. Sfari Burhanuddin, Deputy Minister at the Coordinating Ministry for Maritime Affairs and Investment emphasised on the mission to make local consumers proud of locally caught and farmed seafood with calls to consume local seafood.

Renewable energy in shrimp farming

According to Agus Ciputra, President of BASF Indonesia, among the current challenges in the shrimp sector is the intensive 24/7 use of energy. Electricity from the power grid can be costly and limited in accessibility. The use of renewable energy may address this challenge which can also address the sustainability element for the industry in

Indonesia. Market access is limited, with 90% exported to the US. Access to the EU markets requires a focus on sustainability; ecological and social.

BASF New Business focuses on Aquaculture Solutions AQS – innovations to sustainably electrify Indonesia's shrimp sector. "Our Blue revolution 4.0 hypothesis is that technology exists, but it is not yet available broadly. When used, we see the aquaculture sector in Indonesia moving towards higher productivity, greater efficiency, better sustainability and with higher value products," said Agus. The components are clean energy, digital enablers and automation, disease control and asset financing by partners. Financing has been hard to come by mainly because of sustainability challenges. "The flagship is clean energy from photovoltaic power which costs USD0.05/KWh and is available 24/7," added Agus. This is in comparison to diesel fuel at USD0.35/KWh, which is dependent on oil subsidies, and the grid which cost USD0.07/KWh. However, Agus said that this is key to unlock sustainability and green financing. BASF will work with financing partners and qualified technology providers with automation and digital tools to drive better control of farming, higher biomass with precision feeding and disease control to increase survival rates, reduce costs and risks.

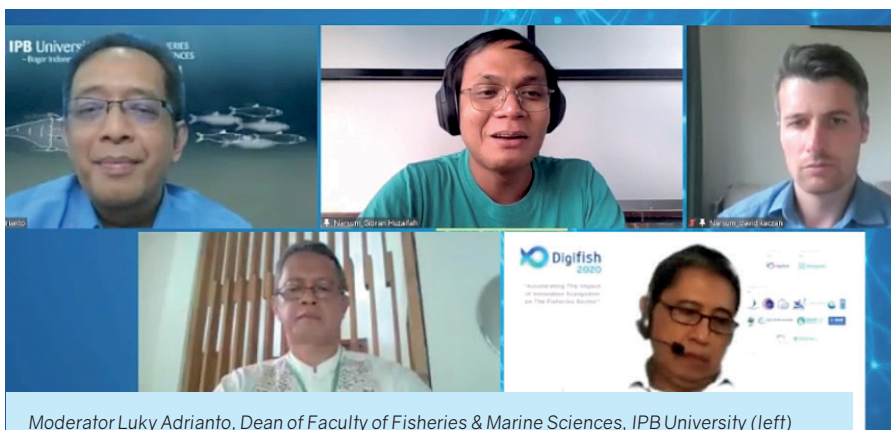
Innovators and startups

AquaEasy Founder Aries Fajar Dwiputera presented its features. This innovation from Bosch Aquaculture has sensors which captures physical and biochemical parameters and AI (artificial intelligence) turns data into decisions and stores them in the cloud for easy accessibility and presents solutions to the farmers. These innovations help to overcome bottlenecks in the industry today comprising shrimp health and disease, maintaining optimal water quality, and recording accurate data. AquaEasy provides daily analysis and recommendations on farm data.

eFishery is the pioneering startup in Indonesian aquaculture; it has its beginnings in Bandung in 2013 with a fish demand feeder which with AI, developed into smart feeding devices for fish and shrimp farming. It has been steadily moving ahead and in 2020, achieved Series B funding led by Go-Ventures and Northstar, participation of Aqua-Spark and Wavemaker Partners. CEO, Gibran Huzaifah gave some insights on the progress to date. In January 2020, Gibran introduced eFisheryFund. Next came the eFisheryFresh, an on-demand marketplace.

A significant contribution during the pandemic was the action taken to help freshwater fish farmers with problems selling their harvests of live fish. "In April 2020, we took 850 tonnes of the unsold freshwater fish and kept them in cold storage. Working with a partner organisation, we donated the fish to Covid-19 frontliners in Indonesia."

Established in 2015, **JALA** is pushing for data driven shrimp farming in Indonesia. Liris Maduningtyas, CEO listed the challenges in shrimp farming: at preproduction with financing and fintech, at production with



Moderator Luky Adrianto, Dean of Faculty of Fisheries & Marine Sciences, IPB University (left) with speakers, clockwise; eFishery CEO, Gibran Huzaifah (centre); David Kaczan, World Bank; Budhi Wibowo, AP5I and Dr Agus Somamihardja.

manually monitoring pond conditions, crop failures, diseases etc, and postproduction with the effects of higher demand than supply, market access and incomplete traceability records. Liris said that as of November 2020, JALA's technology is being used by 14,500 ponds from almost 900 ponds at the end of 2018. In terms of users, there are more than 7,800. It has more than 20 partners and more than 40 B2B clients, local and international in Malaysia, Thailand, Vietnam and China. To date, JALA has raised seed funding from 500 Startups, Hatch, and Conservation International Ventures.

The two-year old startup, **Banoo** has created an affordable technology to improve water quality. This is a system which floats on the water with a microbubble generator and linked to an IoT sensor which turns the generator on/off based on the dissolved oxygen level to improve energy efficiency. **Sgara** has recently launched its Sgara Book in 2020 to improve farmers productivity through data integration, information on water quality, shrimp growth, feeds, chemical use and probiotics combined with feed tray and shrimp disease monitoring, stock and harvests (see article in *Aqua Culture Asia Pacific* September/October 2020).



Azellia Alma Shafira, CEO of Banoo described the work so far with its microbubble generator and linked to an IoT sensor to improve water quality, particularly for small farmers. Banoo showed a proven 40% increase in productivity.

FisTx, a research-based platform provides technology and data driven holistic solutions. Yogi Ariadenta, Head of Business Development said that FisTx is looking at the democratisation of aquaculture: aquaculture for everybody and anywhere. It has the Oasse ponds, a round tank system, which can be easily dismantled and moved, and are suitable as nursery or culture ponds for high density stocking. There is the low cost, easy to use water quality test kits connected to its multifunction app for data input. Results are given in less than 5 minutes and accuracy is 85%. Its four products are now used by farmers in 25 locations, mainly on Java Island.

Minapoli, a startup for information and business in fisheries and aquaculture, strives for a stronger synergy among stakeholders and wishes to integrate all segments in Indonesia's fisheries and aquaculture to contribute to its development. "Minapoli is the first marketplace



Liris Maduningtyas, CEO introduced the latest from JALA, a shrimp trading platform.

for aquaculture; currently it has 36 product categories, essential for shrimp and fish farming. It works with suppliers to offer products at competitive prices. Over the past 10 months in 2020 from January to October, monthly visitors grew 714% at 27,000," said Rully. The coverage is over 47 cities in 18 provinces. It has more than 60 partners, many of them leading companies involved in aquaculture in Asia. "Beginning with Indonesia, we plan to be the leading aquaculture network in the Asia Pacific region."

According to Achmad Jerry, **Venambak** fosters small scale farmers to be partners in the setup of mini recirculating aquaculture systems (RAS) for intensive shrimp farming in urban locations. It will assist in operations: before production, during production and post harvest. The daily challenges are in three areas: water quality management, energy for aeration and other activities, and waste management. Venambak has a hybrid ammonia reducer that requires less energy, produces less wastes and is accessible to smallholder farmers. It has trials in a RAS in Sidoarjo, Java, stocking 300-800 post larvae (PL)/m³ in cooperation with Solidaridad Indonesia and with support from the Walton Foundation.



eFishery's smart feeders are being used in 23 provinces.

2021

April 14-16
VietShrimp Aquaculture
International Fair
Cantho City, Vietnam
<https://vietshrimp.net>

April 13-15 (online)
Aquaculture Europe
(AE2020 Cork)
<https://aquaeas.eu/>

August 18-19
TARS 2021: Shrimp
Aquaculture
Ho Chi Minh City,
Vietnam
www.tarsaquaculture.com

August 23-26
11th Symposium on
Diseases in Asian
Aquaculture (DAA11)
Kuching, Malaysia
www.daa11.org



August 25-27
Vietfish 2021
Ho Chi Minh City
www.vietfish.com.vn

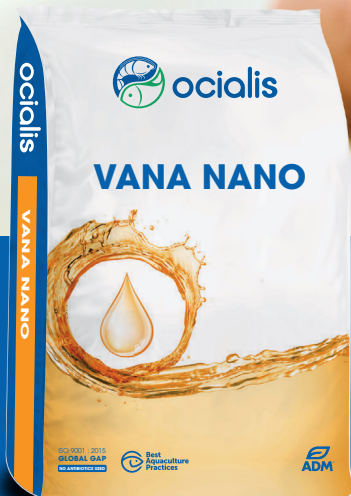
September 14
SPACE 2021
DIGITAL
www.space.fr

September 22-24
VIV Asia
Bangkok, Thailand
www.viv.net

October 4-7
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Micro-extruded pellet for optimal **water quality**
Plant extracts for healthy shrimp juveniles



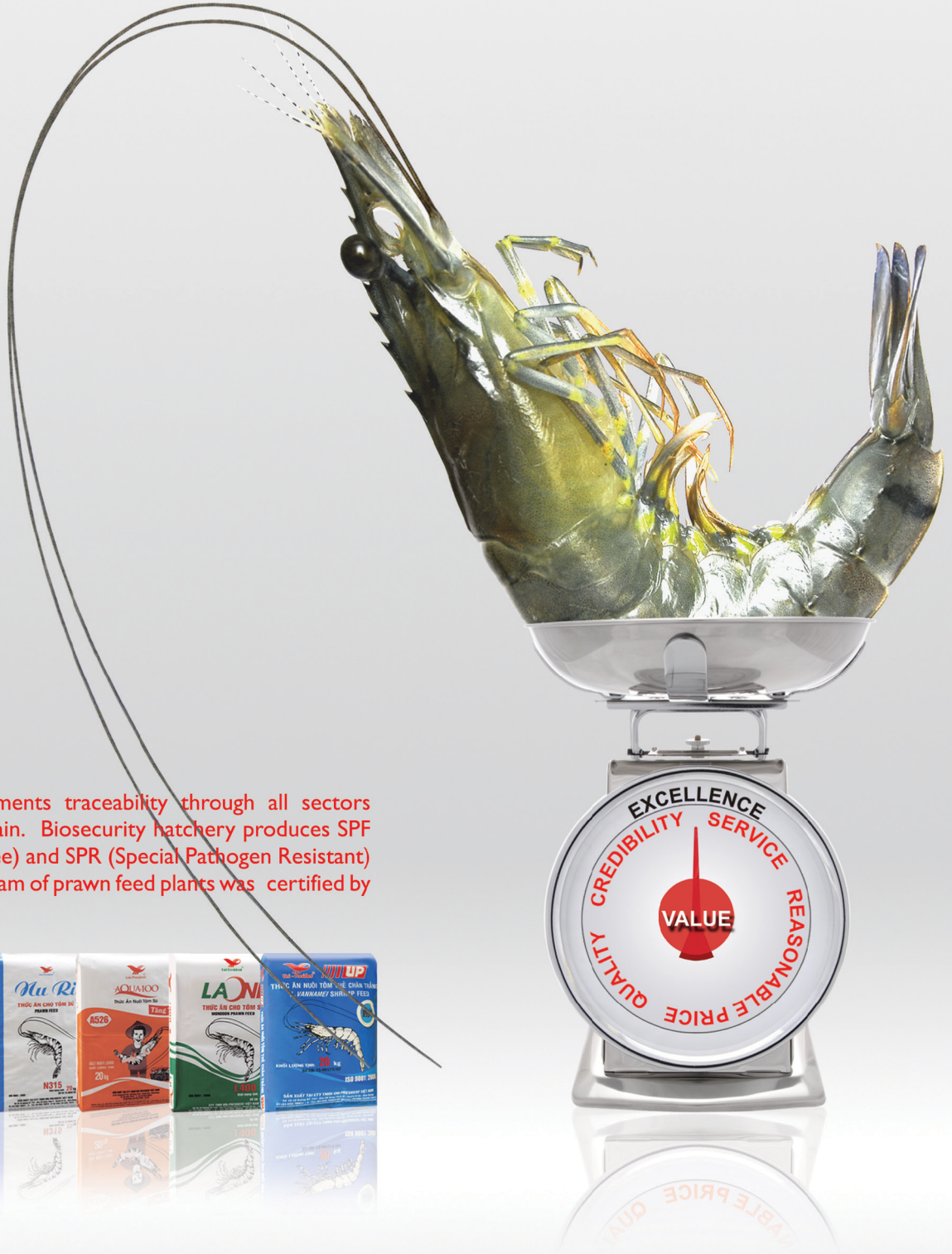
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Uni-President

CREATES THE VALUE OF PRAWN



Uni-President implements traceability through all sectors along with supply chain. Biosecurity hatchery produces SPF (Special Pathogen Free) and SPR (Special Pathogen Resistant) larvae. Quality program of prawn feed plants was certified by ISO 22000 & HACCP.



- No.16-18-20, DT743 Road, Song Than II Industrial Zone, Di An Ward, Di An City, Binh Duong Province, Vietnam
- Tel: +84-274-3790811 (Ext: 1711)
- Fax: +84-274-3790819
- Email: aquafeed@upvn.com.vn



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Establishing a Healthy and Happy Tomorrow