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Aquaculture

Review

Main Factors that Modify the Immune System of Penaeid Shrimps for Sustainable Culture

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ABSTRACT

Introduction: The spread of shrimp diseases worldwide during the last two decades encouraged more researchers to study the factors that modify the immune system. **Aim.** To review the main factors that modify the immune system of penaeid shrimps. **Development:** The factors that affect the immune system of shrimps are classified into abiotic and biotic. Among the abiotic factors are temperature, salinity, the pH, and the presence of nitrogenated compounds in the culture environment. Among the biotic factors are the characterization of the external and internal microbiotas of animals, the analysis of bioactive compounds found in the habitats, and the development of sustainable culture methods, such a as the utilization of probiotics and the biofloc technology. The immune system is particularly susceptible to environmental changes. The microbiota and bioactive components can be important tools to enhance the immune system; however, little is known about how this could be used in practice. **Conclusions:** The utilization of probiotics and the biofloc technology can be very useful to enhance the response of the immune system in practice, and to produce sustainable culture, even when the mechanism activated to produce this effect is unknown.

Keywords: Abiotic factors, biofloc, probiotics, immune response, shrimp (*Source*: *MESH*)

INTRODUCTION

FAO reports say that intensive shrimp culture grew rapidly in the last 10 years, in comparison to the current increase observed in the fishing of naturally grown specimens. Southeast Asia is the

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region with the highest output of aquaculture shrimps, especially China and Indonesia, as the top producers of white shrimp (*Penaeus vannamei*) (FAO, 2018). Intensive culture relies on the creation of an artificial environment with natural conditions; however, high animal stocking densities result in a stressful setting for shrimps, creating ideal conditions for outbreaks of the disease. Consequently, intensive culture comes along with the spread of diseases, posing a permanent challenge to shrimp aquaculture, particularly viral pandemics (mid-1990s), and more recently, bacterial pandemics (2009-2018), causing most disease-related losses (Flegel, 2019), Hence, understanding the immune system of shrimps has been identified as a major target by shrimp-producing companies, including research related to shrimp defenses against the main pathogens reported today, the discovery of immunostimulants, and the description of new technologies for sustainable culture.

The immune system of shrimps undergoes a variation of certain immunological parameters, such as total hemocyte counts (THC), the percentage of each hemocyte population with respect to THC (Ekasari *et al.,* 2014; Kaya *et al.,* 2019; Kumar *et al.,* 2019; Zhao *et al.* 2016), the enzymatic activity of important enzymes linked to the immune system, like catalase (Cardona *et al.,* 2016), phenoloxidase (PO) (Vaillant *et al.,* 2020), peroxidase, lysozyme, and superoxide dismutase (SOD) (Campa-Córdova, Hernández-Saavedra, and Ascencio, 2002). Moreover, the percentage of total hemocyte phagocytosis and the oxidative profile (described through the total antioxidant capacity (TAOC), and the ratio of reduced/oxidized glutathione), are considered important to describe the immunological state of shrimps (Cardona *et al.*, 2016; Xu and Pan 2013; Zhao *et al.,* 2016).

Quite a few reports discuss the effects of environmental factors on the immune system of shrimps under intensive culture conditions. However, most of these studies aree based on short and acute treatments (Knapp *et al.*, 2019). Environmental factors like temperature, dissolved oxygen, the water pH, and salinity levels are known to affect the immune system of crustaceans (Rodrıguez and Le Moullac, 2000). In general, as a result of environmental stress, the expression of genes in shrimps associated with immunity is affected (Aoki *et al.,* 2011). These environmental factors can be divided into abiotic and biotic. The abiotic factors include temperature, salinity, the pH, and the presence of nitrogenated compounds in the culture environment, such as ammonium, nitrites, and nitrates. The biotic factors comprise the microalgae and the microbiota of the exoskeleton and intestine of shrimps, which also modify the response of the immune system of shrimps (Millard *et al.,* 2020).

In recent years, several strategies, including probiotic use, have permitted faster animal growth and survival, along with a drop in the incidence of diseases, and the stressing effects of culturing conditions. Probiotics are defined as living microorganisms that confer benefits to the health of the host when administered in the proper amounts (Hill *et al.*, 2014). Probiotics promote growth and prevent diseases in shrimp culture, becoming an alternative to antibiotics. The knowledge about the mechanism of action of probiotics is limited, though there is strong evidence suggesting that the probiotic effects can be transmitted through competitive exclusion of pathogenic bacteria, the contribution of nutrients and enzymes to shrimp digestion, increased immune response of shrimps, and antiviral effects (Hoseinifar *et al.,* 2018; Knipe *et al.,* 2020; Kumar *et al.,* 2016; Li *et al.,* 2018; Ringø 2020).

Today, the Biofloc system is one of the most commonly used technologies in aquaculture. It is defined as a conglomerate aggregation of microbial communities (floccules) made of phytoplankton, bacteria, and particulate live and dead organic matter suspended in the water of the ponds (Collazos-Lasso and Arias-Castellanos, 2015). These particles engulf particulate organic material on which microalgae develop, along with diverse microscopic microorganisms (protozoa, rotifers, fungi, oligochaetes), constituting a great diversity of heterotrophic bacteria (Avnimelech, 2009). The aim of this paper is to review the main factors that modify the immune system of penaeid shrimps.

DEVELOPMENT

Abiotic factors that modify the immunological response

Temperature

Temperature is an important environmental factor, since it contributes to the success of aquaculture, temperature variations cause fluctuations of the immunological parameters of shrimps. The most commonly cultivated shrimp is *P. vannamei*, which can tolerate a broad range of temperatures between 7.5 and 42.0 ^{0}C (Kumlu, Türkmen, and Kumlu, 2010). However, although temperature is a widely specified parameter, the literature in reference to experimental cultures, which facilitates consensus on the actual range of optimum temperatures, is scarce. Consequently, the wide range of temperatures is part of the broader intensive and extensive aquaculture worldwide (Millard *et al.*, 2020).

In face of temperature fluctuations, the expression of genes related to the expression of components of the immunological system varies. Wang *et al.* (2020) demonstrated that when temperature decreases below 13 ^{0}C, the expression of TLR, IMD, proPO, and Casp3 also decreased significantly, whereas the expression of Muc-3A, Muc-5AC, Muc-17, IAP, p53, HSP70, MT, and Fer increased. Resetting shrimp culture to 28 ^{0}C led to a reinstatement of the expression values of the genes studied at the same level observed before the shrimps had undergone temperature changes (Wang *et al.*, 2020). Moreover, high temperature stress causes a rise in the expression of heat-shock proteins (Hsp70), which are necessary to produce a response against viral pathogens in shrimp, such as the WSSV (Yuan *et al.,* 2017). Nevertheless, other immunological parameters, like total hemocyte counts, phenoloxidase activity, superoxide dismutase activity, and respiratory burst, experienced a significant drop after 24 h at 32 ^{0}C, in *P. vannamei*. Besides, sensitivity to *Vibrio alginathus* increased when the phagocytic capacity of hemocytes was reduced (Cheng, Wang, and Chen, 2005). In these studies, the temperature that produced the highest values of *P. vannamei* immunological parameters was 28 ^{0}C.

Salinity

Penaeid shrimps are a euryhaline species, which means that they can adapt and survive under a broad range of salinity conditions (Mudagandur *et al.*, 2016). The optimum conditions of salinity for penaeid shrimps vary differently among the species. *Penaeus monodon* can withstand low salinity values of 5 psu to high values of 40 psu, with an optimum salinity range of 15-25 psu for the best growth rate. Meanwhile, the juveniles of *P. vannamei* show optimum survival and growth within 33-40 psu *(*Mudagandur *et al.*, 2016).

There is a link between salinity and the presence of hypodermic infections and necrosis in the hematopoietic organs of *P. monodon* tiger shrimps. Low salinity induces changes in the osmotic pressure of the hemolymph, whereas in *M. japonicus,* it causes an increase in oxygen consumption to adjust osmolarity. In *P. schmitti*, a reduction of salinity does not lead to changes in the concentration of total proteins and peroxidase activity, but it decreases glucose concentration and PO activity, and causes gill edema (Lamela *et al.,* 2005). High salinity values (above 25 psu) create more difficulties for shrimp to produce a regular immune response, so they become more susceptible to viral infections like WSSV (Joseph and Philip, 2007). Low salinity values (2.5-5 psu) produced a significant reduction of the immunological parameters in *P. vannamei,* such as PO activity, ROS synthesis, superoxide dismutase activity (SOD), and lysozyme activity (Lin *et al.* 2012). Also, low salinity levels for long exposure periods caused a significant drop of immunological parameters, such as THC, phagocytic and phenoloxidase activities, and resistance to challenges with *Vibrio alginolyticus* (Wang and Chen, 2005). Additionally, in 2010 these data were confirmed with new evidence of a decrease in the parameters of innate immune response of *P. vannamei* under low salinity, in a challenge with *V. alginolyticus* (Li, Yeh, and Chen, 2010)

P. vannamei culture in fresh water or in low salinity conditions is a choice for ponds. The supporters of this type of culture refer to the greater size of the shrimps under low salinity conditions, compared to salty water. The low salinity conditions affect the physiological and immunological state of animals (Lin *et al.,* 2012). Several alternatives can be used to raise the immune response of animals in low salinity conditions, including the utilization of probiotics, particularly *Bacillus* and *Lactobacillus* spp. (Liu *et al.*, 2010; Zheng *et al.*, 2017). Moreover, there is little information about the utilization of biological floccules (biofloc) to improve the immunological conditions of shrimps in low salinity conditions.

Dissolved oxygen

Among the aquatic systems, the concentrations of dissolved oxygen (DO) are heterogeneous, and fluctuate according to physical, chemical, and biological factors in the surrounding area, with daily variations, diminishing at night due to continuous breathing of aquatic life in the absence of photosynthesis. Oxygen is depleted when the demand is higher than the autochthonous production of photosynthetic organisms, and it may be enhanced by anthropogenic factors, including excessive water enrichment with nutrient (for instance, phosphorous compounds), generally through the runoff of ground water from nearby croplands, and later eutrophication (Millard *et al.,* 2020).

In situ measurements of DO concentration in ponds are limited to scale studies, which report DO levels that vary between 2.9 and 5.0 mg mg-L. However, the evidence suggests that the concentrations of DO are significantly reduced after heavy rains (Zhang *et al.,* 2016). *Penaeid* shrimps are oxyregulators, which means that they are capable of maintaining their internal concentration of O_2 actively, regardless of the partial pressure of environmental O_2, to a critical threshold (Herreid II, 1980)**.** Below that threshold, the critical oxygen tension (Pcrit), which depends on temperature and life stage, O_2 consumption becomes the limiting factor of the metabolic rate.

The prolonged absence of sufficient DO also leads to an increase in anaerobic metabolism to maintain survival (Kulkarni and Joshi, 1980)**.** The oxygen-sensitive transcription factors (including the hypoxia induced factors (HIF)) are responsible for triggering a wide range of changes in gene expression when the cell concentrations of O_2 are reduced to improve survival in hypotoxic conditions. To date, HIF-1 in *P. vannamei* (Soñanez-Organis *et al.,* 2009) is associated with the rise of the viral burden in shrimp (Miranda-Cruz *et al.*, 2018) during WSSV infection (Millard *et al.,* 2020).

Shrimps exposed to hypoxia conditions showed low levels of *cMnSOD* transcripts, and lower superoxide dismutase activity (SOD) in the gills and hepatopancreas. Besides, the production of the superoxide anion by hemocytes grew under the same conditions (García-Triana *et al.,* 2010). Dantzler and Burnett (2001) also link the reduction of immune response in hypoxia conditions to inhibition of antibacterial activity of hemocytes (Dantzler, Burnett, and Burnett, 2001), and other immunological parameters like total hemocytes (THC), and antibacterial activity (Jiang and Pan, 2005). The susceptibility against opportunistic pathogens also increases under hypoxia (Burgents *et al.*, 2005), along with an drop in the immune response (Zenteno-Savín *et al.,* 2006).

Hypercapnia and the pH

Hypercapnia refers to a rise in CO_2 levels $^($1.4-1.8 kPa) in aquatic systems, due to complete oxidation of carbonated compounds in biological systems (Metzger *et al.*, 2007). The high levels of CO_2 in the water may cause a reduction of the pH in the medium, with significant physiological alterations, such as insufficient growth. Hence, shrimp culture requires a pH range of 7.8-8.3, thanks to controlled addition of NaOH, CaO, and $Ca(OH)_2$ (ASEAN, 1978).

A low pH under continuous conditions may deteriorate the exoskeleton of crustaceans, making them weak and more susceptible to predators and infections. Milliard *et al.* (2020) remarked the existence of discrepancies as to whether the pH variation can favor the infection process. The contradictions around the pH ranges and their role under pathogenic sensitization are perhaps caused by the different methods of determination used (Millard *et al.*, 2020)**.** A reduction in the expression of genes related to the immunological system was reported during long periods of stress due to low pH, including the anti-polysaccharide factor, lysozyme, *Toll*, and hemocyte protein-glutamine gamma-glutamyl transferase, which were downregulated, in addition to a distortion observed in intestinal tissue (Valencia-Castañeda *et al.*, 2018). Besides, the immunological parameters dropped as a result of long periods of exposure to both low and high pH values. The 8-8.5 interval is the optimum range to achieve the highest values of immune response. Hence, a 0.5 variation due to increase or reduction, leads to a decrease of immunological parameters like THC, phenoloxidase activity, bacteriolytic activity, and antibacterial activity (Lu-Qing, Ling-Xu and Jing-Jing, 2005). Moreover, under hypercapnia conditions, a greater diffusion of *Vibrio campbellii* in the hemolymph and internal *P. vannamei* organs occurred (Burgents *et al.*, 2005). Nevertheless, there are few studies about the influence of this factor on specific parameters of the immune system of shrimps.

Nitrogenated compounds

In the ecosystems of ponds (especially fully closed ponds), the concentrations of ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) usually exceed the natural levels. It occurs mainly due to the degradation of excess feed and metabolic waste excreted by the cultured animals (Nhan *et al*., 2006). Consequently, the highest values of nitrogenated compounds are associated with the feeding time, fertilization, and accumulation of sediments **(**Millard *et al*., 2020**)**

In general, the toxicity of NH_3, NO_2^-, and NO_3^- in crustaceans vary according to the stage of development, stronger, and often, to the greatest differences in the tolerance reported during the larval and juvenile stages. High concentrations of these compounds have similar physiological impacts on penaeid shrimps, including limited feed ingestion, slower development, significantly lower growth rates, despite the more frequent shedding and gill damage including obstruction and blockages, and the loss of structures and functions (Furtado *et al.*, 2015; Millard *et al.*, 2020).

The exposure to prolonged stress brings about immunological responses in some crustaceans, such as the reduction of THC due to oxidative damage, and apoptosis (Xian *et al.*, 2011), and significant changes in the expression of many genes, which is believed to play a role in apoptosis and immunity (Lu *et al*., 2016)**.** These results suggest that prolonged exposure to nitrogenated compounds that may take place in shrimp culture ponds increase susceptibility to infection by WSSV (Kathyayani *et al.*, 2019). Under 24 h exposure to sublethal concentrations of nitrite, the SOD, catalase, and glutathione activities diminished in relation to the standard concentration of nitrite in the medium. In general, these results showed that a balance between pro-oxidant and anti-oxidant forces is one of the toxicity mechanisms of nitrite in shrimps (Wang *et al.*, 2004).

Low pathogen resistance was also observed accompanied by a decrease in the immunological parameters, such as THC and phenoloxidase activity, with high concentrations of nitrite (Tseng and Chen, 2004). Other authors demonstrated the existence of greater susceptibility to bacterial infection (Chand and Sahoo, 2006; Cheng, Liu, and Chen, 2002; Tseng and Chen, 2004)**.**

Among the nitrogenated compounds, ammonium is one of the main limiting factors of shrimp culture systems, with a fast increase of mortality, and ensuing economic losses (Sun *et al.,* 2018). High levels of ammonium (approx. 13.9 mg/L) lead to progressive deterioration of gills, such as edema, inflammation (infiltration of hemolymph and hemocytes), melanization, and necrosis. (Fregoso-López *et al.,* 2018). It is also reported in juveniles that 20 mg/L cause consistent deterioration of the intestinal mucosa that ends in necrosis (Duan *et al.*, 2018). Values above 20 mg/L cause oxidative stress and induction of apoptosis in the hepatopancreas in adult stages (Liang *et al.*, 2016). Moreover, it was reported that high ammonium levels in shrimp culture can suppress the immune response by inhibiting phenoloxidase activity (PO) and phagocytic activity, evidencing a reduction of more than 60% of the normal expression of the *proPO* gene (Sun *et al.*, 2018). Besides, a maintained ammonium stress involves the suppression of other immunological factors such as total hemocyte counts (THC), antibacterial activity using second messenger pathways like AMPc, Calmoduline, and GMPc (Zhang *et al.*, 2018), thus conditioning that stress caused by high ammonium concentrations produces an increase in sensitivity to pathogens (Cui *et al.*, 2017). The severity of bacterial infections increases in the presence of high NH_3 concentrations. For instance, mortality of *P. vannamei* increased following the infection by *V. alginolyticus,* which resulted in a drop of phagocytic activity (Liu and Chen, 2004)**.**

Biotic factors that affect the immunological response

Intestinal microbiota

The intestinal microbiota is described as the new organ in the animal, with important systemic effects (McFall-Ngai *et al.*, 2013), like digestion, nutritional metabolism, and in components of the immune system (Akhter *et al.,* 2015). Because of the constant interaction between the digestive system of shrimps and the surrounding environment (water and sediments), the intestinal microbiota and hepatopancreas must participate in the mechanisms of disease resistance. So far, there are fewer studies of the intestinal microbiota in fishes and shrimps than in other species (Servin Arce *et al.,* 2021). Only in *P. vannamei*, a total of 111 strains from 13 taxonomic groups were isolated and identified using conventional methods (Wan *et al.,* 2006). This microbiota varies in shrimps, as to the composition, depending on the culturing systems and the microbiota of specimens caught in natural environments (Cornejo-Granados *et al.,* 2017).

Progression studies of microbiota composition in *P. vannamei* challenged with pathogens showed that the sick shrimps hosted a more complex bacterial network, a greater connectivity, and more diverse taxons in comparison to the healthy cohorts. The intestinal taxons were associated with *Verrucomicrobiales* and A*lteromonadales* in healthy shrimps, which then changed to

Rhodobacterales, *Vibrionales*, and *Flavobacteriales* in the sick cohorts. Hence, immunity potentials such as SOD levels were associated positively with the relative abundance of key taxons (Dai *et al.*, 2020). However, several other authors suggest that pathogenesis may not be linked to either changes of a specific taxon or a group of them in the population, but to changes in the interactions among the microorganisms that form the microbiota (Holt *et al.*, 2020).

Recently, various studies showed how the bacterial microbiota can be manipulated, either directly or indirectly, through endogenous and exogenous factors to enhance resistance against shrimp pathogens. Among the most commonly described factors are salinity, concentration of Sulphur species, components of the diet, temperature, and the development stage of shrimps (Li *et al.*, 2018). However, several important questions, as how to develop practical methods to achieve an optimum balance in the composition of the microbiota, and therefore, their potential use as a culture tool, are still unanswered.

Bioactive components

Besides being a source of complementary nutrition, microalgae contain bioactive compounds (carotenoids), like fucoxanthin, which helps in animal development, and contribute to the immunological system (Shah *et al.*, 2018). The utilization of commercial diets supplemented with *Dunaliella sp*. in *P. vannamei* raised the immune response of animals challenged with the white spot virus (Anaya-Rosas *et al.*, 2019). Rotifers are primary consumers, and therefore they absorb microalgae pigments, making them available in the higher trophic level (Cezare-Gomes *et al.*, 2019), and can be used as immunostimulants for shrimps (Wang *et al.*, 2015). The extracts of *Sargassum horneri* are also known to stimulate innate immunity, improve growth performance, and up regulate immune genes of *P. vannamei* (Sudaryono, Chilmawati, and Susilowati, 2018).

Probiotics and immune response

By 2020, approximately 20 bacterial genera were reported to have a probiotic effect on shrimps, though most studies included lactic acid *Bacillus* and bacteria (LAB), such as Lactobacillus (Knipe *et al.*, 2021), largely due to their successful prevalence and application as probiotics in mammals and poultry. Some of the administration routes of probiotics are oral, along with the feed (including bioencapsulation with live feed vectors, such as *Artemia*) (Immanuel, 2016); directly in the water as purified cultures or spores (Ringø, 2020); inside a fermented culture medium, like *Bacillus subtilis* (Knipe *et al.*, 2021). Additionally, probiotics can be supplied together with a complementary prebiotic, a non-digestible feed ingredient (Gibson and Roberfroid, 1995), to create treatment known as symbiotic, which is currently regarded as products with an optimum combined action (Li *et al.*, 2018).

Probiotic species often are isolated from shrimp intestines, and from the surrounding water or nearby sediments (Knipe *et al.*, 2021). Additionally, from fruit residue filtrates (Nurliana *et al.*, 2020); curd (Karthik, Hussain, and Muthezhilan, 2014); fermented soybean 'Natto' (Liu *et al.*, 2010); fermented pickles (Zokaeifar, Balcáza, and Saad, 2012); and the intestines of other species. For example, in relation to the intestines of other species, there are reports of Lactobacillus probiotic shrimp species in the digestive tract of chicken (Phianphak *et al.*, 1999), and fish (Doroteo *et al.*, 2018). Commercial probiotic treatments that contain LAB and *Bacillus* spp. to a large extent have probiotic effects on shrimps (Ringø, 2020).

Among the effects sought in previous trials before using probiotics, are easiness of culture, biosafety (including hemolytic activity, and antibiotic susceptibility), as well as the capacity to produce extracellular enzymes and exclude pathogens competitively. However, after isolation, the potential probiotics are selected mainly according to the principle of competitive exclusion (the species that compete over the same limited resources are unable to coexist. Through antagonistic bacterial analysis in which pathogens are exposed directly (co-culture), or indirectly (extracellular products) to candidate bacteria (Knipe *et al.*, 2021).

The role of probiotics as stimulants of zootechnical parameters is known, along with survival in penaeid shrimps, with the first meta-analysis study reports (Toledo *et al.*, 2019). A recent meta-analysis revealed an increase of the enzymatic activity of phenoloxidase, using probiotics from *Bacillus* spp. and *Lactobacillus* spp. (Vaillant *et al.*, 2020). It is also known that probiotics can affect the microbiota in the digestive tract of penaeid shrimps, and play an important role in the prevention of disease (Imaizumi *et al.*, 2021).

Biofloc and the immune system

The shrimp cultured in biofloc systems showed elevated immunological parameters, as evaluated in tests. These systems are characterized by an increase in the density of the culture, with higher productivity by area unit, and lower water consumption, reducing the costs of production (Leite *et al.*, 2017). It is considered an innovating and vanguard technology for superintensive aquaculture productions as part of sustainable productions (Avnimelech, 2009).

Biofloc is one strategy for disease control for its potential as a probiotic (Emerenciano, Gaxiola, and Cuzon, 2013). Bioflocs *have* bioactive compounds, such as carotenoids, chlorophylls, phytosterols, bromophenols, amino sugars, and anti-bacterial compounds (Crab, 2010), which are thought to have mechanisms of fish and shrimp growth; improvement of survival rates; and the development and stimulation of some defense mechanisms of the innate immunological system of fishes and shrimps. The biofloc systems are an interesting alternative to manage the health of these species due to the enhancing effects attributed to the bioactive compounds (Ju *et al.*, 2008).

Various reports point to a joint stimulation of the immunological system of shrimps when they are cultivated in biofloc systems. Among the immunological parameters evaluated, total hematocyte counts evidenced a considerable increase in treatment systems using complex carbon sources, like cassava starch, rice meal, wheat bran (and rice), and flour, to form biofloc, in relation to clear water control (without the addition of carbon sources), and the phagocytic capacity of these hemocytes (Ekasari *et al.*, 2014; Kaya *et al.*, 2019; Kumar *et al.*, 2019; Zhao *et al.*, 2016)**.** Moreover, the enzymatic activity of phenoloxidase increases in biofloc cultures, in comparison to culturing in clear water (Panigrahi, Das, *et al.*, 2020), along with phenoloxidase expression in shrimps (Tepaamorndech *et al.*, 2020).

Other immunological parameters evaluated in Biofloc treatments include the enzymatic activity of superoxide dismutase (Tepaamorndech *et al.*, 2020), enzymatic activity of catalase (Liu *et al.*, 2018), and the enzymatic activity of lysozyme (Liu *et al.*, 2018). All showed increased activity levels using the biofloc technology compared to clear water cultures. The same behavior was observed in more general immunological parameters, such as the total antioxidant capacity (Tepaamorndech *et al.*, 2020), the bacteriolytic activity, antibacterial activity (Liu *et al.*, 2018, and the ratio of reduced/oxidized glutathione (Zhao *et al.*, 2016). However, the mechanism that enhances the immune response using the biofloc technology, has not been fully understood. The utilization of biofloc technology changes the microbiota that surrounds the microorganism, as well as the intestinal microbiota (Cardona *et al.*, 2016).

High culture densities

One of the factors to be considered for intensive shrimp culture is the animal stocking density per volume of water (Kotiya and Vadher, 2021). This variable can reach high values in conventional breeding areas, under adverse and difficult conditions for shrimps due to greater amounts of waste products that raise the concentration of toxic species, and because of a reduction in the quality of water in terms of nutrients and dissolved O_2 (Mena-Herrera *et al.*, 2006). Increased culture density leads to a reduction in total hemocyte counts, phagocytic activity, enzymatic activity of phenoloxidase, lysozyme, catalase, and superoxide dismutase in *Palaemonetes sinensis* (Dong *et al.*, 2018). Furthermore, studies of in *P. vannamei* evidenced a lower activity of enzymes like SOD and CAT, with high levels of culture density, as well as an increase the expression of genes associated with stress, such as HSP70, in the hepatopancreas (Gao *et al.*, 2017). Nevertheless, there is little information about the utilization of different culture densities of penaeid shrimps (Kotiya and Vadher, 2021), particularly about these immunological parameters.

CONCLUSIONS

Studies associated with environmental, biotic, and abiotic factors that affect the immune system of shrimps were more numerous as shrimp culture in several geographical areas increased, which used large densities of animals to generate higher productivity. The main factors include temperature, salinity, concentration of oxygen compounds, the pH, and dissolved oxygen. However, in recent years, the number of studies related to biotic factors like the composition of the intestinal microbiota, the presence of biological production compounds from aquatic microorganisms, the utilization of probiotics, prebiotics, and sustainable technologies like biofloc was higher.

The parameters of the immune system evaluated in the literature reviewed were particularly sensitive to small changes of factors like the pH and temperature. As to salinity, whether there is a preference over low salinity conditions due to greater yields and the size reached by the animals, the immunological parameters are lower than in the sea water. The implementation of new technologies enhances the immune response in low salinity cultures, particularly with the use of prebiotics, probiotics, natural bioactive compounds, and biofloc in low salinity conditions.

Most reports were made after short periods of experimentation using acute treatments; few of them evaluated their effects over long periods of time. There are quite a few papers on the composition and proportion of species that form the microbiota. However, many important questions like how to develop practical methods to achieve an optimum balance in the composition of the microbiota, and therefore, their potential use as a culture tool, still require further elucidation.

Although many probiotics are currently used, it should be noted that the exact mechanism of effect is multifactorial. Furthermore, biofloc technology shows promise as an enhancer of the immunological system of penaeid shrimps.

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AUTHOR CONTRIBUTION

Conception and design of research: LDMR, YCB, MGS, OCF, HCA, AAC; redaction of the manuscript: LDMR, YCB, MGS, OCF, HCA, AAC.

CONFLICT OF INTERESTS

The authors declare the existence of no conflicts of interests.