ARTÍCULO DE REVISIÓN

Technologies to assess and increase the innate immune response to infections in tilapia (*Oreochromis niloticus*). A review

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Abstract. *Within aquaculture, tilapia is one of the most important species, has great physical resistance, is fast growing and adaptable to various growing conditions, in addition to providing meat of excellent nutritional quality, good taste, low spine and affordable price. However, the intensification in its cultivation has implied the appearance of different infectious diseases. This review of the bibliography is aimed at widening the knowledge on the mechanisms associated to the innate immune response of tilapia (Oreochromis niloticus), emphasizing the use of diverse types of immunostimulants for disease control and the techniques used to validate the results of their application, because infectious processes are still the most important limitations in worldwide aquaculture production systems. The review is structured based on three main subjects: innate immunity in bony fish, applicable biotechnology to stimulate innate immunity, and genomic tools related with the assessment of the immune response in fish.*

Keywords: *tilapia, innate immunity, biotechnology, aquaculture, genomic tools.*

Resumen. *Dentro de la acuacultura, la tilapia es una de las especie más importantes, presenta gran resistencia física, es de crecimiento rápido y alta capacidad de adaptación a diversas condiciones de cultivo, además de brindar una carne de excelente calidad nutricional, buen sabor, poca espina y de precio accesible. No obstante, la intensificación en su cultivo ha implicado la aparición de*

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enfermedades infecciosas, mismas que son la mayor limitante en la acuicultura a nivel mundial. La presente revisión bibliográfica tiene como objetivo ampliar el conocimiento sobre los mecanismos asociados a la respuesta inmune innata de la tilapia, enfatizando el uso de inmunoestimulantes de diversos tipos para el control de enfermedades y las técnicas utilizadas para validar los resultados de su aplicación. La revisión se estructura con base a tres temas principales, inmunidad innata en peces óseos, biotecnología aplicable a la estimulación de la inmunidad innata y herramientas genómicas relacionadas con la evaluación de la respuesta inmune en peces.

Palabras clave: *tilapia, inmunidad innata, biotecnología, acuicultura, herramientas genómicas.*

INTRODUCTION

Aquaculture is undoubtedly one of the productive activities with the highest economic and social impact, as it is aimed at satisfying the increasing need of animal protein for human consumption through farming of tilapia, trout, catfish, blue-fin tuna, shrimp, among other species, with the consequent generation of economic revenues and employments, and contributing to improve the quality of life of the populations in different regions of the world (Cuéllar *et al.*, 2018). Tilapia is considered as the second most important species in aquaculture. It is a fish endemic to Africa, and is currently cultivated in the Americas, Southeast Asia, some countries of Europe, and even in Australia, because it has a high physical resistance, which allows it to develop in poorly oxygenated waters, tolerates large salinity ranges, grows fast, has a high reproductive capacity, and adapts well to living in captivity, as well as at high densities. Besides, it offers a high nutritional quality meat, of a good taste, scarce fish bones, and is accessible cost-wise (FAO, 2016). Notwithstanding, intensification of its production has led to the appearance of infectious diseases.

During the past decades, the traditional use of antibiotics was the main strategy to confront these diseases, this practice has been very much questioned due to the potential development of antibiotics-resistant bacteria, the presence of antibiotics in food, destruction of microbial populations in the aquatic environment, and the suppression of the immune system in aquatic animals (Ringø *et al.*, 2018).

In response, an impressive industry of additives has been generated, particularly of immunostimulants for the control and prevention of infectious diseases. According to the FAO, it is expected that the aquaculture additives market will reach more than 22-thousand millions of US-dollars at the end of 2020. Among these additives are immunostimulants (e.g., prebiotics, probiotics, and vaccines), which can be of various origins:

chemical agents, animal and vegetal extracts, fungi, bacteria, and yeasts, among others (Ruiz *et al.,* 2018).

Innate immunity is a very important defense mechanism in fishes, because they require mechanisms to protect themselves against a large variety of microorganisms immersed in their surrounding environment; consequently, this immunity is influenced by diverse factors both internal and external. Regarding internal factors (depending on the organism per se), these are age, nutritional status, stress, hormonal levels, and sexual maturation cycles (Chen *et al.,* 2019). Regarding the external ones (attributable to the environment), these are temperature, salinity, pH, oxygen level, organic load of pollutants in the water, and handling of the organisms (Malmstrøm *et al.,* 2016).

The discovery of the DNA at the end of the XX century and, later on, the studies on the mechanisms that code the genetic information have opened the possibility to understand many of the response processes to environmental alterations or additives in foods by means of molecular tools (e.g., genes expression). Within the molecular tools, the expression of genes allows identifying transcriptional changes as a response to environmental factors or stressors (Zaha *et al.,* 2014). Hence, an efficacious tool is available to identify the key factors for the survival of fishes from their initial developmental states and, with it, to establish the bases to design specific programs for the control of diseases in aquaculture (Plumb, 2018).

Immune system of bony fish

In bony fish, the group to which tilapia belongs, immunity is achieved through two mechanisms: innate immunity and acquired immunity, the latter is considered poorly efficient, because being poikilothermic (cold blooded) organisms they depend strongly on the innate response, hence, the latter is considered uttermost important in fishes (Lizárraga *et al.,* 2018). It must be pointed out that other groups of fishes, like lampreys or hagfishes (Myxini) only present innate immunity.

Regarding differences between the immune system of fishes and mammals, one of the most striking is the lack of bone marrow and lymphatic ganglia. Traditionally, in bony fish, the thymus, kidney, and spleen are considered analogous to the bone marrow and lymphatic ganglia, and as the main organs of the immune system in fishes (Table 1). On the other side, recent research considers the microbiome (the complex formed by the microorganisms of a specific ecological niche) as a new organ/tissue, pointing out the diverse functions related to the immune response in which it participates (Barko *et* *al.*, 2018; Sebastián and Sánchez, 2018). The microbiomes of fishes comprise a diverse community of protists, yeasts, bacteria, and archaea that inhabit the skin, gills, and the intestinal tract (GI) and are influenced by diverse factors like temperature of the water, seasonality, genetics of the fish per se, and the diet (Merrifield and Rodiles, 2015).

Table 1. Main organs of the immune system of bony fish and their functions

Organ	Function related to immunity	Ref
Thymus	Its principal function is the differentiation and selection of T lymphocytes.	1,2
Kidney	Contains many macrophages and B lymphocytes. Due to its large hematopoietic capacity, it is considered analogous to the bone marrow of mammals.	1,2
Spleen	Has similar function to the kidney with emphasis on antigen presentation and induction of the adaptive immune response.	1,2
Mucosa- or gut-associated lymphoid tissue (MALT and GALT)	Tissue considered with important defensive functions; constituted by different cell types associated with the immune response, like lymphocytes, plasmatic cells, macrophages, and some types of granulocytes.	1,2
Microbiome	Participates in endocrine signaling, prevention of colonization by pathogens, and regulation of the immune function.	3

Fernández *et al.*, 2002; Vega *et al.*, 2010, Barko *et al.*, 2018.

Innate immunity

The innate response includes all the components present in the body before the appearance of the pathological agent, among these components are the skin as a physical barrier, the complement system, antimicrobial enzymes, interleukins, interferon, and cells like granulocytes, monocytes, macrophages, and non-specific cytotoxic cells (NCC) (Biller and Urbinati, 2014).

This defense mechanism is characterized by a series of germline-encoded pattern recognition receptors. These receptors recognize two types of molecular patterns: those associated to pathogens (PAMPs) like glycoproteins and lipopolysaccharides (LPS) of bacteria and fungi, bacterial DNA, viral RNA, and other molecules that are not normally on the surface of multicellular organisms; on the other hand, the molecular patterns of the host per se that result from the tissular damage due to an infection or trauma, necrotic changes, or programmed natural cell death, but which are not normally expressed on the cellular surface (Wangkahart *et al.,* 2019).

The innate response is constituted by cellular, humoral, and tissular components; this system acts as the first line of defense against a large variety of external agents, operating in a non-specific way against molecules of both antigenic and immunogenic origin. In inferior invertebrates like fishes, the innate response is highly relevant because the acquired or specific response acts relatively slow when facing an infection (Malmstrøm *et al.,* 2016) The skin, gills, and intestine act consistently as surface barriers against parasites, bacteria, and fungi; in fishes, the humoral innate response acts through several soluble components in body fluids, this includes the production of numerous antibacterial compounds (lysozyme), transferrin. Acute phase proteins (reactive C protein), cytokines like the tumor necrosis factor alpha (TNFα), interleukins (IL), the inflammation process, the complement mainly activated through an alternate pathway, and phagocytosis (Chen *et al.,* 2019). At the cellular defense level, the nonspecific immune system of bony fishes includes mobile phagocytic cells (macrophages and neutrophils), granular eosinophilic cells that are less mobile and are found in mucosal sites like the intestine or gills (analogues to the mast cells in mammals), and the non-specific cytotoxic cells (NCC); the latter are usually described as granular lymphocytes and are equivalent to the natural killer (NK) cells in mammals. The innate response comprises three defense mechanisms: inflammation, phagocytosis, and non-specific cytotoxicity (González *et al.;* 2020)

Although the components of the innate response detect the invading pathogens at the infection site through their pattern recognition receptors, generating antimicrobial and pro-inflammatory responses that slow down the infection, they also start the process of pathogens presentation to the lymphocytes and help to activate the humoral and cellular responses that will try to resolve the infection (Owen *et al*., 2014).

Acquired immunity

In general terms, the acquired immunity is constituted by two elements: the humoral response (involves the production of antibodies), which is mediated by B lymphocytes, and the cellular response mediated by T lymphocytes (Yamaguchi *et al.,* 2019).

The acquired response is characterized by the immunological memory that leads to a faster and more pronounced immune response after a secondary exposure to the same antigen; this exposure leads to the stimulation of a small group of lymphocytes that recognizes that antigen through specific receptors inducing a change in the lymphocytic population, so that, in the next encounters with the antigen, the response will be higher and faster. In fishes, lymphocytes constitute the main population of leukocytes. B lymphocytes are responsible for the production of antibodies and they have been attributed phagocytic function both *in vivo* and *in vitro* in some studies in teleosts (Smith *et al.*, 2019). T lymphocytes are responsible for cellular death and the regulation of the immune response through the secretion of cytokines; both cellular populations are located in tissues, like the kidney, spleen, intestine, heart, and blood

Previously, in fishes, only the presence of the IgM immunoglobulin was known, however, more recent research has reported the presence of IgD and IgT also called IgZ (Yamaguchi *et al.,* 2019); these isotypes have not yet been characterized completely in terms of their function, it is known that IgD could probably functions as a receptor and it is located only in the cell membrane of B cells (Smith *et al.,* 2019). The IgT/IgZ is related to an anti-pathogenic function in the intestine and the mucosal tissue; IgT is expressed as a monomer in the serum of the rainbow trout and as a tetramer in the intestinal mucosa (Zhu *et al.,* 2013).

Applicable technology to the stimulation and study of immunity in aquaculture

Immunostimulants

As the name indicates, immunostimulants strengthen the immune response (mainly the non-specific) against infectious diseases. In general, they are defined as natural or synthetic compounds that modulate the immune system by increasing the host's resistance to diseases that, in most cases, are produced by pathogens (Zhang *et al.,* 2018). They can come from different sources, like chemical agents, bacterial components, animal extracts, vegetal extracts (polyphenols), nutritional factors, cytokines, yeasts, fungi with mycelia

(Ruiz *et al.,* 2018). Many of these immunostimulants are habitual nutrients of the diet, like polysaccharides, lipids, proteins, vitamins, and minerals that, administered at concentrations higher than the normal ones, will produce a stimulating effect (Carbone & Faggio, 2016). Table 2 depicts some examples of immunostimulants used in aquaculture.

The most common method to supply immunostimulants is through their addition in the diet, which offers many advantages as it results less costly and does not imply handling of the specimens (Vásquez *et al.,* 2012); however, their effect will depend on the receptors of the target cells that will recognize them as molecules of high potential risk and will unleash the corresponding defense routes (Peso *et al.,* 2012).

Modulators of the microbiota

The intestinal microbiota is known as the population of microorganisms that inhabit the intestine (Castañeda, 2017). In terrestrial animals, the initial source of bacterial colonization is the maternal microbiota, whereas in aquatic animals it is determined by the contact with the surrounding water, this microbiota influences directly the health and disease vulnerability of fishes (Puello *et al.,* 2018). The GI microbiota plays a critical role in the development and maturation of the gut-associated lymphoid tissue (GALT), which, in turn, mediates a large variety of immune functions in fishes (Wang *et al.,* 2018).

Prebiotics and probiotic microorganisms stand out among the modulators of microbiota used in aquaculture. Prebiotics are ingredients that are not digestible by enzymes, acids and salts produced during the digestion process of animals, and they influence beneficially the intestinal microbiota (Song *et al.,* 2014; Carbone & Faggio, 2016). Some characteristics they must have are: a) to be a natural product, non-hydrolysable nor absorbable in the upper digestive tract, b) be able to modify the composition of the microbiota after being selectively fermented by one or several bacteria; c) be able to stimulate selectively the growth and/or activity of those bacteria that contribute to the health of the host (Castañeda, 2017). Table 2 presents some prebiotics used in aquaculture.

On the other side, the use of probiotic microorganisms is constantly increasing in aquaculture; for it, a definition more agreeable to aquaculture has been developed: "food supplement constituted by living microorganisms that must be supplied at adequate densities and media to produce beneficial effects in the host, modifying the microbial community associated with it or the cultivation environment, ensuring the use of the feed or increasing its nutritional value, improving the response to diseases or the quality of the environment, and all leading to a better growth and higher survival of the animals being cultivated" (Wang *et al.,* 2020; Dawood & Koshio 2016; Ramírez *et al.,* 2019).

Table 2. Examples of immunostimulants used in aquaculture

Immunostimulants and their origin	Action mode	Ref
β-glucans, present in plants, algae, bacteria, fungi, and yeasts	Activate leukocytes, stimulate phagocytosis cytotoxic and antimicrobial activity, modulate production of pro-inflammatory mediators	1
Polyphenols, Green tea, onion extract, grape extract, corn silk	Reduce stress, improve the innate immune system of omnivore, herbivore, and carnivore fishes. Increase lysozyme activity and of the respiratory burst	2,3
Vitamins (C, E, A, D), carotenes, minerals (zinc, copper, manganese, cobalt, iodine, fluorine, among others)	Affect hematological parameters and of the non-specific immune response (phagocytic activity and respiratory burst).	4,5
Pathogens-Associated Molecular Patterns (PAMPs), lipopolysaccharides (LPS), capsule glycoproteins, and muramylpeptides.	They bind to receptors of cells like macrophages or eosinophilic granular cells, this binding activates intracellular signaling pathways and triggers the immune response.	6
Macroscopic fungi containing immunomodulating properties, like lectins, polysaccharides, terpenoid proteins, vitamins, and minerals.	Promote the proliferation of diverse leukocytes, secretion of cytokines, improve the phagocytic activity, production of ROS	7
Prebiotics: manooligosaccharides, fructoligosaccharides, inulin, galactoligosaccharides, polysaccharides.	Regulate the microbiota of the GI and stimulate the immune system.	8,9

Petit & Wiegertjes, 2016; Lizárraga *et al.*, 2018[2]; Catap *et al.*, 2015[3]; Guimarães *et al.*, 2014[4]; Rondón *et al.*, 2004[5]; Vásquez *et al.*, 2012[6]; Ruiz *et al.*, 2018[7]; Song *et al.*, 2014[8]; Tiengtam *et al.*, 2015[9]

Exposure of an organism to a bacterium unleashes a reaction from the immune system. Probiotic bacteria have been shown to have the capacity of stimulating the immune system of fishes (Ringø *et al.*, 2018; Dawood *et al.*, 2019), they have been classified as immunostimulants of biological origin when they increase the secretion of lysozyme (Song *et al.*, 2014), the production of antibodies, the activation of macrophages, and the proliferation of T cells (Panigrahi *et al.*, 2007).

Traditionally, the acid-lactic bacteria like *Lactobacillus casei*, *Lactobacillus farciminis, Lactobacillus plantarum*, *Lactobacillus rhamnosus,* and baccteria of the *Bacillus* genus, like *Bacillus cereus var. toyoi, Bacillus licheniformis, Bacillus subtilis*, *Bacillus megaterium,* have dominated the production and administration in terrestrial organisms and have also been used widely in aquatic organisms (Dawood *et al*., 2019). However, in aquaculture, a diverse range of microorganisms have been considered as potential probiotics, some of them are the *Enterococcus*, *Streptococcus*, *Pediococcus*, *Saccharomyces*, *Weisella, Vagococcus, Rhodococcus*, *Micrococcus*, *Leuconostoc, Vibrio, Carnobacterium*, *Shewanella*, *Aeromonas*, and *Leuconostoc* genera. Although some of these taxa have been associated with pathological events but only some specific strains and conditions; in addition, many of these have been isolated directly from the organism in cultivation (Ringø *et al.*, 2018; Tan *et al.*, 2019).

The mechanisms by which probiotics can modulate some aspects related with the innate immune response are the expression or pro-inflammatory genes and the expression of receptors in the mucosa that trigger the non-specific immune response (Petit & Wiegertjes, 2016), in addition, probiotics increase the levels of cells and proteins related to the non-specific immunity, produce antimicrobial substances, and present antagonism against pathogenic organisms (Kelly & Salinas 2017). Table 3 shows some of the strains used on *O. niloticus* and the effect produced on immune response parameters.

It is considered that the effect of probiotics is more efficient when these microorganisms have been isolated from a species to which they are to be administered, because there is a better adaptation of bacteria in the intestine (Ramos *et al.*, 2017). Although the use of probiotics in aquaculture is promising, more research is to be done to assess their effects as immunostimulants.

Table 3. Some probiotic strains and their effect on the immunity of *O. niloticus*

Strain	Source	Effect	Ref
Bacillus pumilus	Farmed fish	Increases the phagocytic activity and levels of superoxide anions leading to a more effective resistance to streptococcosis.	1
Lactobacillus acidophilus	Not specific	Increases lysozyme secretion and improves neutrophils adherence, as well as resistance against pathogens	2
Lactobacillus rhamnosus	Humans	Increases intraepithelial lymphocytes, acidophilic granulocytes, increases complement activity and TNFα e IL-1.	3
Pediococcus acidilactici	Laboratory	Increases serum levels of lysozyme and leukocytes; competitive effect in the intestine.	4
Lactobacillus plantarum AH 78	Marine environment	Induces a significant regulation of genes of cytokines, IL-4, IL-12, and IFN-γ	5

Srisapoome & Areechon, 2017[1]; Aly *et al.,* 2008[2]; Pirarat *et al.,*2011[3]; Ferguson *et al.,* 2010[4]; Hamdam *et al.,* 2016[5]

Vaccines in aquaculture

Vaccination is an alternative method to control diseases and stimulate the immune system; however, this method is more complex in aquatic animals as compared with other species due to the aquatic environment in which they develop (Kahieshesfandiari *et al.,* 2019).

Vaccines in aquaculture are generally supplied through an intraperitoneal (IP) injection, bath or immersion (BI), or administered orally. The IP is considered the most effective inoculation method, with the disadvantage that its administration is difficult and can damage the fish. In contrast, BI is less efficient and requires a large amount of vaccine to be used (Yao *et al.,* 2019). In the past, oral vaccines were considered poorly effective because of the degradation of the biological material in the gastric compartment of fishes, which gave rise to a short and deficient immune response. Currently, advances in biotechnology have allowed the development of oral vaccines that present several benefits like diminished stress levels in fish, minimal handling of fish, a simple, easy, and adequate administration method for massive vaccination; besides, oral vaccination is applicable to fishes of all sizes, and is more profitable than other methods (Kahies-hesfandiari *et al.,* 2019).Commercial vaccines for aquaculture are available for species of high economic interest like: salmon, trout, channel catfish, European sea bass and sea bream, tilapia, and the Atlantic cod, most of them are focused on fighting bacterial diseases and only a few are focused on viral diseases (Dadar *et al*., 2017).

For tilapia, research has focused on the development of vaccines against *Aeromonas hydrophila* (Bactol *et al.,* 2018) and, mainly, against *Streptococcus agalactiae,* these vaccines can be of different types, attenuated, inactivated, or recombinant DNA (Liu *et al.,* 2016). Regarding the effects of vaccines on the innate immune response of tilapia, Zhu *et al.* (2017) supplied an oral vaccine of recombinant DNA against *S. agalactiae* and reported a significant increase in diverse components of the innate immune response, such as in total serum protein, activity of the superoxide dismutase, lysozyme concentration, concentration of the C3 complement in serum, serum antibacterial activity, and TNFα.

Genomic tools to assess the presence of genes of the innate immune response

Genomic technologies were developed to study the structure, organization, expression, and function of the genome, to select and modify genes of interest to increase the benefits for diverse activities. The fast advance of these molecular tools and the gradual descent in their costs have allowed their use by sciences akin to aquaculture to learn more on the biological processes of the whole genome, about the genetic structure of populations, their local adaptation, evolution, and phylogenetics. They have also allowed the development of eco-toxicological studies, studies on the immunology of fishes, and the genetic expression in different tissues and developmental and/or sexual stages (Kumar and Kocour, 2017).

New generation sequencing (NGS) technologies have been diminishing not only in costs but also in the amount of sample required (DNA, RNA) and the running times, being second generation platforms like HiSeq, NextSeq, and MiSeq (Illumina, Inc.) some of the most utilized; currently NGS are in the third (e.g., Ion Torrent Proton/HeliScope) and fourth (e.g. MinION/GridION) generation of these platforms.

The use of genomic technologies in aquaculture started in the 1990s with the First Workshop on Genomics in Aquaculture, celebrated in Dartmouth, MA, USA. This workshop focused on the research of the genome of six species of commercial interest in the USA: salmonids, catfish, tilapia, stripped bass, oysters, and shrimps (FAO, 2017).

Currently, worldwide efforts have allowed knowing the complete genome of at least a dozen of fish species. The relevance of knowing the sequence of the whole genome is that it allows identifying the genes responsible for the traits of higher yields, these genes can be used to program selective reproduction using assisted selection according to specific markers. Besides, the sequence of the whole genome also helps to know the genetic variation in the form of single nucleotide polymorphisms (SNPs), which is one of the fundamental reasons why individuals of the same species act differently from each other. This knowledge allows researchers to approach better the challenges related to the conservation of wild populations and warrant the sustainability of aquaculture operations (Kumar and Kocour, 2017).

Regarding the genome of tilapia, the complete sequence of this species was reported in 2011 by researchers of the University of Stirling, Scotland, however, the first publication to this respect was done by Brawand *et al.* (2014), later on, a re-sequencing was reported by *Xia et al*. (2015), being these works of utter relevance for the development of aquaculture activities with this species (McAndrew *et al.*, 2016).

Regarding studies on the immunity in fish, techniques like RNA-seq, DNA microarrays, real time PCR, expressed sequence tags (EST), transcriptome profile, and digital gene expression (DGE) allow performing analyses of differentially expressed genes and on the regulation of genetic expression leading to information on their functions. Likewise, these studies allow observing the abundance of genetic expression in the corresponding scenario (for example, environmental conditions, development stages, and treatments).

On the other side, NGS allow exploring not only the taxonomic composition (microbiota) of the microorganisms existing in the GI of fishes, but also of the whole set of microorganisms, their genes and metabolites present in the given ecological niche (microbiome), without having to collect, isolate, and cultivate living microorganisms from a microbial community to be observed in the laboratory. Isolation of the DNA in a sample provides information related with the diversity of the microorganisms that strive in that sample and reveals information related to their roles and biological functions (Martínez and Vargas 2017).

These microorganisms perform diverse and relevant functions, they perform an integral role in the health of the host by stimulating the development of the immunological system, helping to acquire nutrients, and eliminating opportunistic pathogens (Tarknecki *et al.*, 2017). Knowledge of the microbiome allows knowing, on one side, the complex relations host/microorganisms and, on the other side, opens the possibility of manipulating the microbiota aimed at diminishing the susceptibility to diseases (Barko *et al.*, 2018).

These tools have been useful to identify the genes implicated in the innate immune response of the tilapia (Zhang *et al.*, 2013; Quiang *et al.*, 2016; Ken *et al.*, 2017; Rather *et al.*, 2018), and have allowed knowing the genes sensitive to diverse infections and identifying the innate defense genes that are expressed in both the absence and presence of a completely developed adaptive immune system. As shown in Table 4, the immunological functions of diverse tilapia genes have been described in different works.

Table 4. Example of some tilapia genes related with the innate immune response

Gene	Function	Ref
Tumor necrosis factor alpha *(tnf-α)*	Related with the acute inflammatory response, promotes phagocytosis, respiratory burst, as well as recruitment and proliferation of leukocytes.	1
Interleukin 1-beta *(il-1β)*	Plays a critical role in initiating the inflammatory response.	2
Interleukin 10 *(il-10)*	Regulates expression of cytokines with pleiotropic effects in immunoregulation and inflammation.	3
Transforming growth factor-beta *(tgf-β)*	Involved in the signaling pathway of the immune response; it plays an essential role in progression of inflammation, especially in wounds healing.	4
Cycloxigenase-2 *(cox-2)*	Moderator of inflammation through the generation of prostaglandins.	2, 5
Heat shock protein-70 kDa (*hsp70*)	Is expressed when stress occurs in the face of infectious organisms or stress due to overcrowding.	6
Transferrin *(trf)*	Associated with the immune system, this gene participates in iron metabolism, which is crucial for cellular proliferation.	7
Heat shock protein-90 kDa (*hsp90)*	Participates in signal transduction, promotes expression of pro-inflammatory cytokines.	8
Innate immune signal transduction adaptor *(myd88)*	Encodes a cytosolic adaptor protein that plays a central role in the innate and adaptive immune response, this protein regulates the activation of numerous pro-inflammatory genes.	9

Roca *et al.*,2008[1]; Kayansamruaj *et al.*,2014[2]; Standen *et al.*, 2016[3]; Zahran *et al.*, 2019[4]; Zhi *et al .*, 2018[5]; Qiang *et al.*, 2016[6]; Rather *et al.*, 2018[7]; Zhang *et al.*, 2013[8]; Ken *et al.*, 2017[9]

Zhang *et al.* (2013), by means of transcriptome profiling and DGE, identified the over-regulation of diverse genes related with the immune system of tilapia, pointing out the *mhc I* gene and thermal shock proteins genes (e.g., *hsp30, hsp90*), as well as pro-inflammatory cytokine genes and genes related to signal transduction (e.g., *il-1B, C-lectin),* before and after infection with *S. agalactiae.* Otherwise, Standen *et al*. (2013), using real time PCR, identified over-regulation of the *tnf* α gene in tilapias subjected to probiotics-enriched feed.

Identifying genes implicated in the immune response is relevant because it means important advances in search of resistant populations, in genetic improvement of reproducers, and fostering new preventive methods to improve the survival and health of fishes.

The NGS technologies produce a large amount of data and open new research areas and pose important challenges for the analysis and interpretation of data; hence, future technological efforts shall also concentrate in the development of better capacities in bioinformatics and computational genomics (Kumar and Kocour, 2017).

CONCLUSIONS

The understanding of the structure and functions of the immune system of the tilapia is essential for the development of new technologies, products, and strategies that will allow fighting the diseases that affect farming of this species. Innate immunity provides bony fish with defense mechanisms that act rapidly and are effective against several types of pathogens, represents the first line of defense, and is responsible for the elimination of most of the infecting microorganisms. This contention response gives time for the development of adaptive immunity, the innate and acquired response are tightly related, together they make more efficient the immunological response of farmed organisms. Immunostimulants, prebiotics, and probiotics are increasingly used to strengthen the innate immune response and fight against diseases in tilapia farming. The use of molecular techniques in aquaculture has increased importantly in recent years, their use allows understanding in depth the biological processes and evaluating more specifically the response of organisms to the diverse treatments. The use of these tools will aid aquaculture to satisfy the increasing need of animal protein for human consumptions and to become consolidated as a productive activity of high economic and social impact.

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