Review

Development of Fish Immunity and the Role of β-Glucan in Immune Responses

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Abstract: Administration of β-glucans through various routes, including immersion, dietary inclusion, or injection, have been found to stimulate various facets of immune responses, such as resistance to infections and resistance to environmental stress. β-Glucans used as an immunomodulatory food supplement have been found beneficial in eliciting immunity in commercial aquaculture. Despite extensive research involving more than 3000 published studies, knowledge of the receptors involved in recognition of β-glucans, their downstream signaling, and overall mechanisms of action is still lacking. The aim of this review is to summarize and discuss what is currently known about of the use of β-glucans in fish.

Keywords: fish; glucan; immunity; feeding; infection; health

1. Introduction

Aquaculture is quickly becoming a crucial food-producing sector. One of the consistent problems is health management, as ever-increasing fish density results in elevated stress often leading to outbreaks of deadly diseases. Besides antibiotics, which use is increasingly monitored and often prohibited, the need to establish new ways of potentiation of immune reaction is clear. One option is the use of β-glucan, which has been tested in fish for decades.

The first report approaching the β-glucan molecule was published in 1946 by Dimler, et al. [1], and the authors successfully isolated the molecule d-glucosan β (1,4) (1,6) from starch. However, the first robust scientific evidence showing that β-glucan affects the immunity was published almost 20 years later in the journal, Science. Wooles and Diluzio [2] injected mice with β-glucan and observed a higher hyperphagocytic activity of the reticuloendothelial system, and the authors associated this response to an increase in the primary and secondary immune responses of mice to sheep erythrocytes. Ever since, the effects of β-glucan have been extensively studied, and a well-known immunomodulator suitable for bath treatment, injection, and dietary administration in vertebrates. For example, the positive effects of β-glucans in immunity have been shown in humans [3], dogs [4], pigs [4,5], cattle [6], horses [7], sheep [8], chickens [9], frogs [10], fish [11]; invertebrates, such as shrimp [12] and crab [13]; and insects, such as bees [14] and drosophila [15].
The effect of β-glucans in the immunity of a wide number of species in general is related to the conserved pathways of defense reactions among the vertebrate species, more specifically related to recognition of pathogen-associated molecular patterns [16]. In this context, fish as the first vertebrate group appearing in evolution after adaptive radiation during the Devonian Period, play an important role in the evolution of the immune responses, and an apparent crossroads between the innate immune response and the appearance of the adaptive immune response [17].

Glucan plays a particularly important role in aquaculture as demand for fish and shrimp increases. Aquaculture is plagued with several problems from environmental pollution to fish diseases, and it is imperative to improve health of farm animals. This is true not only from the commercial point of view, but also from the point of minimalizing the spread of diseases to the outside world. Farming of species can be a vector for disease proliferation in the wild environment. Disease transfer in salmon aquaculture is perhaps the most reported instance of this phenomenon. The disease, infectious salmon anemia, first appeared in Chile in the 1990s, and has since been noted in other environments around the world. The search for natural substances used as dietary feed supplements is ongoing.

In this review, we approached different aspects of the development of the fish immunity system and how it can be associated with the effects of β-glucan. In addition, we discussed the application and mechanism of action of β-glucans in fish.

2. The Use of Immunomodulators Compounds

The industry of animal protein production is growing exponentially, and it is inevitable that intensive animal production stresses the animals by confinement, transport, and handling, creating a physiological condition characterized by suppressed immunity and consequently higher susceptibility to disease [18]. Particularly, in the aquaculture sector, the farmer cannot always visually assess the fish and very often the perception of disease outbreaks is a challenge. The indiscriminate use of antibiotic to prevent these outbreaks has resulted in the emergence of several resistant pathogens in aquaculture [19], impeding the development and sustainability of the industry worldwide [20]. In this context, many immunostimulant compounds, such as bacterial lipopolysaccharides (LPS), mannoooligosaccharides, vitamins, minerals, and animal and plant extracts, have been widely investigated to enhance fish immunity and protect against disease [18,21,22]. Among these compounds, β-glucans stand out and their role as a biologically active immunomodulator in fish has been well documented.

3. The Molecule of β-Glucans and Their Effects

“Glucan” is the common name given to a group of polysaccharide polymers, classified based on interchain linkages as either α or β linked. β-Glucans obtained from different sources often have different primary structures and conformations. The primary structure is defined by the glycosidic bond type, as well as degrees of branching and polymerization, while the conformation of β-glucans often presents as a random coil, single helix, or triple helix, and is affected by the primary structure, intermolecular force, temperature, and solvent [23].

β-Glucans are widely distributed in bacteria, algae, fungi, and plants, with different structural types (see Barsanti et al. [24]). Their structure is comprised of a main chain of β (1,3) and/or β (1,4)-d-glucopyranosyl units in nonrepeating but nonrandom order, with side chains of varying lengths [25]. In this context, the different β-glucan molecules may differ in their activity/effectiveness as immunomodulator, and even molecules with similar structures, molecular weights, and solution conformations can differ markedly. Many studies have reported correlations between β-glucan effectiveness and molecular structure, size, branching frequency, structural modification, conformation, and their solubility; however, it is risky to make generalizations due the often-contradictory data [26]. In addition, numerous concentrations and routes of administration have been tested including oral applications. Despite extensive investigations, consensus on the source, size, and other biochemical or physicochemical properties of β-glucans has not been achieved.
Basically, the two types of \( \beta \)-glucan molecules, which are based on the glycosidic bonds present in them, are \( \alpha \)-glucan (dextran with 1,6, starch with 1,4 and 1,6 glycosidic bonds) and \( \beta \)-glucan (cellulose with 1,4, zymosan with 1,3, laminarin with 1,3- and 1,6, lichenin with 1,3 and 1,4 glycosidic bond). Because of the complex structure in \( \beta \)-glucans, they have superior ability to activate the immune response and act as biological response modifiers [27]. Certain characteristics of \( \beta \)-glucan, such as ability to function normally on immune system without over-activating them [28], ability to lower the elevated levels of cholesterol [29–31], and ability to reduce sugar levels [32,33], make it unique among immunostimulants.

\( \beta \)-Glucans are responsible for a multitude of actions, which protect and enhance the immune system and provide optimum resistance to any possible health assailants due to its ability to bind directly with macrophages and other white blood cells (neutrophils and natural killer (NK) cells) and activate them [34,35]. When \( \beta \)-glucan receptors are engaged by \( \beta \)-glucan, all immune functions are improved including phagocytosis (ability to engulf foreign cells and particles); release of certain cytokines such as IL-1, IL-6, GM-CSF, and interferons; and the processing of antigens. These cytokines stimulate formation of new white blood cells, providing immunity to \( \beta \)-glucan binding receptors present in all vertebrates ranging from fish to human [36].

4. Overview of Fish Immunity

Like cartilaginous fish, osteichthyes (commonly named ‘bony fish’) evolved through the Paleozoic Era, mainly during the Devonian. Whereas most early elasmobranchs and holocephalans are extinct, the bony fish continued to evolve in an expanding fashion. At present, the bony fish, in comparison to any vertebrate group, have reached the highest level of their evolution and adaptational capacities in an aqueous environment. Adaptationally, they may be considered as the most successful. Selectional pressures of various water environments, which the bony fish invaded, from fresh waters to tropical and polar seas, have induced a wide range of diversities in their body forms and size, so that they are also the most varied of all vertebrates [37–39]. Since the Tertiary Period, bony fish populations, especially the teleosteans (infraclass Teleostei), have increased, and currently form the most numerous group of all vertebrate taxa, nearly 35,000 species (i.e., comprising about one-half of all vertebrate species) [40].

The main cause of this unprecedented growth of various forms among the vertebrates was the emergence of new tissues and organs that enabled the realization of evolutionary novelty, the adaptive specific immunity with immunological memory. The first foundations of this type of defense of internal environment appeared in chondrichthyans (sharks, skates, and rays) together with the appearance of the jaws, which can be considered the most significant revolutionary event in the entire history of vertebrate evolution [41].

The immune system of bony fish is principally the same as in all advanced gnathostomeans. It is composed from two components, the phylogenetically older innate (nonspecific) immunity and the adaptive (specific) immunity with immunological memory. Contrary to higher vertebrates, survival of fish as the free-living organisms practically from the earliest embryonic stage depends mainly on their innate immunity [42], which remains the predominant component of defense throughout adult life.

The components of the innate immunity are divided into humoral molecules that are freely located in body fluids and cellular components. These include the growth inhibitors, lytic enzymes, agglutinins, precipitins (opsonins and primary lectins), cytokines, chemokines, and antibacterial (cationic) peptides [43,44], including components of complement (see below). This type of immunity is crucial in preventing infection due to slow proliferation and differentiation of immunocompetent cells after antigen stimulation, and limited antibody repertoire leading to a delay in the adaptive immune response especially in lower temperatures [45]. Therefore, the innate immune response acts as an alarm that allows the adaptive immune system time to mount a specific response [46]. The cellular components of innate immunity provide a physical barrier in the form of mucus-producing epithelial cells that line the skin and gills and specialized cells protecting the digestive tract, which are
responsible for preventing penetration of pathogens inside the body. They comprise such cellular types like granulocytes, monocytes, macrophages, two types of NK cell homologues characteristic for fish, the non-specific cytotoxic cells and NK-like cells, and also the nonspecific cytotoxic cells, which kill and digest the pathogenic bacterial and viral invaders [47]. Further cellular components of innate immunity form various populations of blood leukocytes, which produce a row of abovementioned humoral substances that are immediately able to kill altered or foreign (allo- and xenogeneic) cells. To them, it is necessary to take into account the components of complement system, which, as seen in evolutionary advanced vertebrates, can be activated in three ways: the classical pathway (triggered by antibody binding to the cell surfaces); the alternative pathway (triggered independently of specific antibodies only by microorganisms); and the lectin pathway (triggered by the binding of a protein complex consisting of mannos/mannan-binding lectin in bacterial cells [43,48,49].

The adaptive immune system is well defined in mammals, and although most of the basic characteristics are also found in fish, the adaptive system is fairly inefficient due to a restricted antibody repertoire and an extensive lag time, up to 12 weeks after infection for activation [44,50]. In addition, due to their evolutionary status, cold-blooded vertebrates, such as fish, lack certain histologically distinct lymphoid architecture, such as follicular dendritic cells (DC) and germinal centers, which explains why fish are heavily reliant on a strong innate system [36,51–53].

It also plays a key role in the acquired immune response through a system of receptor proteins, which recognize pathogen-associated molecular patterns (PAMP), such as LPS and peptidoglycans, including bacterial and viral DNA and RNA.

The fish adaptive immune response takes place thanks to the presence of well-developed and functionally specialized structures and organs, like in higher gnathostomes, the thymus, kidney, spleen and gut-associated lymphatic tissue (GALT), which form a sophisticated network of highly specialized cells, molecular messengers, and effective factors maintaining the homeostasis of an internal milieu [54].

The thymus together with the kidney (anterior and posterior) and spleen are the largest lymphoid organs in teleosteans [55]. Thymic structure, contrary to higher vertebrates, is highly variable and, in many species, is not possible to clearly differentiate between the cortex and the medulla [56].

The kidney in teleost fish is the equivalent of bone marrow in vertebrates and is the largest site of hematopoiesis until adulthood [55]. The main cells found in the anterior kidney are macrophages, which aggregate into so-called melanomacrophage centers, and lymphoid cells, which are found at all developmental stages (mainly B cells) [57].

The spleen is distinctly divided into white and red pulps, even if structurally less organized. This dividing is very variable. In some species, the red pulp prevails and may include the whole organ, whereas in others, it may be composed only of lymphoid cells and macrophages [58,59]. Fish spleen is a main site of erythropoiesis, phagocytosis, and antibody formation. The splenic tissue contains a system of ellipsoids and melanomacrophage centers. In most species, ellipsoids are clustered together and organized around the other two components [60]. The ellipsoids are thick-walled capillaries that open in the pulp and result from the division of the splenic arterioles. The macrophages along the capillaries are engaged on active phagocytosis of foreign material. A similar splenic structure has been described also in other teleosteans. The appearance of immunoglobulin-producing cells in the ellipsoids approximates them to true germinal centers of endothermic vertebrates; they could represent evolutionary predecessors of germinal centers of mammals [61]. In bony fish, it is generally accepted as a main secondary lymphoid organ, in which a plenty of B cells are activated and differentiate into plasma cells. Plasma cells then migrate to the other lymphoid organs such as head-kidney, intestine, skin, and gills. In the intestine, the distribution of B cells is low and variable among different species of fish [62].

In fish, the aggregations of lymphoid cells, plasmacytes, granulocytes, and macrophages present in connective tissue of the mucosa and infiltrating gut epithelia, including the lamina propria, represent, functionally, the GALT, but without organized structures resembling Peyer patches found in the
mammals. These aggregates of immunocytes play the same role as an effective mammalian GALT. Together with the epithelial cells, these accumulations may form a microenvironment for food antigen collecting such as M cells [63]. Accumulations of lymphoid cells and cells producing antibodies have also been found in areas exposed to pathogens such as the skin epidermis, gills and pharynx, heart, liver, and pancreas. Generally, in the abovementioned organs and tissues, the plasma cells and lymphocytes are ultra-structurally similar to those of other ectotherms and endotherms.

Humoral factors are soluble proteins of the plasma and body fluids. These include transferrin, interferons, protease inhibitors (notably C3 and α2-Macroglobulin), lytic enzymes, proteins of the three complement pathways (classical, lytic and alternative), pentraxins, natural antibodies (NAb), cecropins, and a whole host of cytokine and chemokine signaling messengers, notably IL-1β, TNFα, IL-2, IL-4, IL-6, IL-18, IFN1, IFN2, IFNγ, Th1, and possibly Th2 cytokines [43,44,64–67].

Bony fish are also the first vertebrates in which appeared genes of immunoglobulin superfamily molecules, the TCRα/β TCRγ/δ, β2-microglobulin, major histocompatibility complex (MHC) I class and MHC II class. Vβ, Dβ, Jβ, and Cβ regions are also present. It was suggested that fish TCR may be close in shape to the ancestral molecule [68]. Teleostean B-cells produce IgM (tetrameric), IgD, and IgT (also called IgZ) immunoglobulins but not IgA [69–71]. Immunoglobulins of fish are found in the skin, gut, gill mucus, bile, and systemically in the blood plasma [72].

Finally, it should be noted that physiological reactions, including immunological ones, are temperature-dependent in fish as in ectothermic animals, but are also affected by fluctuations in other external stressors such as salinity, photoperiodicity, oxygen concentration, pH changes, and especially by pollution.

Conversely, several food additives and modifiers of biological activity (especially with proven immunostimulants effects) [73], particularly β-glucans and also nucleotides [74–78], and probiotics [79–81] can enhance overall health of especially those fish species that are farmed in aquacultures.

**Mechanism of β-Glucan Action**

β-Glucans have been proven to be highly efficient stimulators of the cellular and humoral branches in mammals and lately in other species, including invertebrates. The best-known effects of β-glucans consist of the augmentation of phagocytosis of granulocytes, macrophages, and DC. In this regard, macrophages are considered the basic effector cells in host defense. Most of the PAMP studied activate antigen-presenting cells together with native T cells into DC and T helper cells [82–85]. During microbial breakdown/degradation, numerous PAMP may be released initiating inflammatory responses upon receptor binding and intracellular activation of signal transducers and transcription factors.

The initial step of β-glucan–macrophage interaction is binding to specific receptors present on a cell membrane. In most animals, several receptors are involved in β-glucan recognition and binding: toll-like receptor 2 (TLR-2) [86], dectin-1 [87], CR3 (complement receptor 3, CD11b/CD18) [88–90], lactosylceramide [91], and less defined scavenger receptors. The binding has been confirmed not only by inhibition via specific antibodies, but also using KO mice [92].

The CR3 receptor, known also as Mac-1 or αMβ2-integrin, is highly promiscuous pattern-recognition receptors recognizing many other ligands, among them β-glucan.

CR3, known as membrane attack complex 1, is mainly expressed on myeloid cells, such as NK cells, DC, macrophages, monocytes and neutrophils, and functions as an eliminator to clear iC3b-opsonized dodderly/apoptotic cells as well as pathogens [93]. CR3 is a dimeric integrin consisting of αMβ2 (CD11b/CD18), two transmembrane proteins, and can recognize and bind to β-glucans through αM [94]. β2 is responsible for transmitting signal of αM to Syk pathway, resulting in CR3-mediated cytotoxicity (CR3-DCC) [95]. As the first observed β-glucan receptor, it is not surprising that most of our knowledge about β-glucan receptor interaction was gained here. The binding is complement-mediated and requires opsonization by iC3b, as confirmed by detection of iC3b and by direct binding (for review,
see Bose et al. [88]). Details of the role of complement and β-glucan receptors in macrophage activation are summarized by Chan et al. [96].

After establishing CR3 as the primary β-glucan receptor, dectin-1 was recognized as another major β-glucan receptor, present on numerous cell types. Using specific anti-dectin-1 antibodies, several studies found that this receptor is almost exclusively responsible for binding of β-glucan and zymosan [97]. Dectin-1 receptor was also shown to be involved in recognition of pathogenic fungi and in secretion of IL-12. Experiments using antifungal response of NK cells showed that dectin-1 response to β-glucan binding starts IL-12 production by antigen-presenting cells with subsequent trigger of NK cells to start IFN-γ production [98]. Major receptors are shown in Figure 1.

**Figure 1.** Illustration of the general mode of action of β-1,3/1,6-glucan on leukocytes (neutrophils, monocytes, natural killer cells, or macrophages). The β-glucan receptors may change according to the vertebrate species and leukocyte type. Figure kindly provided by Biorigin.

Recently, the focus switched from dectin-1 and CR3 receptors to TLRs, which are receptors with important roles in innate immunity. Curdlan (water-insoluble linear beta-1,3-glucan consisting of β-(1,3)-linked glucose residues and forms elastic gel upon heating in aqueous suspension) was found to act on various cell types via binding to TLR-2. This binding was acting through suppression of expression of RANKL [99].

Although the mechanisms are unclear, the interaction among β-glucan and receptors might depend on factors such as solubility, as only insoluble β-glucans cluster dectin-1 receptors with subsequent expulsion of negative regulators such as CD148 or CD45 [100]. β-Glucan has been found to active microglia via dectin-1, but the same group later described that nonsoluble β-glucan acted via TLR-2 and TLR-4 and stimulated reactions, which were unaffected via dectin-1 [101]. The action via TLRs is probably mediated by suppression of NF-kB activation.

Dectin-1 is a well-researched C-type lectin receptor (CLR) that is responsible for β-glucan recognition and plays an important role in antifungal infection [102]. It recognizes β (1,3) and β (1,6) linked β-glucans and the binding strength depends on the size, linkage type, and branching degrees of the β-glucans [103,104]. This receptor can mediate the activation signal to enhance an immune response and is called a β-glucan receptor. It is expressed on numerous cell types including DC, macrophages, monocytes, neutrophils, and T cells. It mainly exists on cell passageways where pathogens can easily invade and can mediate pathogen recognition and phagocytosis, which play an important role in
host defense [105]. The activation of dectin-1 will also result in DC maturation, ligand phagocytosis, respiratory burst, and arachidonic acid metabolization for host defensive immunity [106–108].

To further complicate the situation, some β-glucan can bind to dectin-1 in combination to extracellular TLR [108]. This process might first involve activation of dectin-1 and subsequent complexation of TLR, as described with TLR-2, TLR-6 [34], and TLR-4 [109]. A detailed study using cells coexpressing dectin-1 with TLR-2, TLR-4, or TLR-5 found not only the differences in activity of soluble and nonsoluble β-glucans, but also that the immune effects of β-glucan differed based on the dectin-1/TLR combination [110]. This might explain at least some of the differences in β-glucan activities. Even less clear claims were raised by Su et al. [111], who found that a (1-6)-(1,4)-β-D-glucan inhibits cytokine production and that this activity is mediated via binding to TLR2 but not to dectin-1 or CR3 receptors. This study is confusing not only due to the rather unusual binding patterns, but also due to the first description of inhibition of cytokine synthesis by β-glucan. So far, all β-glucans either stimulated cytokine production or, as seen with betaefectin, had no effects.

Latest experiments suggested the role of programmed cell death protein 1 (PD-1) immuno-checkpoints and the involvement of c-Maf. Treatment with β-glucan reduced c-Maf expression in M2 macrophages together with reduction of some populations of monocytes. In clinical trials, the same treatment decreased the numbers of inflammatory monocytes and increased the numbers of classical “patrolling” monocytes responsible for regulation of tumor metastases. These data suggest the possible benefits of targeting immunosuppressive macrophages and offer a new look at possible mechanisms of β-glucan action [112]. The pathways in which β-glucans mediate their activity in fish are not fully elucidated but, as expected by the well-conserved innate system, so far appear similar to that of mammals.

Complement protein C3 and lectins (possibly dectin-1 homologues or similar) have been identified as β-glucan pattern recognition receptors, as well as a β-glucan pattern recognition receptors on salmon macrophages and catfish neutrophils [43]. In a model of regulation of a gene expression profile, the typical signaling pathway associated with CLR activation and the identification of several candidate β-glucan receptors suggested that immunomodulatory effects of β-glucan in carp macrophages could be a result of signaling mediated by a member of the CLR family [113]. TLR homologues have also been described in Atlantic salmon, Zebrafish, flounder, goldfish, and pufferfish [44].

However, the situation in fish is less clear. So far, no clear homologue of dectin-1 has been found. A detailed study of β-glucan recognition by fish cells suggested possible receptors belonging to the CLR family [113]. An analysis of the carp genome found 239 genes encoding proteins with some C-type lectin domains, but even after additional analysis, no receptor was found on macrophages. Therefore, even when CLR family is the most promising β-glucan-binding moiety, the exact mechanisms of β-glucan recognition in fish are still unclear. However, effects of curdlan, β-glucan known to bind to dectin-1, suggest the presence of a similar binding side. Detailed studies found several candidates with similar protein architecture. Subsequent mining of the zebrafish genome revealed two genes as candidate β-glucan receptors [113]. With respect to the CR3 receptor, there is only indirect proof of the existence of this receptor in fish.

5. Routes of β-Glucan Administration

β-Glucans can be administered internally and externally in a number of different routes such as intravenous, intraperitoneal, or subcutaneous (parenteral) injections; orally; bathing; or as part of a cream [114–116]. Efficacies of different routes (intraperitoneal injection, bathing, and oral administration) have been tested. In a model with Cyprinus carpio, fish were fed with β-glucan and LPS to investigate survival and immune response after challenged with Aeromonas hydrophila. Intraperitoneal injection showed 100% relative percentage survival at all concentrations of β-glucan, whereas oral administration showed high relative percentage survival at higher concentrations (1% β-glucan + 0.25% LPS), but bathing did not improve relative percentage survival levels [117]. In a model of streptococcus caused by Streptococcus iniae, a formalin-killed vaccine was applied in red tilapia by injection, immersion,
and oral vaccination. The result from the study indicated that the best route regarding efficacy was through intraperitoneal injection and that soluble β-glucan increased further the effectiveness of the vaccine [118].

5.1. Injection

The protective effect of β-glucan injection in a dose-dependent response has been demonstrated in different species against several infections [119–121]. The intraperitoneal injection certainly is an effective method to deliver β-glucan and stimulate the immune system but is not the most practical method. For example, a single dose of β-glucan injected intraperitoneal in rainbow trout resulted in a level of protection against infection with the microsporidian, Loma salmonae, similar to the level of protection induced by a 3-week feeding trial using 10 times higher concentrations of β-glucan. Interestingly, the effects of the single intraperitoneal injection could be measured for a prolonged period of up to 9 weeks in vivo [122] and up to 20 days ex vivo (no further time points measured) [123]. This concept may explain the application of β-glucan as a vaccine adjuvant. Glucan does not need to be a direct part of the vaccine, it can serve as an important supplement, as shown by experiments that found significant enhancement of the immersion efficacy on inactivated herpesvirus vaccine in Gibel carp [124].

Phagocytes could be responsible for long-lived effects induced by β-glucans since intraperitoneal injection with β-glucan leads to an increase in oxidative burst, phagocytosis, and lysozyme activity of macrophages in Atlantic salmon [123]. In addition to this, another study showed that increased macrophage activity was still measurable at 10–20 days post-injection, providing clear indications that single intraperitoneal injections with β-glucans can induce long-lived effects in fish [125].

Due to this immunomodulator action, β-glucans have been extensively studied as vaccine adjuvants or as vaccine delivery systems [126–128]. In a model with Vibrio damsela vaccine, turbot (Scophthalmus maximus L.) were injected prior, together, and post-application of yeast β-glucan. The highest activity among all the immune parameters was obtained when β-glucans were injected after the bacterin application. The finding of this study indicates that the sequence of β-glucan administration is critical in order to use β-glucan as a vaccine adjuvant [129].

Apart from the studies about adjuvant activity of β-glucan in vaccine formulation directly, pattern antigens such as ovalbumin (OVA) or bovine serum albumin (BSA) are commonly combined to investigate the adjuvanticity and mechanism of β-glucans. When microparticulate β-glucan (MG) was covalently conjugated to OVA or BSA and administered to animals, the MG-antigen complex was phagocytosed by DC or macrophages via specific receptors that recognize β-glucan, then resided in vesicles and presented by MHC II to activate CD4+ T cells or by MHC I to activate CD8+ T cells through cross-presenting, and the expression of costimulatory molecules, such as CD25, CD69, and B7, were upregulated to strengthen the activation signals [130].

5.2. Dietary

Orally delivered β-glucans make their way to the gastrointestinal tract, where they must first be captured into the circulation before being conducted the bone marrow. The linear β (1,3) backbone ends up undigested in the proximal part of the intestine, where a proportion is captured by M cells in cooperation with neutrophilic granulocytes or macrophages and degraded by the latter under a reactive oxygen species-driven process [131]. Despite the low systemic blood levels of β-glucans (less than 0.5%), significant systemic immunomodulating effects in terms of humoral and cellular immune responses were demonstrated [96]. In addition, the higher part of the non-digestible β-glucans may induce alterations in the composition of the gut microbiota, thereby indirectly influence the local immune system or the bacterial community in the gut. These effects are most probably manifested through decreasing Firmicutes and increasing Akkermansia populations. This bacterial community may help to digest non-digestible oligosaccharides, such as β-glucans, into short-chain fatty acids with a physiological effect of their own [132]. Taken together, these different paths can help explain part of the
previously described effects of β-glucans. In short, in promoting hepatic glycogen synthesis through
improving IRS/Akt insulin signaling pathway, inhibiting the sodium-glucose linked transporter 1
(SGLT-1) expression in intestines and decreasing blood glucose, suppressing macrophage infiltration in
adipose tissues, and decreasing of TNF-α in blood and muscles [133]. A seven-week supplementation
of carp (Cyprinus carpio) with β-glucan did not stimulate expression of bactericidal innate immune genes
but changed bacterial composition in the gut [134]. Some studies suggested that the effects of β-glucan
might not be strong enough to elevate the immune response of fish. However, the simultaneous
use of oxytetracycline with β-glucan supplementation ameliorated the immunosuppressive effects
of antibiotics and help protect fish against bacterial infections [135]. The stimulation resulting from
β-glucan supplementation is dependent on numerous factors including dose, time of supplementation,
water temperature, and species [136]. In vitro experiments showed the high doses of β-glucan inducted
apoptosis in primary cells isolated from carp pronephros, but the doses routinely used in aquaculture
do not induce apoptosis, but stimulate immune system [136]. The short-term feeding with β-glucan
did not change expression of immune genes in striped catfish (Pangasianodon hypophthalmus), but after
subsequent challenge with Edwardsiella infection, the β-glucan-supplemented group showed significant
stimulation of immune genes in all tested organs [137]. These results suggest that the effects of β-glucan
feeding in healthy adult fish are minimum, but this feeding has immediate effects even 24 h after
infection. Similar results were found in case of juvenile pompano (Trachinotus ovatus), where the effects of
β-glucan feeding to healthy individuals were small, but after infection with Streptococcus iniae, β-glucan
offered significant protection [138]. A model of silver catfish (Rhamdia quelen) and Aeromonas hydrophila
infection offered similar results [139]. In general, these studies demonstrated a lack of β-glucan effects
when fish are in resting and a significant and positive effect when fish are exposed to a disturbance in the
homeostasis, usually by stimuli such as stress [140], immunological challenges by pathogens [137,138]
or chemicals (“immunocompromised”) [141]. Therefore, authors should consider this point to discuss
the lack of β-glucan effect in resting.

The orally delivered pathway is much slower and said to have a less profound effect than
injectable methods. However, this is often a more practical method as β-glucans can simply be added
to food/feed [94,115,141–143]. For example, Rodriguez et al. [140] fed salmon a diet supplemented with
β-glucan and found that the β-glucan diet potentiated the immune response to vaccine by increasing
innate and adaptive immune responses through the transcription of key cytokine genes such as INF-γ
and IL-12.

In sea bass fed with β-glucans for 4 or 8 weeks, pyrosequencing of the intestinal microbiota
revealed a transient alteration at the family taxonomic level in the composition of the autochthonous
microbiota [144]. It took a period of 4 weeks to completely shift the dominance within the microbial
communities, which returned to the original composition after another 4 weeks of feeding. The data
presented in these studies imply that effects of oral administration of β-glucans on the microbial
composition in the gut are present but could be transient and require further investigation. In line with
these findings, the effect of long-term feeding with β-glucans on TLR3 expression in the gut of carp
could also be due to an indirect effect of β-glucans on the composition of the microbiota [125].

Studies investigating the effects of β-glucans on maintaining the integrity of the gut have found no
adverse effects and provide evidence for an assumed favorable increase in frequency of mucus-secreting
cells in the epithelial barrier [145,146]. Approaching this subject, oral administration of β-glucans to
rainbow trout appears to downregulate the expression of immunoregulatory genes (e.g., IL-1β and
lysozyme) in the presence of a microbial stimulus [147,148], but upregulate the expression of such
genes (e.g., IL-1β and cathelicidins) in the absence of a microbial stimulus [146,148]. These apparent
contrasting effects of β-glucans on the expression of immunoregulatory genes, in the presence or
absence of a microbial stimulus, could possibly help explain the variable outcomes with respect to
increased resistance against pathogens [125].

In most of the studies performed on bass species, oral administration of β-glucans not only
increased innate immune parameters, such as phagocytic capacity and oxidative burst, lysozyme
and complement activity [149–152], but also protected against challenge with numerous bacterial pathogens including Aeromonas hydrophila and Vibrio alginolyticus [149,151].

Bagni et al. [153] reported duration-dependent effect of dietary application where significant elevation of serum complement activity in sea bass fed with β-glucans at 15 days was seen; however, serum lysozyme, gill, and liver heat shock protein concentrations were enhanced at 30 days. Long-term use had no significant impact on innate and specific immune parameters, survival, growth performances, and conversion index in treated and control fish. Continuous feeding with β-glucans for a number of subsequent days also appears to induce long-lived effects on the immune system of fish. For example, rainbow trout fed with β-glucans for a period of 2 weeks still showed higher antibody responses after vaccination against enteric redmouth disease and higher concanavalin A-induced proliferation of head kidney derived leukocytes 4 weeks after switching back to a control diet [154]. Grouper fed a diet containing a mixture of mushroom-derived β (1,4) (1,3) and β (1,6) glucan for a continuous period of 12 days still showed higher protection against challenge with Vibrio alginolyticus 15 days after switching back to a control diet [149].

Continuous administration of β-glucans generally appears to result in an increased expression of pro-inflammatory genes, with a gradual decline over time depending on, among others, route of administration and immune organ under investigation [155,156]. Oral administration (25 days) of β-glucans can result in the upregulation of anti-apoptotic genes in gut and head kidney, and of both anti- and pro-apoptotic genes in the spleen of common carp [157]. The effects of β-glucans on apoptosis were further investigated and show that, in vitro, β-glucans can have a significant effect on apoptosis, but only at very high concentrations [136]. Taken together, these findings support the notion that oral administration of β-glucans may modulate the intestinal immune response and protect cyprinid fish from an acute (over)reaction [155,158].

In an in vitro study, head-kidney macrophages of pink snapper (Pagrus auratus) pre-incubated with commercial β-glucan (EcoActiva) and subsequently exposed either by phorbol myristate acetate (PMA) or LPS resulted in significant stimulation of superoxide anions and respiratory burst activity compared to induction of macrophage with EcoActiva alone [159]. The result of this study demonstrates that feeding of β-glucan may enhance the recognition of LPS present in the cell wall of Gram-negative fish pathogenic bacteria resulting in improved killing efficiency of macrophages of these pathogens [160]. In another study, oral administration of EcoActiva in pink snapper increased macrophage O2 radicals especially in wintertime, but no enhancement in classical and alternative pathway activities was seen, indicating wintertime to be the most favorable to feed snappers for disease resistance [161].

Yeast β (1,3)(1,6) glucans have been used for in vitro and in vivo experiments to study degranulation of primary granules in fish neutrophils [162]. β-Glucan supplied to nonstress (NS), acute stress (AS), and chronically stressed (CS) fish showed increased degranulation in NS and prevented decrease of degranulation in AS, whereas in CS fish, degranulation reached NS level after 3 days of feeding in fathead minnows (Pimephales promelas, Rafinesque). These results indicate that β-glucan supplementation to fish diet prior to AS and during CS can enhance neutrophils function and increase disease resistance and survival rate [154].

Dietary supplementation of aflatoxin (AFB1) in fish showed reduced immunity with affected biochemical parameters related to organ damage. Nile tilapia immunocompromised with aflatoxin (200 Lg/feed aflatoxin B1) and fed for 21 days with diet supplemented with 0.5% of β (1,3) glucan showed enhanced resistance against Streptococcus iniae and improved non-specific immunity levels compared to AFB1 non-treated fish. Superoxide anion, myeloperoxidase, phagocytic activity, and hemagglutination
were also increased [164,165], and the authors concluded that the use of β (1,3) glucan as feed supplement resulted in enhanced immune response in immunocompromised fish.

Cyclophosphamide, a multifunctional alkylating agent, as well as a cytotoxic drug and a well-known immunosuppressant, was used to induce an immunocompromised state in Asian catfish (Clarias batrachus) [164,165]. The cyclophosphamide-treated fish showed lowered levels of respiratory burst, myeloperoxidase, and phagocytic activities in blood phagocytes, and decreased hemagglutination activity. β-Glucan delivered as feed supplement significantly enhanced these immune parameters. Taken together, the use of β-glucan may have advantages during immunosuppressive states, such as during physiological and environmental stress. The feed manufacturers are also advising to use feed with immunostimulants during such circumstances [166].

Overall, it is becoming clear that oral administration of β-glucans stimulates the innate immune system of cyprinids as it stimulates the innate immune system of salmonid and perciform fish species, suggesting that the capacity to stimulate the innate immune system of fish is a capacity intrinsic to (large molecular weight) β-glucans [125].

Kock et al. (manuscript in preparation) fed Nile tilapia for 0, 15, 30, or 45 days with a diet containing 0.1% of β-glucan (MacroGard), and evaluated the growth performance at the end of the feeding trial, and the innate immune function immediately after the feeding trial, and 7 and 14 days post-treatment (i.e., withdrawal period). The authors found that independent of the administration periods, fish fed with β-glucan had relatively higher innate immune responses, such as lysozyme activity in plasma, liver, and intestine and respiratory burst, compared to control and, overall, these differences became smaller over the withdrawal period. Moreover, at day 10 post-treatment, fish were challenged with bacteria (Aeromonas hydrophila); the control group had early mortalities (2 vs. 4–5 days post-infection, respectively) and lower survival rate (60% vs. 80%, respectively) compared to fish fed with β-glucan for 15 or 30 days, and, interestingly, fish fed for 45 days with β-glucan had no mortality. This study indicates that independent of the administration periods (i.e., 15 to 45 days), the β-glucan improved the innate immune responses and tilapia resistance to disease, and this protection could be observed up to 10 days post-treatment. The most relevant is that long-term administration did not cause immunosuppression as previously hypothesized due to an exhaustion of the immune system, but surprisingly promoted an even better growth and immune performance.

Regarding the period of administration, some studies have proposed that the longer administration may cause an overstimulation or a distress generated by the high energy cost due to prolonged exposure to β-glucan [21,167–169]. However, none of the studies that compared periods of β-glucan administration [149,153,170–175] found evidence that longer administration periods (up to 56 days) negatively impact the immune system. The studies that reported a negative effect used a high dietary inclusion level (e.g., >0.1%) or injected fish. These treatment protocols may have led to an exacerbated/toxic amount of β-glucan [163,167,174,176]. Taken together, these findings indicate that longer administrations periods (i.e., >4 weeks) can be beneficial at a low dose, reinforcing the hypothesis suggested by Ai, Mai, Zhang, Tan, Zhang, Xu and Li [163], Douxfils, Fierro-Castro, Mandiki, Emile, Tort and Kestemont [174], and Do Huu, Sang and Thanh Thuy [175] that immunosuppression may be caused by high dose.

5.3. Bath

A potentially interesting alternative application of immunostimulation induced by β-glucans is provided by the immersion treatment. For example, a short β-glucan bath of 3 min in fertilized eggs or gametes of chum salmon (Oncorhynchus keta) was sufficient to provide significant protection against infection with Saprolegnia spp., [177]. This finding was supported by the observation that both pro- and anti-inflammatory genes were upregulated after immersion of rainbow trout fry in a solution containing β-glucan [178].

It is essential to know the correct dosages of immunostimulants and appropriate administration route to achieve the desired results. Chinook salmon were fed with a diet containing 0%, 0.01%, 0.1%,

Molecules 2020, 25, 5378
and 1.0% of β-glucan for 7 days or immersion administration of β-glucan, and thereafter fish were bath challenged with *Aeromonas salmonicida*. Diet containing 0.1% and 1.0% of VitaStim-Taito glucan resulted in significant protection against *A. salmonicida*, but no significant protection was noted in any of the group bath treated [179].

Administration of β-glucans by immersion, as modulators of mucosal surfaces of the skin or gills, could be a promising new area of research, especially now that tools to reliably measure mucosal immunity are becoming available [180]. Possible explanations for immunostimulating effects of β-glucan immersion baths could be sought, for example, in effects on the composition of microbial communities in the skin mucus [181] or increased local populations of alternatively activated macrophages expressing a healing phenotype [182].

A summary of β-glucan effects on fish is shown in Table 1.
| Species                        | Dose                                      | Trial Duration | Main Effects                                                                                                                                  | Reference                              |
|-------------------------------|-------------------------------------------|----------------|----------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|
| Atlantic salmon (Salmo salar) | 500 or 1000 mg/kg diet                    | 70 days        | MacroGard reduced the number of lice-infested fish by 28%.                                                                                  | Refstie, et al. [183]                  |
| Red tilapia (Oreochromis niloticus x O. mossambicus) | Vaccine with adjuvant, the vaccine was emulsified in an equal volume of 2% | 28 days        | MacroGard increased the effectiveness of vaccine produced from Streptococcus iniae in fish.                                                  | Suanyuk and Itsaro [118]               |
| Common carp (Cyprinus carpio) | 10 mg/kg body weight                     | 14 days        | β-Glucan feeding did show significant effects on both CRP and complement profiles, suggesting that MacroGard stimulated CRP and complement responses to A. salmonicida infection in common carp. | Pionnier, Falco, Miest, Frost, Irnazarow, Shrive and Hoole [156] |
| Persian sturgeon (Acipenser persicus) | 0.1, 0.2, or 0.3% | 6 weeks        | Lysozyme activity and ACH50 were significantly higher in 0.2% and 0.3% β-glucan fed fish. Elevated growth performance (final weight, specific growth rate, and food conversion ratio) was observed in fish fed 0.1; 0.2, or 0.3% β-glucan compared to the control group. | Aramli, Kamangar and Nazari [25]       |
| Pompano fish (Trachinotus ovatus) | 0, 0.5, 1, 2, or 4 g/kg diet             | 8 weeks        | β-Glucan supplementation is effective for improving growth, intestinal Vibrio counts. Fish fed 0.05% or 0.20% β-glucan showed better resistance against salinity. | Do Huu, Sang and Thanh Thuy [175]      |
| Common carp (Cyprinus carpio) | 100 µg/mL (in vitro)                     | Not mentioned  | β-Glucans stimulate carp macrophages to increase the production of reactive oxygen and nitrogen radicals and affect the expression patterns of cytokine genes that can differ among activated pattern recognition receptors. | Pietretti, et al. [184]               |
| Atlantic salmon (Salmo salar) | 0.1%                                      | 35 days        | Results showed that the tested β-1,3/1,6-glucan diets increased the levels of transcripts of key genes involved in innate and adaptive immune response of salmon, potentiating the response to a model vaccine and also antagonizing the effects of hypoxia | Rodriguez, Valenzuela, Farias, Sandino and Imarai [140] |
| Nile tilapia (Oreochromis niloticus) | 0.1% β-glucan + 600 mg vitamin C      | 7, 15, 30, or 45 days before challenge | Diet supplemented with 0.1% of β-glucan and 600 mg of vitamin C/kg fed for at least 15 days is recommended especially when fish are likely to encounter transport-induced stress. | Barros, et al. [185]                  |
| Species                  | Dose                                | Trial Duration          | Main Effects                                                                 | Reference                                      |
|-------------------------|-------------------------------------|-------------------------|------------------------------------------------------------------------------|-----------------------------------------------|
| Nile tilapia (O. niloticus) | 0.1% of each glucan                  | 30 days                 | Different β-glucan samples exhibited biologically differently behaviors, but both increased the resistance against bacterial infection. Specifically, BG01 increased immunostimulation, while BG02 improved growth performance. | Pilarski, Ferreira de Oliveira, Darpossolo de Souza and Zanuzzo [11] |
| Turbot (S. maximus)       | 0.5 g/L MacroGard (Artemia enrichment) | 13 days post hatching  | Mortality was significantly reduced by 15% and an alteration of the larval microbiota was observed. At 11 DPH, gene expression of trypsin and chymotrypsin was elevated in the MacroGard fed fish, which resulted in heightened trypsic enzyme activity. MacroGard induced an immunomodulatory response and could be used as an effective measure to increase survival in rearing of turbot. | Miest, et al. [186]                          |
| Matrinixa (B. amazonicus) | 0.1% β-glucan                        | 15 days                 | β-Glucan modulated the cortisol profile prior to and after the stressor, increasing the number and activity of leukocytes. Our results suggest that β-glucan-induced cortisol increase is one important mechanism to improve the innate immune response in matrinxa. | Montoya, et al. [187]                        |
| Nile tilapia (O. niloticus) | 0.1, 0.2, 0.4, or 0.8% and vitamin C (400 or 600 mg/kg diet) | 60 days                 | 0.1–0.2% β-Glucan and 600 mg/kg vitamin C increased fish resistance to stress. | Barros, et al. [188]                        |
| Nile tilapia (O. niloticus) | 0.1 or 0.2% of β-1,3/1,6-Glucans       | 21 successive days prior to bacterial challenge and during the seven days of sampling | β-Glucan can modulate the antioxidant, inflammation, stress, and immune-related genes in Nile tilapia, moreover, 0.2% β-glucans showed better protective effect with Streptococcus iniae challenge. | Salah, et al. [189]                        |
| Carp (C. carpio)          | 10 g MacroGard kg-1 diet             | 14 days prior bacterial application | In β-glucan fed carp, mucus was quickly released from the intestinal goblet cells and was probably washed out of the gut together with a high number of intestinal bacteria. This could indicate a form of protection against bacteria. | Jung-Schroers, et al. [190]                  |
| Atlantic salmon (S. salar) | 15 mg/kg of fish (intubated fishes)  | Not mentioned           | This study provides some clues on the mechanisms by which the β-glucan evokes response in the fish, at the intestinal level. | Kiron, et al. [191]                        |
Table 1. Cont.

| Species                  | Dose                  | Trial Duration | Main Effects                                                                 | Reference                                      |
|--------------------------|-----------------------|----------------|-------------------------------------------------------------------------------|------------------------------------------------|
| Carp (Cyprinus carpio)   | 1% of feed            | 14             | β-Glucan can boost the host innate immune defense by inducing neutrophil extracellular trap formation and by stabilizing neutrophil extracellular traps against bacterial nuclease degradation, and thereby reduce the severity of an infection of *A. hydrophila*. | Brogden, et al. [192]                          |
| Carp (Cyprinus carpio)   | 20 mg/mL in in vitro head-kidney cells | Not mentioned | β-Glucan stimulation of scratch-wounded fibroblasts cultures did not enhance wound recovery. | Vera-Jimenez and Nielsen [193]                |
| Carp (Cyprinus carpio)   | 20 mg/mL in in vitro head-kidney cells | Not mentioned | Both methods compared during this study, showed the capacity to detect and measure the respiratory burst response of carp head kidney cells after stimulation with β-glucans. | Vera-Jimenez, et al. [194]                    |
| Rainbow trout (Oncorhynchus mykiss) | 0; 0.1; 0.2; 0.5% of feed | 15 × 30 days | Feeding low doses of β-glucans may help to boost immune function in case of a bacterial infection, especially the inflammatory response, while feeding high doses of β-glucans may result in a more or less rapid stress and immune exhaustion or feedback regulation, making appropriate response to subsequent pathogenic threat impossible. Additionally, the effects of β-glucans on the immune-related gene expression mainly concern spleen tissue, both prior and after bacterial infection, suggesting a targeted reinforcement of immune functions in this organ. | Douxfils, Fierro-Castro, Mandiki, Emile, Tort and Kestemont [174] |
| Matrinxã (Brycon amazonicus) | 0.1% on feed         | 15             | Inclusion of β-glucan in fish diet may help to prepare them to face stressful practices in fish farming. | Montoya, et al. [195]                          |
| Carp (Cyprinus carpio)   | 0.1; 1.0; 2.0% of feed | 14 and 28      | Dietary MacroGard may affect the composition of the carp intestinal microbial communities. Furthermore, positive effects on intestinal microvilli length and density were also observed. Indeed, these changes at 1% and 2% MacroGard supplementation might be contributory factors to the improved growth performance recently observed in carp fed 1% and 2% dietary MacroGard. | Kuhlwein, et al. [196]                          |
Table 1. Cont.

| Species                | Dose                        | Trial Duration | Main Effects                                                                                                                                                                                                                                                                                                                                 | Reference                        |
|------------------------|-----------------------------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| Juvenile Pompano       | 0.1; 0.2% of feed           | 21 + 10 challenge | Supplementation of β-glucan in the diet is beneficial in boosting nonspecific immunity, growth performance, survival rate, and tolerance to *Streptococcus iniae* infection of pompano *T. ovatus*. The addition of 0.10% of β-glucan to the pompano diet is recommended to boost disease resistance, immunity, and growth performance. | Do-Huu, Nguyen and Tran [138]    |
| Juvenile pompano       | 0; 0.05; 0.1; 0.2; 0.4; 0.5% of feed | 56             | The results of the present study confirmed that supplementation of β-glucan in the diet could improve the growth, protein content in flesh, feed conversion ratio, feed conversion efficiency, protein efficient ratio, and protein productive value in pompano, *T. ovatus*. It is recommended that supplementation of 0.5–1.0 g/kg β-glucan in the diet to obtain maximal growth, feed utilization and protein utilization of juvenile pompano. | Do-Huu, et al. [197]              |
| Carp (Cyprinus carpio) | 0.1% in vivo                | 42 days        | Application of MacroGard after the third week post hatching resulted in a significant increase in classical complement activity when compared to fish fed the control diet. The results demonstrate that feeding with β-glucan enriched diet enhances the immune defense parameters of juvenile carp.                                                                                     | Sych, et al. [198]                |
| Carp (Cyprinus carpio) | 6 mg/kg in vivo             | 14 days        | β-Glucan supplemented diet administered to common carp decreased the transcript levels of several pro-inflammatory cytokines in gut and head kidney tissues. The infection with *A. salmonicida* did not modify this tendency in gut. Levels of TNFα1, TNFα2, IL-1β, and IL-6 became significantly higher in fish fed β-glucan supplemented diet at 6 h post infection. Such differential effects may reflect the complex interactions between the bacterium and the immunostimulant relationship with the inflammatory response of the host. | Falco, Frost, Miest, Pionnier, Irnazarow and Hoole [155] |
| Carp (Cyprinus carpio) | Not mentioned               | Kidney cells incubated for 30 min. | β-Glucan stimulated the kidney derived neutrophil to produce more neutrophil extracellular traps and entrapped a significantly higher percentage of bacteria than the head kidney derived neutrophil extracellular traps.                                                                                             | Brogden, et al. [199]             |
| Species                         | Dose                                           | Trial Duration                        | Main Effects                                                                 | Reference                      |
|--------------------------------|------------------------------------------------|---------------------------------------|------------------------------------------------------------------------------|--------------------------------|
| Carp (Cyprinus carpio)         | 0–1000 µg incubated for 6, 24, and 48 h (in vitro) | pronephric primary cell culture (in vitro test) | With the concentration higher than 500 µg, MacroGard induces to a higher percentage of apoptosis in vitro. | Miest and Hoole [136]          |
| Pacu (Piaractus mesopotamicus)  | 0.1%                                           | 15 days                               | The results of the present study provide additional evidence that β-glucan modulated not only the immune system, but also the release of cortisol. The β-glucan modulated cortisol levels differently after transport and after inoculation of pacu with Aeromonas hydrophila. Up to 24 h after transport, β-glucan increased the levels of cortisol, while in fish that were additionally inoculated with the bacterium, the elevation of the hormone levels was prevented. In inoculated fish, with reduced levels of cortisol because of β-glucan, we observed a reduction of monocytes (3 h after inoculation) and a reduction of lymphocytes as well as enhanced complement system activity (24 h). | Marinho de Mello, et al. [200] |
| Zebrafish (Danio rerio)        | 12.5 mg/kg BW or 0.35 g/kg of feed             | 14 days                               | Results showed that 1,3–1,6 β-glucans decreased fish mortality rate and enhanced both daily and cumulative regenerated fin area, independent of the β-glucan extraction method used. Based on the mechanisms similarities of the innate immune system and tissue regeneration among different teleost species, these results may likely be extended to species of interest for the aquaculture sector. | Fronte, et al. [201]           |
| Nile tilapia (Oreochromis niloticus) | 100 mg/L (added in water)                  | 8 days                                | Larvae that received the β-glucan treatment were ~20% heavier (10.2 mg—control; 12.3 mg—β-glucan) and ~8.5% longer (0.82 cm—control; 0.89 cm—β-glucan) compared to the control larvae. | de Jesus, et al. [202]         |
| Carp (Cyprinus carpio)         | 0.1 µg/mL (a stock solution was prepared (0.5 g MacroGard/500 mL Milli-Q water) | 14 days                               | The images showed significantly faster wound contraction in both treated groups compared to the control. The obtained results clearly demonstrated that β-glucan enriched bath promotes the closure of wounds in common carp and induced a local change in cytokine expression. | Przybylska-Diaz, et al. [203] |
Table 1. Cont.

| Species | Dose | Trial Duration | Main Effects | Reference |
|---------|------|----------------|--------------|-----------|
| Carp (*Cyprinus carpio*) | 0.1% diet or 10 mg glucan per kg body weight. | 25 days | β-Glucan mediated protection against viral diseases could be due to an increased TLR-3 mediated recognition of ligands, resulting in an increased antiviral activity of macrophages. | Falco, Miest, Pionnier, Pietretti, Forlenza, Wiegertjes and Hoole [158] |
| Rainbow trout (*Oncorhynchus mykiss*) | 0, 0.1, 0.2, and 0.5% in food | 15 versus 30 days | Results suggest that spleen may be a highly responsive organ to dietary β-glucans both in healthy or infected fish, and that this organ may therefore significantly contribute to the immune reinforcement induced by such immunostimulatory diet. Our study further reveals that overdoses of β-glucans and/or prolonged medication can lead to a non-reactive physiological status and, consequently, to a poor immune response. | Douxfils, Fierro-Castro, Mandiki, Emile, Tort and Kestemont [174] |
| Atlantic salmon (*Salmo salar*) | 1 g/kg feed | 12 weeks before vaccination | Dietary supplementation decreased mortality in both unvaccinated and vaccinated *M. viscosa*-challenged fish compared to the non-supplemented groups. Similarly, mortality of infectious salmon anemia virus-challenged fish decreased from 87.5% in vaccinated fish without supplementation to 70.9% in the supplemented and vaccinated group (RPSend 26.4). | Filho, et al. [204] |
| Pacu (*Piaractus mesopotamicus*) | 0.1% β-glucan or diet containing 1% β-glucan | 7 days before inoculation | Feeding β-glucan up to 7 days significantly increased resistance against *A. hydrophila*, as well the leukocytes production and lysozyme activity of pacu suggesting benefits of the use of this immunostimulant in the farming of this species. | Biller-Takahashi, et al. [205] |
| Mirror carp (*Cyprinus carpio L.*) | 0% (control), 0.1%, 1%, or 2% MacroGard | 8 weeks | High dietary inclusion levels of β-glucan can enhance growth performance and localized intestinal leukocyte infiltration in the anterior intestine of mirror carp without detrimental effects on carcass composition, intestinal morphology, or the hemato-immunological parameters investigated. | Kuhlwein, et al. [206] |
| Carp (*Cyprinus carpio*) | 6 mg/kg live weight | 25 days | The 25-day period of β-glucan oral administration induced and enhanced an immune response in carp, and subsequent lipopolysaccharides and polyinosinic-polycytidylic acid injections significantly affected carp C-reactive protein and complement responses. | Pionnier, et al. [207] |
| Species            | Dose                                      | Trial Duration | Main Effects                                                                 |
|--------------------|-------------------------------------------|----------------|-------------------------------------------------------------------------------|
| Nile Tilapia       | 18 MacroGard/kg diet and then switching   | 14 days (as a bath) | Improved weight gain and feed efficiency than those fed the control diet uninterupted or switched from the β-glucan. Feeding tilapia β-glucan for 4 w and then switching to the basal diet for 2 w caused a significant increase in the respiratory burst. No differences in survival to S. iniae infection occurred between dietary groups. | Welker, et al. [210] |
| Rainbow trout      | 0.1 mg MacroGard/L (in vitro study, macrophage stimulation) | 14 days | Prolonged healing dynamics of rainbow trout muscle wounds and a very limited response to stimulation with β-glucans. | Schmidt, et al. [209] |
| Common carp        | 25 mg/L, (in vitro study, macrophage stimulation) | 28 days | The identification of several candidate β-glucan receptors suggests that immune-modulatory effects of β-glucan in carp macropages could be a result of signaling mediated by a member of the C-type lectin receptor family. | Petit, Bailey, Wheeler, de Oliveira, Forlenza and Wiegertjes [113] |
| Silver catfish     | 0.1% (0.1 mg/L) or 0.5% (0.5 mg/L)        | 28 days         | Results indicate that in silver catfish, wound healing occurs rapidly and improves greatly at a daily bathing with β-glucan. | Dos Santos Voleski, et al. [211] |
| Silver catfish     | 0.01% of β-glucan or 0.1%                 | 28 days         | The addition of β-glucan to the diet improved natural complement hemolytic activity, reduced bacterial levels and, most importantly, increased fish resistance to challenge with A. hydrophila. | Di Domenico, Canova, Costa, Nied, Costa, Fradlosede and Carlos [139] |
| Common carp        | 0.01% of β-glucan or 0.1%                 | 28 days         | The addition of β-glucan to the diet improved natural complement hemolytic activity, reduced bacterial levels and, most importantly, increased fish resistance to challenge with A. hydrophila. | Jung-Schoeters, et al. [208] |
| Nile Tilapia       | 0.1% of β-glucan or 0.1%                 | 28 days         | The addition of β-glucan to the diet improved natural complement hemolytic activity, reduced bacterial levels and, most importantly, increased fish resistance to challenge with A. hydrophila. | Jung-Schoeters, et al. [208] |
| Nile Tilapia       | 0.1% of β-glucan or 0.1%                 | 28 days         | The addition of β-glucan to the diet improved natural complement hemolytic activity, reduced bacterial levels and, most importantly, increased fish resistance to challenge with A. hydrophila. | Jung-Schoeters, et al. [208] |
| Nile Tilapia       | 0.1% of β-glucan or 0.1%                 | 28 days         | The addition of β-glucan to the diet improved natural complement hemolytic activity, reduced bacterial levels and, most importantly, increased fish resistance to challenge with A. hydrophila. | Jung-Schoeters, et al. [208] |
| Nile Tilapia       | 0.1% of β-glucan or 0.1%                 | 28 days         | The addition of β-glucan to the diet improved natural complement hemolytic activity, reduced bacterial levels and, most importantly, increased fish resistance to challenge with A. hydrophila. | Jung-Schoeters, et al. [208] |
6. New Insights about the Use of β-Glucan in Aquaculture

β-glucan seems to affect more physiological conditions than “only” the immune system. A study of rainbow trout (Oncorhynchus mykiss) given a food supplement for 60 days and using proteomic analysis, found changed expression of structural muscle proteins. The authors speculate that these alterations might be responsible for improved growth rate in fish [212].

Among direct effects on improvement of various immune reactions, β-glucan supplementation can have additional nutritional effects including amelioration of toxic effects caused by deltamethrin. Experiments using Nile tilapia showed improved cortisol levels and significantly reversed inflammatory and transcriptomic damages caused by the toxin [213]. In addition, β-glucan feeding ameliorate cold stress-related mortality in Pangasianodon hypophthalmus [214], but glucose and cortisol levels remained unchanged. Environmental stress caused by either overcrowding or by environmental pollution, is one of the problems the current aquaculture suffers from. Food supplementation with β-glucan was found to improve ammonia-related stress in Oreochromis mossambicus via improvements of cellular, humoral, and antioxidant response [215].

Recent insights in the field of innate immunity provide indications that β-glucans could also have effects for a longer period, possibly explained by the phenomenon of ‘trained immunity’ [125]. At present, the strict absence of a form of memory for innate immune responses is challenged by a new concept named trained immunity, which is characterized by three criteria: (i) it can be induced after a primary infection or immunization and subsequently provide protection against a secondary infection in a T- and B-lymphocyte independent manner; (ii) it may be less specific than the adaptive immune response but still confers increased resistance upon reinfection of the host; and (iii) innate cell types, such as macrophages and NK cells, are key players in the mechanism, which involves improved pathogen recognition and an increased inflammatory response [216]. A concept of trained immunity is shown in Figure 2.

![Figure 2. The concept of “trained innate immunity” adapted from Alvarez-Errico et al. [217] and Petit and Wiegertjes [125].](image)

Another possibility of glucan action is the potential effect on neuroendocrine axis. It is well established that the neuroendocrine and immune systems communicate bidirectionally via numerous cytokines acting as auto/paracrine or endocrine factors regulating pituitary development, cell proliferation, hormone secretion, and feedback control of the hypothalamic-pituitary-adrenal axis. However, the information on these possible effects of glucan in fish is still lacking.

Effects induced by vaccination with Bacille Calmette-Guerin (BCG) [218,219], prepared from attenuated live Mycobacterium bovis, support the proposed benchmarks of trained immunity that it can elicit cross-specific protection in a T- and B-cell independent manner with innate immune cell types,
such as macrophages, acting as key players [216]. Of evolutionary interest, long before the recent discussions on the presence of trained immunity in humans and mice [220], similar cross-specific protection was observed in plants [221–223] and invertebrates [224], which, typically without T and B lymphocytes, can build up a form of immunity to protect the organism from a secondary exposure. Owing to the basal position of teleost fish as early vertebrates, it makes evolutionary sense to expect that trained immunity could be an important mechanism determining immunostimulation of fish by β-glucans [125].

There are a few studies providing evidence for the presence of a form of trained immunity in fish, primarily based on experiments with mycobacteria. Olivier et al. [225] observed a long-lived increase in phagocytic activity of peritoneal macrophages from brook trout (Salvelinus fontinalis), for a period up to 33 days after intraperitoneal injection with modified Freund complete adjuvant containing killed Mycobacterium butyricum. Only macrophages from trout injected with modified Freund complete adjuvant showed a significantly higher bactericidal activity. Vaccination of Japanese flounder (Paralichthys olivaceus) with BCG resulted in an upregulation of pro-inflammatory cytokines and conferred protection against Mycobacterium sp. [226]. Moreover, vaccination of Amberjack (Seriola dumerili) with BCG led to protection against challenge with Mycobacterium sp. [226]. Importantly, these researchers could measure cross-specific protection, one of the proposed benchmarks of trained immunity. The cross-specific protection could be induced in Japanese flounder by BCG, shown by challenge with Nocardia seriolae, and was possibly mediated by bacteriolytic activity of the serum [227].

Cross-specific protection occurring in a T- and B-cell independent manner [216] was also studied in fish. Exposure of Rag-KO zebrafish to a sublethal infection with Edwardsiella ictaluri significantly protected the same animals from a subsequent lethal infection with the same bacteria. Importantly, protection could be transferred to native Rag-KO individuals by injection with kidney leukocytes from animals pre-exposed to the sublethal infection [228].

According to Petit and Wiegertjes [125], it remains to be investigated if trained immunity has the predicted, pronounced role in the immune defense of fish, and is indeed mediated by innate immune cell types, such as macrophages.

7. Conclusions

Currently, more than 3000 papers have reported the effect of β-glucan on immune responses in fish; however, several questions remain. Detailed knowledge of the receptors involved in recognition of β-glucans and of their downstream signaling is missing for teleosts, leaving obscure whether the observed potentiation should be attributed to direct effects on leukocytes or to indirect effects on, for example, the composition of microbial communities in the gut. Typically, studies investigating the effects of β-glucans have mostly focused on relatively short-lived effects, in the order of days up to a few weeks, but recent insights in the field of innate immunity provide indications that β-glucans could also have effects for a longer period of time, possibly explained by the phenomenon ‘trained immunity’.

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References

1. Dimler, R.J.; Davis, H.A.; Hilbert, G.E. A new anhydride of d-glucose: d-glucosan <1,4>beta<1,6>. J. Am. Chem. Soc. 1946, 68, 1377–1380. [CrossRef] [PubMed]
2. Wooles, W.R.; Diluzio, N.R. Reticuloendothelial function and the immune response. Science 1963, 142, 1078–1080. [CrossRef] [PubMed]
3. Murphy, E.A.; Davis, J.M.; Carmichael, M.D. Immune modulating effects of beta-glucan. Curr. Opin. Clin. Nutr. Metab. Care 2010, 13, 656–661. [CrossRef] [PubMed]
4. Vetvicka, V.; Oliveira, C. beta(1-3)(1-6)-β-Glucans modulate immune status in pigs: Potential importance for efficiency of commercial farming. *Ann. Transl. Med.* 2014, 2, 16. [CrossRef] [PubMed]

5. Vetvicka, V.; Vannucci, L.; Sima, P. The effects of beta-glucan on pig growth and immunity. *Open Biochem J.* 2014, 8, 89–93. [CrossRef]

6. Eicher, S.D.; Patterson, J.A.; Rostagno, M.H. β-Glucan plus ascorbic acid in neonatal calves modulates immune functions with and without *Salmonella enterica* serovar Dublin. *Vet. Immunol. Immunopathol.* 2011, 142, 258–264. [CrossRef]

7. Paap, P.; Roberti, F. Race horses perform better with beta-glucans. *Health Nutr.* 2014, 22, 500–502.

8. Waller, K.P.; Colditz, I.G. Effect of intramammary infusion of beta-1,3-glucan or interleukin-2 on leukocyte subpopulations in mammary glands of sheep. *Am. J. Vet. Res.* 1999, 60, 703–707.

9. Tian, X.; Shao, Y.; Wang, Z.; Guo, Y. Effects of dietary yeast β-glucans supplementation on growth performance, gut morphology, intestinal *Clostridium perfringens* population and immune response of broiler chickens challenged with necrotic enteritis. *Anim. Feed. Sci. Technol.* 2016, 215, 144–155. [CrossRef]

10. Crumlish, M.; Inglis, V. Improved disease resistance in *Rana rugulosa* (Daudin) after beta-glucan administration. *Aquac. Res.* 1999, 30, 431–435. [CrossRef]

11. Pilarski, F.; Ferreira de Oliveira, C.A.; Darposso de Souza, F.P.B.; Zanuzzo, F.S. Different beta-glucans improve the growth performance and bacterial resistance in Nile tilapia. *Fish Shellfish Immunol.* 2017, 70, 25–29. [CrossRef] [PubMed]

12. Wu, Y.S.; Liau, S.Y.; Huang, C.T.; Nan, F.H. Beta 1,3\(\beta\)-Glucan plus ascorbic acid in neonatal calves modulates white shrimp immune response (*Litopenaeus vannamei*). *Fish Shellfish Immunol.* 2016, 57, 269–277. [CrossRef]

13. Zhang, X.; Zhu, Y.T.; Li, X.J.; Wang, S.C.; Li, D.; Li, W.W.; Wang, Q. Lipopolysaccharide and beta-1,3-glucan binding protein (LGBP) stimulates prophenoloxidase activating system in Chinese mitten crab (*Eriochetin sinensis*). *Dev. Comp. Immunol.* 2016, 61, 70–79. [CrossRef] [PubMed]

14. Mazzei, M.; Fronte, B.; Sagona, S.; Carrozza, M.L.; Forzan, M.; Pizzurro, F.; Bibbiani, C.; Miraglia, V.; Abram, F.; Millanta, F.; et al. Effect of 1,3-1,6 beta-glucan on natural and experimental deformed wing virus infection in newly emerged honeybees (*Apis mellifera ligustica*). *PLoS ONE* 2016, 11, e0166297. [CrossRef]

15. Kim, Y.S.; Ryu, J.H.; Han, S.J.; Choi, K.H.; Nam, K.B.; Jang, I.H.; Lemaitre, B.; Brey, P.T.; Lee, W.J. Gram-negative bacteria-binding protein, a pattern recognition receptor for lipopolysaccharide and beta-1,3-glucan that mediates the signaling for the induction of innate immune genes in *Drosophila melanogaster* cells. *J. Biol. Chem.* 2000, 275, 32271–32277. [CrossRef]

16. De Oliveira, C.A.F.; Vetvicka, V.; Zanuzzo, F.S. β-Glucan successfully stimulated the immune system in different jawed vertebrate species. *Comp. Immunol. Microbiol. Infect. Dis.* 2019, 62, 1–6. [CrossRef]

17. Tort, L.; Balasch, J.C.; Mackenzie, S. Fish immune system. A crossroads between innate and adaptive responses. *Immunology* 2003, 22, 277–286.

18. Sohn, K.S.; Kim, M.K.; Kim, J.D.; Han, I.K. The role of immunomodulators in monogastric animal and fish-Review. *Asian Australas. J. Anim. Sci.* 2000, 13, 1178–1187. [CrossRef]

19. Scholz, U.; Garcia Diaz, G.; Ricque, D.; Cruz Suarez, L.E.; Vargas Albores, F.; Latchford, J. Enhancement of vibriosis resistance in juvenile *Piaractus mesopotamicus* by supplementation of diets with different yeast products. *Aquaculture* 1999, 176, 271–283. [CrossRef]

20. Bondad-Reantaso, M.G.; Subasinghe, R.P.; Arthur, J.R.; Ogawa, K.; Chinabut, S.; Adlard, R.; Tan, Z.; Shariff, M. Disease and health management in Asian aquaculture. *Vet. Parasitol.* 2005, 132, 249–272. [CrossRef]

21. Sakai, M. Current research status of fish immunostimulants. *Aquaculture* 1999, 172, 63–92. [CrossRef]

22. Zanuzzo, F.S.; Sabioni, R.E.; Montoya, L.N.F.; Favero, G.; Urbinati, E.C. Aloe vera enhances the innate immune response of pacu (*Piaractus mesopotamicus*) after transport stress and combined heat killed *Aeromonas hydrophila* infection. *Fish Shellfish Immunol.* 2017, 65, 198–205. [CrossRef] [PubMed]

23. Wang, Q.; Sheng, X.; Shi, A.; Hu, H.; Yang, Y.; Liu, L.; Fei, L.; Liu, H. β-Glucans: Relationships between modification, conformation and functional activities. *Molecules* 2017, 22, 257. [CrossRef] [PubMed]

24. Barsanti, L.; Passarelli, V.; Evangelista, V.; Frassanito, A.M.; Gualtieri, P. Chemistry, physico-chemistry and applications linked to biological activities of beta-glucans. *Nat. Prod. Rep.* 2011, 28, 457–466. [CrossRef]

25. Aramli, M.S.; Kamangar, B.; Nazari, R.M. Effects of dietary beta-glucan on the growth and innate immune response of juvenile Persian sturgeon, *Acipenser persicus*. *Fish Shellfish Immunol.* 2015, 47, 606–610. [CrossRef]
26. Chen, J.; Seviour, R. Medicinal importance of fungal beta-(1→3), (1→6)-glucans. *Mycol. Res.* 2007, 111, 635–652. [CrossRef]

27. Miura, N.N.; Ohno, N.; Aketagawa, J.; Tamura, H.; Tanaka, S.; Yadomae, T. Blood clearance of (1→3)-beta-n-glucan in MRL lpr/lpr mice. *FEMS Immunol. Med. Microbiol.* 1996, 13, 51–57. [CrossRef]

28. Chihara, G. Recent progress in immunopharmacology and therapeutic effects of polysaccharides. *Dev. Biol. Stand.* 1992, 77, 191–197.

29. Behall, K.M.; Scholfield, D.J.; Hallfrisch, J. Effect of beta-glucan level in oat fiber extracts on blood lipids in men and women. *J. Am. Coll. Nutr.* 1997, 16, 46–51. [CrossRef]

30. Bell, S.; Goldman, V.M.; Bistrian, B.R.; Arnold, A.H.; Ostroff, G.; Forse, R.A. Effect of beta-glucan from oats and yeast on serum lipids. *Crit. Rev. Food Sci. Nutr.* 1999, 39, 189–202. [CrossRef]

31. Braaten, J.T.; Wood, P.J.; Scott, F.W.; Wolynetz, M.S.; Lowe, M.K.; Bradley-White, P.; Collins, M.W. Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *Eur. J. Clin. Nutr.* 1994, 48, 465–474. [PubMed]

32. Pick, M.E.; Hawrysh, Z.J.; Gee, M.I.; Toth, E.; Garg, M.L.; Hardin, R.T. Oat bran concentrate bread products improve long-term control of diabetes: A pilot study. *J. Am. Diet. Assoc.* 1996, 96, 1254–1261. [CrossRef]

33. Wood, P.J. Physicochemical properties and physiological effects of the (1→3)(1→4)-beta-n-glucan from oats. *Adv. Exp. Med. Biol.* 1990, 270, 119–127. [CrossRef] [PubMed]

34. Gantner, B.N.; Simmons, R.M.; Canavera, S.J.; Akira, S.; Underhill, D.M. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J. Exp. Med.* 2003, 197, 1107–1117. [CrossRef] [PubMed]

35. Herre, J.; Gordon, S.; Brown, G.D. Dectin-1 and its role in the recognition of beta-glucans by macrophages. *Mol. Immunol.* 2004, 40, 869–876. [CrossRef] [PubMed]

36. Raa, J. *The Use of Immune-Stimulants in Fish and Shellfish Feeds*; Cruz-Suarez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Olvera-Novoa, M.A., Civera-Cerecedo, R., Eds.; Available online: http://www.aquatech.com/ve/pdf/raa.pdf (accessed on 16 November 2020).

37. Helfman, G.; Collette, B.B.; Facey, D.E.; Bowen, B.W. *The Diversity of Fishes: Biology, Evolution, and Ecology*, 2nd ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2009.

38. Nelson, J.S. *Fishes of the World*, 4th ed.; John Wiley: Hoboken, NJ, USA, 2006.

39. Carroll, R.L. *Vertebrate Paleontology an Evolution*; WH Freeman and Co.: New York, NY, USA, 1988.

40. Fricke, R.; Eschmeyer, W.N.; van der Laan, R. *Eschmeyer’s Catalog of Fishes: Genera, Species, References*; Eschmeyer, W.N., Ed.; California Academy of Sciences: San Francisco, CA, USA, 2019.

41. Colbert, E.H. *Evolution of Vertebrates*; Birkhauser Verlag: Basel, Switzerland; Boston, FL, USA, 1998; p. xi. 196p.

42. Rombout, J.H.; Huttenhuis, H.B.; Picchietti, S.; Scapigliati, G. Phylogeny and ontogeny of fish leucocytes. *Icel. Agric. Sci.* 1998, 12, 191–206. [CrossRef]

43. Magnadottir, B. Innate immunity of fish (overview). *Fish Shellfish Immunol.* 2006, 20, 137–151. [CrossRef]

44. Magnadottir, B. Immunological control of fish diseases. *Mar. Biotechnol.* 2010, 12, 361–379. [CrossRef]

45. Magnadottir, B. Comparison of immunoglobulin (IgM) from four fish species. *Ict. Agric. Sci.* 1998, 12, 47–59.

46. Fearon, D.T.; Locksley, R.M. The instructive role of innate immunity in the acquired immune response. *Science* 1996, 272, 50–53. [CrossRef] [PubMed]

47. Fischer, U.; Koppan, E.O.; Nakanishi, T. Teleost T and NK cell immunity. *Fish Shellfish Immunol.* 2013, 35, 197–206. [CrossRef] [PubMed]

48. Holland, M.C.; Lambris, J.D. The complement system in teleosts. *Fish Shellfish Immunol.* 2002, 12, 399–420. [CrossRef] [PubMed]

49. Sakai, D.K. Repertoire of complement in immunological defense mechanisms of fish. *Annu. Rev. Fish Dis.* 1992, 2, 223–247. [CrossRef]

50. Uribe, C.; Folch, H.; Enriquez, R.; Moran, G. Innate and adaptive immunity in teleost fish: A review. *Vet. Med.* 2011, 56, 486–503.

51. Du Pasquier, L. The immune system of invertebrates and vertebrates. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 2001, 129, 1–15. [CrossRef] [PubMed]

52. Sima, P.; Vetvicka, V. *Evolution of Immune Functions*; CRC Press: Boca Raton, FL, USA, 1990.

53. Větvička, V.; Sima, P. *Evolutionary Mechanisms of Defense Reactions*; Birkhauser Verlag: Basel, Switzerland; Boston, FL, USA, 1998; p. xi. 196p.
54. Firdaus-Nawi, M.; Zamri-Saad, M. Major components of fish immunity: A review. *Pertanika J. Trop. Agric. Sci.* 2016, 39, 393–420.

55. Zapata, A.; Diez, B.; Cejalvo, T.; Gutierrez-de Frias, C.; Cortes, A. Ontogeny of the immune system of fish. *Fish Shellfish Immunol.* 2005, 19, 413–427. [CrossRef] [PubMed]

56. Bowden, T.J.; Cook, P.; Rombout, J.H. Development and function of the thymus in teleosts. *Fish Shellfish Immunol.* 2005, 19, 413–427. [CrossRef] [PubMed]

57. Press, C.M.; Dannevig, B.H.; Landsverk, T. Immune and enzyme histochemical phenotypes of lymphoid and nonlymphoid cells within the spleen and head kidney of Atlantic salmon (*Salmo salar* L.). *Fish Shellfish Immunol.* 1994, 4, 79–93. [CrossRef]

58. Manning, M.J. Fishes. In *The Immune System: Comparative Histophysiology*; Zapata, A.G., Cooper, E.L., Eds.; John Wiley and Sons: Chichester, UK, 1990; pp. 69–100.

59. Zapata, A.G.; Cooper, E.L. *The Immune System: Comparative Histophysiology*; John Wiley and Sons: Chichester, UK, 1990.

60. Ferguson, H.W.; Dukes, T.W. *Systemic Pathology of Fish: A Text and Atlas of Comparative Tissue Responses in Diseases of Teleosts*, 1st ed.; Iowa State University Press: Ames, IA, USA, 1989; p. ix. 263p.

61. Imagawa, T.; Hashimoto, Y.; Kon, Y.; Sugimura, M. Immunoglobulin containing cells in the head kidney of carp (*Cyprinus carpio* L.) after bovine serum albumin injection. *Fish Shellfish Immunol.* 1991, 1, 173–185. [CrossRef]

62. Salinas, I.; Zhang, Y.A.; Sunyer, J.O. Mucosal immunoglobulins and B cells of teleost fish. *Dev. Comp. Immunol.* 2011, 35, 1346–1365. [CrossRef] [PubMed]

63. Fuglem, B.; Jirillo, E.; Bjerkas, I.; Kiyono, H.; Nochi, T.; Yuki, Y.; Raida, M.; Fischer, U.; Koppang, E.O. Antigen-sampling cells in the salmonid intestinal epithelium. *Dev. Comp. Immunol.* 2010, 34, 768–774. [CrossRef] [PubMed]

64. Ellis, A.E. Innate host defense mechanisms of fish against viruses and bacteria. *Dev. Comp. Immunol.* 2001, 25, 827–839. [CrossRef]

65. Gomez, G.D.; Balcazar, J.L. A review on the interactions between gut microbiota and innate immunity of fish. *FEMS Immunol. Med. Microbiol.* 2008, 52, 145–154. [CrossRef] [PubMed]

66. Robertsen, R.; Engstad, E.; Jorgensen, J.B. β-Glucans as immunostimulants in fish. In *Modulators of Fish Immune Responses*; Stolen, J.S., Fletcher, T.C., Eds.; SOS Publications: Fair Haven, NJ, USA, 1994; pp. 83–99.

67. Rombout, J.H.; Abelli, L.; Picchietti, S.; Scapigliati, G.; Kiron, V. Teleost intestinal immunology. *Fish Shellfish Immunol.* 2011, 31, 616–626. [CrossRef]

68. Matsunaga, T.; Rahman, A. What brought the adaptive immune system to vertebrates?—The jaw hypothesis and the seahorse. *Immunol. Rev.* 1998, 166, 177–186. [CrossRef]

69. Danilova, N.; Bussmann, J.; Jekosch, K.; Steiner, L.A. The immunoglobulin heavy-chain locus in zebrafish: Identification and expression of a previously unknown isotype, immunoglobulin Z. *Nat. Immunol.* 2005, 6, 295–302. [CrossRef] [PubMed]

70. Solem, S.T.; Stenvik, J. Antibody repertoire development in teleosts—A review with emphasis on salmonids and *Gadus morhua* L. *Dev. Comp. Immunol.* 2006, 30, 57–76. [CrossRef]

71. Flajnik, M.F.; Kasahara, M. Origin and evolution of the adaptive immune system: Genetic events and selective pressures. *Nat. Rev. Genet.* 2010, 11, 47–59. [CrossRef]

72. Morrison, R.N.; Nowak, B.F. The antibody response of teleost fish. *Semin. Avian Exot. Pet Med.* 2002, 11, 46–54. [CrossRef]

73. Anderson, D.P. Immunostimulants, adjuvants, and vaccine carriers in fish: Applications to aquaculture. *Ann. Rev. Fish Dis.* 1992, 2, 281–307. [CrossRef]

74. Burrells, C.; Williams, P.D.; Forno, P.F. Dietary nucleotides: A novel supplement in fish feeds: 1. Effects on resistance to disease in salmonids. *Aquaculture* 2001, 199, 159–169. [CrossRef]

75. Burrells, C.; Williams, P.D.; Southgate, P.J.; Wadsworth, S.L. Dietary nucleotides: A novel supplement in fish feeds: 2. Effects on vaccination, salt water transfer, growth rates and physiology of Atlantic salmon (*Salmo salar* L.). *Aquaculture* 2001, 199, 171–184. [CrossRef]

76. Li, P.; Lewis, D.H.; Gatlin, D.M., 3rd. Dietary oligonucleotides from yeast RNA influence immune responses and resistance of hybrid striped bass (*Morone chrysops × Morone saxatilis*) to *Streptococcus iniae* infection. *Fish Shellfish Immunol.* 2004, 16, 561–569. [CrossRef] [PubMed]
77. Sakai, M.; Taniguchi, K.; Mamoto, K.; Ogawa, H.; Tabata, M. Immunostimulant effects of nucleotide isolated from yeast RNA on carp, *Cyprinus carpio*. *L. J. Fish Dis.* 2001, 24, 433–438. [CrossRef]

78. Leonardi, M.; Sandino, A.M.; Klempau, A. Effect of a nucleotide-enriched diet on the immune system, plasma cortisol levels and resistance to infectious pancreatic necrosis (IPN) in juvenile rainbow trout (*Oncorhynchus mykiss*). *Bull. Eur. Assoc. Fish Pathol.* 2003, 23, 52–59.

79. Gatesoupe, F.J. The use of probiotics in aquaculture. *Aquaculture* 1999, 180, 147–165. [CrossRef]

80. Wang, T.; Holland, J.W.; Carrington, A.; Zou, J.; Secombes, C.J. Molecular and functional characterization of IL-15 in rainbow trout (*Oncorhynchus mykiss*): A potent inducer of IFN-gamma expression in spleen leukocytes. *J. Immunol.* 2007, 179, 1475–1488. [CrossRef]

81. Yanbo, W.; Zirong, X. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. *Anim. Feed Sci. Technol.* 2006, 127, 283–292. [CrossRef]

82. Aderem, A.; Ulevitch, R.J. Toll-like receptors in the induction of the innate immune response. *Nature* 2000, 406, 782–787. [CrossRef]

83. Bricknell, I.; Dalmo, R.A. The use of immunostimulants in fish larval aquaculture. *Fish Shellfish Immunol.* 2005, 19, 457–472. [CrossRef] [PubMed]

84. O’Hagan, D.T.; MacKichan, M.L.; Singh, M. Recent developments in adjuvants for vaccines against infectious diseases. *Biomol. Eng.* 2001, 18, 69–85. [CrossRef]

85. Schijns, V.E. Mechanisms of vaccine adjuvant activity: Initiation and regulation of immune responses by vaccine adjuvants. *Vaccine* 2003, 21, 829–831. [CrossRef]

86. Underhill, D.M.; Ozinsky, A.; Hajjar, A.M.; Stevens, A.; Wilson, C.B.; Bassetti, M.; Aderem, A. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* 1999, 401, 811–815. [CrossRef] [PubMed]

87. Brown, G.D.; Herre, J.; Williams, D.L.; Willment, J.A.; Gordon, S. Dectin-1 mediates the biological effects of beta-glucans. *J. Exp. Med.* 2003, 197, 1119–1124. [CrossRef] [PubMed]

88. Bose, N.; Chan, A.S.; Guerrero, F.; Maristany, C.M.; Qiu, X.; Walsh, R.M.; Ertelt, K.E.; Jonas, A.B.; Gorden, K.B.; Dudney, C.M.; et al. Binding of soluble yeast beta-glucan to human neutrophils and monocytes is complement-dependent. *Front. Immunol.* 2013, 4, 230. [CrossRef]

89. Elder, M.J.; Webster, S.J.; Chee, R.; Williams, D.L.; Hill Gaston, J.S.; Goodall, J.C. beta-glucan size controls dectin-1-mediated immune responses in human dendritic cells by regulating IL-1beta production. *Front. Immunol.* 2017, 8, 791. [CrossRef]

90. McGreal, E.P.; Miller, J.L.; Gordon, S. Ligand recognition by antigen-presenting cell C-type lectin receptors. *Curr. Opin. Immunol.* 2005, 17, 18–24. [CrossRef]

91. Zimmerman, J.W.; Lindermuth, J.; Fish, P.A.; Palace, G.P.; Stevenson, T.T.; DeMong, D.E. A novel carbohydrate-glycosphingolipid interaction between a beta-(1–3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. *J. Biol. Chem.* 1998, 273, 22014–22020. [CrossRef]

92. Xia, Y.; Vetvicka, V.; Yan, J.; Hanikyrová, M.; Mayadas, T.; Ross, G.D. The beta-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J. Immunol.* 1999, 162, 2281–2290.

93. Bajic, G.; Yatime, L.; Sim, R.B.; Vorup-Jensen, T.; Andersen, G.R. Structural insight on the recognition of surface-bound opsonins by the integrin domain of complement receptor 3. *Proc. Natl. Acad. Sci. USA* 2013, 110, 16426–16431. [CrossRef] [PubMed]

94. Goodridge, H.S.; Wolf, A.J.; Underhill, D.M. Beta-glucan recognition by the innate immune system. *Immunol. Res.* 2009, 230, 38–50. [CrossRef] [PubMed]

95. Jin, Y.; Li, P.; Wang, F. β-Glucans as potential immunoadjuvants: A review on the adjuvanticity, structure-activity relationship and receptor recognition properties. *Vaccine* 2018, 36, 5235–5244. [CrossRef] [PubMed]

96. Chan, G.C.; Chan, W.K.; Sze, D.M. The effects of beta-glucan on human immune and cancer cells. *J. Hematol. Oncol.* 2009, 2, 25. [CrossRef]

97. Brown, G.D.; Taylor, P.R.; Reid, D.M.; Willment, J.A.; Williams, D.L.; Martinez-Pomares, L.; Wong, S.Y.; Gordon, S. Dectin-1 is a major beta-glucan receptor on macrophages. *J. Exp. Med.* 2002, 196, 407–412. [CrossRef]
Heinsbroek, S.E.; Taylor, P.R.; Rosas, M.; Willment, J.A.; Williams, D.L.; Gordon, S.; Brown, G.D. Expression of low molecular-weight curdlan, (1→3)-beta-glucan suppresses TLR2-induced RANKL-dependent bone resorption. *Biol. Pharm. Bull.* 2018, 41, 1282–1285. [CrossRef]

Goodridge, H.S.; Reyes, C.N.; Becker, C.A.; Katsumoto, T.R.; Ma, J.; Wolf, A.J.; Bose, N.; Chan, A.S.; Magee, A.S.; Danielson, M.E.; et al. Activation of the innate immune receptor Dectin-1 upon formation of a ‘phagocytic synapse’. *Nature* 2011, 472, 471–475. [CrossRef]

Shah, V.B.; Williams, D.L.; Keshvara, L. *β*-Glucan attenuates TLR2- and TLR4-mediated cytokine production by microglia. *Neurosci. Lett.* 2009, 458, 111–115. [CrossRef]

Ferwerda, G.; Netea, M.G.; Joosten, L.A.; van der Meer, J.W.; Romani, L.; Kullberg, B.J. The role of Toll-like receptors and C-type lectins for vaccination against *Candida albicans*. *Vaccine* 2008, 28, 614–622. [CrossRef] [PubMed]

Heinsbroek, S.E.; Taylor, P.R.; Rosas, M.; Willment, J.A.; Williams, D.L.; Gordon, S.; Brown, G.D. Expression of functionally different dectin-1 isoforms by murine macrophages. *J. Immunol.* 2006, 176, 5513–5518. [CrossRef] [PubMed]

Sahasrabudhe, N.M.; Doktor-Fokkens, J.; de Vos, P. Particulate beta-glucans synergistically activate TLR4 and Dectin-1 in human dendritic cells. *Mol. Nutr. Food Res.* 2016, 60, 2514–2522. [CrossRef] [PubMed]

Branz, N.; Lubojemska, A.; Hardison, S.E.; Wang, Q.; Gutierrez, M.G.; Brown, G.D.; Papayannopoulos, V. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nat. Immunol.* 2014, 15, 1017–1025. [CrossRef] [PubMed]

Baert, K.; Sonck, E.; Goddeeris, B.M.; Devriendt, B.; Cox, E. Cell type-specific differences in beta-glucan recognition and signalling in porcine innate immune cells. *Dev. Comp. Immunol.* 2015, 48, 192–203. [CrossRef] [PubMed]

Huang, J.H.; Lin, C.Y.; Wu, S.Y.; Chen, W.Y.; Chu, C.L.; Brown, G.D.; Chuu, C.P.; Wu-Hsieh, B.A. *CR3 and dectin-1 collaborate in macrophage cytokine response through association on lipid rafts and activation of Syk-JNK-AP-1 pathway.* *PLoS Pathog.* 2015, 11, e1004985. [CrossRef] [PubMed]

Sahasrabudhe, N.M.; Tian, L.; van den Berg, M.; Bruggeman, G.; Bruininx, E.; Schols, H.A.; Faas, M.M.; de Vos, P. Endo-glucanase digestion of oat *β*-glucan enhances Dectin-1 activation in human dendritic cells. *J. Funct. Foods* 2016, 21, 104–112. [CrossRef] [PubMed]

Ferwerda, G.; Meyer-Wentrup, F.; Kullberg, B.J.; Netea, M.G.; Adema, G.J. *Dectin-1 synergizes with TLR2 and TLR4 for cytokine production in human primary monocytes and macrophages.* *Cell. Microbiol.* 2008, 10, 2058–2066. [CrossRef]

Kanjan, P.; Sahasrabudhe, N.M.; de Haan, B.J.; de Vos, P. Immune effects of *β*-glucan are determined by combined effects on Dectin-1, TLR2, 4 and 5. *J. Funct. Foods* 2017, 37, 433–440. [CrossRef]

Su, C.H.; Lu, M.K.; Lu, T.J.; Lai, M.N.; Ng, L.T. A (1→6)-Branch(1→4)-beta-β-glucan from *Grifola frondosa* inhibits lipopolysaccharide-induced cytokine production in RAW264.7 macrophages by binding to TLR2 rather than Dectin-1 or CR3 receptors. *J. Nat. Prod.* 2020, 83, 231–242. [CrossRef]

Liu, M.; Tong, Z.; Ding, C.; Luo, F.; Wu, S.; Wu, C.; Albeituni, S.; He, L.; Hu, X.; Tieri, D.; et al. Transcription factor c-Maf is a checkpoint that programs macrophages in lung cancer. *J. Clin. Invest.* 2015, 130, 2081–2096. [CrossRef]

Petit, J.; Bailey, E.C.; Wheeler, R.T.; de Oliveira, C.A.F.; Ferlenza, M.; Wiegertjes, G.F. Studies into beta-glucan recognition in fish suggests a key role for the C-type lectin pathway. *Front. Immunol.* 2019, 10, 280. [CrossRef]

Novak, M.; Vetvicka, V. Beta-glucans, history, and the present: Immunomodulatory aspects and mechanisms of action. *J. Immunotoxicol.* 2008, 5, 47–57. [CrossRef]

Vetvicka, V.; Vetvickova, J. A comparison of injected and orally administered *β*-glucans. *J. Am. Nutr. Assoc.* 2008, 11, 42–49.

Volman, J.J.; Ramakers, J.D.; Plat, J. Dietary modulation of immune function by beta-glucans. *Physiol. Behav.* 2008, 94, 276–284. [CrossRef] [PubMed]

Selvaraj, V.; Sampath, K.; Sekar, V. Adjuvant and immunostimulatory effects of beta-glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with *Aeromonas hydrophila*. *Vet. Immunol. Immunopathol.* 2006, 114, 15–24. [CrossRef] [PubMed]
Molecules 2020, 25, 5378

118. Suanyuk, N.; Itsaro, A. Efficacy of inactivated Streptococcus iniae vaccine and protective effect of beta-(1,3/1,6)-glucan on the effectiveness of vaccine in red tilapia Oreochromis niloticus × O. mossambicus. Songklanakarin J. Sci. Technol. 2011, 33, 143–149.

119. Anderson, D.P.; Siwicki, A.K. Duration of protection against Aeromonas salmonicida in brook trout immunostimulated with glucan or chitosan by injection or immersion. Prog. Fish Cult. 1994, 56, 258–261. [CrossRef]

120. Misra, C.K.; Das, B.K.; Mukherjee, S.C.; Pattnaik, P. Effect of multiple injections of beta-glucan on non-specific immune response and disease resistance in Labeo rohita fingerlings. Fish Shellfish Immunol. 2006, 20, 305–319. [CrossRef]

121. Selvaraj, V.; Sampath, K.; Sekar, V. Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (Cyprinus carpio) infected with Aeromonas hydrophila. Fish Shellfish Immunol. 2005, 19, 293–306. [CrossRef] [PubMed]

122. Guselle, N.J.; Speare, D.J.; Markham, R.J.F.; Patelakis, S. Efficacy of intraperitoneally and orally administered ProValence, a Yeast β-(1,3)/(1,6)-glucan product, in inhibiting Xenoma formation by the microsporidian Loma salmonae on rainbow Trout gills. N. Am. J. Aquac. 2010, 72, 65–72. [CrossRef]

123. Paredes, M.; Gonzalez, K.; Figueroa, J.; Montiel-Eulefi, E. Immunomodulatory effect of prolactin on Atlantic salmon (Salmo salar) macrophage function. Fish Physiol. Biochem. 2013, 39, 1215–1221. [CrossRef]

124. Yan, Y.; Huo, X.; Ai, T.; Su, J. beta-glucan and anisodamine can enhance the immersion immune efficacy of inactivated cyprinid herpesvirus 2 vaccine in Carassius auratus gibelio. Fish Shellfish Immunol. 2020, 98, 285–295. [CrossRef] [PubMed]

125. Petit, J.; Wiegertjes, G.F. Long-lived effects of administering beta-glucans: Indications for trained immunity in fish. Dev. Comp. Immunol. 2016, 64, 93–102. [CrossRef] [PubMed]

126. Bazan, S.B.; Breinig, T.; Schmitt, M.J.; Breinig, F. Heat treatment improves antigen-specific T cell activation after protein delivery by several but not all yeast genera. Vaccine 2014, 32, 2591–2598. [CrossRef] [PubMed]

127. De Gregorio, E.; D’Oro, U.; Wack, A. Immunology of TLR-independent vaccine adjuvants. Curr. Opin. Immunol. 2009, 21, 339–345. [CrossRef]

128. Miyamoto, N.; Mochizuki, S.; Sakurai, K. Designing an immunocyte-targeting delivery system by use of beta-glucan. Vaccine 2018, 36, 186–189. [CrossRef]

129. Figueras, A.; Santarem, M.M.; Novoa, B. Influence of the sequence of administration of beta-glucans and a Vibrio damselae vaccine on the immune response of turbot (Scophthalmus maximus L.). Vet. Immunol. Immunopathol. 1998, 64, 59–68. [CrossRef]

130. Berner, V.K.; duPre, S.A.; Redelman, D.; Hunter, K.W. Microparticulate beta-glucan vaccine conjugates phagocytized by dendritic cells activate both naive CD4 and CD8 T cells in vitro. Cell. Immunol. 2015, 298, 104–114. [CrossRef]

131. Hino, S.; Kito, A.; Yokoshima, R.; Sugino, R.; Oshima, K.; Morita, T.; Okajima, T.; Nadano, D.; Uchida, K.; Matsuda, T. Discharge of solubilized and Dectin-1-reactive beta-glucan from macrophage cells phagocytizing insoluble beta-glucan particles: Involvement of reactive oxygen species (ROS)-driven degradation. Biochem. Biophys. Res. Commun. 2012, 421, 329–334. [CrossRef]

132. Swennen, K.; Courtin, C.M.; Delcour, J.A. Non-digestible oligosaccharides with prebiotic properties. Crit. Rev. Food Sci. Nutr. 2006, 46, 459–471. [CrossRef]

133. Cao, Y.; Zou, S.; Xu, H.; Li, M.; Tong, Z.; Xu, M.; Xu, X. Hypoglycemic activity of the Baker’s yeast beta-glucan in obese/type 2 diabetic mice and the underlying mechanism. Mol. Nutr. Food Res. 2016, 60, 2678–2690. [CrossRef] [PubMed]

134. Harris, S.J.; Bray, D.P.; Adamek, M.; Hulse, D.R.; Steinhagen, D.; Hoole, D. Effect of beta-1,3,1/6-glucan upon immune responses and bacteria in the gut of healthy common carp (Cyprinus carpio). J. Fish Biol. 2020, 96, 444–455. [CrossRef] [PubMed]

135. Lee, P.T.; Liao, Z.H.; Huang, H.T.; Chuang, C.Y.; Nan, F.H. Beta-glucan alleviates the immunosuppressive effects of oxytetracycline on the non-specific immune responses and resistance against Vibrio alginitolyticus infection in Epinephelus fuscoguttatus × Epinephelus lanceolatus hybrids. Fish Shellfish Immunol. 2020, 100, 467–475. [CrossRef]

136. Miest, J.J.; Hoole, D. Time and concentration dependency of MacroGard(R) induced apoptosis. Fish Shellfish Immunol. 2015, 42, 363–366. [CrossRef] [PubMed]
137. Sirimanapong, W.; Thompson, K.D.; Ooi, E.L.; Bekarta, M.; Collet, B.; Taggart, J.B.; Bron, J.E.; Green, D.M.; Shinn, A.P.; Adams, A.; et al. The effects of feeding beta-glucan to *Pangasianodon hypophthalmus* on immune gene expression and resistance to *Edwardsiella ictaluri*. *Fish Shellfish Immunol.* 2015, 47, 595–605. [CrossRef] [PubMed]

138. Do-Huu, H.; Nguyen, T.H.N.; Tran, V.H. Effects of dietary beta-glucan supplementation of growth, innate immune, and capacity against pathogen *Streptococcus iniae* of juvenile pompano (*Trachinotus ovatus*). *Isr. J. Aquac.* 2019, 71, 1622–1632.

139. Di Domenico, J.; Canova, R.; Soveral, L.; Nied, C.; Costa, M.; Frandoloso, R.; Carlos, K. Immunomodulatory effects of dietary beta-glucan in silver catfish (*Rhamdia quelen*). *Pesqui. Vet. Bras.* 2017, 37, 73–78. [CrossRef]

140. Akramiene, D.; Kondrotas, A.; Didziapetriene, J.; Kevelaitis, E. Eects of dietary beta-glucans on the immune system. *Medicina (Kaunas)* 2007, 43, 597–606. [CrossRef] [PubMed]

141. Rice, P.J.; Adams, E.L.; Ozment-Skelton, T.; Gonzalez, A.J.; Goldman, M.P.; Lockhart, B.E.; Barker, L.A.; Mora, N.; Garcia, S.; Rodas, H.; et al. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. *J. Pharmacol. Exp. Ther.* 2005, 314, 1079–1086. [CrossRef] [PubMed]

142. Thompson, I.J.; Oyston, P.C.; Williamson, D.E. Potential of the beta-glucans to enhance innate resistance to biological agents. *Expert Rev. Anti-Infect. Ther.* 2010, 8, 339–352. [CrossRef] [PubMed]

143. Schmitt, P.; Wacyk, J.; Morales-Lange, B.; Rojas, V.; Guzman, F.; Dixon, B.; Mercado, L. Immunomodulatory effect of cathelicidins in response to a beta-glucan in intestinal epithelial cells from rainbow trout. *Dev. Comp. Immunol.* 2015, 51, 160–169. [CrossRef]

144. Djordjevic, B.; Skugor, S.; Jorgensen, S.M.; Overland, M.; Mydland, L.T.; Krasnov, A. Modulation of splenic immune responses to bacterial lipopolysaccharide in rainbow trout (*Oncorhynchus mykiss*) fed lentinan, a beta-glucan from mushroom *Lentinula edodes*. *Fish Shellfish Immunol.* 2009, 26, 201–209. [CrossRef]

145. Djordjevic, B.; Skugor, S.; Jorgensen, S.M.; Overland, M.; Mydland, L.T.; Krasnov, A. Modulation of splenic immune responses to bacterial lipopolysaccharide in rainbow trout (*Oncorhynchus mykiss*) fed lentinan, a beta-glucan from mushroom *Lentinula edodes*. *Fish Shellfish Immunol.* 2009, 26, 201–209. [CrossRef]

146. Thompson, I.J.; Oyston, P.C.; Williamson, D.E. Potential of the beta-glucans to enhance innate resistance to biological agents. *Expert Rev. Anti-Infect. Ther.* 2010, 8, 339–352. [CrossRef] [PubMed]

147. Breuel, K.F.; Deponti, W.K.; Kalbfleisch, J.H.; et al. Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. *J. Pharmacol. Exp. Ther.* 2005, 314, 1079–1086. [CrossRef] [PubMed]

148. Djordjevic, B.; Skugor, S.; Jorgensen, S.M.; Overland, M.; Mydland, L.T.; Krasnov, A. Modulation of splenic immune responses to bacterial lipopolysaccharide in rainbow trout (*Oncorhynchus mykiss*) fed lentinan, a beta-glucan from mushroom *Lentinula edodes*. *Fish Shellfish Immunol.* 2009, 26, 201–209. [CrossRef]

149. Shinn, A.P.; Adams, A.; et al. The eects of orally administered immunostimulants on inflammatory gene expression and sea lice (*Lepeophtheirus salmonis*) burdens on Atlantic salmon (*Salmo salar*). *Aquaculture 2012, 366–367, 9–16. [CrossRef] [PubMed]

150. Covello, J.M.; Friend, S.E.; Purcell, S.L.; Burk, J.F.; Markham, R.J.F.; Donkin, A.W.; Groman, D.B.; Fast, M.D. Eects of dietary supplementation of *β*-1,6-glucan and probiotic *Euglena gracilis* beta-1,3-glucan from mushroom *Lentinula edodes* in rainbow trout (*Oncorhynchus mykiss*) fed lentinan, a beta-glucan from mushroom *Lentinula edodes*. *Fish Shellfish Immunol.* 2009, 26, 201–209. [CrossRef]

151. Fast, M.D. Eects of orally administered immunostimulants on inflammatory gene expression and sea lice (*Lepeophtheirus salmonis*) burdens on Atlantic salmon (*Salmo salar*). *Aquaculture 2012, 366–367, 9–16. [CrossRef] [PubMed]

152. Shinn, A.P.; Adams, A.; et al. The eects of orally administered immunostimulants on inflammatory gene expression and sea lice (*Lepeophtheirus salmonis*) burdens on Atlantic salmon (*Salmo salar*). *Aquaculture 2012, 366–367, 9–16. [CrossRef] [PubMed]

153. Finoia, M.G.; Abelli, L.; Scaglioni, G.; Tiscar, P.G.; Sarti, M.; Marino, G. Short- and long-term effects of a dietary yeast beta-glucan (Macrogard) and alginate acid (Ergosan) preparation on immune response in sea bream (*Dicentrarchus labrax*). *Fish Shellfish Immunol.* 2005, 18, 311–325. [CrossRef] [PubMed]
154. Verlhac, V.; Obach, A.; Gabaudan, J.; SchÜEp, W.; Hole, R. Immunomodulation by dietary vitamin C and glucan in rainbow trout (Oncorhynchus mykiss). *Fish Shellfish Immunol.* 1998, 8, 409–424. [CrossRef]

155. Falco, A.; Frost, P.; Miest, J.; Pionnier, N.; Irrnazarow, I.; Hoole, D. Reduced inflammatory response to *Aeromonas salmonicida* infection in common carp (*Cyprinus carpio* L.) fed with beta-glucan supplements. *Fish Shellfish Immunol.* 2012, 32, 1051–1057. [CrossRef] [PubMed]

156. Pionnier, N.; Falco, A.; Miest, J.; Frost, P.; Irrnazarow, I.; Shrive, A.; Hoole, D. Dietary beta-glucan stimulate complement and C-reactive protein acute phase responses in common carp (*Cyprinus carpio*) during an *Aeromonas salmonicida* infection. *Fish Shellfish Immunol.* 2013, 34, 819–831. [CrossRef]

157. Miest, J.J.; Falco, A.; Pionnier, N.P.; Frost, P.; Miet, J.; Falco, A.; Pionnier, N.; Pietretti, D.; Forlenza, M.; Wiegertjes, G.F.; Hoole, D. The influence of dietary beta-glucan, PAMP exposure and *Aeromonas salmonicida* on apoptosis modulation in common carp (*Cyprinus carpio*). *Fish Shellfish Immunol.* 2012, 33, 846–856. [CrossRef] [PubMed]

158. Falco, A.; Miest, J.J.; Pionnier, N.; Pietretti, D.; Forlenza, M.; Wiegertjes, G.F.; Hoole, D. β-Glucan-supplemented diets increase poly(I:C)-induced gene expression of Mx, possibly via Tlr3-mediated recognition mechanism in common carp (*Cyprinus carpio*). *Fish Shellfish Immunol.* 2014, 36, 494–502. [CrossRef]

159. Cook, M.T.; Hayball, P.J.; Hutchinson, W.; Nowak, B.; Hayball, J.D. The efficacy of a commercial beta-glucan preparation, EcoActiva, on stimulating respiratory burst activity of head-kidney macrophages from pink snapper (*Ptrasurus*). *Sparidae*. *Fish Shellfish Immunol.* 2001, 11, 661–672. [CrossRef]

160. Meena, D.K.; Das, P.; Kumar, S.; Mandal, S.C.; Prusty, A.K.; Singh, S.K.; Akhtar, M.S.; Behera, B.K.; Kumar, K.; Pal, A.K.; et al. Beta-glucan: An ideal immunostimulant in aquaculture (a review). *Fish Physiol. Biochem.* 2013, 39, 431–457. [CrossRef]

161. Cook, M.T.; Hayball, P.J.; Hutchinson, W.; Nowak, B.F.; Hayball, J.D. Administration of a commercial immunostimulant preparation, EcoActiva as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Ptrasurus*). *Sparidae* (Bloch and Schneider)) in winter. *Fish Shellfish Immunol.* 2003, 14, 333–345. [CrossRef]

162. Palic, D.; Andreasen, C.B.; Herolt, D.M.; Menzel, B.W.; Roth, J.A. Immunomodulatory effects of beta-glucan on neutrophil function in fathead minnows (*Pimephales promelas Rafinesque*, 1820). *Dev. Comp. Immunol.* 2006, 30, 817–830. [CrossRef]

163. Ai, Q.; Mai, K.; Zhang, L.; Tan, B.; Zhang, W.; Xu, W.; Li, H. Effects of dietary beta-1,3 glucan on innate immune response of large yellow croaker, *Pseudocentra crocea*. *Fish Shellfish Immunol.* 2007, 22, 394–402. [CrossRef]

164. Kumari, J.; Sahoo, P.K. Dietary immunostimulants influence specific immune response and resistance of healthy and immunocompromised Asian catfish *Clarias batrachus* to *Aeromonas hydrophila* infection. *Dis. Aquat. Organ.* 2006, 70, 63–70. [CrossRef] [PubMed]

165. Kumari, J.; Sahoo, P.K. Non-specific immune response of healthy and immunocompromised Asian catfish (*Clarias batrachus* to several immunostimulants. *Aquaculture* 2006, 255, 133–141. [CrossRef]

166. Dalmo, R.A.; Bogwald, J. β-Glucans as conductors of immune symphonies. *Fish Shellfish Immunol.* 2008, 25, 384–396. [CrossRef] [PubMed]

167. Álvarez-Rodriguez, M.; Pereiro, P.; Reyes-López, F.E.; Tort, L.; Figueras, A.; Novoa, B. Analysis of the long-lived responses induced by immunostimulants and their effects on a viral infection in Zebrafish (*Danio rerio*). *Front. Immunol.* 2018, 9. [CrossRef] [PubMed]

168. Sahoo, P.K. Role of immunostimulants in disease resistance of fish. CAB Rev. 2007, 2. [CrossRef]

169. Sabioni, R.E.; Zanuzzo, F.S.; Gimbo, R.Y.; Urbanati, E.C. beta-glucan enhances respiratory activity of leukocytes suppressed by stress and modulates blood glucose levels in pacu (*Piaractus mesopotamicus*). *Fish Physiol. Biochem.* 2020, 46, 629–640. [CrossRef]

170. Misra, C.K.; Das, B.K.; Mukherjee, S.C.; Pattnaik, P. Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. *Aquaculture* 2006, 255, 82–94. [CrossRef]

171. Lauridsen, J.H.; Buchmann, K. Effects of short- and long-term glucan feeding of rainbow trout (*Salmonidae*) on the susceptibility to *Ichthyophthirius multifiliis* infections. *Acta Ichthyol. Piscat.* 2010, 40, 61–66. [CrossRef]

172. Lin, S.; Pan, Y.; Luo, L.; Luo, L. Effects of dietary beta-1,3-glucan, chitosan or raffinose on the growth, innate immunity and resistance of koi (*Cyprinus carpio koi*). *Fish Shellfish Immunol.* 2011, 31, 788–794. [CrossRef]

173. Kumari, J.; Sahoo, P.K. Dietary beta-1,3 glucan potentiates innate immunity and disease resistance of Asian catfish, *Clarias batrachus* (L.). *J. Fish Dis.* 2006, 29, 95–101. [CrossRef] [PubMed]
174. Douxfils, J.; Fierro-Castro, C.; Mandiki, S.N.; Emile, W.; Tort, L.; Kestemont, P. Dietary beta-glucans differentially modulate immune and stress-related gene expression in lymphoid organs from healthy and Aeromonas hydrophila-infected rainbow trout (Oncorhynchus mykiss). *Fish Shellfish Immunol.* 2017, 63, 285–296. [CrossRef] [PubMed]

175. Do Huu, H.; Sang, H.M.; Thanh Thuy, N.T. Dietary β-glucan improved growth performance, Vibrioc counts, haematological parameters and stress resistance of pompano fish, *Trachinotus ovatus Linnaeus*, 1758. *Fish Shellfish Immunol.* 2016, 54, 402–410. [CrossRef] [PubMed]

176. Adloo, M.N.; Soltanian, S.; Hafezieh, M.; Ghadimi, N. Effects of long term dietary administration of beta-Glucan on the growth, survival and some blood parameters of striped catfish, *Pangasianodon hypophthalmus* (Siluriformes: Pangasiidae). *Iran. J. Ichthyol.* 2015, 2, 194–200.

177. Kiseleva, M.; Balabanova, L.; Elyakova, L.; Rasskazov, V.; Zvyagintseva, T. Dietary beta-glucan (MacroGard(R)) improves growth performance, Vibrio counts in juvenile chinook salmon Oncorhynchus tshawytscha. *Dis. Aquat. Organ.* 1993, 17, 191–196. [CrossRef]

178. Zhang, Z.; Swain, T.; Bogwald, J.; Dalmo, R.A.; Kumari, J. Bath immunostimulation of rainbow trout (Oncorhynchus mykiss) fry induces enhancement of inflammatory cytokine transcripts, while repeated bath induce no changes. *Fish Shellfish Immunol.* 2009, 26, 677–684. [CrossRef]

179. Nikl, L.; Evelyn, T.P.; Albright, L.J. Trials with an orally and immersion-administered beta-1,3 glucan as an immunoprophylactic against *Aeromonas salmonicida* in juvenile chinook salmon Oncorhynchus tshawytscha. *J. Fish Dis.* 2014, 37, 3–10. [CrossRef]

180. Zhang, Z.; Swain, T.; Bogwald, J.; Dalmo, R.A.; Kumari, J. Bath immunostimulation of rainbow trout (Oncorhynchus mykiss) fry induces enhancement of inflammatory cytokine transcripts, while repeated bath induce no changes. *Fish Shellfish Immunol.* 2009, 26, 677–684. [CrossRef]

181. Lam, K.-L.; Chi-Keung Cheung, P. Non-digestible long chain beta-glucans as novel prebiotics. *J. Biol. Chem.* 2018, 293, 5359–5369. [CrossRef] [PubMed]

182. Miest, J.J.; Arndt, C.; Adamek, M.; Steinhagen, D.; Reusch, T.B. Dietary beta-glucan (MacroGard(R)) enhances survival of first feeding turbot (*Scophthalmus maximus*) larvae by altering immunity, metabolism and microbiota. *Fish Shellfish Immunol.* 2014, 39, 188–195. [CrossRef] [PubMed]

183. Pietretti, D.; Vera-Jimenez, N.I.; Hoole, D.; Wiegejttes, G.F. Oxidative burst and nitric oxide responses in carp macrophages induced by zymosan, MacroGard(R)) and selective dectin-1 agonists suggest recognition by multiple pattern recognition receptors. *Fish Shellfish Immunol.* 2013, 35, 847–857. [CrossRef] [PubMed]

184. Barros, M.M.; Falcon, D.R.; Orsi, R.O.; Pezzato, L.E.; Fernandes, A.C.; Fernandes, A.; de Carvalho, P.L.P.F.; Padovani, C.R.; Sartori, M.M. Non-specific immune parameters and physiological response of Nile tilapia fed beta-glucan and vitamin C for different periods and submitted to stress and bacterial challenge. *Fish Shellfish Immunol.* 2014, 39, 188–195. [CrossRef] [PubMed]

185. Wiegertjes, G.F.; Wentzel, A.S.; Spaink, H.P.; Elks, P.M.; Fink, I.R. Polarization of immune responses in fish: The ‘macrophages first’ point of view. *Mol. Immunol.* 2016, 69, 146–156. [CrossRef]

186. Wiegertjes, G.F.; Wentzel, A.S.; Spaink, H.P.; Elks, P.M.; Fink, I.R. Polarization of immune responses in fish: The ‘macrophages first’ point of view. *Mol. Immunol.* 2016, 69, 146–156. [CrossRef]

187. Refstie, S.; Baeverfjord, G.; Seim, R.R.; Elvebø, O.E. Dietary beta-glucan (MacroGard(R)) enhances Vibrio counts in nile tilapia (*Oreochromis niloticus* C) after feeding with beta-1,3/1,6-glucan. *Fish Shellfish Immunol.* 2017, 63, 285–296. [CrossRef] [PubMed]

188. Barros, M.M.; Falcon, D.R.; Orsi, R.O.; Pezzato, L.E.; Fernandes, A.C.; Fernandes, A.; de Carvalho, P.L.P.F.; Padovani, C.R.; Guimaraes, I.G.; Sartori, M.M.P. Immunomodulatory effects of dietary -glucan and vitamin C in nile tilapia, *Oreochromis niloticus*, subjected to cold-induced stress or bacterial challenge. *J. World Aquac. Soc.* 2015, 46, 363–380. [CrossRef]

189. Salah, A.S.; El Nahas, A.F.; Mahmoud, S. Modulatory effect of different doses of beta-1,3/1,6-glucan on the expression of antioxidant, inflammatory, stress and immune-related genes of *Oreochromis niloticus* challenged with Streptococcus iniae. *Fish Shellfish Immunol.* 2017, 70, 204–213. [CrossRef]

190. Jung-Schroers, V.; Adamek, M.; Harris, S.; Syakuri, H.; Jung, A.; Irmazarow, I.; Steinhagen, D. Response of the intestinal mucosal barrier of carp (*Cyprinus carpio*) to a bacterial challenge by *Aeromonas hydrophila* intubation after feeding with beta-1,3/1,6-glucan. *J. Fish Dis.* 2018, 41, 1077–1092. [CrossRef]
191. Kiron, V.; Kulkarni, A.; Dahle, D.; Vasanth, G.; Lokesh, J.; Elvebo, O. Recognition of purified beta 1,3/1,6 glucan and molecular signalling in the intestine of Atlantic salmon. *Dev. Comp. Immunol.* **2016**, *56*, 57–66. [CrossRef]

192. Brogden, G.; von Kockritz-Blickwede, M.; Adamek, M.; Reuner, F.; Jung-Schoers, V.; Naim, H.Y.; Steinhagen, D. Beta-glucan protects neutrophil extracellular traps against degradation by *Aeromonas hydrophila* in carp (*Cyprinus carpio*). *Fish Shellfish Immunol.* **2012**, *33*, 1060–1064. [CrossRef]

193. Vera-Jimenez, N.I.; Nielsen, M.E. Carp head kidney leukocytes display different patterns of oxygen radical production after stimulation with PAMPs and DAMPs. *Mol. Immunol.* **2013**, *55*, 231–236. [CrossRef] [PubMed]

194. Vera-Jimenez, N.I.; Pietretti, D.; Wiegerjtjes, G.F.; Nielsen, M.E. Comparative study of beta-glucan induced respiratory burst measured by nitroblue tetrazolium assay and real-time luminol-enhanced chemiluminescence assay in common carp (*Cyprinus carpio*). *Fish Shellfish Immunol.* **2013**, *34*, 1216–1222. [CrossRef] [PubMed]

195. Montoya, L.N.F.; Favero, G.C.; Zanuzzo, F.S.; Urbinati, E.C. Distinct beta-glucan molecules modulates differently the circulating cortisol levels and innate immune responses in matrix (Brycon amazonicus). *Fish Shellfish Immunol.* **2018**, *83*, 314–320. [CrossRef] [PubMed]

196. Kuhlwein, H.; Emery, M.J.; Rawling, M.D.; Harper, G.M.; Merrifield, D.L.; Davies, S.J. Effects of a dietary beta-(1,3)(1,6)-n-glucan supplementation on intestinal microbial communities and intestinal ultrastructure of mirror carp (*Cyprinus carpio* L.). *J. Appl. Microbiol.* **2013**, *115*, 1091–1106. [CrossRef]

197. Do-Huu, H.; Lam, H.S.; Nguyen, C.V. Beta-glucan supplemented diet stimulates C-reactive protein and complement immune acute phase responses following PAMPs injection. *Fish Shellfish Immunol.* **2014**, *38*, 1–13. [CrossRef] [PubMed]

198. Sych, G.; Frost, P.; Irnazarow, I. Influence of beta-glucan (Macrogard®) on innate immunity of carp fry. *Bull. Vet. Inst. Pulawy* **2013**, *57*, 219–223. [CrossRef]

199. Brogden, G.; Krimmling, T.; Adamek, M.; Naim, H.Y.; Steinhagen, D.; von Kockritz-Blickwede, M. The effect of beta-glucan on formation and functionality of neutrophil extracellular traps in carp (Cyprinus carpio L.). *Dev. Comp. Immunol.* **2014**, *44*, 280–285. [CrossRef]

200. Brogden, G.; Krimmling, T.; Adamek, M.; Naim, H.Y.; Steinhagen, D.; von Kockritz-Blickwede, M. The effect of beta-glucan on formation and functionality of neutrophil extracellular traps in carp (Cyprinus carpio L.). *Dev. Comp. Immunol.* **2014**, *44*, 280–285. [CrossRef] [PubMed]

201. Kuhlwein, H.; Merrifield, D.L.; Rawling, M.D.; Foey, A.D.; Davies, S.J. Effects of dietary beta-(1,3)(1,6)-n-glucan supplementation on growth performance, intestinal morphology and haemato-immunological profile of mirror carp (*Cyprinus carpio* L.). *J. Anim. Physiol. Anim. Nutr. (Berl.)* **2014**, *98*, 279–289. [CrossRef] [PubMed]

202. Pionnier, N.; Falco, A.; Miest, J.J.; Shrive, A.K.; Hoole, D. Feeding common carp *Cyprinus carpio* with beta-glucan supplemented diet stimulates C-reactive protein and complement immune acute phase responses following PAMPs injection. *Fish Shellfish Immunol.* **2014**, *39*, 285–295. [CrossRef] [PubMed]
208. Jung-Schroers, V.; Adamek, M.; Jung, A.; Harris, S.; Döza, Ö.S.; Baumer, A.; Steinhagen, D. Feeding of β-1,3/1,6-glucan increases the diversity of the intestinal microflora of carp (Cyprinus carpio). *Aquac. Nutr.* 2016, 22, 1026–1039. [CrossRef]

209. Schmidt, J.G.; Andersen, E.W.; Ersboll, B.K.; Nielsen, M.E. Muscle wound healing in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol.* 2016, 48, 273–284. [CrossRef]

210. Welker, T.L.; Lim, C.; Yildirim-Aksoy, M.; Klesius, P.H. Use of diet crossover to determine the effects of beta-glucan supplementation on immunity and growth of nile tilapia, *Oreochromis niloticus*. *J. World Aquac. Soc.* 2012, 43, 335–346. [CrossRef]

211. Dos Santos Voloski, A.P.; de Figueiredo Soveral, L.; Dazzi, C.C.; Sutili, F.; Frandoloso, R.; Kreutz, L.C. β-Glucan improves wound healing in silver catfish (*Rhamdia quelen*). *Fish Shellfish Immunol.* 2019, 93, 575–579. [CrossRef]

212. Ghadri, G.; Keyvanshokooh, S.; Mohammadi Azarm, H.; Akhlaghi, M. Proteomic analysis of muscle tissue from rainbow trout (*Oncorhynchus mykiss*) fed dietary beta-glucan. *Iran. J. Vet. Res.* 2016, 17, 184–189.

213. Dawood, M.A.O.; Abdo, S.E.; Gewaily, M.S.; Moustafa, E.M.; SaadAllah, M.S.; AbdEl-Kader, M.F.; Almalki, A.; Khaled, J.M.; Almanaa, T.N.; Vaseeharan, B. Effects of BCG vaccination on both heterologous Th1/Th17 and Th1/Th1 responses and innate trained immunity. *J. Innate Immunol.* 2014, 6, 152–158. [CrossRef]

214. Divya, M.; Gopi, N.; Iswarya, A.; Govindarajan, M.; Alharbi, N.S.; Khaled, J.M.; Almanaa, T.N.; Vaseeharan, B. β-Glucan extracted from eukaryotic single-celled microorganism Saccharomyces cerevisiae: Dietary supplementation and enhanced ammonia stress tolerance on Oreochromis mossambicus. *Microb. Pathog.* 2020, 139, 103917. [CrossRef] [PubMed]

215. Kleinnijenhuis, J.; Quintin, J.; Preijers, F.; Joosten, L.A.; Irim, D.C.; Saeed, S.; Jacobs, C.; de Jong, D.; Stunnenberg, H.G.; et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific inflammatory and histopathology disorders caused by deltamethrin toxicity in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.* 2020, 98, 301–311. [CrossRef] [PubMed]

216. Netea, M.G.; Quintin, J.; van der Meer, J.W. Trained immunity: A memory for innate host defense. *Cell Host Microbe* 2011, 9, 355–361. [CrossRef]

217. Alvarez-Errico, D.; Vento-Tormo, R.; Sieweke, M.; Ballestar, E. Epigenetic control of myeloid cell differentiation, identity and function. *Nat. Rev. Immunol.* 2015, 15, 7–17. [CrossRef]

218. Kleinijenhuis, J.; Quintin, J.; Preijers, F.; Benn, C.S.; Joosten, L.A.; Jacobs, C.; van Loenhout, J.; Xavier, R.J.; Aaby, P.; van der Meer, J.W.; et al. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. *J. Innate Immunol.* 2014, 6, 152–158. [CrossRef]

219. Kleinnijenhuis, J.; Quintin, J.; Preijers, F.; Joosten, L.A.; Irim, D.C.; Saeed, S.; Jacobs, C.; van Loenhout, J.; de Jong, D.; Stunnenberg, H.G.; et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc. Natl. Acad. Sci. USA* 2012, 109, 17537–17542. [CrossRef]

220. Netea, M.G.; Latz, E.; Mills, K.H.; O’Neill, L.A. Innate immune memory: A paradigm shift in understanding host defense. *Nat. Immunol.* 2015, 16, 675–679. [CrossRef]

221. Conrath, U. Systemic acquired resistance. *Plant Signal. Behav.* 2006, 1, 179–184. [CrossRef]

222. Ryals, J.A.; Neuenschwander, U.H.; Willits, M.G.; Molina, A.; Steiner, H.Y.; Hunt, M.D. Systemic acquired resistance. *Plant Cell* 1996, 8, 1809–1819. [CrossRef]

223. Vernooij, B.; Friedrich, L.; Goy, P.A.; Staub, T.; Kessmann, H.; Ryals, J. 2,6-Dichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. *Mol. Plant Microbe Interact.* 1995, 8, 228–234. [CrossRef]

224. Kurtz, J. Specific memory within innate immune systems. *Trends Immunol.* 2005, 26, 186–192. [CrossRef] [PubMed]

225. Olivier, G.; Eaton, C.A.; Campbell, N. Interaction between *Aeromonas salmonicida* and peritoneal macrophages of brook trout (*Salvelinus fontinalis*). *Vet. Immunol. Immunopathol.* 1986, 12, 223–234. [CrossRef]

226. Kato, G.; Kondo, H.; Aoki, T.; Hirono, I. BCG vaccine confers adaptive immunity against *Mycobacterium* sp. infection in fish. *Dev. Comp. Immunol.* 2010, 34, 133–140. [CrossRef] [PubMed]
227. Kato, G.; Kondo, H.; Aoki, T.; Hirono, I. Mycobacterium bovis BCG vaccine induces non-specific immune responses in Japanese flounder against Nocardia seriolae. *Fish Shellfish Immunol.* 2012, 33, 243–250. [CrossRef] [PubMed]

228. Hohn, C.; Petrie-Hanson, L. Rag1−/− mutant zebrafish demonstrate specific protection following bacterial re-exposure. *PLoS ONE* 2012, 7, e44451. [CrossRef] [PubMed]

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