

# Dietary functional feed additives and their influence on the physiological development of crab species: current status, prospects, and future trends

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## Abstract

The increasing global demand for high-quality seafood has accelerated the growth of aquaculture, leading to the widespread adoption of intensive farming systems aimed at boosting production and reducing pressure on wild stocks. However, these systems face sustainability challenges stemming from their dependence on fishmeal and soybean meal as primary protein sources, escalating feed costs, nutrient-rich effluents, and the overuse of antibiotics to control disease outbreaks. Such practices contribute to antimicrobial resistance, environmental degradation, and chemical residues in farmed organisms, underscoring the urgent need for more sustainable and health-oriented alternatives. In recent years, functional feed additives have emerged as promising substitutes for conventional ingredients. These additives, encompassing nutrient-based compounds (e.g., vitamins, minerals, essential fatty acids, carotenoids, and functional amino acids), and non-nutrient bioactives (e.g., chitosan,  $\beta$ -glucans, probiotics, prebiotics, plant and seaweed extracts, organic acids, essential oils, and natural binders), have been shown to significantly ( $p < 0.05$ ) improve growth performance, immune responses, and antioxidant capacity in various crab species compared to control diets. Furthermore, several studies report enhanced gut morphology, stress resistance, and disease tolerance in treated groups ( $p < 0.05$ ), highlighting their potential role in mitigating the adverse effects of fishmeal replacement and environmental stressors. This review critically synthesizes current evidence on the physiological impacts of dietary functional feed additives in crab aquaculture, elucidating their mechanisms of action, and evaluating their comparative efficacy based on statistical outcomes across studies. It also identifies existing knowledge gaps and challenges related to formulation optimization, additive standardization, cost-effectiveness, and large-scale application. Ultimately, this work underscores the prospects and future trends of functional feed additives as key tools for improving crab health, performance, and sustainability in modern aquaculture.

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## Introduction

Crab aquaculture, a sustainable and eco-friendly method of seafood production, is gaining popularity as a means of meeting the growing demand for crab meat, while reducing the strain on wild crab populations. As global consumption and production of this species increase, producers face mounting pressure to improve growth rates and maintain proper health while minimizing disease outbreaks<sup>[1]</sup>. Feed plays a crucial role in this species and is formulated using various ingredients to meet the nutritional needs of cultured organisms. Fish meal (FM), and fish oil (FO) are commonly used in feed formulations due to their rich supply of highly digestible amino acids, long-chain polyunsaturated fatty acids (LC-PUFAs), and phospholipids<sup>[2]</sup>. However, since the 2000s, the costs of FM and FO have more than doubled, and since 2012, they have consistently remained high<sup>[2]</sup>, posing a significant challenge to the economic sustainability of crab farming.

In response to these rising costs and sustainability concerns, aquaculture nutritionists and feed manufacturers have significantly reduced FM and FO inclusion in feed formulations. However, this shift has often led to compromised growth, reduced feed efficiency, and immune suppression in crab species. To offset these negative effects, attention has turned toward alternative protein sources—such as plant-based proteins, animal by-products, and insect meals—and the integration of functional feed additives to support performance and health in FM-replaced diets.

The incorporation of functional feed additives in aquaculture offers a promising strategy to mitigate the drawbacks of FM reduction. Unlike conventional feed components that merely fulfill basic nutritional requirements, functional feed additives are incorporated to enhance growth, improve immunity, boost stress resilience, and reduce disease incidence<sup>[3]</sup>. These additives are particularly important when using low-FM or FM-free diets, which may be deficient in certain nutrients or contain anti-nutritional factors (ANFs). In such

diets, functional feed additives have been shown to improve nutrient bioavailability, counteract ANFs, and support gut and immune health<sup>[4]</sup>, making them a critical component of successful FM replacement strategies.

Among the wide range of functional additives, medicinal plants and their bioactive derivatives have attracted increasing interest due to their natural origin, safety, and multifunctional health benefits. Medicinal plant extracts and essential oils contain diverse secondary metabolites such as alkaloids, saponins, flavonoids, tannins, phenolic acids, terpenoids, and glycosides that exhibit antimicrobial, antioxidant, anti-inflammatory, and immunostimulatory activities<sup>[3]</sup>. Several plant-derived additives—such as *Aloe vera*, *Curcuma longa* (turmeric), *Glycyrrhiza glabra* (licorice), *Moringa oleifera*, *Azadirachta indica* (neem), *Ocimum gratissimum* (African basil), and *Allium sativum* (garlic) have been reported to enhance disease resistance, elevate antioxidant enzyme activity, and promote growth in aquatic animals<sup>[3]</sup>. These phytochemicals act by stimulating immune-related gene expression, improving intestinal morphology, and maintaining oxidative balance, thereby offering eco-friendly alternatives to synthetic antibiotics<sup>[3]</sup>. Furthermore, essential oils derived from plants such as oregano, thyme, and clove are rich in phenolic compounds (e.g., carvacrol, thymol, and eugenol) that contribute to gut health, feed preservation, and immune modulation, supporting overall robustness in cultured crabs.

Probiotics represent another crucial category of functional additives widely studied in crustacean nutrition. They are live beneficial microorganisms—commonly strains of *Bacillus*, *Lactobacillus*, *Enterococcus*, or *Saccharomyces*—that, when administered in adequate amounts, confer health benefits on the host by improving microbial balance in the gut. In crabs and other crustaceans, probiotic supplementation has been shown to enhance digestive enzyme secretion, improve nutrient absorption, and inhibit the colonization of pathogenic bacteria through competitive exclusion and bacteriocin production. Probiotics also modulate immune-related gene expression and increase resistance to bacterial pathogens such as *Vibrio* spp. and *Aeromonas* spp., which are major threats in crab aquaculture systems.

In addition, prebiotics such as mannan-oligosaccharides, inulin, and  $\beta$ -glucans serve as selective substrates that promote the proliferation of beneficial gut bacteria. When combined, probiotics and prebiotics form synbiotics, which synergistically improve intestinal function, immune responses, and disease resistance. Recent advances have also introduced paraprobiotics and postbiotics—non-viable microbial cells and their metabolic products—which can deliver similar health-promoting effects without the viability concerns associated with traditional probiotics.

In addition to synthetic or purified additives, fermented feed ingredients have gained attention as novel functional components in aquaculture. Fermentation of plant-based or agro-industrial by-products using beneficial microbes such as *Lactobacillus*, *Bacillus*, or yeast can enhance the nutritional quality of the substrate, reduce anti-nutritional factors, and generate bioactive metabolites with immunomodulatory and probiotic-like effects<sup>[3]</sup>. In other crustacean species, fermented feed ingredients have been shown to improve growth performance, gut morphology, digestive enzyme activity, and disease resistance. However, their application in crab diets remains underexplored, and their potential as both protein replacers and functional enhancers in FM-reduced formulations warrants further investigation.

Currently, a wide range of functional feed additives is being explored in crab aquaculture, encompassing both nutrient-based supplements and non-nutrient bioactive compounds<sup>[5]</sup>. Nutrient-based additives include vitamins, minerals, carotenoids (e.g., astaxanthin,  $\beta$ -carotene), essential amino acids, and omega-3 PUFAs, which

support key physiological processes including growth, immune function, antioxidant defense, and reproduction. Non-nutrient compounds such as chitosan,  $\beta$ -glucans, peptidoglycan, plant and seaweed extracts, probiotics, prebiotics, organic acids, essential oils, and binders are increasingly used to enhance gut health, modulate microbiota, boost immune responses, and increase resilience to stress and disease.

The specific effects and mechanisms of these additives vary widely, and their inclusion is typically tailored to address particular challenges in intensive farming systems<sup>[5]</sup>. Nevertheless, despite a growing interest, the scientific understanding of how these additives perform specifically in FM-reduced or FM-free diets is still limited, particularly for crabs. Furthermore, many producers have yet to adopt these strategies, possibly due to inconsistent research outcomes, limited cost-effectiveness data, and the lack of standardized guidelines for formulation and application.

This review aims to consolidate and critically evaluate current knowledge on the effects of various functional feed additives—both conventional and emerging—on the physiological performance and health of crab species. Special attention is given to their application within FM-replaced diets and the potential of fermented feed ingredients as functional tools for sustainable crab aquaculture. By addressing existing knowledge gaps and identifying future research priorities, this work seeks to guide the development of nutritionally balanced, cost-effective, and environmentally responsible feed strategies for the crab farming industry.

## Cultured species

FAO data<sup>[6]</sup> showed that the top three farmed crab species are the giant mud crab (*Scylla serrata* Forsskal, 1775), with 249,000 tons annually, the Chinese mitten crab (*Eriocheir sinensis* Milne-Edwards, 1853), with 160,000 tons, and the green mud crab (*Scylla paramamosain* Estampador, 1949), with 160,000 tons. Swimming crabs (*Portunus trituberculatus* Miers, 1876), blue swimming crabs (*Portunus pelagicus* Linnaeus, 1758), orange mud crabs (*Scylla olivacea* Herbst, 1796), purple mud crabs (*Scylla tranquebarica* Fabricius, 1798), camtschaticus Tilesius, 1815, Dungeness crabs (*Metacarcinus magister* Dana, 1852), and flatback mud crabs (*Eurypanopeus depressus* Smith, 1869) are economically significant crabs that are either farmed or caught. Other crab species include: the Japanese horseshoe crab (*Tachypleus tridentatus* Leach, 1819), mangrove horseshoe crab (*Carcinoscorpius rotundicauda* Latreille, 1802), blue crab (*Callinectes sapidus* Rathbun, 1896), sesarmid crab (*Episesarma singaporense* Tweedie, 1936), spider crab (*Maja brachydactyla* Balss, 1922), red king crab (*Paralithodes camtschaticus* Tilesius, 1815), Dungeness crab (*Metacarcinus magister* Dana, 1852), and flatback mud crab (*Eurypanopeus depressus* Smith, 1869).

## Current status of dietary functional additives for crab farming

Feed additives encompass a wide range of substances incorporated into animal feed to achieve various objectives, including promoting growth, enhancing reproduction, improving feed shelf life, mitigating anti-nutrient effects, optimizing nutrient absorption, boosting product quality, and supporting the overall health of cultured organisms<sup>[7]</sup>. Over the years, aquaculture has relied on a variety of feed additives, such as feeding attractants, immunostimulants, prebiotics, probiotics, acidifiers, and phytochemicals. These functional feed additives offer health benefits that extend beyond basic nutritional requirements. Functional feed additives are categorized into two main types: (1) nutrient-based supplements, including vitamins, minerals, carotenoids, amino acids, and omega-3 polyunsaturated fatty acids (n-3 PUFAs), which not only meet

essential nutritional needs but also promote health and stress resistance; and (2) non-nutrient compounds, such as chitosan,  $\beta$ -glucans, peptidoglycan, plant extracts, seaweed extracts, probiotics, prebiotics, organic acids, essential oils, and binders, which provide health benefits by influencing various physiological processes<sup>[5]</sup>. In the course of the review, the focus will be on vitamins, minerals, carotenoids, amino acids, and omega-3 polyunsaturated fatty acids (n-3 PUFAs) as nutrient-based supplements, while chitosan, plant extracts, probiotics, prebiotics, organic acids, etc., will be considered as non-nutrient compounds.

## Nutrient-based functional feed additives

### Vitamins and minerals

Vitamins and minerals are essential micronutrients for fish and shrimp, supporting development, reproduction, and overall health. As most cannot be synthesized by animals, they must be provided through the diet to meet nutritional needs<sup>[8]</sup>. These nutrients serve as cofactors and substrates that are essential for the proper functioning of physiological defense mechanisms. Conversely, deficiencies in specific vitamins and minerals can lead to various health issues and immunosuppression, making dietary supplementation crucial. Vitamins and minerals are usually added as premixes in aquafeeds, although they may also be included individually in specific formulations. In studies on aquatic animals, vitamins C, E, and A, as well as iron and selenium, have been particularly noted for their significant functional roles<sup>[8]</sup>.

Several fundamental nutritional needs of farmed crabs are typically met when FM is utilized in aquafeeds as the primary or only source of protein. Historically, high FM formulations have provided a foundation for nutrient supplementation<sup>[8]</sup>. However, as crab diets evolve to include less FM, and more alternative ingredients such as plant proteins, complex carbohydrates, etc., the nutritional composition of these feeds will also change, thus affecting the dietary concentrations and bioavailability of nutrients, including vitamins and minerals. The contribution of endogenous vitamins also changes as feed composition varies, given that different ingredients contain varying levels of vitamins.

A study on mud crab (*Scylla serrata*) farming assessed the effects of three diets and a mono-sex culture on growth and production. Males had a higher final body weight than females, but no significant differences were found in other growth metrics or survival between the sexes. Crabs fed with fish bycatch supplements exhibited significantly higher body weight compared to the non-supplemented group, whereas those receiving vitamins and minerals showed no statistically significant differences from either group<sup>[9]</sup>. In a separate study, dietary vitamin A improved lipid peroxidation and optimized lipid metabolism in *Eriocheir sinensis* fed a high-fat diet for 8 weeks, promoting better lipid utilization<sup>[10]</sup>. Additionally, supplementing with 9,000 IU/kg of vitamin D3 enhanced growth, antioxidant capacity, molting, and immunity in juvenile Chinese mitten crabs. It also influenced protein processing, steroid biosynthesis, and antigen presentation, while increasing levels of vitamin D receptors, retinoic acid receptors, and C-type lectins. Serum 1 $\alpha$ , 25-dihydroxy vitamin D3 levels were higher in crabs fed 3,000–9,000 IU/kg of vitamin D3<sup>[11]</sup>. Further research on *Eriocheir sinensis* showed that adding vitamin A to a palm oil-based diet improved fatty acid utilization, reduced lipid accumulation in the hepatopancreas, and enhanced growth. A study on folic acid supplementation (ranging from 0 to 16 mg/kg) found that crabs fed at least 2.0 mg/kg folic acid showed significantly improved growth, feed efficiency, and antioxidant enzyme activity, with minimized malondialdehyde (MDA) levels. No additional benefits were observed at higher doses. Crabs receiving 2.0 mg/kg also exhibited

enhanced immune responses and reduced mortality. The optimal dietary folic acid requirement for *Eriocheir sinensis* was found to be between 2.29 and 2.90 mg/kg<sup>[12]</sup>. Finally, vitamin C and E supplementation have been shown to reduce the risk of hepatopancreatic necrosis in farmed crabs. These vitamins protect the hepatopancreas from injury caused by oxidized fish oil, maintaining a stable fatty acid profile. This combination also enhanced antioxidant status and modulated immune responses, mitigating the harmful effects of oxidized fish oil. The study suggests that feeding rancid forage fish or diets with highly oxidized oil may lead to hepatopancreatic necrosis, but this risk can be reduced with vitamins C and E supplementation<sup>[13]</sup>.

Manganese sulfate monohydrate (MnSO<sub>4</sub>·H<sub>2</sub>O) was added to a semi-purified basal diet to produce six diets with Mn levels ranging from 2.12 to 66.18 mg/kg. Over 50 d, Mn supplementation significantly enhanced weight gain rate (WGR), feed efficiency ratio (FER), and the activities of hepatic Mn-SOD and T-SOD in juvenile *Eriocheir sinensis*. Crabs fed the basal diet showed elevated serum malondialdehyde (MDA) levels, indicating higher oxidative stress. Moreover, Mn-SOD gene expression in the hepatopancreas increased with Mn levels up to 22.45 mg/kg, after which it plateaued<sup>[14]</sup>. These benefits are attributed to manganese's essential role as a cofactor for Mn-SOD, an enzyme critical for neutralizing free radicals generated during metabolism. Complementing these findings, Hu et al.<sup>[15]</sup> also reported significant effects of dietary manganese on growth and antioxidant function in *Portunus trituberculatus*. In their study, the highest post-weight gain (PWG) and specific growth rate (SGR) were observed at 38.2 mg/kg Mn. Tissue analysis revealed that Mn accumulation peaked in the muscle and carapace at 123.6 mg/kg and in the hepatopancreas at 65.6 mg/kg. Crabs fed the lowest Mn level (9.8 mg/kg) exhibited reduced antioxidant enzyme activities and gene expression, both of which were highest at 38.2 mg/kg. Based on these results, the optimal Mn requirement for juvenile *P. trituberculatus* was estimated to be between 48.02 and 53.30 mg/kg.

Another study by Li et al.<sup>[16]</sup> found that dietary supplementation with 20 mg/kg of zinc (Zn) resulted in the greatest weight gain in Chinese mitten crabs (*Eriocheir sinensis*). While hepatopancreatic Zn levels increased with higher supplementation, the fatty acid composition in the hepatopancreas varied depending on the Zn levels. Crabs were fed Zn at levels of 0, 10, 20, 40, and 80 mg/kg. Another study demonstrates that optimum dietary phosphorus could enhance growth, antioxidant capacity, and reduce hepatopancreatic lipid accumulation in juvenile *E. sinensis*, and the range of available P in the diet was suggested to be 1.16%–1.51%<sup>[17]</sup>. Based on another study, dietary selenium can improve both antioxidant capacity and immune response and alter the protein and carbohydrate metabolism of *E. sinensis* under nitrite stress<sup>[18]</sup>.

Studies on common feedstuffs<sup>[19]</sup> indicate that plant-based feeds contain lower concentrations of certain B vitamins compared to fish-meal (FM). For example, riboflavin, vitamin B12, and niacin levels are often reduced in plant-derived protein sources. Pea meals and corn gluten meals specifically have low amounts of vitamin B6 and pantothenic acid, respectively. Howlader et al.<sup>[20]</sup> reported that soybean meal (SBM) naturally has only half the riboflavin content found in FM and other animal byproducts. The increasing substitution of FM with SBM and other plant proteins in diets for crab species underscores the need to revise recommended intakes of vitamins and minerals for diets.

### Functional amino acids

Amino acids are the fundamental components of proteins and are long chains of amino acids. The animal body contains thousands of distinct proteins, each with a specific amino acid sequence that determines its



shape and function<sup>[21]</sup>. Twenty amino acids are essential for the proper functioning of animals, including fish. These amino acids are categorized as either essential or non-essential, based on whether the body can synthesize them. Nutritional studies on crab farming have shown that certain amino acids, such as arginine, leucine, taurine, methionine, cystine, proline, glutamate, glutamine, tryptophan, tyrosine, and aspartic acid, are involved in key metabolic pathways related to health, reproduction, development, growth, antioxidant defense, and survival. These are referred to as functional amino acids (FtAA)<sup>[22]</sup>. Research has demonstrated that supplementing diets with FtAA can improve the overall health of various aquatic animals. However, fish fed diets high in plant-based ingredients may experience stunted growth and poor health due to deficiencies in these FtAA.

Methionine, often the first limiting essential amino acid in plant proteins, is vital for crabs, as it alone or with cystine, fulfills sulfur amino acid requirements. It also serves as a cystine precursor and enhances immune responses, including complement and lysozyme activity, leukocyte function, IgM levels, and plasma bactericidal and peroxidase activities in fish<sup>[23]</sup>. A 6-week study on crabs examined the effects of dietary methionine levels (0.49%, 1.29%, 2.09%) on metabolism, antioxidant capacity, apoptosis, and immunity under normal (24 °C), and high (30 °C) temperatures. Chronic heat stress increased growth, molting, and protein efficiency but reduced survival. Higher methionine levels improved survival, reduced lipid accumulation, increased protein content, and enhanced antioxidant and immune responses under heat stress. A methionine level of 1.72% was identified as optimal for mitigating the negative effects of elevated temperatures in *Eriocheir sinensis* farming<sup>[24]</sup>.

The diverse roles of taurine as an antioxidant and growth-promoting supplement in aquatic animals have been extensively studied. It is found in fishmeal but is absent from plant-based feed ingredients<sup>[17]</sup>. Taurine is classified as a conditionally essential amino acid (CEAA) in some species, while in others, it is considered an essential amino acid (EAA)<sup>[18]</sup>. Taurine supplementation plays a vital role in improving feed conversion ratio (FCR), final weight gain (FWG), and specific growth rate (SGR) in crabs. It also helps regulate immune-related enzymes such as acid phosphatase (ACP), phenoloxidase (PO), lysozyme (LZM), alkaline phosphatase (AKP), and total hemocyte count (THC). Additionally, taurine boosts the expression of gut immune genes and antimicrobial peptides, along with enhancing antioxidant capacity, as indicated by increased SOD, GSH-PX, and total antioxidant capacity (T-AOC). Based on a 65-d feeding trial, the optimal dietary taurine level for *Eriocheir sinensis* was estimated to be between 0.4% and 0.8%<sup>[25]</sup>.

Arginine is an amino acid crucial for regulating vascular function and blood flow. As one of the most versatile EAA, it is often considered a limiting amino acid because of its low urea cycle activity<sup>[26]</sup>, making it a valuable addition to functional diets. Arginine deficiency can lead to reduced weight gain, poor feed utilization, and even mortality in severe cases, whereas arginine supplementation—particularly through its involvement in polyamine metabolism—can enhance immune function by stimulating macrophages to secrete cytokines essential for fish immunity<sup>[27]</sup>. For instance, dietary arginine has been shown to reduce inflammation and antioxidant stress, hence enhancing the immunity of juvenile Chinese mitten crabs under high pH stress in contrast to controls<sup>[27]</sup>. A study on Chinese mitten crabs (*Eriocheir sinensis*) by Qi et al.<sup>[28]</sup>, further highlights the potential of dietary supplements in enhancing reproductive health. After 11 weeks of feeding a diet containing 2.52% arginine, the crabs showed improved vitellogenesis and ovarian maturation. This effect was mediated through the regulation of vitellogenin (Vg) production via the cGMP/cAMP pathway, as well as a reduction in VIH levels.

Building on the idea of enhancing crab health through nutrition, Yang et al.<sup>[29]</sup> explored the effects of L-tryptophan on the survival and immune response of Chinese mitten crabs. When fed L-tryptophan at levels of 0.36%, 0.47%, 0.73%, and 1.05%, the diet significantly improved survival rates after a 96-h challenge with *Aeromonas hydrophila*. Moreover, the highest survival rates were observed in the 1.05% L-tryptophan group, which also exhibited enhanced serum catalase (CAT) and alkaline phosphatase (AKP) activity. Notably, this diet increased gut microbiota richness and diversity, promoting beneficial bacteria such as *Actinobacteria*, *Firmicutes*, and *Proteobacteria*, which are known to support immune function.

In a similar vein, Han et al.<sup>[30]</sup> investigated the impact of N-acetylcysteine on mitigating the negative effects of  $\beta$ -conglycinin in crab diets. After 8 weeks of feeding at concentrations of 0.5 and 1 g/kg, N-acetylcysteine effectively alleviated growth suppression, inflammation, and oxidative damage. These findings emphasize the importance of optimizing diet formulations to support crab health and growth, particularly in scenarios where the diet is deficient in fishmeal (FM) and fish oil (FO). These studies collectively highlight the need for strategic dietary interventions to ensure both the health of the crabs and the quality of the final product, ultimately catering to consumer preferences.

## Fatty acids (n-3 PUFAs)

In recent decades, research interest in n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), potential health benefits commonly known as omega-3 LC-PUFAs, has led to extensive research on their functional roles in aquatic animals<sup>[31]</sup>. Marine fish and fish oils are the primary sources of n-3 LC-PUFAs, particularly EPA and DHA. Fish, depending on whether they are oily or lean, synthesize very little or no of these fatty acids themselves; thus, their EPA and DHA contents largely depend on their diet, including their natural prey or formulated feeds in aquaculture<sup>[31]</sup>. Adequate dietary n-3 PUFA levels are essential for enhancing the immune function of fish. Deficiencies in n-3 PUFAs, especially in high-plant protein diets, can cause digestive deformities, gut morphology issues, weakened immunity, and reduced tolerance to pathogens<sup>[32]</sup>. As aquaculture production has increased and the demand for formulated feeds has grown, the reduced availability of FM and FO has led to the greater use of plant-based and alternative ingredients in feeds. The effect of these ingredients on crab performance varies based on their inclusion levels, feeding habits, and species differences.

An 8-week study using mud crabs (*Scylla paramamosain*) examined the effects of dietary n-3 LC-PUFA levels on growth, lipid metabolism genes, and tissue fatty acid profiles. The highest WG and SGR were seen in crabs that received diets containing 19.8 mg/g n-3 LC-PUFA with 7% lipid and 13.2 mg/g n-3 LC-PUFA with 12% lipid. Elevated n-3 LC-PUFA levels significantly affect lipid metabolism-related genes' expression. According to Wang et al.<sup>[33]</sup>, the optimal n-3 LC-PUFA levels were determined to be 20.1 mg/g for diets with 7% lipid and 12.7 mg/g for diets with 12% lipid. In another study on crabs (*Scylla paramamosain*), the effects of n-3 PUFA sources—fish oil (FO), krill oil (KO), and linseed oil (LO). KO and FO had similar effects, but KO led to significantly higher weight gain (WG), specific growth rate (SGR), and feed efficiency (FER) than LO. FO raised MDA and LPO levels, while KO boosted T-SOD and CAT activity. FO and KO increased ALA in tissues, while LO raised EPA and DHA in muscle. KO also upregulated genes linked to lipid metabolism (*srebp1*, *srebp2*, *hsl*, *cpt1*, *fas*, *6pgd*)<sup>[34]</sup>.

Hu et al.<sup>[35]</sup> investigated the effects of varying dietary DHA/EPA ratios (0.70–1.25) on juvenile swimming crabs (*Portunus trituberculatus*)

over 8 weeks. Results showed that lower DHA/EPA ratios (0.70 and 0.84) significantly improved growth, feed utilization, and survival, with the 0.70 ratio producing the highest weight gain compared to the 1.25 ratio. Building on the theme of fatty acid influence on crab physiology, another 8-week study on mud crabs (*Scylla paramamosain*) found that a 1.18% dietary ARA level led to the highest final body weight, weight gain, and growth rate. While survival and molting were unaffected, higher ARA reduced EPA content. Antioxidant activity (SOD, T-AOC) peaked, and MDA was lowest at 1.18% ARA, indicating that moderate ARA improves growth and antioxidant capacity, while excess may cause oxidative stress. The optimal ARA/EPA ratio was 1.82<sup>[36]</sup>.

The aforementioned research confirms that crab species can experience increased n-3 LC-PUFA levels from novel sources of EPA and DHA without significant negative effects on physiological parameters. Consequently, these novel sources can serve as valuable alternatives to FO, not only in meeting the fish's requirements for essential fatty acids and energy but also—importantly—in elevating EPA and/or DHA levels to amounts suitable for human consumption. This, in turn, reaffirms the significance of cultured fish as essential components of a healthy and balanced diet.

### Carotenoid/astaxanthin

Carotenoids, especially astaxanthin, play a crucial role in aquaculture nutrition by improving the growth, health, and market quality of farmed species<sup>[37]</sup>. Astaxanthin, a red-orange pigment and potent antioxidant, is found in fish, crustaceans, and algae. Astaxanthin supplementation supports pigmentation, immunity, growth, and overall health in species like salmon, shrimp, and crabs, which may lack sufficient natural production; it also reduces oxidative stress by neutralizing free radicals, protecting cells, and enhancing reproductive performance<sup>[38]</sup>. It also enhances immune responses, helping aquatic species fight pathogens, reduce infections, and lower mortality rates, ultimately promoting healthier populations in aquaculture.

Astaxanthin boosts immune function, growth, and feed efficiency, leading to faster growth, better nutrient absorption, and improved productivity and sustainability in aquaculture. It also enhances stress resistance against environmental changes and handling, resulting in higher survival rates and consistent growth. Additionally, astaxanthin improves reproductive health by increasing fertility, egg quality, and hatching success, supporting sustainable breeding<sup>[39]</sup>. Furthermore, it boosts disease resistance, particularly against pathogens like *Vibrio* species, reducing the need for antibiotics and promoting more eco-friendly practices in aquaculture.

In crab farming, recent studies have explored the benefits of carotenoid supplementation in juvenile crabs. For instance, a balanced 1:1 ratio of astaxanthin to beta-carotene, with a total carotenoid content of 75 mg/kg, has proven to be a cost-efficient alternative to diets containing only astaxanthin. This formulation has been shown to provide optimal quality and resilience in juvenile *Eriocheir sinensis*<sup>[40]</sup>. To further assess the impact of different carotenoid sources, an experiment was conducted with six diets: a basal diet, three with varying levels of *Haematococcus pluvialis* powder (0.2%, 0.4%, 0.6%), and two with different concentrations of synthetic astaxanthin (0.13%, 0.16%). The results indicated that *H. pluvialis* powder outperformed synthetic astaxanthin in enhancing astaxanthin accumulation and improving carotenoid composition, as well as the astaxanthin isomer profiles in *E. sinensis*. These findings suggest that *H. pluvialis* is a more effective source of carotenoids than synthetic alternatives, offering significant potential for improving the nutritional profile of crab diets.

$\beta$ -Carotene supplementation had no significant effect on body weight or feed efficiency, suggesting no growth enhancement in adult

*Portunus trituberculatus*. However, supplementation with 50–100 mg/kg of  $\beta$ -carotene improved antioxidant and immune markers in the hepatopancreas and hemolymph. Additionally, higher levels of  $\beta$ -carotene intensified the red coloration of the ovaries and hepatopancreas after 30 d of feeding<sup>[41]</sup>. Moving to a different aspect of crab development, another study found that an appropriate feeding frequency, combined with  $\beta$ -carotene enrichment, could enhance the survival and growth of small blue swimming crab larvae. Specifically, for the megalopa stage, the optimal feeding frequency was three times per day<sup>[42]</sup>. In contrast, dietary supplementation with astaxanthin (AX) did not significantly impact growth performance or feed efficiency in *Portunus trituberculatus*. However, a diet containing 30–60 mg/kg of AX enhanced coloration, nutritional value, and antioxidant status, suggesting that this range is optimal for this crab species after 56 d of feeding<sup>[43]</sup>.

Shifting focus to *Eriocheir sinensis*, another study indicated that moderate  $\beta$ -carotene supplementation increased carotenoid, fatty acid, and amino acid levels in the ovaries, enhancing total carotenoids, fatty acids, and specific amino acids. While its coloring effect was minimal,  $\beta$ -carotene significantly improved flavor. Supplementing with 100 mg/kg promoted aroma precursors and key components (e.g., 2-methylbutanal, hexanal). However, excessive supplementation (300 mg/kg) negatively impacted metabolism and flavor after 70 d of feeding<sup>[44]</sup>. Further testing of  $\beta$ -carotene at inclusion levels of 3.06, 52.40, and 103.59 mg/kg for Day 35 and Day 70 in pre-adult male *Eriocheir sinensis* revealed the following results: (1)  $\beta$ -carotene supplementation did not significantly enhance growth or feed utilization; (2) antioxidant and immune markers (T-AOC, MDA, AKP, ACP) improved with 50–100 mg/kg of  $\beta$ -carotene; (3) in a high-pH stress test, Diet 1 had the highest mortality, while Diet 3 had the lowest; and (4) carapace and hepatopancreas redness significantly increased with higher  $\beta$ -carotene levels by Day 30<sup>[45]</sup>.

Switching to another carotenoid, a study on *Eriocheir sinensis* fed astaxanthin at 68 mg/kg for 4 weeks demonstrated that the crabs experienced oxidative damage due to prolonged exposure to high pH levels. However, including AX in their diet not only reduced oxidative damage but also improved the crabs' body pigmentation<sup>[46]</sup>. Moreover, adult *Eriocheir sinensis* fed defatted *Haematococcus pluvialis* improved coloration, boosted antioxidant and immune capacities, and lowered the cost of fattening diets compared to the control<sup>[47]</sup>. Supplementation with *Haematococcus pluvialis* powder (0%, 0.2%, 0.4%, and 0.6%) did not notably influence ovarian development, but it did improve coloration, antioxidant activity, and protein content in the ovaries and muscles of female *Eriocheir sinensis*. These findings suggest that the optimal dietary level of natural astaxanthin is around 0.6% at both 30 and 60 d<sup>[47]</sup>. Additionally, microalgal astaxanthin has been shown to enhance antioxidant capacity, nonspecific immune responses, tissue astaxanthin levels, and resistance to ammonia-N stress. These results indicate that the ideal dietary concentration of microalgal astaxanthin for juvenile *Eriocheir sinensis* is around 60 mg/kg after 40 d of feeding<sup>[48]</sup>.

Dietary *Haematococcus pluvialis* had no significant effect on survival, weight gain, or gonadal development in male *Eriocheir sinensis*, but it enhanced coloration by increasing carotenoid levels, deepening carapace redness, and lightening the hepatopancreas. Antioxidant responses varied: 0.6% inclusion resulted in lower SOD and POD but higher GSH-Px and LDH, with MDA levels reduced at higher doses. The control group (0%) showed the highest SOD, POD, and GSH-Px in the hepatopancreas. For immune indices, 0.4% and 0.6% diets showed the highest ACP and  $\gamma$ -GT activities, while the 0% diet had the highest ACP and ALP. The lowest ALP and ACP levels were observed in the 0.2% and 0.4% groups, respectively<sup>[49]</sup>.

These studies collectively highlight the varying effects of carotenoids like  $\beta$ -carotene and astaxanthin on the health, pigmentation, and immune functions of different crab species, emphasizing the importance of specific concentrations and supplementation strategies.

## Non-nutrient-based functional feed additive

### Probiotic bacteria

Probiotic bacteria are increasingly used as functional feed in crab farming, offering a range of benefits that enhance health, growth, and productivity. As live microorganisms, they improve gut health, boost immunity, and promote disease resistance by enhancing the digestive microbiota, which in turn improves nutrient utilization, feed conversion, and growth rates<sup>[50]</sup>. Additionally, probiotics outcompete harmful pathogens, balancing gut flora and reducing gastrointestinal infections. Probiotics also strengthen immunity by stimulating immune enzymes and enhancing antimicrobial peptides. Bacteria like *Bacillus subtilis*, *Lactobacillus*, and *Rhodobacter* increase enzymes such as superoxide dismutase (SOD), lysozyme, and catalase, improving resistance to pathogens like *Aeromonas*, *Vibrio*, and *Choleraesuis*<sup>[51]</sup>. Probiotics also produce antimicrobial compounds that inhibit harmful microorganisms, further protecting crabs from diseases.

Probiotics provide additional digestive enzymes, improve larval development and feed conversion ratios (FCR), and prevent gut diseases. During larval development, probiotic bacteria can colonize the digestive tract of larvae, breaking down carbohydrates and producing enzymes, such as amylase, lipase, and protease<sup>[51]</sup>. A combination of *Lactobacillus* species offers added protection during stressful conditions and against pathogenic bacteria in *Portunus pelagicus* larvae<sup>[52]</sup>. The enhanced defense observed in multiple probiotic species is likely due to the unique benefits offered by each species, resulting in an increased range of positive effects. The highest enzyme activity in *P. pelagicus* larvae was achieved with a mixture of three *Lactobacillus* species at  $1 \times 10^3$  CFU/mL, followed by  $5 \times 10^2$  CFU/mL<sup>[53]</sup>. Probiotics likely enhance digestion by increasing beneficial bacterial populations, boosting enzyme activity, balancing intestinal bacteria, and improving nutrient absorption, which in turn increases survival rates and enhances the ability of larvae to combat pathogens.

Dan & Hamasaki<sup>[32]</sup> investigated the probiotic potential of five bacterial strains in *Scylla serrata* larvae, including strains from *Alteromonadaceae*, *Flavobacteriaceae*, and *Aeromonadaceae*. Their study showed that these probiotics effectively reduced necrosis and improved larval survival up to the Z4 stage, but their efficacy declined after the Z5 stage. This suggests that while probiotics can be beneficial in early developmental stages, their impact may diminish as larvae mature. In contrast, a study by Gunarto et al.<sup>[54]</sup> tested the commercial probiotic product Rica-1, containing *Brevibacillus brevis*, on *Scylla paramamosain* larvae. This product was found to inhibit *Vibrio* growth in *Artemia*, but when tested on *S. paramamosain*, it performed less effectively than antibiotics (erythromycin) and the antibacterial agent nitrofurantoin. All treatments were applied at 15 mg/L in rearing tanks, and results showed that Rica-1 was less effective in both crablet production and controlling *Vibrio harveyi* infection during the megalopa stage. Notably, no crablets were produced by day 20 in Rica-1-treated tanks, and larvae succumbed to the infection, suggesting that stress from the thinning process between tanks may have contributed to the reduced efficacy<sup>[54]</sup>. On a more positive note, Yeh et al.<sup>[55]</sup> conducted a 28-d study on *Scylla paramamosain*, assessing the effects of dietary supplementation with *Bacillus subtilis* E20,

*Lactobacillus plantarum* 7–40, or their combination. While no significant differences were observed in growth, respiratory bursts, or immune markers, crabs fed *B. subtilis* E20 exhibited notable improvements in phagocytic activity, PO activity, and disease resistance compared to the control and mixed probiotic groups. Crabs on the *B. subtilis* E20 diet also had lower mortality rates than those on *L. plantarum* 7–40. These findings suggest that *B. subtilis* E20 can significantly enhance immune responses and disease resistance in *Scylla serrata*.

Further supporting the benefits of probiotics, a study by Dan & Hamasaki<sup>[32]</sup> demonstrated that crabs supplemented with *Bacillus pumilus* ( $10^5$  CFU/g) for 30 d showed improved immune responses and better disease resistance against *Vibrio parahaemolyticus*. This was accompanied by a reduction in the growth of harmful bacteria such as *Sphingomonas paucimobilis*, *Acinetobacter lwoffii*, and *Bacillus* species in hermit crabs. Similarly, Wu et al.<sup>[56]</sup> observed that *Bacillus subtilis* supplementation ( $10^5$  CFU/g) in juvenile *S. paramamosain* improved immune responses, respiratory burst activities, and resistance to *Vibrio parahaemolyticus*. Additionally, Yang et al.<sup>[57]</sup> found that supplementing mud crabs with *Enterococcus faecalis* ( $10^9$  CFU/g) for 6 weeks significantly enhanced growth, serum enzyme activities, and resistance to *Vibrio parahaemolyticus*, resulting in higher survival rates compared to controls.

These studies collectively underscore the potential of probiotics, particularly *Bacillus* and *Enterococcus* strains, to enhance growth, immune responses, and disease resistance in mud crabs, offering promising avenues for improving aquaculture practices. Similarly, in blue swimming crabs (*Portunus pelagicus*), probiotics have shown positive effects on larval health and survival. For instance, *Lactobacillus plantarum* supplementation improved survival rates, enhanced protease and amylase activities, and helped improve water quality while reducing bacterial counts, including *Vibrio*, after 14 d<sup>[58]</sup>. This suggests that probiotics like *Lactobacillus* can be effective in enhancing larval development and managing bacterial infections, including *Vibrio*.

Expanding on the benefits of probiotics in crab aquaculture, a study on *Scylla paramamosain* larvae demonstrated that supplementation with *Bacillus* species (PondPlus®, Novozymes®) for 40 d effectively suppressed the growth of green *Vibrio* bacteria, significantly improving larval survival at bacterial concentrations of  $1 \times 10^4$  and  $2 \times 10^4$  CFU/mL<sup>[55]</sup>. This highlights the role of *Bacillus* spp. in managing harmful bacterial infections and improving survival rates in crab larvae.

These findings collectively emphasize the positive effects of probiotics, particularly *Bacillus* and *Enterococcus* strains, in improving the growth, immune responses, and disease resistance of crabs, further reinforcing the potential for probiotics in sustainable aquaculture practices. In line with this, recent studies have provided further evidence of the benefits of probiotic supplementation in enhancing crab health and resilience.

For instance, a study by Liu et al.<sup>[59]</sup> demonstrated that crabs fed *Bdellovibrio* powder (7.5 g/kg) exhibited significantly higher activities of immune-related enzymes such as lysozyme, SOD, CAT, ACP, and AKP in their blood and hepatopancreas compared to the control group. These crabs also showed a substantial decrease in MDA levels and a significantly higher survival rate (81.25%) against *Aeromonas veronii* after 50 d of feeding. This supports the idea that probiotics can enhance immune function and disease resistance in crabs. Similarly, Jin et al.<sup>[60]</sup> investigated the effects of *Bacillus subtilis* B10 ( $1 \times 10^9$  CFU/kg) on male crabs and found improvements in growth and antioxidant capacity, including the enhancement of antioxidant genes like SOD and PRX1. This treatment also regulated fat metabolism,



contributing to better adaptation to environmental changes. Interestingly, while no growth improvement was observed in female crabs, the treatment did enhance their antioxidant capacity and immune resilience. This highlights the varied effects of probiotics on different genders and the broader potential for improving crab farming outcomes.

Expanding on these findings, a study by Cao et al.<sup>[61]</sup> on *Rhodobacter azotoformans* ( $6.0 \times 10^5$  to  $6.0 \times 10^8$  CFU/g diet) showed positive effects on growth performance, immune enzyme activity, and intestinal microbiota diversity. Crabs fed this probiotic exhibited significant protection against *C. freundii* infections, with survival rates ranging from 70% to 100% after seven days. Similarly, *Bacillus licheniformis* supplementation ( $6.0 \times 10^5$  to  $6.0 \times 10^8$  CFU/g) resulted in dose-dependent improvements in antioxidant capacity and immune function, demonstrating enhanced resistance to *C. freundii* infections.

Further supporting the potential of probiotics, Yang et al.<sup>[62]</sup> found that *Lactobacillus rhamnosus* supplementation (2% and 5% of the diet) significantly improved body weight gain, immune enzyme activities, and gut microbiota composition in crabs. This study also showed that the probiotic supplementation increased the abundance of beneficial bacteria, such as *Proteobacteria* and *Firmicutes*, while decreasing harmful bacteria like *Actinobacteriota*. This indicates that probiotics can not only boost growth and immunity but also promote a healthier gut microbiome, which is essential for overall crab health. In another study, probiotics enhanced both growth performance and immune function in *Eriocheir sinensis* by increasing body weight and Ecdysone levels, while also improving antioxidant capacity and reducing harmful metabolites like MDA. The probiotic treatments led to a higher abundance of beneficial gut bacteria, such as *Tyzzelerella*, *Pseudorhodobacter*, and *Rhodobacter*, while reducing potential pathogens like *Vibrio*. These results underscore the dual benefits of probiotics in enhancing both immune responses and intestinal health<sup>[63]</sup>.

Finally, He et al.<sup>[64]</sup> demonstrated that supplementation with *Bacillus subtilis* ( $10^9$  CFU/kg) in cottonseed meal improved growth, redox defense, and nonspecific immunity in *E. sinensis*. This study also observed increased transcription of immune-related genes, such as *mtmsod*, *prx6*, and *propo*, as well as a reduction in harmful MDA levels. These findings further highlight the role of probiotics in strengthening the immune system and improving the overall health and productivity of crabs.

## Prebiotics

Prebiotics are indigestible food ingredients that positively influence gut bacterial growth and activity, thereby improving host health<sup>[65,66]</sup>. To be classified as a prebiotic, a substance must: (a) resist digestion and absorption in the gastrointestinal tract; (b) be fermentable by gut microbiota; and (c) promote the growth and/or activity of beneficial bacteria<sup>[67]</sup>. This process involves the fermentation of dietary fibers by anaerobic gut bacteria, producing short-chain fatty acids (SCFAs) like lactic acid, acetate, butyrate, and propionate, which aquatic animals directly benefit from. Prebiotics also impact the innate immune system in two key ways: by directly enhancing immune responses or by altering gut microbiota composition<sup>[66]</sup>. The former occurs when prebiotics like  $\beta$ -glucan interact with cell surface receptors (e.g., dectin-1), activating immune cells and signaling pathways such as nuclear factor kappa B (NF- $\kappa$ B). The latter involves the modulation of gut microbiota, which has been shown to improve beneficial bacteria populations, as seen in studies on shrimp<sup>[68]</sup>.

In aquaculture, prebiotics are gaining attention for their ability to enhance immune responses and resistance to pathogens. For instance, Lu et al.<sup>[69]</sup> studied the effects of mannose oligosaccharides (MOS) on

*Eriocheir sinensis* crabs, using MOS alone or in combination with  $\beta$ -glucan or inulin. The study found that the combination of MOS with  $\beta$ -glucan or inulin was most effective, leading to the greatest improvements in weight gain (WG) and specific growth rate (SGR). Crabs fed these diets also exhibited increased trypsin activity, particularly in the hepatopancreas, along with elevated antioxidant enzyme activities (AKP, ACP, LYM, and phenoloxidase). Additionally, the MOS +  $\beta$ -glucan diet boosted immune-related gene expression and improved survival rates following a challenge with *Aeromonas hydrophila*.

Similarly, Jia et al.<sup>[70]</sup> found that fructooligosaccharides (FOS) supplementation significantly enhanced growth, antioxidant capacity, immune response, and disease resistance in *Eriocheir sinensis*. This supplementation also up-regulated the mRNA expression of peroxinectin (*px*) and Interleukin Enhancer-Binding Factor 2 protein (*ilf2*) while down-regulating lipopolysaccharide-induced TNF factor (*litaf*) expression. Furthermore, FOS supplementation increased the abundance of beneficial bacteria, such as *Bifidobacteriales* and *Bacteroides*, suggesting a positive impact on gut microbiota composition. These findings further demonstrate the potential of prebiotics to improve growth and immunity in aquaculture species.

Expanding on the role of prebiotics, a study by Tran et al.<sup>[71]</sup> explored the effects of resistant starch and galacto-oligosaccharides (GOS) on mud crab gut microbiota. Both prebiotics altered gut microbiota composition and diversity, increasing the production of butyrate and other short-chain fatty acids (SCFAs). The GOS group showed lower bacterial diversity, dominated by *Bacteroidetes*, whereas the resistant starch group had a higher diversity with a prevalence of *Tenericutes*. This suggests that both GOS and resistant starch may offer prebiotic potential for improving gut health in mud crabs.

Together, these studies demonstrate the effectiveness of prebiotic supplementation in enhancing growth, immune function, and disease resistance in crabs, emphasizing the importance of gut microbiota modulation for aquaculture improvement.

## Plant extracts as functional feed additives in crab farming

Natural products like plant extracts effectively control microbial growth due to their complex chemistry. Rich in flavonoids and polyphenols from herbs, spices, vegetables, and fruits, they are considered safe from extensive use and research, and are commonly added to foods for their antimicrobial and antioxidant benefits<sup>[72]</sup>. The phytochemical composition of plant extracts, especially polyphenols, varies by plant and extraction method, influencing their antioxidant activity, which relies on their hydroxyl groups to chelate metal ions, donate hydrogen, quench oxygen, inhibit lipid oxidation, and scavenge free radicals, with effectiveness depending on the number and position of these groups<sup>[73]</sup>.

Given their beneficial properties, plant extracts are increasingly used as functional feed additives in crab aquaculture, offering a cost-effective, non-toxic alternative to antibiotics. Research has shown that these extracts can enhance growth, improve digestive enzyme activity, bolster immune response, and boost antioxidant capacity in aquatic animals. For example, a study on Chinese mitten crabs (*Eriocheir sinensis*) found that dietary supplementation with icariin (ICA) at doses of 0, 50, 100, and 200 mg/kg, followed by a challenge with 400  $\mu$ g/kg body weight LPS for 6 h, revealed that a 100 mg/kg dose of ICA significantly enhanced resistance to oxidative stress and apoptosis in *E. sinensis* under LPS challenge<sup>[74]</sup>.

One study explored the effects of icariin supplementation on glycolipid and energy metabolism in the hepatopancreas of female *Eriocheir sinensis*. The results showed that icariin positively influenced both glycolipid and energy metabolism by activating relevant metabolic

genes. This finding highlights the potential of plant-based compounds to modulate metabolic processes in crustaceans<sup>[75]</sup>.

Shifting the focus to molting and survival, one study examined the effects of phytoecdysteroid extracts from *Vitex glabrata* on the blue swimming crab (*Portunus pelagicus*). The study found that phytoecdysone supplementation at doses of 0.4 and 0.5 µg/g body weight in post-molt and inter-molt stages significantly reduced molting periods, while higher doses at pre-molt stages inhibited molting. Additionally, injections during post-molt and inter-molt stages increased survival rates, underscoring the importance of timing and dosage in improving survival outcomes<sup>[76]</sup>.

Lastly, a study investigated the efficacy of mangrove leaf extract in treating vibriosis in mud crabs. *In vitro*, the extract inhibited *Vibrio harveyi* growth, with inhibition zones ranging from 11.37 to 13.67 mm. *In vivo*, after bacterial injection, crabs treated with mangrove leaf extract showed improved survival, healing, and histopathological conditions. The 900 mg/L concentration was most effective, enhancing recovery and survival 14 d post-infection<sup>[77]</sup>. This suggests that natural extracts can play a crucial role in treating infections and improving the health of crustaceans.

Plant extracts offer considerable promise for improving growth and health in aquaculture, positioning them as a valuable area for research aimed at optimizing alternative proteins in plant-based diets. Nonetheless, caution is required, as excessive or prolonged use of certain plant extracts can be harmful to crustaceans and fish species. To ensure the safe and effective integration of plant extracts into aquaculture, further research is needed to develop standardized guidelines for their use in plant-based feeds. The effect of plant extract on crab species is presented in Table 1.

## Organic acids as functional feed additives in crab farming

Organic acids, commonly used as feed additives in animal and aquaculture diets, are naturally found in fruits, vegetables, and fermentation products, and are known to improve growth, health, and performance by enhancing digestion, feed efficiency, and overall health; acids such as formic, acetic, citric, and lactic lower gut pH, promote beneficial microbes, inhibit harmful pathogens, and improve nutrient absorption<sup>[78]</sup>. By creating a more acidic gut environment, organic acids

**Table 1.** Effect of plant extracts on crab species development.

Crab species	Plant extract	Dosages	Experimental findings	Ref.
Mud crab ( <i>Scylla olivacea</i> )	<i>Melastoma malabathricum</i> leaf	0.25, 0.5, 1 mg/g	At 1 mg/g, ovary weight and gonadosomatic index (GSI) were highest; oocytes were larger and more advanced histologically.	[77]
Blue swimming crab ( <i>Portunus pelagicus</i> L.) larvae	Mulberry ( <i>Morus alba</i> ) extract	0 (control), 1, 2, and 4 mg/100 g	Only at 4 mg/100 g did larvae molt fully through to megalopa and crab stages; lower doses halted development at earlier larval stages.	[78]
Chinese mitten crab ( <i>Eriocheir sinensis</i> )	Yeast extract	2.5, 5, and 10 g/kg yeast extract	Doses ≥ 5 g/kg increased edible viscera index, catalase (CAT) activity, muscle/viscera protein content, serum immunity, and antioxidant status.	[79]
Orange mud crab ( <i>Scylla olivacea</i> )	Amaranth extract ( <i>Amaranthus</i> spp.)	17, 29, 42, 54, and 67 µg mL	At 42 µg/mL: optimized molting rate, duration, weight gain, survival and elevated hemolymph ecdysteroids.	[80]
Mud crab ( <i>Scylla paramamosain</i> )	Guava leaf extract	0, 80, 160, 320, and 640 mg/kg	320 mg/kg improved growth, survival, enzyme activities (lipase, pepsin, lysozyme, SOD, ACP, GSH), with enhanced gene expression (GPx3, CAT, SOD, GST, p53).	[81]
<i>Portunus pelagicus</i> (Linnaeus, 1758) broodstock females	Amaranth extracts	250, 500, 750 ng/g	All doses improved absolute weight gain (AWG), carapace length (ACL), and carapace width (ACW) relative to control.	[82]
Chinese mitten crabs ( <i>Eriocheir sinensis</i> )	Bamboo leaf flavonoids	0, 500, and 1,000 mg/kg	BLF enhanced growth, gut microbiota balance, serum phenoloxidase and lysozyme, hepatopancreas antioxidant indices, and activated Toll/NF-κB pathway.	[83]
Mud crab ( <i>Scylla serrata</i> )	<i>Solanum ferox</i> extract	P2-20 mL/kg of A, P3-30 mL/kg of A, P4-20 mL/kg of B, and P5-30 mL/kg of B	Formula A (30 mL/kg) most effectively improved molting rate and survival; formula B improved growth and survival, but not significantly.	
Chinese mitten crab ( <i>Eriocheir sinensis</i> )	<i>Ophiopogon japonicus</i>	100 mg/kg	Reduced White Spot Syndrome Virus (WSSV) loads, inhibited ie1, modulated JAK-STAT, increased autophagy markers and antioxidants (SOD, CAT, GSH), and improved survival.	
Chinese mitten crabs ( <i>Eriocheir sinensis</i> )	Garlic powder	1,000, 2,000 mg/kg	Enhanced final weight, WG, SGR, immune enzymes (PO, lysozyme), antioxidant enzymes (CAT, SOD, GPX), reduced MDA, improved antioxidant/immunity gene expression, modulated gut microbiota.	
Chinese mitten crab ( <i>Eriocheir sinensis</i> )	Honeysuckle stem	5.0, 10.0, and 20.0 g/kg	After 30 d: increased hemocyte count, lysozyme, PO activity; limited antibacterial effect, but suppressed inflammatory gene expression and boosted immunity markers.	
Fresh water crab ( <i>Oziotelphusa senex senex</i> )	<i>Ricinus communis</i> L. leaf extract	0.05 mL of 80%	Improved immune defense against microbial infections by elevating haemocyte responses.	
Chinese mitten crab ( <i>Eriocheir sinensis</i> )	Icariin	50, 100, and 200 mg/kg I	At 100 mg/kg: enhanced WG, SGR, antioxidant enzymes (SOD, CAT, GPX), immune enzymes (PO, lysozyme, ACP, AKP), upregulated protective genes, and downregulated inflammation-related genes.	
Chinese mitten crab ( <i>Eriocheir sinensis</i> )	Combined extracts ( <i>Rhizoma coptidis</i> , <i>Mosla</i> , <i>Caesalpinia sappan</i> , <i>Punica granatum</i> )	5, 10, and 15 g/kg diet	Dose-dependent increases in WG, SGR, ACP, AKP, CAT, lysozyme, SOD, improved gut bacterial diversity, and protected against <i>Aeromonas veronii</i> infection.	
Swimming crab ( <i>Portunus trituberculatus</i> )	<i>Quillaja saponaria</i>	150, 300, and 450 mg/kg	At 300–450 mg/kg: improved average body weight, weight gain, SGR, hemocyte counts, phenoloxidase, antioxidant enzymes; respiratory burst unchanged.	
Burrowing crab ( <i>Cardiosoma guanhumi</i> )	<i>Moringa oleifera</i> . Aqueous extract	0.5–2.0 mL/kg	Hemocyte profiles shifted (granulocytes, monocytes); at 2.0 mL/kg: highest serum enzyme activity (SOD, CAT, GPX, MDA).	



help reduce diseases such as *Salmonella* and *E. coli*; certain acids like formic and propionic also possess antimicrobial properties that suppress harmful bacteria, decreasing the reliance on antibiotics, while improving mineral digestibility, supporting bone health, growth, and metabolic efficiency in aquatic species, and stimulating the immune system to act as a barrier against pathogens<sup>[79]</sup>.

In aquaculture, organic acids optimize digestion and nutrient absorption, leading to better growth and feed conversion, especially in high-density systems. Their antimicrobial properties reduce disease, mortalities, and support gut health. Organic acids also boost immune response and resilience to environmental stressors<sup>[58]</sup>. Additionally, they improve feed efficiency, reducing waste and nutrient runoff. They are typically added to feed at 0.5% to 2% concentrations, depending on species and health goals. For example, citric acid improves digestion and reduces microbial contamination in shrimp, while acetic and lactic acids enhance feed conversion in fish.

A study by Sukor et al.<sup>[80]</sup> explored the effects of dietary organic acids on blue swimmer crab juveniles (*Portunus pelagicus*). Over 20 d, the study tested organic acid salts (sodium acetate, sodium citrate, sodium butyrate, and sodium propionate) at 2% on survival, growth, and hepatopancreatic health. All treatments accelerated molting and improved growth, with sodium acetate and sodium propionate showing the highest specific growth rates for weight. However, hepatopancreatic histopathology showed no significant structural changes. This study highlights the need for more research on organic acids in crabs compared to fish.

### Chitosan as a functional feed additive in crab farming

Chitosan, derived from crustacean shells like shrimp and crabs, is increasingly used in aquaculture for its growth-promoting, antimicrobial, antioxidant, and immunostimulant properties. Applied through feed or water, it enhances immune function, reduces disease, improves digestion and nutrient absorption, and boosts feed efficiency, leading to faster growth and higher productivity<sup>[81]</sup>. Moreover, they aid in environmental management by decreasing organic waste and nitrogen levels in water, thereby enhancing water quality. As a sustainable and eco-friendly substitute for synthetic chemicals, chitosan presents an encouraging option for healthier and more sustainable aquaculture methods.

In the context of crab farming, a study showed that crabs fed a diet with 5% or 10% chitin exhibited fewer cultivatable bacteria in the hepatopancreas compared to those on the basal diet. However, chitin supplementation had no significant effect on serum protein, glucose, or glycogen levels. The total number of circulating hemocytes remained unchanged, but crabs fed 10% chitin had more hyaline hemocytes at week 6. Despite these changes, the phagocytic activity of hemocytes and susceptibility to *Vibrio alginolyticus* were similar across all dietary groups<sup>[82]</sup>. In a separate study, a chitosan level of 2% was found to significantly improve growth performance in rice-field crabs, increasing final weight gained, weight gain, average daily growth rate, specific growth rate, and survival rate, though it did not affect feed conversion ratio, where the control group performed best. Based on these findings, a 2% chitosan level in the diet is recommended for optimal rice-field crab culture<sup>[83]</sup>. This highlights the potential of chitosan supplementation in improving the performance of different crab species under varying farming conditions. However, further research is needed on the effects of chitosan supplementation in crab nutrition, particularly regarding its impact on growth and overall health. Additionally, exploring the potential benefits of chitosan diets for maintaining healthy microbiomes in crabs also deserves the attention of researchers.

## Conclusions

This study emphasizes the benefits of functional feed additives in aquaculture and their contribution to overcoming sustainability issues. According to the literature reviewed, these additives help alleviate stress, improve digestion, boost growth and water quality, increase survival rates after infections, reduce parasitic infestations, and minimize the environmental footprint of aquaculture. By providing these advantages, functional feed additives enhance profitability, decrease reliance on antibiotics, and reduce related expenses. Although these benefits underscore the value of functional feed additives in aquaculture, further research is required to determine the most effective combinations and dosages to optimize their benefits.

## Future directions and recommendations

The adoption of FFAs in aquaculture represents a significant advancement, yet it does not completely resolve all sustainability challenges within the industry. As FFAs are mainly preventative rather than therapeutic; additional research is needed to find natural alternatives for disease management that could reduce the reliance on antibiotics and chemotherapeutics. Future investigations should aim to refine the optimal combinations, dosages, and application periods of FFAs to maximize their efficacy. Furthermore, identifying affordable FFAs is essential, as high costs can hinder their widespread use. Overcoming these challenges will require coordinated efforts among researchers, feed manufacturers, regulatory agencies, and aquaculture professionals. To address these challenges, and promote the sustainable use of functional feeds, ongoing research and innovation in feed technology, and aquaculture techniques are essential.

## Ethical statements

Not applicable.

## Author contributions

The authors confirm their contributions to the paper as follows: conceptualization, writing – original draft: Sayed NM; writing – review and editing: Desouky HE; writing – review and editing: Abasubong KP; review and editing: Seddik HE; supervision, funding acquisition, writing – review and editing: Zhang Z. All authors reviewed the results and approved the final version of the manuscript

## Data availability

The datasets generated during and/or analyzed in the current study are available from the corresponding author on reasonable request.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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