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Review article

Fructooligosaccharides (FOS) as Immunostimulant Additives in *Penaeus vannamei* Shrimp Culture, a Review

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ABSTRACT

Background: Sustainable food additives used as diet supplements can have beneficial effects on crustacean growth and immunity. **Aim.** This paper aims to characterize the inclusion of oligofructosaccharides as immunostimulant additives in *Penaeus vannamei* shrimp culture. **Development:** Prebiotics are non-digestible food ingredients that bring benefits to the host, stimulating growth and/or the activity of certain groups of bacteria in the digestive tract selectively. Prebiotics are food additives studied as potential growth promoters in aquatic organisms. In aquaculture, both diet FOS and ScFOS have gained increasing interest due to their growth-promoting factors in several aquatic species, including decapod crustaceans. The effect of several concentrations of fructooligosaccharide (FOS) additives in the food stimulates the immune system and microbiota of these crustaceans and can be important tools to boost the immune system. **Conclusions:** The utilization of fructooligosaccharides as immunostimulant additives in *P. vannamei* shrimp culture improves their physiological state thanks to positive changes in the microbiota, and consequently, the displacement of pathogenic microorganisms. It also enhances innate immune response mechanisms through cell response stimulation, particularly an increase in the number of enzymes in the immune system, and greater microbial recognition in lectin titers.

Keywords: shrimp, fructooligosaccharide, immune response, microbiota (Source: *AGROVOC*)

INTRODUCTION

Aquaculture is the main link of food production. In the last five years, the percentage of aquatic animal production is over 40% (FAO, 2020). The world aquaculture production in 2020 accounted for 122.6 million tons, including 87.5 million tons of aquatic animals, whose

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contribution hit a record of 49.2%. That year, the same trend continued despite the COVID-19 pandemic, though there were differences among regions and countries in each region (FAO, 2022).

Shrimp culture in Cuba and the world faces the risks of large financial losses produced by diseases that cause high mortality, as well as a drop in production quality. The infectious agents affecting shrimp are viruses, fungi, protozoa, and bacteria (Gram-negative and Gram-positive), being the Vibrio genus the most commonly occurring (Thitamadee *et al.,* 2016). Because of the continuous development and propagation of diseases in shrimp culture systems, there is a need to identify the effect of environmental factors on the immune response of penaeid shrimps (Martín *et al.*, 2022).

The introduction of antibiotics in prophylactic treatments constituted an effective strategy to control bacterial diseases, but uncontrolled use entails the development of bacterial resistance (Lulijwa *et al.*, 2019). Recently, the effect of molecules and whole bacterial cells and yeasts has had a promising and controversial effect (Mastan, 2015). Their beneficial effects depend on several factors, such as the type of immunostimulant (prebiotics), administration route, dose, shrimp species, and culture conditions.

Prebiotics are foods metabolized by bacteria selectively in the digestive tract since they promote diet modulation of the intestinal microbiota, thus improving animal health. (Glenn, *et al*, 2017). Moreover, they do not create dependence or negative impacts on the culture organisms or the medium. Crustaceans do not require much energy to metabolize these substances, thus growth is not affected even after repeated use (Otero, 2018).

Among the prebiotics used in aquaculture are fructooligosaccharides (FOS) which are prebiotics defined as non-digestible feed ingredients that affect the host and promote growth and the activity of beneficial bacteria in the digestive tract. Other examples of possible benefits that promote health include harmful bacterium growth inhibition, better absorption of essential nutrients, the synthesis of certain vitamins, and stimulation of immunity functions. FOS application has demonstrated improvements in gastrointestinal flora, digestion, growth, and disease resistance (Mustafa *et al.*, 2019).

Considering the importance for Cuba, shrimp culture breeding requires the study of immunostimulant additives that enhance the response of the immune system against the main pathogens affecting shrimp. Recently the related literature has become more extensive. Accordingly, this paper aims to characterize the inclusion of oligofructosaccharides as immunostimulant additives in *Penaeus vannamei* shrimp culture.

DEVELOPMENT

Shrimp culture was developed in the mid-1900s and it is currently the second largest animal production sector in terms of values, with a permanent increase of the demand by the industrial countries. (FAO, 2020). The Latin American, southeast, and far east Asian countries have most of the production of these species (Roy *et al.*, 2020), where consumption has increased quickly since the proportion of aquaculture products grew five-fold the amounts consumed 60 years ago (FAO, 2022).

There are several species with remarkable productive potential, but the genus *Penaeus* is the most widely exploited in shrimp farms thanks to their fast growth and adaptation (FAO, 2018). The black tiger shrimp *Penaeus monodon* dominated world production at the beginning but faced reproductive problems, difficult domestication, viral diseases, and increased competence in the market compared to other *Penaeus* (Jerry, 2016). Therefore, the industry moved its productions to the Pacific white shrimp *Penaeus vannamei.*

Penaeus vannamei is a euryhaline species with adaptability to a broad range of saline conditions. They have increased tolerance to a high population density, greater availability of domesticated breeders, and resistance to viral pathogens selected genetically (Reyes, 2021). In Cuba, it is the main culture species thanks to its adaptability to captivity, handling stress and disease resistance, and culture indicators, with much larger literature and genetic lines (Espinosa, 2021).

Pacific white shrimp

The origins of its culture date back to the 1970s when French researchers in Tahiti developed raising and intensive breeding techniques in more than one species of penaeid shrimps, including *P. japonicus, P. monodon P. stylorostris*, and *P. vannamei* (Núñez, 2022). One of the main species is the Pacific white shrimp (*P. vannamei*), accounting for 70% of the world population of shrimp culture (Asche *et al*., 2021).

This species is a decapod that usually lives in aquatic settings where the mean temperature is 20 °C throughout the year, and they can tolerate salinity values between 2 and 40 practical units (spu), reaching optimum development at 35 spu. Usually, the females grow faster than the males. The juvenile and pre-adult shrimps live in estuaries and coastal lagoons, whereas the adults live in aquatic settings with sandy floors, and are omnivorous in their natural environment (Quintino, 2022).

During the larval stage, penaeids go through three different phases known as nauplii, zoea, and mysis, before they turn into larvae. In that period, the organisms undergo different morphological and physiological changes associated with the development of motor and metabolic functions (Romero, 2020; Coello and Román, 2020; Quintino, 2022). The health of these animals is determined by the interactions of environmental parameters, microbial communities, and the host.

Main negative effects of white shrimp culture

The shrimp culture industry reports large annual economic losses caused by outbreaks of infectious diseases, especially diseases caused by bacteria of the genus *Vibrio* (Vibriosis), which are linked to about 40% of the incidence for this species worldwide (Licona, 2022). The Asian shrimp industry underwent annual losses of up to four billion due to diseases affecting shrimp between 2009 and 2018. It was estimated that 60% of the losses in shrimp production were caused by the White Spot Syndrome Virus (WSSV), whereas 20% was caused by bacteria, particularly *Vibrio* spp. The characteristics and incidence of vibriosis in shrimp culture are known to be a permanent threat. Species like *Vibrio harveyi,* which causes the so-called shrimp luminescence, are one of the pathogens attacking shrimp that can cause massive mortality of penaeid shrimps in breeding areas and ponds (Amatul *et al*., 2020).

Excessive and uncontrolled use of antibiotics and chemicals to treat or prevent outbreaks has led to a quick propagation of drug-resistant pathogens in aquaculture settings and residual antibiotics in aquatic products (Tan *et al.,* 2019), therefore, there are restrictions to their use and cut the impact on aquaculture production as a direct effect (Amatul *et al*., 2020). The identification of risks associated with antimicrobial use encourages the search for more environmentally friendly strategies, such as the inclusion of prebiotics and probiotics as immunostimulant additives (Wang *et al*., 2015; Mustafa, 2019, Jamal *et al*., 2019) The interest in preventing diseases via immunostimulation has increased, immunostimulants send an alert to the immune system of organisms, improving their responses to infections in an efficient immunological state.

Shrimp’ s immune system

Overall, crustaceans offer an immunological response based on the capacity of the exoskeleton and the peritrophic membrane to act as physical barriers and hemostatic mechanisms, cellular and humoral responses (Miccoli *et al.*, 2021). Through many years of research, the immune system of crustaceans was thought to lack any form of immune memory like that of vertebrates. However, in recent years, reports have been made on a type of immunological memory, so Roy *et al.*, (2020a) suggest the term trained immune response, rather than adaptative immune memory, due to differences in several aspects of the latter, especially the time of persistence in the memory and cellular and molecular mechanisms involved.

The shrimp’ s immune system depends on the innate response and its two components: cellular and humoral (Martín *et al,* 2023). The cellular part is directly influenced by hemocytes, which have a cytotoxic capacity, and intercellular communication, which permits them to perform recognition, phagocytosis, coagulation, melanization, node formation, and encapsulation. Humoral defense involves the production of antimicrobial peptides, oxygen, and nitrogen-free radicals, and a complex enzymatic cascade that regulates coagulation or melanization of the hemolymph. (Reyes, 2021). The activation of innate immunity in shrimps is done through pattern-recognition receptors (PRRs), including type Toll receptors (TLRs) (Licona, 2022).

The mechanisms of the active response of the immune system in penaeid shrimps rely, in the first stage, on the recognition and molecular associations among antigens, pathogens, and recognition molecules (Liu *et al.*, 2020), and a second stage that comprises the activation of enzymes like peroxidase (PX), superoxide dismutase (SOD), and the pro-phenoloxidase system (proPO) to the recognition mechanism, cytotoxic and cellular mechanisms, among which are coagulation proteins, lectins, peptides, antimicrobials, peroxinectins, O_2 generation, quinones, opsonization, phagocytosis, and nodulation, used to eliminate the pathogenic agent (Miccoli *et al.*, 2021). In these mechanisms, hemocytes have a critical role in the production and release of proteins and bioactive species and more complex processes like cell-cell interaction (Kulkarni *et al.*, 2021)

Crustaceans have an open circulatory system with three cellular types (hemocytes), which have been identified in this system, granule-free hyalin cells, semigranular cells with a variable number of small granules, and granular cells, with an abundant number of granules. Hemocytes act as the main recognition system of self and foreign molecules, they stimulate the elimination of pathogens and trigger different defense mechanisms, such as phagocytosis, agglutination, encapsulation, activation of the respiratory burst, and the production of antimicrobial peptides (Licona, 2022).

Hemocytes are cellular effectors, which are critical for these immunity reactions (Liu *et al*,2020, Martin *et al*., 2022; Licona, 2022). They are formed in the cellular fraction of the hemolymph, and are generally classified into three types: hyaline, with no granules and a thin basophil cytoplasm and a broad nucleus in the middle, capable of phagocytosis, and intervention in coagulation; semi-granular due to their spherical or horseshoe-like nucleus with many round granules involved in phagocytosis, encapsulation, and release of the proPO system, they synthesize and release antimicrobial peptides; and granular, with a high cytoplasm-nucleus association, they store enzymes that form the proPO system, even at a larger scale than the semi-granular, and y in the process of encapsulation (Camacho *et al.*, 2019). In these animals, hemocytes not only take part in cellular immunity, but also humoral immunity by storing and releasing immune factors (Liu., *et al*; 2020,),

The humoral response involves the synthesis and discharge of molecular mediators triggering this response in the hemolymph. Some of them had been previously described as agglutinins, coagulation factors and proteins, lectins, antimicrobial peptides, α2M, peroxinectins, lysozymes, and the pro-phenoloxidase (proPO) system components. Lectins are nonenzymatic proteins or glycoproteins, which are regarded as recognition molecules capable of detecting carbohydrates that stimulate the activation of various components of the immune system in crustaceans, such as the activation of proPO. Recently, an increase in the number of functions performed by type C lectins (CTLs) in the immune response has been identified and characterized in crustaceans. These CLTs not only take part in the binding and recognition but they are also involved in other immune responses that include microorganism agglutination, as well as other bactericidal and antiviral functions, like increased opsonization and cellular encapsulation (Martin *et al*., 2022).

Antimicrobial peptides (AMPs) are low molecular weight proteins that are critical for organisms with no adaptative immunity and act as a defense mechanism against pathogens. They have multiple types of isoforms and broad antibacterial, antifungal, and antiviral activity against several different shrimp pathogens (Tassanakajon *et al.*, 2013). They can be released by exocytosis following stimulation with microorganisms (Tassanakajon *et al*., 2018).

Another significant aspect is the activation of the immune system when circular antimicrobial peptide and other protein secretion increases to fight the infection (Tassanakajon *et al*., 2018). Two signal pathways of the immune system are involved in this response, known as Toll (TLR) pathways and Imf (immunodeficiency), which regulate AMP expression. The routes that include TLR receptors and immunodeficiency receptors are essential signaling pathways that induce genes linked to the innate immune system during a pathogenic infection (Licona, 2022). The interest in preventing diseases via immunostimulation has increased, immunostimulants send an alert to the immune system of organisms, improving their responses to infections in an efficient immunological state.

Immunostimlant additives

Today, many of the immunostimulants available are regular nutrients in the diet, such as polysaccharides, proteins, or lipids, which, at high concentrations, can produce an accelerated stimulating effect (Costavalo, 2021). Disease resistance through specific or unspecific mechanisms of immunological response is a primary prophylactic agent, so their limitations within immunostimulation depend on the development stage of the organism' s immunological system.

Méndez *et al*. (2021) conceptualized the criterion that the " utilization of biostimulants in the diet for fish is a promising strategy to reduce the use of antibiotics, enhance biochemical and immune responses that boost production, and reduce economic losses. Other authors (Cavalcante *et al*., 2020) claim that the association of technology applied to nutrition and animal health maintenance is well-accepted in aquaculture. Modern studies clearly show the capacity of nutrients and feed additives to stimulate fish immunity and protect them against the most frequent pathogens.

The inclusion of these additives is associated with improvements in unspecific immunity, higher disease resistance, and the promotion of animal growth. This term includes chemical agents, bacterial components, polysaccharides, animal or alga extracts, nutritional factors, cytokines, and others. The most commonly used in aquaculture are β-glucans, lipopolysaccharides, and beneficial bacteria, known as probiotics (Gutiérrez, 2021). The addition of beneficial components to the diet is important for the intestinal flora, which is responsible for maintaining good health in individuals; the health of these microbes is directly influenced by prebiotic ingestion (Gutiérrez, 2021).

Prebiotics

So far, there are quite a few definitions of prebiotics, but most of them refer to man and mammals. Prebiotics are " nondigestible food ingredients that bring benefits to the host by stimulating growth selectively and/or the activity of one or a limited number of bacterial species in the colon, thus improving health" , according to the International Scientific Association of Prebiotics and Probiotics (2017). Prebiotics require a selective use of the host by living microorganisms, not mere enzymes or bioactive chemical products, in a way that supports or restores the host’ s health. Generally, they are low digestible carbon hydrate substrates like oligosaccharides or diet fiber, which contribute to the proliferation of bacteria in the intestinal flora of animals, improving health and production yields in addition to functioning closely to probiotics, as part of the food of probiotic bacteria, since they are not degraded by the direct action of the digestive tract (Wee *et al.,* 2022).

López and Torres (2022) dealt with the term prebiotic about the “ ingredients of non-digestible foods that bring benefits to the host by selectively stimulating growth and/or the activity of a type or limited number of bacteria in the digestive tract.” In other words, a prebiotic is an ingredient fermented selectively, which permits specific changes in the composition and/or activity in the gastrointestinal microflora, promoting animal well-being and health. For a food ingredient to be considered a prebiotic, it must meet three main criteria: resistance to gastric acidity, hydrolysis by mammal enzymes, and gastrointestinal absorption, followed by fermentation by intestinal microflora, and finally, selective growth stimulation and/or the activity of intestinal bacteria that contribute to health and wellbeing (Mustafa *et al.,* 2019). Despite the many definitions of prebiotics with common elements, this paper will follow the one described by the Consensus of the International Association, where the proper dose is implicit, as enough to generate a prebiotic effect, but not too high to induce undesired or adverse effects, such as the excessive formation of gases or nonselective utilization. The “ proper” dose will vary depending on the microbial system and the related metabolic effects.

The utilization of prebiotics in aquaculture is recent when compared to studies conducted in other ground species (Gutiérrez, 2021); their inclusion in the diet has advantages since they do not require authorization as food additives. Originally, they were used to stimulate *Bifidobacteria* and *Lactobacilli* in the human microbiota (Carbone and Faggio, 2016), when their capacity to change the microbial community in the gastrointestinal tract was reported as a way to enhance unspecific immune responses. Oligosaccharides are a substrate for the growth and proliferation of anaerobic bacteria, particularly *Bifidobacteria*, which inhibit the growth of pathogenic bacteria that cause rotting in the colon (Mancilha and Mussatto, 2007).

Prebiotic activity in the intestinal tract and the organism

Prebiotics are viable alternatives to fight antimicrobial resistance (Elshaghabee and Rokana, 2021). Their main action in the digestive system consists of a potential change of the intestinal bacterial community into a known community of beneficial bacteria, thus favoring the inhibition of colonization by pathogens. According to the literature, these substances (Song, *et al*., 2014; Glenn, 2017; Cavalcante, *et al*., 2020; Elshaghabee *et al*., 2021; Anacona *et al*., 2021) are not digested but generate changes in the gastrointestinal microbiota, and alter their composition or activity to improve the host’ s general health.

They stimulate beneficial bacterial growth reported in fish, mollusks, and crustaceans, such as *Lactobacillus*, while the presence of potentially pathogenic bacteria like *Vibrio*, *Aeromonas*, and *Streptococcus* is limited. They may be considered as having an indirect action because they are mediated by changes staged in the composition and/or activity of the intestinal microbiota (Anacona, 2021). Prebiotics act as food complements, they do not substitute the already-established shrimp diet. Therefore, the nutritional adjustment of the host is still a previous requisite so prebiotics release all their potential since they mainly feed the microbial population in the organisms, not the host itself (Hu *et al*., 2019).

Prebiotics in shrimp culture

The most widely used prebiotics are fructans, carbohydrates with fructose units that form polysaccharides such as inulin or oligosaccharides, and fructooligosaccharides (Di Primio *et al*. 2021). Henríquez (2021) noted that the International Scientific Association for Probiotics and Prebiotics (ISAPP) describes prebiotics as a " substrate used selectively by microorganisms in the host, conferring health benefits" . Prebiotics, including inulin (Li *et al.*, 2018); β-glucanes (Li *et al.*, 2019), mannanooligosaccharides (MOS) (Li *et al.*, 2018), fructooligosaccharides (Zhou *et al.*, 2007), isomalto oligosaccharides (IMO) (Li *et al.*, 2009), xylooligosaccharides (XOS) (Wang *et al.*, 2010), were studied in shrimp, due to their capacity to stimulate growth, feed use, the positive effects on the intestine, morphology, intestinal microbiota, immune system, and disease resistance (Yousefian and Amiri, 2009). Accordingly, compounds like short-chain FOS (scFOS) and MOS, function as immunostimulant saccharides by activating innate immune responses directly, thus promoting shrimp health. (Sivasankar *et al.*, 2017).

Fructooligosaccharides

These are prebiotics known as oligofructose, oligofructans, fructose oligomers, fructans, or glucofructans with glycosidic bonds β (2 → 1) fructosyl-fructose linkages (Sánchez *et al*., 2020). Some reports (Di Primio *et al*. 2021) claim that FOS are fermented by certain bacteria of the genus *Lactobacillus* and *Bifidobacteria*. The bifidogenic nature of these compounds is related to the fact that most bifidobacteria have nutritional advantages over other groups of microorganisms in the intestinal tract.

The fructans in the diet that get to the colon may, therefore, benefit the growth and the activity of the population of native bifidobacteria since they have a D-fructans-fructans intracellular hydrolase that hydrolyzes the fructan molecules into an assimilable substrate (Di Primio *et al*. 2021). The diet supplementation with FOS has been reported to improve the growth rate of some aquatic animals (Yousefian and Amiri, 2009), along with feed rates and conversions in shrimp juveniles, including a reduction in the counts of opportunistic pathogens, such as *V. parahemolyticus, Aeromonas hydrophila, Lactobacillus spp.* and *Estreptococo spp* in feces found in the shrimp gut (Zhou *et al*., 2007).

Enzymes of the immune system

Phenolooxidase

PO is a metal-like protease that depends on Cu and catalyzes multiple reactions: quinone production, orto-hydroxylated phenols, L-DOPA, L-DOPA quinone, and melanin. It is an essential enzyme that provides immune response of invertebrates since its activity not only deals with the production of bactericidal action but also helps repair wounds and cuticle sclerotization due to its microbicidal effects (Vaseeharan *et al*., 2016).

Martin *et al*., (2022) noted that PO from various crustaceans catalyze tyrosinase-type reactions, such as hydrolyzation of monophenols and the oxidation of o-diphenols into quinones, and they are not membrane proteins of a specific organelle. The final result of all the reactions is the formation of toxic metabolites with antimicrobial activity. quinones and phenols are involved in the generation of several cytotoxic metabolites like superoxides and hydroxyl radicals, as well as the covalent overlapping of adjacent molecules that form melanin in the sites affected and around the invading organisms. PO activity is therefore an indicator used as a reference for the analysis of the immunological state in crustaceans.

Lysozymes

Lysozymes are part of the family of antibacterial peptides for their low molecular weight and powerful unspecific bacteriostatic power. They perform the hydrolysis of glycosidic bonds associated with the bacterial cell wall. These proteins are synthesized in hemocytes, mainly the granular type. Besides, they are well characterized in penaeid shrimps, where they have lytic activity against a wide number of Gram-positive and Gram-negative bacteria, including pathogenic bacteria *Vibrio* spp. When hemocytes are recruited quickly to the infection sites, they release lysozymes together with the rest of the immunological effectors as antimicrobial peptides, transglutaminases, and others. There are three types of lysozymes, c-lysozyme, g-lysozyme, and i-lysozyme, of which the first and third have been reported in P. vannamei, both having a powerful antimicrobial activity (Hu *et al*., 2022).

Peroxidase

Peroxidase (PX) activity is closely related to the generation and removal of reactive oxygen species (ROS) generated during oxidative metabolism when the phagocytosis of foreign particles takes place, along with cytotoxic activity against foreign cellular types that invade the organism, thus conferring an immune function (Martin *et al*., 2022). Peridoxinectin has a peroxidase functional domain, so peroxidase activity includes and is closely related to, peroxinectin activity (Licona, 2022).

Peroxinectins are synthesized in granulated and semi-granulated hemocytes inactively, and they are released into the hemolymph through exocytosis as a response to stimuli from pathogenic invasion. Peroxinectins have several biological activities, such as activation of the proPO system, adherence and degranulation of hematocytes, promotion of encapsulation, opsonizing, and peroxidase activity (Martin *et al*., 2022).

Superoxide-dismutase

SOD has the capacity of reducing the superoxide anion that generates water and hydrogen peroxide. They are made up of three major groups depending on the metallic ion they have. Mn-

SOD is present in the mitochondria, Fe-SOD in bacteria, and Cu/Zn-SOD in eukaryotes. Extracellular SOD cooperates in the destruction of parasites ingested or encapsulated during the respiratory burst following phagocytosis (Li *et al*., 2019). Besides, environmental factors like hypoxia and high temperatures cause an increase in SOD expression in shrimp (Lin *et al*., 2010).

Extracellular SOD cooperates in the destruction of parasites ingested or encapsulated during the respiratory burst following phagocytosis (Li *et al*., 2019). This distribution is closely related to the antioxidant function and the places where the respiratory burst takes place, thus confirming its role in the prevention and removal of oxidative damage (Wang *et al.*, 2007).

Lectins

The lectins in the hemolymph of crustaceans also have antimicrobial activity. They are glycoproteins with no catalytic activity, which are present in most living creatures, with one or more conserved domains for carbohydrate recognition, which can discriminate and bind mono and polysaccharides alternatively in a solution or the cellular surface (Sánchez *et al*., 2017). They have the ability of specific carbohydrate binding at the surface of different cells. Because they are generally divalent (having at least two specific binding sites), they can bind cells and therefore agglutinate them.

Sánchez *et al*. (2017) said that lectins are present in most organisms and they are attributed to several biological functions, such as carbohydrate transport in the cells and between tissues, cytolytic and cytotoxic factors, as well as adherence, migration, and cell apoptosis. Lectins work like a receptor for pattern recognition and the variability of carbohydrate recognition could represent a broad range of identifications for different pathogens. Despite the main role of lectins in the recognition of foreign matter and opsonization, they also appear to be involved in neutralization. Some evidence shows that they may also be engaged in coagulation and proPO systems (Martin *et al*., 2022).

FOS as immunostimulants

Immunostimulants like oligosaccharide prebiotics like FOS are used to stimulate immune response, stress and disease resistance through direct immune cell interaction and their activation (Di Primio *et al*., 2021). As previously mentioned, FOS, like other prebiotic oligosaccharides, promote the inhibition of harmful bacterial growth, improved essential nutrient absorption, synthesis of certain vitamins, and therefore, stimulation of immune functions according to the interactions observed (Li *et al*., 2007).

In aquaculture, both diet FOS and ScFOS have garnered increasing interest due to their growth-promoting factors in several aquatic species, including the white shrimp *Penaeus vannamei* (Zhou *et al.*, 2007). The inclusion of FOS in the diet supports growth selectively, as well as the survival of these bacteria in the gastrointestinal tract. As FOS change the microbial communities in shrimp, their capacity to start an immune response in *P. vannamei* can be explained (Li *et al.*, 2007).

Studies done by Li *et al.* (2007) and Mustafa *et al*. (2019) report the effect of scFOS diet supplementation on the beneficial microbiota of the white shrimp. They demonstrated that FOS have already identified characteristics in other prebiotics, which upon supplementation in the diet of *P. vannamei* shrimps, could modify the intestinal microbiota and trigger a sequence of higher immune responses.

Some authors (Song *et al*., 2014) have stated that prebiotics like FOS are also classified as immunosaccharides due to their capacity to improve unspecific immune responses directly through reactions with PRR. Moreover, the beneficial intestinal bacteria could interact with the lymphoid tissue associated with the gut (GALT), which can be translated as a stimulus to the immune system, and improvements in the host' s capacity to prevent infections.

The analysis of the findings of Zhou *et al.,* (2007) shows that supplementation with scFOS in the diet not only improves the growth rate and survival but also promotes the growth of beneficial bacteria and the production of immune and digestive enzymes. Furthermore, greater stimulation of defensive cells is observed due to the growth of beneficial bacteria in the guts, which may lead to an over-expression of lectins, understood as a better physiological condition in bacterial recognition (Bi *et al*., 2020).

Safari *et al.,* (2014) demonstrated that the administration of FOS may stimulate and improve the hemocyte proliferation rate; an increased number of these cells explains high PO activity because they are the main producers of this enzyme. Consequently, the specific enzymatic activity of phenoloxidase is not the only one with higher values, also superoxide dismutase increases, which could influence the survival rate of *P. vannamei* favorably (Dong and Wang, 2013).

CONCLUSIONS

The utilization of fructooligosaccharides as immunostimulant additives in *P. vannamei* shrimp culture improves their physiological state thanks to positive changes in the microbiota, and consequently, the displacement of pathogenic microorganisms.

The inclusion of this prebiotic in the diet of shrimp *P. vannamei* enhances innate immune response mechanisms through cell response stimulation, particularly an increase in the number of enzymes in the immune system, and greater microbial recognition in lectin titers.

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Research conception and design: YCB, YLZ, LDMR, AAC; data analysis and interpretation: YCB, YLZ, LDMR, AAC; redaction of the manuscript: YCB, YLZ, LDMR, AAC.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.