## Efficacy of a phytobiotic-based additive to reduce the severity of EHP-WFS outbreaks in field conditions

A series of field trials in Indonesia, Malaysia, India and China, over a six-year period assessed the effectiveness of the additive in reducing the severity of WFS and EHP infections.

By I-Tung Chen, Martha Mamora, Maria Mercè Isern-Subich and Waldo G. Nuez-Ortín

White faeces syndrome (WFS) is a multifactorial syndrome affecting farmed shrimp. It results from the interaction of various factors, including poor-quality nutritional sources, environmental stressors and multiple infectious agents. WFS is characterised by the white discolouration in the shrimp gastrointestinal tract and the appearance of floating white fecal strings on the pond water. Other clinical signs include loss of appetite, slow growth, high size variation, and softshell. WFS has become a significant challenge to aquaculture, especially in major shrimp-producing regions across Asia.

Recent studies indicate a unique combination of pathogenic Vibrio spp. and Enterocytozoon hepatopenaei (EHP), an intracellular microsporidian parasite infecting shrimp hepatopancreas, is required to induce WFS in the whiteleg shrimp, Penaeus vannamei (Aranguren Caro et al., 2021). EHP plays a critical role as a primary pathogen that intensifies the impact of opportunistic bacteria like Vibrio spp. resulting in WFS.

Disease prevention strategies are mainly focused on implementing pond management and strict biosecurity measures to minimise the introduction of diseases. Also, an important preventive approach is functional nutrition aimed to reduce the load of EHP and Vibrio in the digestive tract of shrimp.

Sanacore<sup>®</sup>GM (Adisseo) is a functional additive based on a synergetic blend of phytobiotic extracts with broad spectrum inhibitory activity against bacteria and parasites. The mode of action of the additive also includes the modulation of the dysbiosis generated by infection and antiinflammatory action. Sanacore<sup>®</sup>GM can be administered via feed to reduce the severity of infection of EHP-WFS.

# Fields trials on preventive and corrective strategies

The present article describes various field tests demonstrating the efficacy of Sanacore<sup>®</sup>GM against EHP and WFS under different farming conditions e.g., climates,

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Ponds at the test farm in Lampung, Indonesia

pond designs, stocking densities and disease history. Over a period of six years (2016-2022), a series of field trials were conducted in Indonesia, Malaysia, India and China to assess the effectiveness of the additive in reducing the severity of WFS and EHP infections. Disease outbreaks were associated with the presence of other pathogens, including white spot disease (WSD) and acute hepatopancreatic necrosis disease (AHPND, Table 1).

Location		Disease History	
Indonesia	Subang-West Java	WFS, EHP, WSSV, IMNV	Failure for the last 2 crops
Indonesia	Bratasena-Lampung	WFS, EHP, Vibriosis	Outbreak at DOC 30-40, SR max 50% with FCR 1.7 for last 4 crops
Malaysia	Penang	WFS, AHPND, WSSV	Outbreak at DOC 40-60
India	Balasore-Odisha	WFS, WSSV	Outbreak at DOC 30-60; SR 30-40%
China	Guangdong	Vibriosis	Failure for the last 2 crops, SR 10-20%

**Table 1.** History of disease outbreaks (white faeces syndrome (WFS), Enterocytozoon hepatopenaei (EHP), white spot syndrome virus (WSSV), infectious myonecrosis virus (IMNV) in the selected test farms. DOC=days of culture; SR=survival rate; FCR=feed conversion ratios.

Three strategies, namely preventive, corrective, and preventive plus corrective, were evaluated (Table 2). The preventive strategy involved adding a preventive continuous dose of the additive in feed through the production cycle, while the corrective dosage was supplemented for a short period at the appearance of disease symptoms.

We summarised the effects of Sanacore<sup>®</sup>GM supplementation under farming conditions across various locations in Southeast Asia and India are in Figures 1-3. Under infection, shrimp fed diets not supplemented with the functional additive exhibited poor performance, including low survival rates, high feed conversion ratios (FCR), and frequent emergency harvests due to growth retardation.

On the contrary, the dietary supplementation Sanacore<sup>®</sup>GM under preventive and corrective strategies led to improved

survival, feed conversion ratios (FCRs), and recovery in growth rates. The survival rate of shrimp in the control ponds ranged from 35% to 55%. However, shrimp that received additive supplementation exhibited delayed disease outbreaks, despite showing gross signs of infection. Notably, the preventive plus corrective supplementation led to a significant recovery of daily growth from infection, with survival rates reaching up to 66% to 99%. Moreover, the additive supplement reduced the FCR by 25% to 33% and increased total crop yields by an average of three times compared to the ponds without functional additives supplementation. It was observed that the corrective dosage at the start of the outbreak helped to eliminate WFS symptoms and stabilise growth, while the preventive dosage supports growth recovery to pre-outbreak rates. These findings are explained by the capacity of the additive to disrupt the cycle of EHP and to inhibit vibrio proliferation, reducing hepatopancreatic damage and the impact of infection.

Several in vitro and in vivo studies have corroborated the capacity of the additive to inhibit EHP germination, a critical step to invade host cells, and reduce EHP copies after a period of supplementation. By reducing the viability of EHP spores in the shrimp gastrointestinal tract, it is possible to prevent the parasite from entering the hepatopancreas, where it can multiply rapidly and cause hepatopancreatic microsporidiosis (HPM). This condition leads to the destruction of tubule epithelial cells and the development of lesions. Likewise, Sanacore<sup>®</sup>GM was proven to interrupt the quorum sensing regulation of *V. parahaemolyticus* through a depressive effect on the signaling pathways determining the bacterial density and toxin production.



Figure 1. The effects on survival rate (%) with the dietary supplementation of Sanacore<sup>®</sup>GM to support farmed shrimp in dealing with EHP-WFS across regions. The shrimp that were not supplemented with functional additives had lower survival rates and experienced recurrent disease outbreaks, as well as growth retardation, which required more frequent emergency harvesting.



**Figure 2.** The effects on feed conversion ratio (FCR) with the dietary supplementation of Sanacore<sup>®</sup>GM to support farmed shrimp in dealing with EHP-WFS across regions. Shrimp that received additive supplementation exhibited a delayed onset of disease outbreaks, despite displaying gross signs of infection, which resulted in a rebound in growth and a decrease in FCR.

Location		Pond size	Stocking density	Application strategies
Indonesia	Cubara Mash Jawa	3000 m <sup>2</sup>	100 PL/m <sup>2</sup>	Corrective dose during disease outbreak
Indonesia	Subang-west Java			Preventive + Corrective during disease outbreak
		1000 m <sup>2</sup>	100 PL/m <sup>2</sup>	Control
Indonesia	Bratasena-Lampung			Corrective dose during disease outbreak
				Preventive + Corrective during disease outbreak
		4000 m <sup>2</sup>	110 PL/m <sup>2</sup>	Control
Malaysia	Penang			Preventive + Corrective during disease outbreak
India		7500 m <sup>2</sup>	65 PL/m <sup>2</sup>	Control
	Balasore-Odisha			Preventive + Corrective during disease outbreak
		3000-4000 m <sup>2</sup>	67 PL/m <sup>2</sup>	Control
China	Guangdong			Corrective dose during disease outbreak

Table 2. Application strategies of Sanacore® GM to support shrimp growth and survival across trials.



Figure 3. The effects on total biomass (tonnes/ha) with the dietary supplementation of Sanacore®GM to support farmed shrimp in dealing with EHP-WFS across regions. Applying the preventive plus corrective strategy eliminated disease symptoms while facilitating growth recovery to pre-outbreak levels. This, in turn, led to a significant increase in total crop yield during the production cycle.

### Conclusion

Shrimp diseases are caused by multifactorial interactions. Field trials contemplate all production variables and are essential in determining the efficacy of functional additives to promote shrimp health under real conditions.

Sanacore<sup>®</sup>GM has shown efficacy under different application strategies and production conditions. Clearly, it supports shrimp health and reduces the severity of EHP-WFS. The preventive use of the additive can be a strategy to recover growth rates to pre-outbreak levels and optimize farm profitability. Pond management measures must go hand in hand with feed supplementation of Sanacore<sup>®</sup>GM.

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