Husbandry and Nutrition

Review

Immunostimulant Additives in the Diet of Tilapia (*Oreochromis ssp.*)

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ABSTRACT

Background: Some diet additives have become alternatives to achieve higher yields within the productively sustainable, organic, and ecological margins. Hence, nutrients and microorganisms can be added to the diet for fish immunity improvements, by using the nutrients in the feed, which lead to a positive animal performance. Accordingly, the aim of this paper was to gather information related to the utilization of additives as immunostimulants in tilapia (*Oreochromis ssp.*), and their physiological performance in this species. **Development:** Many of these immunostimulants are microorganisms or habitual nutrients in the diet, such as polysaccharides, proteins or lipids, which, at high concentrations are capable of creating a stimulating effect, speeding disease resistance through specific or non-specific mechanisms of immunological response, thus becoming primary prophylactic agents. **Conclusions:** The use of additives as immunostimulants helps prevent the occurrence of common diseases in tilapia culture, by modulating the intestinal system in the different species of genus *Oreochromis*, thus enhancing the non-specific immune response, and, in turn, some processes like individual growth, feeding efficiency, and stress tolerance can be favored.

Key words: stimulating effect, additives, tilapia (*Source*: *AGROVOC*)

INTRODUCTION

Aquaculture development in recent decades required improvements of the main aspects of production, such as, genetic breeding, good management practices, health, nutrition, and production systems. This fact led to an increase in the production of the faster growing Nile tilapia (*Oreochromis niloticus*), with bigger and better filets, resulting in a higher demand of consumers seeking more balanced diets and better nutrition (Braz, 2022).

The culturing technology for the Nile tilapia has garnered particular attention in recent years, though other major areas still need further research, such as the strategic utilization of nutritional

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additives that help the fish cope with unfavorable environmental conditions for growing, and are impacted by the most common etiological agents (Zambrano, 2021). However, there is ongoing search for alternatives that contribute to better production yields and a stable nutritional profile, with fewer diseases and higher income, making production more organic and sustainable (Costavalo, 2021).

Nutritional additives are usually added to the fish diet as an alternative to improve fish immunity, by using the nutrients in the feed for satisfactory animal performance. One example of this group of additives is probiotics, which are supplied as feeds for probiotic bacteria, along with the synbiotics, a combination of probiotics and prebiotic (Braz, 2022).

Since this line of research related to biotechnology is particularly helpful to aquaculture in Cuba, the aim of this paper was to gather information related to the utilization of immunostimulant additives in tilapia (*Oreochromis ssp.*), and their physiological performance in the organism.

DEVELOPMENT

Aquaculture

Aquaculture is a productive activity that provides considerable amounts of food to the world population, and has contributed to global development in that it favors rural area development with new jobs. In the present-day scenario, most nations underwent the negative effects of COVID-19. The United Nations Food and Agriculture Organization (FAO) is trying to assess the overall impact of the pandemic, both on production and consumption, and fishing and aquaculture sales (Gutiérrez, 2021). Even though, several authors have referred to the fact that the "scientific advances of the last 50 years have caused improvements in the know-how of aquatic systems, and the general awareness of the need to manage them sustainably" (Méndez *et al*., 2021; FAO, 2020).

Throughout the history of economics, the growing demand for foods has continued despite the impressive production increases of proteins from aquatic species in the last 40 years. There is an apparent remarkable contrast between the developed and developing countries in terms of technological advances, production efficiency, and the reduction of environmental effects (Pérez *et al*., 2020). Tan *et al*. (2019) said that the aquaculture activities have the "potential of contributing to the world economy, and meet part of the growing demand of seafoods."

According to Ramírez *et al*. (2018), the Cuban freshwater aquaculture began with the construction of dams and the introduction of exotic species with a high commercial value, as the country ranked third in Latin America by 1990. For decades, new development programs were designed in order to increase the quality of production. However, these initiatives were strictly centralized and lacked comprehensive feasibility studies and a strategic vision of the business setting capable of adjusting development policies to the real and complex social problems.

In Cuba, freshwater aquaculture is represented by cyprinids, catfish, and tilapias. Several authors have referred to the gross capture volume in the sector, around 60 900 tons, including freshwater fish as well as marine species. Hence, intensive (ponds) and extensive aquaculture (dams) provide more than 20 tons every year, on average, though the domestic demand is still unmet, nor close to the potential of the region (Méndez *et al*., 2018).

Tilapia Basic information

Tilapia has gained relevance for human nutrition in this century thanks to a relatively shorter growing time compared to other species, high adaptability to different environments, and low cost. Tilapia is one of the most commonly sold species internationally for its lean meat, easy filet preparation, few bones, mild taste, and cooking versatility. Considering the high protein deficiency of humanity, and the fact that there are sanctuary markets that demand low-cholesterol diets, tilapia has become a viable alternative for its productivity and nutritional composition (Méndez *et al*.,2018).

Some authors (Zavala-Leal and Ortega, 2021) refer that tilapia is the colloquial name given to several species of genera *Oreochromis and Tilapia*, though in some regions they are also known as bream. These fishes grow in fresh water and originated in Africa and Asia, particularly in the Middle East. In the nineteenth century tilapia was grown in Africa and Asia, especially in Malaysia. Then, its culture extended progressively.

In the 1970s, aquaculture began to grow gradually, contributing to the world nutritional stability, as tilapia is the second most relevant set of fishes worldwide, behind the Chinese carps (Opiyo *et al.,* 2019; Andrade, 2021). Tilapia is grown in more than 100 countries, and it is second in terms of production yields. Despite the easy conditions for tilapia aquaculture, and that this species is relatively tolerant to environmental stress compared to other species, the incidence of diseases caused by bacteria is frequent, and is rising as a result of the worsening conditions of water quality due to intensive culture (Tan *et al.,* 2019; Shourbela *et al.,* 2021).

Basic aspects of the immune response

The immune response of bony fishes is well developed and integrated; it usually works efficiently, though as any other physiological system, the immune system of an individual is affected when their health status is deficient. In population terms, when the conditions are adverse, the risks of infection are higher, threatening the health of a group of specimens. Proper response can be influenced by a series of elements influence, and on occasions it is significantly blocked.

In keeping with the rationales of various authors, Del Barco (2020) referred that the immune system of fishes is generally similar to that of the higher vertebrates, though there are some significant differences. The immune response of all vertebrates, including fishes, may be split into two: innate or non-specific response, which consists of a series of ancient phylogenetic mechanisms that can remove the pathogens from the body or block their entry in an unspecified manner; and the combined or specific immune response that may be induced, and requires the presence of a group of cells with a specific reaction to the inducer antigen, and the main cells of this mechanism are the lymphocytes.

Fishes, like the rest of vertebrate organisms, conserve ancient humoral immune mechanisms for natural or innate immunity. Some of these mechanisms are the capacity to produce antimicrobial peptides, proteolytic enzymes, acute phase proteins, and the complementary system. Moreover, unlike invertebrates, from the fish on, the classic cells of innate immunity can be identified, such as monocytes/macrophages, neutrophils, and eosinophils, which have similar action mechanisms to mammals, like phagocytosis, peptide exocytosis, and other mediators, along with the release of extracellular neutrophil traps (Dawood *et al.*, 2020).

Besides the tissues and organs with specific functions linked to chemotaxis, fishes have other defense mechanisms. In that sense, Cavalcante *et al*. (2020) referred that the intestinal microbiota plays a significant role in the immunological system, modulating the maturation of the lymphoid tissue associated to the intestine, and that surface acts as the first line of defense against the invasion of pathogens through the B and T lymphocytes, macrophages, dendritic and calceiform cells, granulocytes, and mastocytes with eosinophil granules.

Nowadays, the tools to employ these mechanisms efficiently are prophylaxis and biostimulation. The variability of etiological agents is higher, so among the factors that hinder aquaculture is the absence of prophylactic immunotherapeutic tools that can reduce the effect of diseases on the cultures, that not only favor the functions of the immune system, but also the metabolic factors and growth rate, and are environmentally friendly (Méndez *et al*., 2021).

Innate immune system

Moreover, unlike invertebrates, from the fish on, the classic cells of innate immunity can be identified, such as monocytes/macrophages, neutrophils, and eosinophils, which have similar action mechanisms to those of mammals, like phagocytosis, peptide exocytosis, and other mediators, along with the release of extracellular neutrophil traps (Girón and Toledo, 2019). Furthermore, Braz (2022) claimed that the intestine may act as the first barrier against infections caused by pathogens, since there is a microbial community made of facultative and/or compelling aerobial and anaerobial bacteria that collaborate to strengthening the immune system, besides being the place where most nutrients, ions, and water of the diet concentrate, along with the digestion products, which remain in a solution, thus enabling absorption.

Adaptative immune system

The second line of defense, as in any other vertebrate, is adaptative immunity, though the fishes lack bone marrow or lymphoid nodules, the thyme and spleen take over this role. Adaptative immunity may be split into cellular and humoral, and it is T and B lymphocyte dependent, respectively (Andrade, 2021).

Factors that cause aquaculture tilapia diseases

As in other countries, in Cuba, tilapia is cultured in intensive and semi-intensive systems, usually in river or dam water, where the nutritional requirements are met through man-made diets and other local additives, but under high sowing densities and limited water space. Accordingly, the species are under constant stress, which means low growth rates, nutritional deficiency, and low survival (Méndez *et al*., 2021).

In Cuba and other countries that grow tilapia, intensive culture is the most appropriate approach, though intensive cultured fishes may be stressed out as a result of poor water quality, hypoxia, bacterial infection, etc. Consequently, the immune system is suppressed, creating higher risks under these conditions (Abdel *et al*., 2019).

The predisposing causes of high-stocking rates may lead to the emergence of microorganisms like *Aeromonas hydrophila* and *Streptococcus iniae,* which as referred to by Cavalcante *et al*. (2020), are typical pathogens that cause hemorrhagic septicemia in tilapia, among other fish diseases. In recent decades, these diseases have caused significant mortality in cultured fishes, and significant economic losses to developing countries like Cuba, and therefore, they have become a significant potential threat to sustainability and development of tilapia aquaculture.

IMMUNOSTIMULANT ADDITIVES

Physiological impact of immunostimulant additives

Many of the existing immunostimulants are habitual nutrients in the diet, such as polysaccharides, proteins or lipids, which at high concentrations can produce a stimulating effect. These additives stimulate certain physiological mechanisms that speed disease resistance through specific or unspecific mechanisms of immune response, making them primary prophylactic agents, so the flaws of immunostimulation depend on the development stage of the immune system associated to target organisms (Costavalo, 2021).

In the history of animal growing systems, additives have played a major role in their diets. Traditionally, antibiotics and chemicals have been commonly used to treat or prevent disease outbreaks in aquaculture species. However, overuse of antibiotics and chemicals have led to a rapid propagation of drug-resistant pathogens in aquaculture environments, and residual antibiotics in aquatic products. Besides, the inclusion of antibiotics for prophylaxis and treatment causes intestinal dysbiosis and induces resistant bacterial populations in fish, which might result in the reduction of nutrient metabolism, immunity, and disease resistance (Tan *et al.,* 2019).

Presently, there are restrictions to the utilization of antibiotics, thus preventing possible damage to aquaculture productions and human health as a direct effect. An alternative to the issue has been reported in several studies that recognized the appearance of new additives, and to evaluate the feasibility of prebiotic and probiotic use in the diet. When these additives are used in the correct amounts, they improve the health of the organisms, with a higher production performance (Tachibana *et al*., 2020; Alvarenga, 2021).

Méndez *et al*. (2021), noted that "the utilization of biostimulants in the fish diets is a promising strategy to reduce antibiotic use, and to promote biochemical and immune response, thus contributing to higher productive yields and lower economic losses." In the same direction, Cavalcante *et al*. (2020) explained that the association of technologies applied to nutrition and animal health maintenance are well accepted in aquaculture, as modern studies have demonstrated the capacity of nutrients and feed additives to stimulate fish immunity clearly, and to protect them against the most commonly occurring pathogens.

The inclusion of these immunostimulant additives is linked to an improvement of non-specific immunity, higher disease resistance, and enhanced growth rates. Chemical agents, bacterial compounds, polysaccharides, animal or alga extracts, nutritional factors, and cytokines can be included within this term. Among the most commonly used in aquaculture are β-glucans, lipopolysaccharides, and bacteria with beneficial properties, named probiotics (Gutiérrez, 2021).

The addition of beneficial components to the diets plays a significant role in the manipulation of the intestinal flora responsible for maintaining the health of individuals. The health of microbes is directly influenced by the ingestion of prebiotics. Functional ingredients such as probiotics and prebiotics improve growth, nutritional efficiency, stress tolerance, disease resistance, and the health status of fishes, and therefore, they are increasingly being used to produce feeds for aquaculture fishes (Ali and El-Feky, 2019).

Gutiérrez (2021) stated that temperature changes, management, stress, and high stocking rates could suppress the innate or non-specific immune system of fishes, whereas feed additives and immunostimulants could improve their efficiency significantly. Hence, there is a growing interest in developing natural nutritional additives to overcome the foreseen shortcomings, and maintain ecological fish culture at the production facilities. Feed quality must be enhanced with the inclusion of these natural additives that can favor growth, health, immunity, and productivity (Abdel et al., 2019).

Probiotics

According to various authors (Pérez *et al.*, 2020; Gutiérrez, 2021), probiotics are microscopic agents that were first studied during the second half of the twentieth century, with a significant contribution in terms of preventive and therapeutical uses. The term probiotic comes from the Greek *"pro*" and "*bios*", which mean *for life*. It was first introduced in 1965, and defined as *substances secreted by a microorganism that stimulates another organism's growth.* Eventually, the term acquired a broader meaning, to what is known today as *organisms and substances that contribute to the intestinal microbial balance.*

The enormous potential of probiotics as promoters of fish health relies on their multiple action mechanisms, such as competition against pathogens over adhesion sites, the production of antimicrobial substances in the gastrointestinal lumen to prevent the growth of opportunistic pathogenic microorganisms, the competition over essential nutrients for pathogen growth, and the stimulation of the immune system of the host. One of the numerous common defense actions of probiotics that keep fish health is their role in the modulation of the immune system through the action of cytokines (Tan *et al.,* 2019).

Probiotics act under different modes to stimulate the innate or non-specific immune response through the modulation of humoral immune responses, and the inhibition of growth of pathogenic microbes, that can be used in the strategy for the treatment of bony fishes. These microorganisms are capable of producing inhibiting substances or compounds, mainly chemical substances that can be toxic/bactericidal to other microorganisms, like hydrogen peroxide, bacteriocins, lysozymes, and other similar compounds. The inhibitory compounds are capable of suppressing, or even eliminating common and unusual pathogens. Some of these beneficial bacteria produce organic acids and volatile fatty acids (lactic, acetic, butyric, and/or propionic) that reduce the pH in the gastrointestinal tract, and therefore, inhibit the proliferation of opportunistic pathogens (Xia *et al.,* 2020; Gutiérrez, 2021).

Accordingly, some specialists (Pérez *et al*., 2020) have demonstrated that when these antimicrobial compounds are synthesized and excreted, they become antibiotics, short-chain fatty acids (formic, acetic, propionic, butyric, and lactic), hydrogen peroxide, iron siderophores (chelating compounds), bacteriolytic enzymes (lysozyme), proteases, amylases, bacteriocin-like antimicrobial compounds (BLIS), and bacteriocins, though there are other little studied metabolites with a high antimicrobial activity. The action mechanisms attributed to probiotics are mainly two: 1) The synthesis of antibacterial molecules like bacteriocins: and 2) competitive exclusion, either by inhibiting the adhesion of potentially-pathogenic bacteria to the intestinal epithelial cells (IEC), or by blocking toxin binding.

Through the years and evolution of scientific knowledge, the prebiotics commonly used in aquaculture belong to different groups, generally being lactic acid bacteria or bacterial strain from genera Vibrio, Bacillus, and Pseudomonas. Likewise, though less interesting, genera Aeromonas, Alteromonas, and Flavobacterium, and unicellular algae and yeasts, have been described (Gutiérrez, 2021). Other authors refer that the popularity and efficiency of the most commonly known genera are milk derivatives, such as cheese or other ground sources, like *Bacillus spp*. Y *Lactobacillus spp* (Van *et al.,* 2018, Waiyamitra *et al.,* 2020).

Prebiotics

Prebiotics are generally low digestible carbon hydrate substrates, such as oligosaccharides or diet fiber, which contribute to the proliferation of bacteria in the intestinal flora of animals, resulting in better health and increased productive responses, besides acting closely together with probiotics, the food of probiotic bacteria, since they are not degraded by the direct action of the digestive tract (Mariluz,2020; Alvarenga, 2021; Braz, 2022; Weeet *al.,* 2022).

Triviño (2020) referred to the term prebiotic in relation to the ingredients of non-digestible foods with beneficial effects on the host, creating a selective growth stimulus and/or activity of a limited number of bacteria in the colon. This definition coincides in part with that of diet fiber, though it adds the selectivity of prebiotics over certain types of microorganisms. A prebiotic is a selectively fermented ingredient that permits specific changes in the composition and/or activity present in the gastrointestinal flora, which promotes individual wellbeing and health. So that a food ingredient can be considered a prebiotic, it must comply with three main criteria: initially, resistance to gastric acidity, to enzyme hydrolysis in mammals, and gastrointestinal absorption; followed by fermentation by the intestinal flora; and lastly, the selective stimulation of growth and/or the activity of intestinal bacteria that contribute to health and wellbeing.

According to Anacona (2021), prebiotics can be considered a beneficial diet supplement to improve growth, as it enhances the activity of digestive enzymes, and promotes immune response and increased stress resistance. They are also regarded as a non-digestible compound that, by means of microorganic metabolization in the intestine, can modulate the composition and/or activity of the intestinal microbiota, which confers beneficial effects to the host, like enhanced growth, and nutritional efficiency.

They are carbohydrates classified according to their molecular size, being monosaccharides, polysaccharides, or oligosaccharides. Some examples of prebiotics are inulin or β-glucan, fructoolisaccharides (FOS), oligofructose, and xylooligodsaccharides. Its main role consists in the potential replacement of the intestinal bacterial community by one dominated by beneficial bacteria, thus favoring the colonization of pathogenic organisms. The utilization of prebiotics in aquaculture is recent, when compared to studies done on other ground species. The literature often refers to these substances as non-digested, but that cause changes in the gastrointestinal microbiota, modifying its composition or activity to improve the general health of the host (Gutiérrez, 2021).

Most prebiotic effects take place directly on immunity, as they do not require a specific antigenic response; their effects are attributed to the change in the microbiota, which enable non-specific immunity against a wide variety of pathogens. They act by modulating the composition of the intestinal microbiota, stimulating the growth of beneficial bacteria (Lactobacilli) reported in fishes, mollusks, and crustaceans, while the presence of potentially pathogenic bacteria, such as Vibrio, Aeromonas, and Streptococcus, is limited. Regarding the modulation of the immune system, the effects are indirect, since they mediate changes in the composition and/or activity of the intestinal microbiota (Anacona, 2021).

Among the most commonly used prebiotics effectively in aquaculture as immunostimulants, according to Dawood *et al*. (2020) are β-glucan, frutooligosaccharides, and mannanoligosaccharides. β-glucan has demonstrated promising immune results, favoring phagocytosis, the production of superoxide anions, and lysozyme activity. The utilization of BG diet is a common economic practice that can be adopted by aquaculture farmers at small and large scales, with numerous advantages that go from enhancing growth to increasing immune responses.

Synbiotics

Upon the definition of prebiotics and probiotics, a third element should be defined: synbiotics. They are products in which the prebiotic components act in favor of the probiotic components. The administration of synbiotics benefits the strains supplied, with greater opportunities for colonization and survival in the host's digestive system. Likewise, diverse beneficial effects on the intestinal biota trigger the release of short-chain volatile fatty acids, and perform a protective activity by inhibiting pathogenic microorganisms. Overall, the application of these additives in aquaculture is linked to the biological control of infectious diseases and improvements in the quality of the system's water. It is also seen in animals with a better performance, greater enzymatic activity, and enhanced stress response (Alvarenga, 2021; Prieto, 2021).

Cavalcante *et al*. (2020) claimed that the utilization of probiotics and prebiotics together, named synbiotics, can improve the survival rates, and the modulation of the intestinal microbiota. The positive effect caused by the combination of two or more additives in the diet results in three patterns: activity, synergism, and enhancement. In that sense, the action of probiotic bacteria could be increased by prebiotics, due to the contribution of this component to growth, metabolism, and activation.

The utilization of a symbiotic additive in the animal diet permits increased numbers of probiotic bacteria in the intestine of the host, which, in turn, exerts control over the pathogenic bacteria, improving the immune system and nutrient use. This association enables better enzymatic digestion of the host, in addition to increasing the production of acetic, lactic, and butyric acids (from the fermentation of prebiotic and probiotic bacteria), and the activation of the innate immune system. This mix may act competitively on pathogens, modulating the intestinal environment, and the inflammatory and immune responses (Braz, 2022).

Recent studies on synbiotics suggest greater enzymatic digestion of the host, along with higher production of acetic, lactic, and butyric acids (from the fermentation of prebiotic and probiotic bacteria), and the activation of the innate immune system. Hence, based on the arguments of several scholars, since 2012 the greatest trend in the application of synbiotics in aquaculture has correlated to the positive effects of growth performance. For instance, growth and weight gain, as well as immunostimulant effects. DBA® is a commercial probiotic contaning bacteria *Bifidobacterium*s *spp, Lactobacillus acidophilus* and *Enterococcus faecium,* which can promote several beneficial effects, such as the production of bacteriocins against antimicrobial-resistant pathogens, the stimulation of innate immunity, resulting from greater myeloperoxidase activity of the host, and as a growth promoter (Cavalcante *et al*., 2020).

Knowing the different and complex non-specific immunological mechanisms of bony fishes, especially the commercial species of genus Oreochromis, as a major interest for aquaculture in the world, allows researchers to explore the traits of its physiology more efficiently. The optimization of such mechanisms through immunostimulant additives may generate a positive impact on the production of these species in aquaculture, with improvements in yields and efficiency.

CONCLUSIONS

The inclusion of immunostimulant additives, contributes to the prevention of common tilapia diseases, by means of a modulation of the intestinal environment that enhances the non-specific immune response.

Individual growth, nutritional efficiency, and stress tolerance are favored with the application of immunostimulant additives, thanks to the intestinal microbial balance created in the different species of genus Oreochromis.

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AUTHOR CONTRIBUTION STATEMENT

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

CONFLICT OF INTEREST STATEMENT

The author declares the existence of no conflicts of interests.