

Immunostimulants and Innate Immunity in Crustaceans

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Shrimp and other crustaceans are farmed intensively in many tropical and subtropical regions, and infectious disease is one of the main threats to production. Viral, bacterial, and parasitic infections can spread quickly in dense culture systems, and animals that are stressed by crowding, poor water quality, or handling are more susceptible. Unlike fish and other vertebrates, crustaceans do not produce immunoglobulins. Their defense depends on innate immunity: recognition proteins, blood cells (hemocytes), enzyme cascades, and antimicrobial molecules that respond within minutes to hours [1, 2]. Because there is no antibody-based memory, vaccines of the type used in vertebrates cannot be applied in the same way, and disease control relies on biosecurity, good husbandry, selective breeding, and immunostimulation. An immunostimulant is a substance that enhances the non-specific defense mechanisms of an animal, usually by mimicking molecular patterns of microbes without causing disease. The aim is not to create memory but to raise the readiness of the innate system, so that the animal responds faster or more strongly when a real pathogen arrives.

The Crustacean Innate Immune System

Overview of components

Crustacean immunity combines physical barriers, cellular responses in the hemolymph (the crustacean equivalent of blood), and humoral factors dissolved in it. Table 1 summarizes the main components.

Table 1. Main components of the crustacean innate immune system

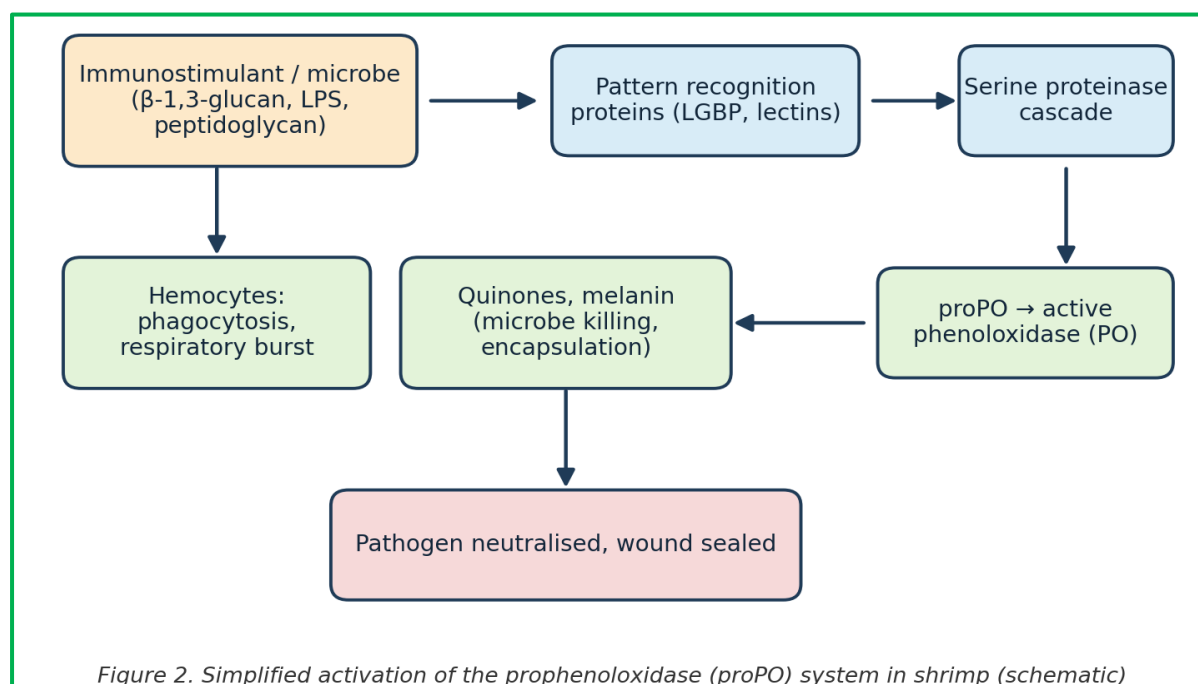
Component	Main function	Notes
Cuticle and gut linings	Physical barrier against invasion	First line of defense; breaks in it allow infection
Hemocytes	Phagocytosis, nodulation, encapsulation of invaders	Several hemocyte types circulate in the hemolymph; counts vary with stress and health
Pattern recognition proteins (e.g., LGBP, lectins)	Detect microbial LPS, peptidoglycan, and β -1,3-glucan	Link recognition to effector responses [3]
Prophenoloxidase (proPO) system	Produces phenoloxidase, melanin, and toxic intermediates	Central effector; PO activity is a common immune indicator [1, 4]
Hemolymph clotting	Seals wounds and traps microbes	Works together with the proPO system
Antimicrobial peptides and lysozyme	Kill or inhibit bacteria, fungi, and some viruses	Include penaeidins and crustins in penaeid shrimp [2]
Reactive oxygen intermediates and antioxidant enzymes	Microbe killing and protection of host tissue	Respiratory burst measured in hemocytes [5, 6]

Pattern recognition

Innate responses begin when recognition proteins detect conserved microbial structures that the host does not produce, including lipopolysaccharide (LPS) from Gram-negative bacteria, peptidoglycan, and β -1,3-glucan from fungal cell walls. In *Penaeus monodon*, an LPS- and β -1,3-glucan-binding protein (LGBP) binds both ligands, increases phenoloxidase activity of hemocyte preparations in their presence, and is needed for normal function of the proPO system, as shown by gene silencing [3]. This is the molecular basis for the action of β -glucan immunostimulants.

The prophenoloxidase (proPO) system

Once a microbial pattern is recognized, a cascade of serine proteinases converts the inactive zymogen prophenoloxidase into active phenoloxidase (PO). PO produces quinones and melanin, which contribute to killing microbes and to encapsulating them [1, 4]. Two proPO forms have been shown to contribute to the survival of *P. monodon* challenged with *Vibrio harveyi* [7]. Figure 2 shows a simplified version of the pathway.



Hemocytes, peptides, and oxidative defenses

Hemocytes engulf and encapsulate invaders and generate reactive oxygen intermediates through the respiratory burst; methods to measure this in penaeid hemocytes and the effect of stimulation on microbicidal substances have been described [5, 6]. Humoral effectors include antimicrobial peptides such as penaeidins and crustins, as well as lysozyme [2]. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase help limit damage to the animal's own tissues during these reactions.

Classes of Immunostimulants

A wide range of substances has been tested in crustaceans. Table 2 lists the main classes and what is known about them.

Table 2. Main classes of immunostimulants tested in crustaceans

Class	Examples	Comments	Key refs
β -Glucans	Yeast, fungal, and algal β -1,3/1,6-glucans	Best studied; effect depends on glucan structure and purity	[8, 9, 10, 11]
Bacterial cell-wall components	Peptidoglycan, LPS	Directly engage recognition proteins	[3]

Class	Examples	Comments	Key refs
Nucleotides and yeast products	Dietary nucleotides, brewer's yeast	Improved growth and immune responses in some trials, alone or with glucan	[12, 13]
Plant-derived compounds	Glycyrrhizin	Tested against β -glucan; gave less sustained immune enhancement in one trial	[14]
Seaweed polysaccharides	Compounds from brown and red algae	Emerging area; evidence still mainly experimental	—

Evidence from Shrimp and Prawn Studies

β -Glucans and virus resistance

In black tiger shrimp (*P. monodon*), dietary β -1,3-glucan improved immunity and survival after challenge with white spot syndrome virus (WSSV) [9]. Earlier work examined the effect of dietary β -1,3-glucan in *P. monodon* brooders, showing that immunostimulation is also studied in breeding stock [15]. In *L. vannamei*, β -glucan derivatives were evaluated for their effects on immunity and resistance to WSSV [11]. Reviews of β -glucan in aquaculture summarize its effects on innate responses and disease resistance across many species [8].

Glucan structure matters

Not every glucan works equally. In the Indian white shrimp *Fenneropenaeus indicus*, an alkali-insoluble glucan fraction containing about 85% (1 $\rightarrow$ 3)- β -D-glucan raised proPO and reactive oxygen activity, whereas an alkali-soluble fraction made mostly of (1 $\rightarrow$ 3)- α -glucan did not produce a significant response [10]. The extraction method changed the structure and, with it, the biological activity, which means that "glucan" on a product label is not enough information.

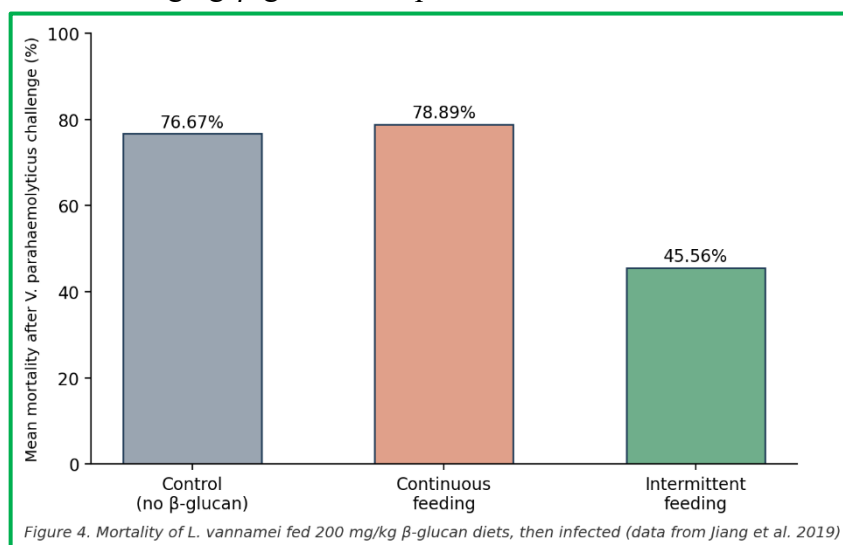
Nucleotides and yeast

Combined dietary β -glucan and nucleotides improved growth, survival, and immune responses of *L. vannamei* [12]. In the giant freshwater prawn *Macrobrachium rosenbergii*, brewer's yeast was examined for effects on immune response and resistance to white muscle disease [13].

Immune fatigue: more is not always better

One of the most practical findings is that continuous feeding of an immunostimulant can lose its effect. In a 6-week trial with *L. vannamei*, continuous β -glucan feeding raised immune indices at first, but by day 27 total hemocyte count, superoxide anion, and SOD activity were no longer significantly higher than in controls, and PO activity followed by day 35; the authors described this as immunity fatigue [14]. β -Glucan maintained higher immunity than glycyrrhizin during the trial.

A later 60-day trial with 200 mg/kg β -glucan compared continuous and intermittent feeding. Neither schedule improved growth, but intermittent feeding raised hepatopancreas SOD, alkaline phosphatase, and acid phosphatase, and increased expression of genes for LGBP, SOD, lysozyme, penaeidin-3a, and catalase. After artificial infection with *Vibrio parahaemolyticus*, mean mortality was 76.67% in controls, 78.89% with continuous feeding, and 45.56% with intermittent feeding [16] (Figure 4).



Routes and Schedules of Administration

Immunostimulants can be given in the feed, by immersion in the water, or by injection; dietary and immersion routes are the only practical ones on farms, and injection is used mainly in experiments [8, 17]. The route of immunostimulation has been shown to affect survival and growth of *P. monodon* postlarvae [18]. Dose, duration, life stage, and the rearing environment all influence the outcome, and results from one species or system should not be transferred to another without testing. Schedule is as important as dose. The fatigue results above suggest that alternating periods of immunostimulant feeding with periods of normal feed may preserve responsiveness better than continuous use [14, 16]. The best schedule likely differs between species, products, and farm conditions.

How Immune Status Is Assessed

Researchers use a panel of indicators to judge whether an immunostimulant is working. Table 3 lists the ones most often reported.

Table 3. Common indicators of immune status in shrimp studies

Indicator	What it reflects	Examples in the literature
Total hemocyte count (THC)	Number of circulating immune cells	[14]
Phenoloxidase (PO) activity	Activation of the proPO system	[10, 14]
Respiratory burst / superoxide anion	Microbicidal capacity of hemocytes	[5, 6, 10]
SOD, catalase, ALP, ACP	Antioxidant and lysosomal enzyme activity	[14, 16]
Immune gene expression	Transcription of LGBP, lysozyme, penaeidin, etc.	[16]
Disease challenge test	Survival after pathogen exposure	[9, 16]

Raised immune indicators are useful signals, but they do not guarantee better survival on a real farm. A survival benefit in a challenge test is the stronger evidence.

Practical Guidelines for Farmers and Hatchery Managers

The research points to several practical rules of thumb. They should be checked against local conditions and advice from a fish health professional.

- Ask what the product actually contains. For glucans, β -1,3 content and purity matter more than the word "glucan" [see Section 4.2].
- Do not assume continuous feeding is best. Trials in *L. vannamei* suggest intermittent schedules may keep the response from fading [see Section 4.4].
- Use immunostimulants as a supplement to biosecurity, clean water, sound nutrition, and healthy seed, not as a replacement.
- Match the product and schedule to the species and life stage, and test on a small scale before large-scale use.
- Keep records of feed, water quality, survival, and disease events so the benefit can be judged honestly.
- Consider cost. Glucan treatments are not yet routine in many farms and feed mills, mainly for economic reasons [17].

Limitations and Research Gaps

- Results vary widely with species, life stage, product source, dose, duration, and culture conditions.
- Laboratory improvements in immune indicators do not always translate into higher survival under farm conditions.
- Growth benefits are inconsistent; in one 60-day trial neither continuous nor intermittent β -glucan improved growth [16].

- Optimal doses and schedules have been worked out for only a few species and products.
- Long-term effects on animal health, product quality, and the environment need more study.

Conclusion

Immunostimulants, particularly β -1,3-glucans, can enhance innate immune parameters and disease resistance in crustaceans by engaging recognition proteins and the proPO cascade. Their effectiveness depends on structure, dose, timing, and context, and continuous use may lead to immune fatigue. They are best used as one part of integrated health management rather than as a stand-alone solution.

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