

Phytobiotic-based health additive reduces the severity of vibriosis through immunocompetence and gut integrity

The additive provides a reliable health strategy to reduce the severity of AHPND and other infections in juvenile vannamei shrimp

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Acute hepatopancreatic necrosis disease (AHPND), also known as early mortality syndrome (EMS), remains one of the most serious bacterial threats in shrimp aquaculture. It has caused significant production losses in whiteleg shrimp (*Penaeus vannamei*), particularly across Asia and Latin America (Estrada-Perez et al., 2020). The disease is primarily caused by virulent strains of *Vibrio parahaemolyticus* (VpAHPND) that carry specific toxin genes. Once these bacteria colonise the shrimp stomach, they release potent toxins that lead to severe necrosis of the hepatopancreatic tubules (Kumar et al., 2020). Other *Vibrio* species, including *V. harveyi* and *V. campbellii*, may also cause AHPND if they acquire these toxin genes through horizontal gene transfer (Dong et al., 2017). Therefore, an effective strategy to reduce the severity of AHPND involves controlling pathogenic bacteria and mitigating the tissue damage caused by the toxins.

Feeding trial and *Vibrio* AHPND challenge

Sanacore GM (Adisseo) is a phytobiotic-based health additive that delivers broad spectrum protection to shrimp including inhibitory activities against bacteria and parasites, overall immunocompetence, and digestive health. A recent peer-reviewed study conducted in collaboration with the Yellow Sea Fisheries Research Institute (YSFRI) in Qingdao, China, evaluated the effectiveness of Sanacore GM in improving shrimp health and performance under AHPND challenge conditions.

The study involved a 60-day feeding period followed by a 7-day challenge with a virulent *V. parahaemolyticus* (Figure 1). Juvenile whiteleg shrimp, with an average initial weight of 4.5g, were randomly assigned to three groups. The control group received a standard diet without additive, while the treatment groups were fed with the same basal diet but supplemented with Sanacore GM at 3g/kg (SNGM-3) and 5g/kg (SNGM-5). Shrimp were reared under stable recirculating aquaculture system (RAS) conditions and fed to satiation four times daily. After 60 days of feeding, shrimp were subjected to bacterial challenge by immersion, and survival was monitored over 7 days.

Support for growth

After the initial feeding phase, Sanacore GM improved final body weight by 10–14%, FCR by 9%, and survival rate by 20% (Figure 2). Histological analysis revealed that shrimp supplemented with the additive had villi that were 13% and 12% taller in the SNGM-3 and SNGM-5 groups, respectively, compared to the control (Figure 3), indicating increased surface area for nutrient absorption and better gut barrier function.

These structural improvements indicated enhanced digestive efficiency and a stronger physical defense against bacterial invasion. This implied that this phytobiotic-based health additive could support growth in the absence of disease and prepare shrimp for upcoming disease or stress challenges.

 <i>P. vannamei</i> IBW: 4.5g 100 shrimp/ 300L 24-27°C 18-20 ppt n = 4	Day	60 days	7 days
	Phase	PRE -FEEDING	VpAHPND challenge
	Diet	SNGM 0.3 % or SNGM 0.5 %	

Figure 1. Experimental design of the Sanacore GM feeding and challenge trial. Shrimp were fed for 60 days with a control diet supplemented with the additive at 3g/kg (SNGM-3) or 5g/kg (SNGM-5). After the feeding phase, shrimp were challenged by immersion with *Vibrio parahaemolyticus* and monitored for 7 days.

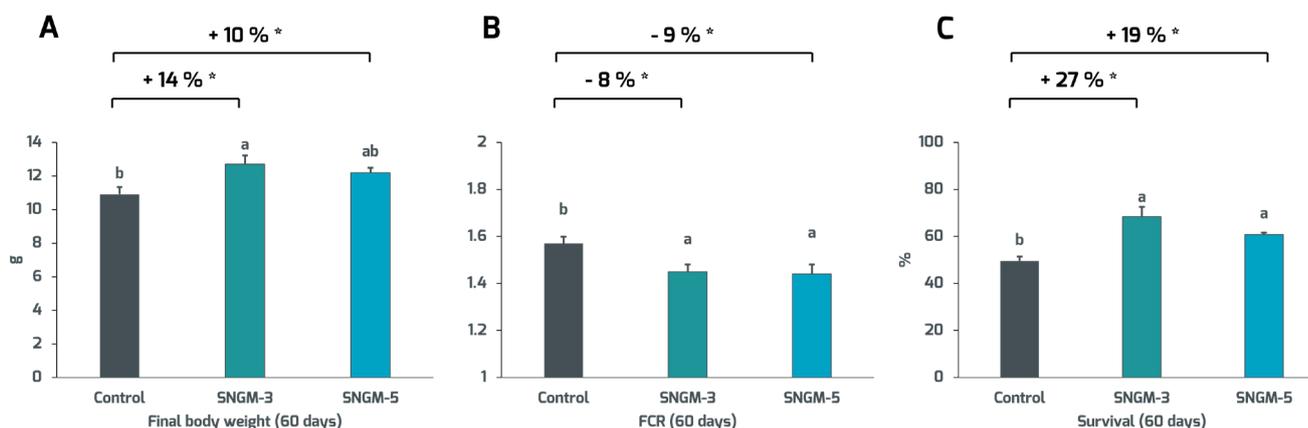


Figure 2. Growth performance of shrimp after 60 days of feeding. Sanacore GM supplementation improved final body weight (A), reduced FCR (B), and increased survival during the pre-feeding phase (C) compared to the control. Values are means \pm SEM and different letters indicate significant difference ($P < 0.05$) between groups.

Additionally, shrimp in the supplemented groups displayed better nutritional status as demonstrated by higher levels of circulating total protein, albumin, and glucose. These changes indicated improved metabolic condition and energy availability, which could be explained by a more efficient digestion and nutrient uptake, and improved capacity for an effective immune response and post-stress recovery (Table 1).

A healthier hepatopancreatic structure

Following challenge with *V. parahaemolyticus*, shrimp in the control group exhibited typical signs of AHPND, including a pale, sloughed, and necrotised hepatopancreas, as confirmed by histopathological examination (Figure 4). In contrast, shrimp fed with Sanacore GM maintained healthier hepatopancreatic structure and exhibited significantly less tissue damage. The ability to alleviate tissue necrosis helped shrimp resist the destructive effects of the bacterial toxins,

resulting in 26% and 28% improvement in survival rates in the SNGM-3 and SNGM-5 groups, respectively, compared to the control group.

Key indicators of immune function and physiological stress

To better understand the mechanism behind this protection, key indicators of immune function and physiological stress were analysed in the haemolymph. Shrimp that received the additive exhibited increased phenol oxidase (i.e. indicator of pathogen encapsulation and melanisation capacity), lysozyme (i.e. indicator of bacterial cell wall degrading capacity), and superoxide dismutase (i.e. indicator of antioxidant capacity) activities. Markers of oxidative damage, including malondialdehyde (MDA) and protein carbonyls, remained stable in SNGM-supplemented shrimp (Table 1). These activities demonstrated superior capacity to deal with bacterial infections due to enhanced immunocompetence.

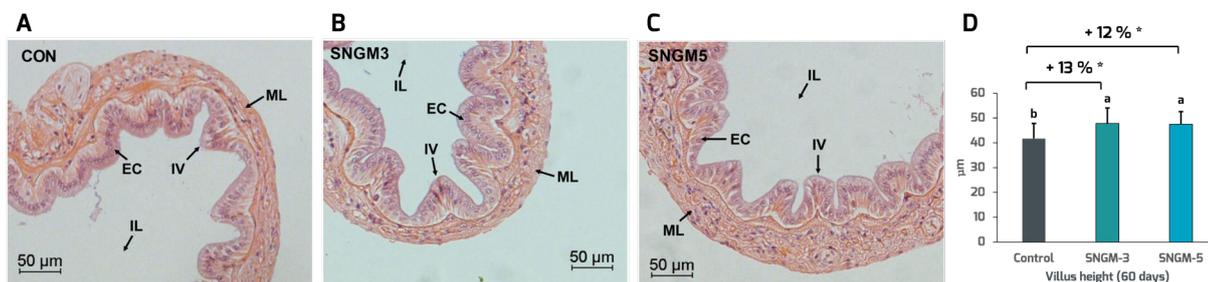


Figure 3. Effects of Sanacore GM on intestinal morphology and villus height in shrimp. Histological sections of the shrimp midgut after 60 days of feeding with the control diet or diets supplemented with the additive at 3g/kg or 5g/kg (A-C). The bar chart quantifies villus height (µm) between groups (D). EC: enterocyte; IV: intestinal villus; IL: intestinal lumen; ML: muscle layer. Scale bar = 50 µm. Different letters indicate statistical differences ($p < 0.05$).

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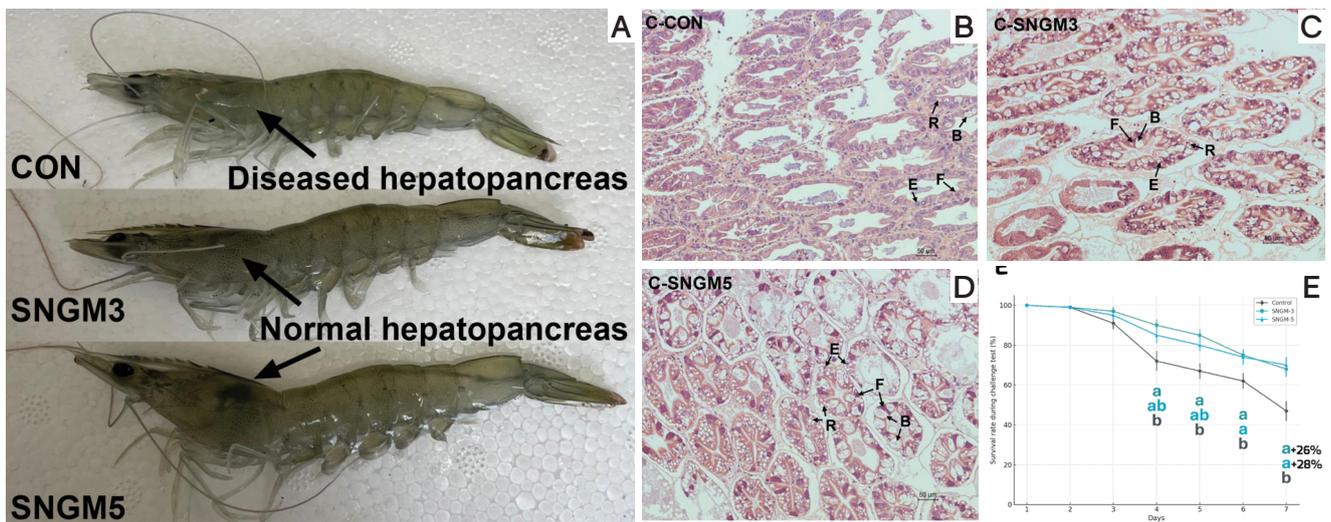


Figure 4. Hepatopancreas observations and survival of shrimp following AHPND challenge. Gross appearance of shrimp hepatopancreas post-challenge (A). Histological analysis (H&E staining) of the hepatopancreas (B-D). Black arrows indicate the four hepatopancreatic cell types: basophilic cells (B-cell), resorptive cells (R-cell), fibrillar cells (F-cell), and embryonic cells (E-cell). Scale bar = 50 µm. Cumulative survival curves over 7 days post-challenge (E). Different letters indicate statistical differences ($p < 0.05$).

In summary, Sanacore GM enhanced gut structure, metabolic resilience, and immunocompetence. In line with published field efficacy (Chen et al., 2023), these results demonstrated that this phytobiotic-based health additive was able to reduce the severity of AHPND and other infections.

Chen, I. T., Mamora, M., Isern-Subich, M. M., Nuez-Ortina, W. G. (2023). Efficacy of a phytobiotic-based additive to reduce the severity of EHP-WFS outbreaks in field conditions. *Aqua Culture Asia Pacific*, May/June 2023, pp 33-35.

	Feeding phase (60 days) (SNGM vs CON)	Vibrio challenge (7 days) (SNGM vs CON)
Nutritional Markers		
Total protein	Improved	Improved
Albumin	Improved	Improved
Glucose	Improved	Improved
Immune/Health Markers		
Phenol oxidase activity	Improved	Improved
Lysozyme activity	Stable	Improved
Alkaline phosphatase activity	Improved	Improved
Superoxide dismutase activity	Stable	Improved
Oxidative Stress Markers		
Malonaldehyde	Stable	Stable
Protein carbonyl	Stable	Stable

Table 1. Summary of shrimp haemolymph markers during the feeding and challenge phases.

References

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