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The response of fish to immunostimulant diets.

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1. Fish Immunostimulation through dietary manipulation

The aquaculture sector has shown a rapid growth over the last 30 years with an associated increase in disease problems as result of rapid expansion and amongst other factors high stocking densities. In order to maintain fish health and to improve performance immunostimulants have been used as dietary additives to improve weight gain, feed efficiency, and/or disease resistance in cultured fish.

An immunostimulant is a natural or chemical substance that stimulates the immune system by specific (vaccines or antigens) or non-specific (irrespective of antigenic specificity) routes. In Aquaculture, non-specific immunostimulants have been widely used probably due to the limited knowledge of the immune response in fish and the ease of their application. In this review we will focus on recent studies on: (1) plant, herbs and algae; and (2) PAMPs (Pathogen Associated Molecular Patterns), as immunostimulants administered in diet to fish.

2. Most frequently evaluated immunological parameters in fish fed with supplemented diets from the immune response perspective.

The immunostimulant effect of dietary supplements in fish has been focused mainly on the evaluation of non-specific immune parameters and, therefore, on the consequences of these treatments on the innate immune system. The innate immune system has both cellular and humoral components by which it carries out its protective function. The major components of the innate immune system at cellular level are leukocytes, mainly monocytes, macrophages and granulocytes [1,2]. Among the granulocytes, neutrophils are the most abundant cell-type and their presence has been described in Salmoniformes, Cypriniformes and Perciformes [3]. Neutrophils and macrophages are responsible for the production of bioactive molecules for pathogen recognition and destruction, cellular communication and activation, initiation of an adaptive immune response and later, resolution of an inflammatory response and tissue repair. Furthermore, these cell types are responsible in the majority for phagocytosis [4], one of the main mediators of innate immunity to remove pathogens such as bacteria, viruses, and parasites. For this reason, these immune cell types are also called phagocytes. This microbe/killing mechanism triggers diverse antimicrobial processes that use a wide variety of mechanisms including cellular activation,

production of oxidative radicals, and the production of cytokines driving the inflammatory response amongst others.

Two of the most important antimicrobial systems of phagocytic cells are the NADPH phagocyte oxidase and inducible nitric oxide synthase (iNOS) pathways, which are responsible for the generation of superoxide (O_2^-) and nitric oxide (NO) radicals, respectively. NADPH oxidase, a multi-subunit complex, catalyses a one-electron reduction of molecular oxygen into superoxide anion (O_2^-), also referred to as reactive oxygen species (ROS), which is either spontaneously converted to H_2O_2 or enzymatically by superoxide dismutase (SOD). In comparison to neutrophils, the size of the respiratory burst is much reduced in macrophages [5]. Since O_2^- is the first product to be released from the respiratory burst, the measurement of O_2^- has been accepted as a direct and accurate way of measuring respiratory burst activity [6]: the reduction of ferricytochrome c to determine extracellular O_2^-, and the reduction of the nitroblue tetrazolium (NBT) redox dye to determine intracellular O_2^- [7]. On the other hand, iNOS is responsible for NO production and its derivatives, which are collectively known as reactive nitrogen species (RNS). Unlike ROS, macrophages generally produce considerably more RNS than neutrophils [8]. iNOS is activated by interferon gamma (IFN-γ) or by tumor necrosis factor (TNF) [9]. Also, NO has been demonstrated to activate nuclear factor-kappaB (NF-κB) in peripheral blood mononuclear cells, an important transcription factor in iNOS gene expression in response to inflammation [10]. The ROS and RNS antibacterial activity have been widely discussed [11].

Although less studied, myeloperoxidase (MPO) is a lysosomal protein stored in azurophilic granules also involved in antimicrobial mechanisms and is also produced by phagocytes. It is most abundantly expressed in neutrophils although is also present in circulating mammalian monocytes but is lost as these mature into macrophages [12]. It possesses antimicrobial activity via hypohalous acid production [13] and is released into the extracellular space during degranulation [14].

Among the immune cell parameters, red blood cell (RBC) count is a frequently used parameter to evaluate possible undesired collateral effects (anaemia) provoked by immunostimulant administered in supplemented feed. Interestingly, RBC's has have received increased attention over the last few years due to the reported participation of these cells in the rainbow trout immune response [15].

In addition to the cellular response, humoral elements also participate in the innate immune response including lysozyme or the complement system [1,2]. IgM is the most common immunoglobulin in serum and mucus and is the key player in systemic immune responses [16]. For this reason, the total immunoglobulin and total protein level (an indirect antibody level measurement) are frequent among the immune parameters evaluated in immunostimulant supplemented diets. IgM also participates in the opsonization of pathogens, facilitating their phagocytosis. In this ambit, complement is a vital component of innate immunity and represents one of the major effector mechanisms of the innate immune system [17]. It initiates with the identification of pathogenic surfaces and leads to the generation of potent pro-inflammatory mediators (anaphylatoxins), opsonization (coating) of the pathogenic surface through various complement opsonins (such as C3b), and targeted lysis of the pathogenic surface through the assembly of membrane-penetrating pores known as the membrane attack complex (MAC). The complement system can be activated through three major pathways: classical (antigen: antibody immune

complexes), lectin (PAMP recognition by lectins), and the alternative pathway (spontaneous hydrolysis/pathogenic surfaces) [17].

Various lytic enzymes, acting alone or in cascade, are also important in the defense against pathogens. Without any doubt, lysozyme is one of the most analyzed lytic enzyme activities to evaluate immunostimulant-enhanced improvement of innate immunity. Lysozyme is bactericidal, hydrolyzing β-[1,4] linked glycoside bonds of both Gram positive and negative bacterial cell wall peptidoglycans resulting in lysis [2]. As with the innate components described above, it is also present in the fish mucosa [16].

Finally, at the gene expression level pro-inflammatory (IL-1, IL-6, TNF-α) and anti-inflammatory or immunosuppressive (IL-10, TGF-β) cytokines have been evaluated in fish fed with immunostimulant supplemented diets. Thus, the limited available information addressing gene expression modulation does not allow a direct understanding the possible pathways and immunological functions stimulated by the administration of immunostimulants in a global context.

3. Fish mucosal immunity as a target of immunostimulant diets.

As described above, one of the main goals of immunostimulant diets is to confer resistant to pathogens by potentiating the immune system. Many studies have therefore focused upon the innate immune response at a cellular and humoral level but not on their consequences on other sites in direct contact with the immunostimulants such as the mucosa. One integral mechanism of fish resistance against pathogens is primarily centered in the portals of entry, i.e., the surfaces that are in contact with the external environment: gills, nose, gastrointestinal tract, and skin. Non-self stimuli will be recognized firstly in these mucosal tissues and as a consequence will produce local alterations that may also produce messenger substances (hormones, cytokines, peptides) that will activate the overall physiological response [16] promoting the immune response at systemic level.

As immunological sites, the mucosal tissues are capable of mounting a robust immune response against pathogens [18,19]. In teleosts, four mucosal-associated lymphoid tissues (MALT), responsible for the immune response at mucosal sites have been described: nose-associated lymphoid tissue (NALT), skin-associated lymphoid tissue (SALT), gill-associated lymphoid tissue (GIALT), and gut-associated lymphoid tissue (GALT) [20]. These lymphoid tissues have four main characteristics: (1) the lack of organized lymphoid structures, such as lymphoid nodes or germinal centers, that lead to a disperse location of leukocytes; (2) the presence of secretory Igs in the mucus, which are transported into the lumen through a polymeric Ig receptor (pIgR); (3) the presence of a specialized mucosal Ig class, IgT/Z; and 4) the presence of commensal bacteria, some of them coated by Igs [16].

At an immunological level, GALT has resident granulocytes, macrophages, lymphocytes, and plasma cells (lamina propria leukocytes, LPLs), and T and B cells among epithelial cells (intraepithelial lymphocytes, IELs). These cells together with epithelial cells, goblet cells, and neuroendocrine cells produce and regulate gut immune responses [16].

Taking into account that the main portals of entry, and therefore the primary immunological barrier, are SALT and GIALT there exists an urgency to generate knowledge that allows understanding of the influence immunosupplement diets in these tissues The skin is the is the outermost organ of the body and the first line of defense from external aggressions [21]. The presence of mucus-secreting cells in the fish epidermis defines this site as a mucosal tissue. The innate immune response is represented by lysozyme, complement, lectins, and proteolytic enzymes [22], while secreted IgM and IgT have also been detected [21,23]. On the other hand, GIALT has a particular relevance due its continuous contact with aquatic antigens and, at the same time, is a direct portal of entry to the bloodstream. Lymphocyte cell aggregation in the interbranchial lymphoid tissue (ILT) [24] mainly T cells and some scattered B cells [25] are present.

Thus, in the future it may be desirable to choose specific immunostimulants administered as dietary supplements depending of the nature and MALT target as a portal of entry to each specific pathogen.

4. Toll like receptors (TLR) and their role in fish fed with immunostimulant diets.

TLRs are type I transmembrane proteins associated to the innate immune response that are involved in the sensing of microbial-specific structures - or also danger-associated molecular patterns (PAMPs and DAMPs, respectively) by pattern-recognition receptors (PRRs). This molecular recognition may occur in various cellular compartments including the plasma membrane, endosomes, lysosomes and endolysosomes [26]. TLRs activate pathways through a variety of TIR-domain-containing adaptor proteins responsible of the cell signaling cascade activation, such as MyD88 and TRIF, which activate transcription factors (NF-kB, IRFs) and mitogen-activated protein kinases (MAPKs) to promote a pro-inflammatory response [27], thus modulating innate and adaptive immune response. In teleost, there are more than 17 TLRs identified [28] with evolutionary conservation of key components of the TLR-signaling pathway [29]. However, the fish TLRs also exhibit very distinct features and large diversity [30]

In the last decade there has been a growing interest in the use of plant, herbs and algae extracts as immunostimulant dietary supplements in fish. However, in most cases the mechanisms responsible of the physiological outcome in fish are still unknown [31]. Although many civilizations have used natural extracts for centuries, several studies in mammals have shown that plants extracts modulate TLRs [reviewed in [32]]. Thus, some plants and algae compounds can down-regulate mRNA expression [33–35] and induction [36,37] of TLRs, suppressing the TLR/NF-kB signalling pathway [38]. Conversely, other extracts promote the pro-inflammatory response [39] and enhance TLR9 and IRF7 activation inducing IFN-β protein expression [40]. These extracts also elicit the M1 and M2 macrophage responses through the TLR/NF-kB signaling pathway [41]. In fish, the effects of plant, herbal and algae extracts has been poorly described. Only one study using a zebrafish model suggests an anti-inflammatory effect of leuropein (primary phenolic compounds present in olive leaf) by inhibiting TLR and MAPK signaling [41]. In general, the phytochemical subcomponents of plant-derived extracts are diverse, ranging from phenolics, terpenoids, polysaccharides, and proteins. Thus, the extraction solvent is important in determining the final composition of the extract. In this ambit has been demonstrated while aqueous extracts reduce the activation of TLRs, ethanolic extracts from the same plant increased activation of the same TLRs [42–46]. Therefore, it seems that not only the nature of the plant-derived extract is important to determine the immunomodulatory effect, but also the extraction method employed. In conclusion, it

appears that it is essential to address the implications of the extraction method for plant-derived compounds. The nature of the potential regulatory ligands also requires further study in order to understand the linkage between the extracts and functional TLR outputs whether stimulatory or inhibitory. This area has significant potential reaching beyond fish alone with potential impact upon human health.

One of the most commonly used strategies to stimulate the immune system in fish is the administration of purified immunostimulants in the diet. This strategy follows the premise of recognition, identification and response to PAMPs can be used to modulate immunological activity. To date, the most commonly used PAMPs in fish immunostimulant diets are lipopolysaccharide (LPS), peptidoglycan (PGN) and β-glucans. In mammals LPS, a component of the outer membrane of Gram-negative bacteria, recognized by TLR4 forms a complex with co-receptors lymphocyte antigen 96 (MD2), and CD14 on the cell surface that serves as the main LPS-binding component leading to activation of multiple signalling components and the subsequent production of pro-inflammatory cytokines [47]. The utilization of LPS as an immunostimulant for fish seems to be contradictory because fish are highly resistant to the toxic effects of LPS [reviewed in [48]], due to the significant evolutionary differences observed between these two groups in terms of TLR4. The vast majority of fish do not express TLR4 and neither CD14 nor MD2 have been isolated [28]. Thus, it has been proposed for fish that (1) LPS interacts with alternative factors such as β2-integrins which are abundantly expressed on rainbow trout mononuclear phagocytes after stimulations with high concentrations of LPS [49]; and (2) TLR4 negatively regulates the MyD88-dependent TLR pathway [50]. PGN is the basic component of the bacterial cell wall. In mammals has reported that PGN activates both the TLR2 and NOD pathways however does not activate TLR4-mediated signal transduction [51,52]. In rainbow trout macrophages PGN stimulation induced the expression of MyD88, IRAK and TNF activated factor (TRAF6), suggesting TLR involvement in PGN-mediated inflammatory response in trout [53]. Interestingly both compounds are likely to be present in most PAMP preparations unless ultra-pure compounds are used. This is unlikely and not economical for IS diet production thus a mixture of potentially antagonist effects upon TLR signalling are likely occurring in parallel. The most commonly used PAMP included in the diets as an immunostimulant are the β-glucans, the major structural components of yeast and fungal cell walls. β-glucans consist of a heterogeneous group of glucose polymers also named β-1,3/1,6-glucans. In mammals, although various receptors e.g. complement receptor C3 and TLR1/6 have been described [54], dectin-1 is considered as the main β-glucan receptor [55]. However, dectin-1 has not been identified in fish and it has been suggested that β-glucan could be detected by TLRs however the actual target receptor is unknown [56]. Despite the growing knowledge of the mechanisms involved in the TLR-mediated fish innate immune response, more efforts are needed to determine the underlying effects upon the recognition and activation of fish immune response by PAMP-associated immunostimulants. The complex composition of immunostimulant extracts from plant, algal and microbial sources makes the identification of specific activation/suppression pathways and receptor-ligand relationships particularly difficult. When economic constraints are also taken into consideration for application of a complex IS diet to the farm the understanding of the underlying immune response is a significant challenge.

5. Plant, herbs and algae extracts as immunostimulant dietary supplements in fish.

Diverse efforts have been made in order to evaluate the immunostimulant effects of algae, herbs and plant extracts in various fish species. The immunostimulants presented here will be introduced according to the fish Order in which their effects have been evaluated (Tables I and II). This facilitates a vision of the different dietary supplements used to date in fish that share common physiological and genetic characteristics.

5.1 Acipenseriformes. The effects of Vitacel, a pure raw fiber composed of cellulose and hemicelluloses, has been evaluated in giant sturgeon (*Huso huso*) fed with 1.3% Vitacel per kg food. The results showed the increase in plasma lysozyme activity after 15 and after 90 days, and also an increase in number of neutrophil and eosinophil cells after 90 days of feeding [57]. Thus, the administration of Vitacel may enhance the innate immune system and growth performance in juvenile sturgeon. This improvement in the innate immune response is in agreement with the results observed in rainbow trout [58], as it will be mentioned below.

***5.2 Anguilliformes*.** In the Japanese eel the immunostimulant effect of Korean mistletoe, a semi-parasitic woody perennial commonly found growing in deciduous trees which possess activity as immunoadjuvant mainly reported to be derived from lectins [59,60], was evaluated by measuring the induction of cytokines, and stimulation of natural killer (NK) cell activity [61–65]. An increase in lysozyme and phagocytic activity in doses of 0.1, 0.5 and 1% and also a dose-dependent increase in the total survival rate in eels challenged against *A. hydrophila* was reported [66], thus probably could be implicated in potentiating the defense mechanism against bacterial infections.

5.3 Cypriniformes*.* The immunostimulant effects of several Chinese herbs have been evaluated: *Astragalus* root *(Astragalus radix*, *AR)*, a plant that contains polysaccharides, alkaloids and volatile oils that modulate the function of immune system relevant cells types including T cells, B cells, NK cells and macrophages [67,68]; *Ganoderma lucidum (GL)*, a mushroom whose polysaccharides have been reported to be effective in modulating immune responses inhibiting tumor growth, preventing oxidative damage and activating B lymphocytes [69–71]; Angelica root (*Angelicae sinensis, AS*), whose polysaccharides possess biological activities such as hematopoietic activity, immunomodulation, antitumor, antioxidant, radioprotection and hypoglycemic activity [72]; Herba Epimedii, the aerial parts of species of many Epimedium species (Berberidaceae) with immunostimulating effects [73]; *Rehmannia glutinosa* (RG) (also known as Di-Huang in China) which belongs to the family of Scrophulariaceae; and *Ficus carica* polysaccharide (FCP), obtained from a plant which belongs to the largest genus of the Moraceae family with anti-inflammatory, antitumor and antioxidant properties [74–76]. These herbs showed an increase in plasma lysozyme activity and leukocyte phagocytic activity in carp (*Cyprinus carpio*) [69,77] and in Chinese sucker (*Myxocyprinus asiaticus*) [78]. In Jian carp (*Cyprinus carpio* var. Jian) fed with AS the number of NBT-positive cells (blood), and lysozyme and complement activity (serum) was also registered [79]. At the gene expression level, an up-regulation of IL-1β, TNF-α and iNOS and a down-regulation of IL-10 and TGF-β has been detected in carp [77] while in FCP-fed grass carp (*Ctenopharyngodon idella*) the up-regulation of IL-1 and TNF-α with HSP70 down-regulation has been registered [80]. An increase in

respiratory burst activity but also in phagocytic activity of isolated blood cells and plasma lysozyme activity was observed when fish were immunostimulated with AR+GL and vaccinated against *A. hydrophila*/ *A. salmonicida* (Yin et al. 2009). A high survival rate in carp challenged with *A. hydrophila* in RG-treated fish [77], and high resistance to *Flavobacterium columnare* in grass carp fed with FCP [80] was observed, indicating a potential value of the immune response for these immunostimulants in aquaculture.

The immunostimulant effect of some Indian plants has also been evaluated. The Indian medicinal plant *Eclipta alba* (L.), a herb belonging to Asteraceae, has been reported to confer anti-inflammatory and anti-microbial properties [81,82]. In tilapia, *Eclipta alba* showed an increase of the non-specific humoral (lysozyme, antiprotease and complement) and cellular response (myeloperoxidase content, production of reactive oxygen and nitrogen species), with improved survival against *A. hydrophila* [83]. Increased protection against *A. hydrophila* in *Labeo rohita* fed with *Ocimum sanctum* (Tulsi, "Queen of plants") has been reported accompanied with enhanced non-specific immune (super oxide anion production, lysozyme activity, total protein, Ig) and hemato-immune parameters (total RBC/WBC counts, hemoglobin content) [84]. The effect of azadirachtin, a high-value carotenoid from an Indian plant (*Azadirachta indica*) responsible for its antibacterial property [85], has been evaluated in goldfish (*Carassius auratus*) registering high NBT activity, serum lysozyme, erythrocyte and leukocyte counts [86]. The dietary effect of andrographolide, the main medicinal compound of *Andrographis paniculata* native to India and Sri Lanka with antimicrobial, antioxidant, anti-inflammatory, and immunomodulator properties [87–90] had a stimulatory effect on non-specific immune parameters in *Labeo rohita* [91], a similar effect to that observed in fish infected with *Aphanomyces invadans* fed with *Rauvolfia tetraphylla* supplemented diets [92], a plant of the family *Apocynaceae* distributed in tropical countries including India. The effect of guava (*Psidium guajava L.*) leaves, colloquially known as the "poor man's apple of the tropics" and widely distributed throughout Asia, including India, have reported anti-microbial and anti-oxidant activities [93,94]. This treatment has shown not only better growth and immune parameters in immunostimulated groups, but also changes in the expression levels of immune-related genes of *Labeo rohita*: up-regulation of IL-1β and TNF-α, and down-regulation of IL-10, TGF-β, inducible nitric oxide synthase (iNOS), cyclooxigenase-2 (COX-2) and NF-κB [95]. Furthermore increased resistance against *A. hydrophila* [86,91,95] and *Aphanomyces invadans* [92] was reported.

The evaluation of changes in the modulation of genes associated with the immune system has not been a routine practice in evaluating the immunostimulant effects in diets. Moreover, three recent studies have evaluated the gene expression profile in fish fed with immunostimulant diets making an effort to complement the general and systemic information provided in these types of studies such as growth, non-specific humoral and cellular innate immune parameters, and cumulative mortality against pathogens. Based on the limited existing information, it worthy of mention the up-regulation of IL-1β and TNF-α [77,80,95] and the down-regulation of IL-10 and TGF-β [77,95] has been observed suggesting the expression of these genes as potential candidates contributing to the observed immunomodulation in fish fed with different immunostimulant diets. Further studies evaluating the transcriptomic response of fish fed with immunostimulant diets are needed to confirm this hypothesis.

Algae-derived supplements as potential immunostimulants have received increasing attention in recent years. In spotted wolffish (*Anarhichas minor*) was evaluated the effect of alginate, a polysaccharide found in brown algae cell walls composed of M- and G-blocks [96], observing a SGR increase only in the 0.01% alginate feeding group but not in the 0.06% and 0.1% [97]. Despite the differences observed in SGR, no significant differences were found in mortalities between any groups when spotted wolffish were infected with atypical *A. salmonicida* [97]. Another algae-derived compound evaluated as an immunostimulant in fish is astaxanthin, a high-value carotenoid produced from microalgae with anti-inflammatory activity, antioxidant benefits, and enhances the IL-1 and TNF-α release [98–100]. In carp fed with an astaxanthin-supplemented diet formulation an increase in RBC and WBC, hemoglobin, hematocrit, and a better survival curve was registered against *A. hydrophila* [101].

A traditional medicine herb and one of the most widely used in both eastern and western tradition is *Mentha piperita* (also known as peppermint), a perennial herb belonging to the Lamiaceae family with reported antioxidant, antiviral and antibacterial properties, amongst others [102]. Although an increases in hematological and both mucosal and systemic immune system parameters were reported, a decrease in the number of lymphocytes was observed in fry of the Caspian white fish (*Rutilus frisii kutum*) fed with peppermint supplemented diets [103]. Another plant used as an immunostimulant in fish diets is the stinging nettle (*Urtica dioica*), a herbaceous perennial flowering plant native to Europe, Asia, northern Africa, and western North America with reported immunostimulatory, anti-inflammatory, antioxidant, antiviral, antibacterial, and antifungal activities [104–106]. Together with the increase in hematological and immunological parameters, it was noted that plasma cortisol and glucose decreased with increasing *U. dioica* in the diet of juvenile and adult Victoria Labeo (*Labeo victorianus*) after challenge with *A. hydrophila* [107]. The cortisol and glucose response to immunostimulant administration has not as general biomarkers been extensively explored. Based on changing dietary composition over the last years (i.e. vegetal protein source instead animal protein), using cortisol and glucose measurements to evaluate the effects of dietary administration of new immunostimulant could be important not only for the effect on the stress response but also for the consequences upon the host-pathogen response as intimate regulation between endocrine and immune system is a central requirement for efficacious responses [108].

Coffee is one of the most popular drinks in the world with *Coffea arabica* (coffee bean, Rubiaceae family) representing 75-80 percent of the world's coffee production. Caffeine has been reported to improve defense against different stressors [109]. In carp, coffee bean dietary administration showed that roasted coffee bean (RCB) did not improve fish growth [110]. Along the same lines, RCB in Nile tilapia does not improve growth performance [110] and even an adverse effect at a concentration higher than 1 g kg^{-1} diet has been reported in seabream [111]. However, RCB was reported to improve some immune parameters in carp [110] opening the possibility to the use of non-conventional immunostimulants in fish diets.

5.4 Gadiformes. As was mentioned above, in the last years an increasing interest have focused on algae-derived supplements as potential immunostimulants. In Atlantic cod (*Gadus morhua*) fed with 0.01, 0.06, and 0.1% of alginate was observed an increase in the specific growth rate (SGR) in all groups compared to fish fed with control diet [97]. However this result contrast with that reported in Perciformes, specifically in the spotted wolffish (*Anarhichas minor*), in which the SGR increased only in lower doses of algae-derived alginate (0.01%) [97]. This suggests that the immunostimulant effect may be species-specific.

***5.5 Perciformes*.** The influence of the traditional Chinese medicine has also tested in *Perciformes*. The effect of *Astragalus* root and in combination with *Angelica* root was evaluated in large yellow croaker (*Pseudosciaena crocea*) with a significantly enhancement of respiratory burst activity of phagocytic cells, phagocytosis and lysozyme activities in plasma [112]. In *Cypriniformes*, a similar effect in common carp fed with *Astragalus* and *Ganoderma* was reported [69]. Similar non-specific immune parameters enhanced including SOD, peroxidase (POD) activity and a reduced mortality following *A. hydrophila* challenge were obtained in tilapia (*Oreochromis niloticus*) supplemented with a Chinese herbal mixture composed of *Astragalus*, *Angelica*, hawthorn, Licorice root and honeysuckle [113]. Also, the up-regulation of IL-1 and TNF-α was reported [113], confirming them as candidates genes contributing to the observed immune modulation capabilities of different immunostimulant diets as mentioned above. In our opinion this further highlights the need to further identify the underpinning mechanisms at a molecular level contributing to the observable impact of immunostimulants..

The historical and traditional use of *Echinacea purpurea*, a flowering plant that belongs to Asteraceae family has been subject to investigation. *Echinacea* activates macrophages and stimulates phagocytic-function [114]. The effect of Echinacea extract was further evaluated in Nile tilapia (*Oreochromis niloticus*) and shown to positively impact upon body gain, SGR, monocytes, neutrophil adherence, and survival rate against *A. hydrophila* [115].

Dihydroquercetin obtained from deodar (*Cedrus deodara,* family *Pinaceae*), a traditional plant used in Hindu medicine native to the Indian subcontinent with a broad spectrum of action [116], was evaluated in gilthead seabream (*Sparus aurata*). These studies detected an increase in cellular (phagocytosis and respiratory burst activities) and humoral (seric complement activity, antiprotease, total protein, peroxidase, bactericidal activity and IgM level) activities with the highest parameter increases related to the the lowest doses [117]. *Rhizophora apiculata* (family of *Rhizophoraceae*) is one of the widely distributed mangrove tree species in tropical countries, like India, with a reported antimicrobial and antiviral activity [118,119]. Survival rates were higher in clownfish (*Amphiprion sebae*) infected with *Vibrio alginolyticus* [120] and, interestingly, the same survival rate (although with different immunostimulant doses) was observed when diets were supplemented with *Avicennia marina* [121], another mangrove tree widely distributed along tropical and subtropical coastlines with antioxidant, antibacterial and antiviral activity [122–124]. Another tree mainly cultivated in subtropical regions is the sweet orange peel (*Citrus sinensis*), a member of the *Citrus* family with antimicrobial and antifungal properties [125,126]. In tilapia (*Oreochromis mossambicus)* fed with essential oil an increase in weight gain, specific growth rate (SGR), serum biochemical and hemato-immunological parameters and survival against *Streptococcus iniae* infection was reported with a concommitant decrease in feed conversion rate (FCR), albumin, and mean cell hemoglobin (MCH) [127].

Aloe barbadensis, also called *Aloe vera* (family *Xanthorrhoeaceae*) is a plant frequently used in herbal medicine with several properties such as antiviral and immunomodulatory activity, amongst others [128,129]. In a study in Nile tilapia fed with *Aloe vera* supplemented diet and propolis no significant differences were found [130]. However, an increase in growth performance but few and slight changes in RBC and WBC count, hemoglobin and hematocrit were observed and no changes in glucose and cortisol were observed in tilapia (GIFT) after challenge with *S. iniae* [131]. These differences may be related to

differences in the *A. vera* concentration used: in both studies fish were fed with 0.5%, 1%, and 2% of the supplemented diet however in the case of Nile tilapia the *A. vera* was equally mixed with propolis. Overall, no significant favorable health status changes were observed in tilapia (GIFT) fed with *A. vera* supplemented diet. Similarly, no differences were observed in Nile tilapia fed with a propolis supplemented diet, although in 1% propolis-ethanolic-extract increased the monocyte count and decreased neutrophils 28 days after treatment [132].

Green tea (*Camellia sinensis* L., GT) is a medicinal herb with non-oxidized and unfermented leaves, which have anti-inflammatory, antioxidative, antiproliferative, antibacterial, and antiviral properties [133–135]. In Nile tilapia fed with GT experimental diet for 12 weeks a higher growth performance, hemato-immune parameters and cumulative survival against *A. hydrophila* was observed [136], while in yellowtail (*Seriola quinqueradiata*) fed with GT polyphenols supplemented diet no significant differences were observed [137].

Another immunostimulant used as an additive is the marine diatom *Navicula* sp., a boated-shaped algae belonging to the family *Naviculaceae* rich in antioxidant carotenoids and vitamins [138]. Silage microalgae *Navicula* sp enriched with *Lactobacillus sakei* enhanced the immunity in gilthead seabream [139]. This effect was evaluated in separate diets in a different fish species, Pacific red snapper (*Lutjanus peru*), showing increased growth rate, humoral immune response and antioxidant capabilities in fish fed supplemented with *Navicula* + *L. sakei* (a probiotic) or *L. sakei* alone [140].

5.6 Pleuronectiformes. Efforts have focused on the evaluation of algal derivates mainly administered to fish through artemia and rotifers [141–144]. In this fish order the only study whose administration strategy is not by artemia and rotifers was done in *Senegalese sole* using lyophilized red algae as an immunostimulant (*Porphyridium cruentum*) in a commercial diet routinely used on farms; no statistical differences were found for the respiratory burst activity of phagocytes [145]. A similar result was observed when fish larvae were immunostimulated with high-M alginate with artemia in halibut (Skjermo and Bergh 2004) and turbot (Skjermo et al. 1995). However, Conceição et al. (2001) reported that turbot larvae fed with rotifers enriched with alginate capsules containing 86% mannuronic acid polymers (FMI) had a three fold higher protein turnover compared to the control group. The authors suggested this may contribute to higher larval viability and survival in case of environmental/disease stress [144]. The rich alginate compounds contributed to an improved survival rate against *Vibrio anguillarum* both in juvenile turbot [143] and halibut larvae [142]. The high-M alginate has a stimulatory effect on human monocytes inducing the expression of TNF-α [146,147]. TNF-α production was induced by TLR2 and TLR4/MD-2 receptor complexes and membrane CD14 in humans and mice [148] with response intensity correlating to the molecular weight of high-M alginate [149]. Thus, alginate-dependent TNF-α production may be involved in the observed increase in survival of halibut and turbot larvae against *V. anguillarum*.

5.7 Salmoniformes. The majority of studies have evaluated immunostimulant effects in the rainbow trout (*Oncorhynchus mykiss*). As previously mentioned several studies have used medicinal plants to evaluate their efficacy as dietary supplement. In rainbow trout fed with a diet containing a 1% aqueous extract of powdered ginger roots for three weeks a significant non-specific immune response increase was recorded including extracellular respiratory burst activity and phagocytosis of blood leukocytes. These processes

considered to be one of the most important mechanisms involved in the bactericidal activity of macrophages; and an increase in plasma protein levels [7], indicate that humoral factors may enhance phagocytosis in fish [150]. Also, a proliferation in the number of neutrophils, macrophages and lymphocytes, and enhanced phagocytic, respiratory burst, lysozyme, bactericidal, anti-protease activities, and an augmented relative percent survival in fish challenged with *A. hydrophila* were observed in rainbow trout fed with ginger [151]. Ginger (*Zingiber officinale*) has anti-inflammatory and anti-oxidative activity and its effect upon a range of bacterial, fungal and parasitic conditions has been reported [152–156]. A higher survival rate was also observed in rainbow trout dietary supplemented with garlic [157].

Among the medicinal plants, stinging nettle (Quercetin) and black cumin seed oil (*Nigella sativa*) have been also evaluated. While black cumin has antibacterial, antioxidant and anti-inflammatory effects [158–160], stinging nettle possess antimicrobial activity with effectiveness against a wide range of microorganisms [104]. These supplements induced an increase in lysozyme, myeloperoxidase and antiprotease activities, and total serum protein and IgM levels [161]. Tetra (*Cotinus coggyria*) is a medicinal plant with antimicrobial and antibacterial effects [162] that in rainbow trout fed with a 1% dose increased extracellular and intracellular respiratory burst activity, phagocytic and lysozyme activity, and total protein level was observed [163]. However, not all the medicinal plant-derivatives have an effect on fish. Dietary *Aloe vera* inclusion had no effect on growth, non-specific immune parameters, the expression of several immune-related genes, and the immune response to formalin-killed atypical *Aeromonas salmonicida* in steelhead rainbow trout (*Oncorhynchus mykiss*, Walbaum) [164]. The authors suggest that prolonged feeding with *A. vera* may cause this undesirable effect in salmonids however no significant differences were found in Nile tilapia fed for 2 weeks [130] and few and slight changes were observed in hemato-immune parameters (RBC, WBC) in tilapia (GIFT) challenged with *S. iniae* that were fed for 8 weeks prior to challenge [131]. Thus, further studies are needed to evaluate the effective impact of *A. vera* as an immunostimulant in fish.

Green tea (GT) extracts have also been evaluated in rainbow trout and a decaffeinated GT extract showed a higher lysozyme and peroxidase content [165]. However when rainbow trout were fed with an Epigallocatechin-3-gallate (EGCG) supplemented diet, a very potent antioxidant derived from GT, no significant differences were observed [166]. These antecedents are consistent with the results observed in Nile tilapia fed with GT with changes in immune parameters [136] but no significant differences were observed with a GT polyphenol supplemented diet [137].

Spirulina platensis, which belongs to the cyanobacteria (blue-green algae) family can up-regulate IL-1β and TNF-α mRNAs, increase phagocytic activity and superoxide anion production in carp leukocytes [167] and in rainbow trout increases in hemato-immune parameters (RBC, WBC, total protein) and decreases in plasma cortisol and glucose were observed [168]. The decreases in the latter parameters have been previously reported [107], confirming the need to further investigate the effects and mechanisms of immunostimulants on the stress response. *S. platensis* does not have a cellulose cell wall and therefore fish can digest it [169]; however, some non-digestible components such as dietary fiber have been introduced as diet supplements. Vitacel, a pure raw fiber composed of cellulose and hemicelluloses, has shown in rainbow trout fed with 10 g kg^{-1} Vitacel the increase serum lysozyme, ACH50, bactericidal activity. The increase in plasma lysozyme has been also reported in sturgeon, as was mentioned above, although with a

426 greater amount administrated to fish (13 g kg^{-1}) [57]. Also, feeding with Vitacel decreased the cumulative
427 mortality after challenge with *A. hydrophila* [58]. Importantly, HSP70 gene expression was down-
428 regulated [58]. The down-regulation of HSP70 has been reported in fish fed with supplemented diet [80],
429 thus the effect of immunostimulant diets on the expression of stress-related genes may be an interesting
430 route of study.

431 The medicinal mushroom *Lentinula edodes* extract as a supplement in trout diets induced an increase in
432 the number of total leukocytes (percentage of monocytes and neutrophils was higher but lymphocytes
433 was lower), phagocytic, lysozyme activity, and serum IgM levels. When fish were challenged against
434 *Lactococcus garvieae* a higher survival was observed in fish fed with the *L. edodes* extract [170].

435 *6. Immunostimulant diets using PAMPs.*

436 The activation of the immune response in the presence of pathogens requires that the host recognizes
437 specific and structurally conserved components that are produced by the pathogen. These conserved
438 components are called PAMPs and are typically lipopolysaccharide (LPS), peptidoglycan (PGN),
439 lipoproteins, flagellin, CpG DNA and muramyl dipeptide (MDP) from bacteria, fungal beta-glucans (β-
440 glucan) and zymosan, and double-stranded RNA (dsRNA) from virus.

441 PAMP recognition is mediated by pathogen recognition receptors (PRRs) that are structurally and
442 functionally conserved between vertebrates, invertebrates and plants, and allow the host to distinguish
443 among different classes of pathogen in order to mount an appropriate immune response. This immune
444 response is mediated through the induction of inflammatory cytokines, chemokines and co-stimulatory
445 molecules that facilitate leukocyte recruitment to the site of the infection, activation of adaptive immunity
446 and activation of antimicrobial effectors (Reviewed in Boltaña et al., 2011; Janeway and Medzhitov, 2002).

447 Throughout the last 15 years the use of PAMPs as immunostimulants has been reported upon for different
448 species (Table III and Table IV). As was mentioned before, one of the commonly used PAMPs in
449 immunostimulant diets are β-glucans. This major structural component of yeast and fungal cell walls is
450 widely used in fish diets as additive in commercial supplements such as Biosaf, DVAQUA, Ecoactiva,
451 Ergosan, Fibosel, MacroGard, and VitaStim, and also, chitin, LPS, mannan-oligosaccharides (MOS), PGN,
452 and yeast extract (Table III), which have also been included in this review.

453 ***6.1 Cypriniformes.*** The most used PAMP in dietary immunostimulants in cypriniformes is β-glucan. β-
454 glucan has been directly studied as a direct extract from *S. cerevisiae* or in one of its commercial form
455 (MacroGard, Sigma). The effect of MacroGard dietary immunostimulation upon the expression of selected
456 inflammatory genes in carp has been studied (TNF-α1, TNF-α2, IL-1b, IL-6family and IL-10) in gut and
457 head kidney after 14 days of feeding. TNF-α2 was significantly down-regulated in gut and head kidney,
458 and IL-10 was down-regulated in gut, and a posterior challenge with *A. salmonicida* stimulated the
459 production of tnfα1 and TNF-α2 at 6 hpi in head kidney [173]. Also, in carp fed with MacroGard for 25
460 days it was observed that most all of the selected cytokines analysed (IL-1b, IL-10, TNF-α1, TNF-α2 and
461 CXCa) were down-regulated, but the expression of Mx transcripts was increased in liver and mid-gut
462 [174]. A posterior challenge with poly (I:C) did not affect the expression of the cytokines, but was found to
463 induce an up-regulation of Mx in liver, head kidney, spleen and mid-gut [174]. A down-regulation of

complement-related mRNA transcripts at 7 and 25 days was observed in liver and head kidney in carp fed with MacroGard in conjunction with a high serum CRP level after 7 days. An increase in alternative complement activity at 25 days of feeding was also detected [175]. In the mid gut at 7 days, Bf/C2, C3 and MAP2 were up-regulated, and CRP2 and C3 were also up-regulated at 25 days of feeding. A following LPS or poly (I:C) challenge study was also reported showing regulation of CRP and complement related gene expression profiles, with a significant effect in fish fed with β-glucan; however for CRP levels, and serum complement activity, the effect was lower than in control fish. The authors suggested that β-glucan immunostimulation was sufficient enough to reduce the effects PAMP challenge [175].

The use of β-glucan (Sigma) on a basal diet for 1 week has also been reported and no significant differences reported for orally administered β-glucan compared to control for bacterial killing, O_2^- production/NBT, or survival to *A. hydrophila* infection [176]. In contrast in Rohu fingerlings four different diets containing 100, 250 or 500 mg of Sigma β-glucan kg^{-1} diet for 56 days impacted upon immune parameters including leukocytes count, phagocytic ratio, phagocytic index, lysozyme activity, complement activity and serum bactericidal activity. The highest levels were reported 42 days after feeding with the 250 mg of β-glucan kg−1 diet furthermore a significant reduction in mortality compared to control during *A. hydrophila* and *E. tarda* infection was reported [177]. Sahoo and Mukherjee (2001) described that fish fed for 1 week with β-glucan at a doses of 0.1% showed an increase in bacterial agglutination, hemagglutination and hemolysin titre, bactericidal activity, serum phagocytic ratio, serum phagocytic index and serum leukocrit compared with control and an increase in survival to *A. hydrophila* challenge was reported. These data suggest that in Rohu administration of Sigma β-glucan has a protective effect against bacterial pathogens.

The effect of a combined diet of β-glucan and LPS (0.1% β-glucan + 0.025% LPS, 0.5% β-glucan + 0.125% LPS, 1% β-glucan + 0.25% LPS) administered at days 1, 7 and 14, were analysed after 16 days, observing an increase on the bactericidal activity compared with control. Also an increase in survival against *A. hydrophila* was observed in the fish fed with the highest dose of β-glucan plus LPS [179]. Interestingly administration of a LPS only diet at doses of 1, 2.5 y 5 mg did not show any immunostimulant effect or survival or increased antibody titre to an *A. hydrophila* challenge [180].

The effect of baker's yeast extract (*S. cerevisiae*) a supplement containing nucleotides and β-glucan upon immune parameters in carp after 10 days of feeding was reported [181]. The expression of inflammatory cytokines as IL-1β, TNF-α and IL-12p35, IL-12p40 and IFN-γ2 was significantly increased in the head kidney after 1 day and the expression CXC-chemokines was increased after 1, 5 and 7 days of feeding interestingly a reduction of IL-10 gene expression was reported. O_2^- production and phagocytic activity in head kidney leukocytes highlighted increased O_2^- production and phagocytic index at 3 days in fish fed with the IS diet, and the phagocytic activity increased at 1 and 3 days. In parallel a increase in fish resistance to bacteria was observed at 6 hours post injection of *A. hydrophila* in the fish fed with the IS diets [181]

A longer trial, 60 days, was reported using microbial levan at 0.25%, 0.5% 0.75%, 1.0% and 1.25% in juvenile Rohu. Within the treatment groups albumin/globulin ratios decreased with a smaller decrease in lower levan-supplemented groups. On the other hand the hemoglobin content, total leucocyte count and

serum total protein were increased with supplementation at 1% or more. As levan supplementation concentration was increased there was a gradual concomitant increase in serum lysozyme activity and respiratory burst activity with the highest activity in the 0.75% and 1.0% levan groups. A reduction of mortality rate for all levan diets was observed compared to control in Rohu challenged with *A. hydrophila* [182].

6.2 Perciformes. In this order several studies using different PAMPs as immunostimulants have been reported. The most frequently studied have been diet additives derived from yeast cultures; by adding the whole yeast [183–186], the yeast cell walls [187] and also extracts such as MOS [188], chitin [185,189], and β-glucan either extracted directly [190,191] or using those commercially available as Biosaf [190], VitaStim [192], Sanictum [193], EcoActiva [194], Fibosel [192], and MacroGard [192,195,196].

Using whole yeast as the immunostimulant at doses of 1, 5 y 10 g/Kg of feed for 4 weeks, the main effects observed were associated with the highest doses highlighting increased respiratory burst activity, phagocytic ability, natural cytotoxic activity and MPO content in the head kidney of seabream leukocytes [184]. Increases in serum IgM content after 2 week of treatment was also reported [185]. On the other hand, Rodriguez et al (2003) evaluated the effect of a modified strain of *S. cerevisiae* (fsk-1) which has a lower glucan and higher chitin composition in the cell wall. They found that doses of 10 g/kg of feed increased levels of serum lysozyme activity, leucocyte phagocytic ability and leucocyte phagocytic capacity and decreased serum complement activity and peroxidase content after 6 weeks of the diet [183]. In Japanese sea bass yeast cell wall (YCW) supplementation to diets at doses of 250, 500, 1000, 2000 and 20000 mg/Kg were examined throughout a 72 days trial. No significant effect was observed for C3, IgM, MPO, NBT or Anti-O_2^- content however 500 mg/kg of YCW did the cumulative survival of sea bass challenged with *A. veronii*. [187]

The commercial product DVAQUA (product of fermentation of *S. cerevisiae*) was used in doses of 0.125, 0.25, 0.50, 1.0 and 2.0 g/kg of feed in hybrid tilapia (*Oreochromis niloticus* ♀ *x O. aureus* ♂) for 8 weeks. An increase in serum lysozyme activity, serum C3 and C4 content and macrophage phagocytic activity isolated from head kidney at the end of the 8 week was reported for all dosages whereas an increase in head kidney macrophage respiratory burst activity was evident at 0.125, 0.25 and 1.0 g/Kg of DVAQUA [186].

Chitin (poly [1→4]-β-N-acetyl-D-glucosamine), which is an insoluble polysaccharide present in the exoskeleton of shellfish, insects and in the cell walls of fungi has been used and tested as an immunostimulant. The synthetic compound (Sigma) was used to test immunostimulatory action in seabream with doses of 25, 50 and 100 mg/kg of feed for 6 weeks. All doses showed an increase after 2 weeks in natural hemolytic complement activity and head kidney-leukocyte natural cytotoxic activity. Furthermore an increased head kidney-leukocyte respiratory burst activity after 4 weeks compared to the control was also reported [189]. Interestingly, with a concentrations of 25 mg/kg an increase in the total content of serum IgM was identified after 6 weeks [185]. Other derivatives of yeast have also been used such as MOS (extracted from *S. cerevisiae*) which when administered at 4% in the diet for 9 weeks showed an increase in phagocytic macrophage HK-activity in sea bass This same diet when contrasted in a *V.*

alginolyticus cohabitation challenge model produced a significant reduction in infected fish at doses of 2% and no infected fish were found at 4% of MOS [188].

The most commonly used additive has been β-glucan, extracted directly or from commercial extracts. Ai et al [191] used a β-glucan extract in the diet of large yellow croaker in doses of 0.09% and 0.18% for 8 weeks and it was observed that the lower doses produced an increase in serum lysozyme content and phagocytic and respiratory burst activity in head kidney macrophages. Furthermore a reduction in the mortality rate was reported after *V. harveyi* challenge [191]. In addition, in Nile tilapia fed with 0.1% β–glucan an increase in the above mentioned parameters was reported in addition to increases in serum bactericidal activity, serum NO and the lymphocyte transformation index after 21 days of diet [190]. In tilapia fed with 10g/kg of feed of Biosaf (commercial extract) an increase in the same immunological parameters was reported with the exception the lymphocyte transformation index which had no significant differences [190]. Again after challenge with *A. hydrophila* a reduction in the mortality rate was reported [190].

The effect of EcoActiva, another commercial β-glucan, in Snapper diets at 0.1% in winter and summer seasons during 84 days was reported by [194]. During the winter season an increase in respiratory burst activity of head kidney macrophages was observable even at 56 days post administration of the diet, however when administrated in the summer season this parameter increased only at day 28 suggesting important temperature related effects upon diet efficacy..

The commercial extracts Fibosel and VitaStim (1 g/Kg of feed, 10 g/Kg of feed) have been tested in sea bream and highlight an increase in spleen macrophage respiratory burst activity at lower doses with both supplements and at higher doses with Fibosel whereas no effect was observed with VitaStim. Lower doses of Fibosel induced an increased spleen macrophage phagocytosis. Importantly both immunostimulants caused a significant reduction in mortality rate after *P. damselae piscicida* bath challenge [192].

Sea bass fed with 2% MacroGard for 2 weeks every 3 months showed increased serum complement activity and plasma lysozyme activity at the end of 3 cycles [195]. Further studies have shown that MacroGard at 0.1% for 15 days also increased serum complement activity, serum lysozyme activity and gills HSP70 content at 30 days from the end of the treatment period. In contrast when the diet was administrated over a longer term (4 cycles of 15 days every 60 days) no effect was found [196]. In seabream fed for one week with MacroGard (1 g/Kg of feed and 10 g/Kg of feed) an increase in head kidney macrophage phagocytic activity with the lower doses and an increase of spleen macrophage respiratory burst and phagocytic activity for the highest doses was detected and a reduction of the mortality rate in a challenge with *P. damselae* [192] similar to results obtained for sea bass.

SALMONIFORMES. In Salmoniformes, as in Perciformes, most of the reports have focused on evaluating the immunostimulant effect of β-glucan. However the capacity of β-glucan in diet to stimulate the immune system is not clear. In 1994, Siwicki described the effect of different β-glucan containing feeds including MacroGard 0.2g/100g, *C. utilis* 2.7g/100g, *S. cerevisiae* 2.7g/100g and a deacylated chitin 0.5g/100g on rainbow trout for 7 days. In all diets increased respiratory burst activity of blood, phagocytic index, MPO activity, serum total Ig and blood bactericidal killing activity were reported after 1 week [197]. However,

Kunttu et al. (2009) administered MacroGard in doses of 0.2, 0.6 and 1.8%, and were able to report an increase in blood-respiratory burst activity at the two higher doses after 21 days of diet administration. In another parallel study in rainbow trout MacroGard at a dose of 4.5 g/kg of feed and a β-glucan extracted from *H. vulgare* at 12.2, 16.7 and 26.4 g/kg of diet were compared over for 9 weeks. None of the treatments were able to immunostimulate at any measured parameter [199].

An interesting study was carried out by Ghaedi et al (2015) in which they fed 4 Kg female broodstock for 3 months prior to spawning with 0.1% and 0.2% of MacroGard and a control diet. In continuation the fry were fed the same doses of the immunostimulant diet for 2 months [200]. Brood fish fed with 0.2% β-glucan diet showed the highest levels of ACH50 and lysozyme activity. The total serum Ig and IgM content was significantly higher for both doses. The descendent fry fed with the immunostimulant diets for 2 months showed an increase in the levels of ACH50, lysozyme, total Ig and IgM and also a significant increase in survival, but the fry descending from IS diets fed broodstock did not promote the immunity in the fry. [200].

In rainbow trout peptidoglycan (PG) enriched diets have also been evaluated. The expression of antimicrobial peptides (AMP) was analysed in fish fed with PG for 14 days and at higher PG doses, a sequential expression over the time, where defensins were typically expressed early and cathelicidins and LEAPs later on in the course of the experiment [201]. A withdrawal of PG diets at day 14 resulted in a fall in expression level of this AMP that was particularly apparent with cathelicidins and LEAP expression whereas, defensins remained at high levels [202].

In rainbow trout fed with 20µg/kg of BW day LPS extracted from *P. agglomerans* for 93 days bactericidal activity, lysozyme activity, blood hemolytic activity and NBT were found to increase in comparison to controls[203]. Moreover, in Atlantic salmon fry LPS extracted from *A. salmonicida* at a concentration of 0.1% in feed for 62 days was not able to increase the plasma immunoglobulin level [204].

6.3 Siluriformes. Finally, in Siluriforms diets with β-glucan and synthetic levamisole (Sigma) were evaluated and again increased blood-phagocytes respiratory burst activity and leucocyte myeloperoxidase content were reported at 31 days post diet for both treatments [205].

In conclusion, researchers have mostly carried out different studies to assess the effect of dietary immunostimulants in fish from derivatives of algae, herbs and plant extracts in a non-specific manner based upon: (1) tradition and folklore transferred through generations, (2) their biological properties mainly evaluated *in vitro* or, in some cases, in experimental animals. The results indicate that there are few studies in which it is possible to observe a clear and direct dose-dependent immune stimulatory effect. There are many experimental design variations, many different fish species and a diverse array of immunostimulant preparations designed for the full array of aquacultural practices. Also, it seems clear that all the studies are focused on to evaluate the innate immune response analyzing almost the same non-specific (both humoral and cellular) and hemato-immune parameters and, hence, the ability of the immunostimulant to confer non-specific immune protection against fish pathogens. This limits the knowledge regarding the scope of treatment. Another critical limitation is the arbitrary use of dose and timing of administration making difficult the comparison and integration of results. Therefore, it is a

priority to generate a consensus on this matter. Finally, more efforts are needed using high-throughput screening tools to elucidate the transcriptome and proteome response to assess the scopes of the dietary supplementation of immunostimulants in fish in order to establish in the future dietary supplemented immunostimulant according to the specific fish requirements.

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References

[1] C.J. Secombes, T.C. Fletcher, The role of phagocytes in the protective mechanisms of fish, Annu. Rev. Fish Dis. 2 (1992) 53–71. doi:10.1016/0959-8030(92)90056-4.

[2] B. Magnadóttir, Innate immunity of fish (overview), Fish Shellfish Immunol. 20 (2006) 137–51. doi:10.1016/j.fsi.2004.09.006.

[3] E.A. Flerova, L. V. Balabanova, Ultrastructure of granulocytes of teleost fish (Salmoniformes, Cypriniformes, Perciformes), J. Evol. Biochem. Physiol. 49 (2013) 223–233. doi:10.1134/S0022093013020126.

[4] M.T. Silva, M. Correia-Neves, Neutrophils and macrophages: The main partners of phagocyte cell systems, Front. Immunol. 3 (2012) 2008–2013. doi:10.3389/fimmu.2012.00174.

[5] K.E. Iles, H.J. Forman, Macrophage signaling and respiratory burst, Immunol. Res. 26 (2002) 95–105. doi:10.1385/IR:26:1-3:095.

[6] C.J. Secombes, G. Olivier, Host—Pathogen Interactions in Salmonids, 1997. doi:DOI: 10.1016/B978-012093040-1/50014-2.

[7] S.K. Dügenci, N. Arda, A. Candan, Some medicinal plants as immunostimulant for fish, J. Ethnopharmacol. 88 (2003) 99–106. doi:10.1016/S0378-8741(03)00182-X.

[8] C. Nathan, M.U. Shiloh, Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 8841–8848. doi:10.1073/pnas.97.16.8841.

[9] S.J. Green, C.A. Nacy, R.D. Schreiber, D.L. Granger, R.M. Crawford, M.S. Meltzer, A.H. Fortier, Neutralization of gamma interferon and tumor necrosis factor alpha blocks in vivo synthesis of nitrogen oxides from L-arginine and protection against *Francisella tularensis* infection in *Mycobacterium bovis* BCG-treated mice, Infect. Immun. 61 (1993) 689–698.

[10] M. Kaibori, K. Sakitani, M. Oda, Y. Kamiyama, Y. Masu, M. Nishizawa, S. Ito, T. Okumura, Immunosuppressant FK506 inhibits inducible nitric oxide synthase gene expression at a step of NF-kappaB activation in rat hepatocytes, J. Hepatol. 30 (1999) 1138–1145.

[11] F.C. Fang, Antimicrobial reactive oxygen and nitrogen species: concepts and controversies, Nat. Rev. Microbiol. 2 (2004) 820–832. doi:10.1038/nrmicro1004.

[12] R.M. Locksley, C.S. Nelson, J.E. Fankhauser, S.J. Klebanoff, Loss of granulemyeloperoxidase during in vitro culture of human monocytes correlates with decay in antiprotozoa activity, Am. J. Trop. Med. Hyg. 36 (1987) 541–548.

[13] S.J. Klebanoff, Myeloperoxidase : friend and foe, J. Leukoc. Biol. 77 (2005) 598–625. doi:10.1189/jlb.1204697.1.

[14] J.K. Spitznagel, E. Martin, M. Kinkade, Differential distribution of distinct forms of myoloperoxidase in different azuropholic granule subpopulations from human neutrophils, Biochem. Biophys. Res. Commun. 114 (1983) 296–303.

[15] D. Morera, N. Roher, L. Ribas, J.C. Balasch, C. Donate, A. Callol, S. Boltana, S. Roberts, G. Goetz, F.W. Goetz, S.A. MacKenzie, RNA-Seq reveals an integrated immune response in nucleated erythrocytes, PLoS One. 6 (2011) e26998.

[16] D. Parra, F.E. Reyes-Lopez, L. Tort, Mucosal immunity and B cells in teleosts: Effect of vaccination and stress, Front. Immunol. 6 (2015) 1–12. doi:10.3389/fimmu.2015.00354.

[17] J.R. Dunkelberger, W.-C. Song, Complement and its role in innate and adaptive immune responses, Cell Res. 20 (2010) 34–50. doi:10.1038/cr.2009.139.

[18] I. Salinas, Y.-A. Zhang, J.O. Sunyer, Mucosal immunoglobulins and B cells of teleost fish, Dev. Comp. Immunol. 35 (2011) 1346–65. doi:10.1016/j.dci.2011.11.009.

[19] D. Gomez, J.O. Sunyer, I. Salinas, The mucosal immune system of fish: The evolution of tolerating commensals while fighting pathogens, Fish Shellfish Immunol. 35 (2013) 1729–1739. doi:10.1016/j.fsi.2013.09.032.

[20] I. Salinas, The mucosal immune system of teleost fish, Biology (Basel). 4 (2015) 525–539. doi:10.3390/biology4030525.

[21] Z. Xu, D. Parra, D. Gómez, I. Salinas, Y.-A. Zhang, L.V.G. Jørgensen, R.D. Heinecke, K. Buchmann, S. LaPatra, J.O. Sunyer, Teleost skin, an ancient mucosal surface that elicits gut-like immune responses, Proc. Natl. Acad. Sci. 110 (2013) 13097–13102. doi:10.1073/pnas.1304319110.

[22] A.K. Nigam, U. Kumari, S. Mittal, A.K. Mittal, Comparative analysis of innate immune parameters of the skin mucous secretions from certain freshwater teleosts, inhabiting different ecological niches, Fish Physiol. Biochem. 38 (2012) 1245–1256. doi:10.1007/s10695-012-9613-5.

[23] J.L. Maki, H.W. Dickerson, J.L. Maki, H.W. Dickerson, Systemic and cutaneous mucus antibody responses of Channel catfish immunized against the protozoan parasite *Ichthyophthirius multifiliis*, Clin. Diagn. Lab. Immunol. 10 (2003) 876–881. doi:10.1128/CDLI.10.5.876.

[24] E. Haugarvoll, I. Bjerkås, B.F. Nowak, I. Hordvik, E.O. Koppang, Identification and characterization of a novel intraepithelial lymphoid tissue in the gills of Atlantic salmon, J. Anat. 213 (2008) 202–209. doi:10.1111/j.1469-7580.2008.00943.x.

[25] E.O. Koppang, U. Fischer, L. Moore, M. a. Tranulis, J.M. Dijkstra, B. Köllner, L. Aune, E. Jirillo, I. Hordvik, Salmonid T cells assemble in the thymus, spleen and in novel interbranchial lymphoid tissue, J. Anat. 217 (2010) 728–739. doi:10.1111/j.1469-7580.2010.01305.x.

[26] S. Akira, S. Uematsu, O. Takeuchi, Pathogen recognition and innate immunity, Cell. 124 (2006) 783–801. doi:10.1016/j.cell.2006.02.015.

[27] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, Nat. Immunol. 11 (2010) 373–84. doi:10.1038/ni.1863.

[28] M. Tanekhy, The role of Toll-like Receptors in innate immunity and infectious diseases of teleost, Aquac. Res. 47 (2016) 1369–1391. doi:10.1111/are.12607.

[29] M.K. Purcell, K.D. Smith, L. Hood, J.R. Winton, C. Jared, Conservation of Toll-like receptor signaling pathways in teleost fish, Comp Biochem Physiol Part D Genomics Proteomics. 1 (2007) 77–88. doi:10.1016/j.cbd.2005.07.003.

[30] Y. Palti, Toll-like receptors in bony fish: From genomics to function, Dev. Comp. Immunol. 35 (2011) 1263–1272. doi:10.1016/j.dci.2011.03.006.

[31] M. Reverter, N. Bontemps, D. Lecchini, B. Banaigs, P. Sasal, Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives, Aquaculture. 433 (2014) 50–61. doi:10.1016/j.aquaculture.2014.05.048.

[32] D.S. Chahal, R.K. Sivamani, R. Rivkah Isseroff, M.R. Dasu, Plant-based modulation of toll-like receptors: An emerging therapeutic model, Phyther. Res. 27 (2013) 1423–1438. doi:10.1002/ptr.4886.

[33] R.C. Robertson, F. Guihéneuf, B. Bahar, M. Schmid, D.B. Stengel, G.F. Fitzgerald, R.P. Ross, C. Stanton, The anti-inflammatory effect of algae-derived lipid extracts on lipopolysaccharide (LPS)-stimulated human THP-1 macrophages, Mar. Drugs. 13 (2015) 5402–5424. doi:10.3390/md13085402.

[34] N. Arora, K. Shah, S. Pandey-Rai, Inhibition of imiquimod-induced psoriasis-like dermatitis in mice by herbal extracts from some Indian medicinal plants, Protoplasma. 253 (2016) 503–515. doi:10.1007/s00709-015-0829-y.

[35] X. Zhong, Y. Shi, J. Chen, J. Xu, L. Wang, R.C. Beier, X. Hou, F. Liu, Polyphenol extracts from *Punica*

granatum and *Terminalia chebula* are anti-inflammatory and increase the survival rate of chickens challenged with *Escherichia coli*, Biol. Pharm. Bull. 37 (2014) 1575–1582. doi:10.1248/bpb.b14-00163.

[36] S. Granica, J.P. Piwowarski, A.K. Kiss, Ellagitannins modulate the inflammatory response of human neutrophils *ex vivo*, Phytomedicine. 22 (2015) 1215–1222. doi:10.1016/j.phymed.2015.10.004.

[37] C.T. Nguyen, T.T. Luong, S.Y. Lee, G.L. Kim, H. Kwon, H.G. Lee, C.K. Park, D.K. Rhee, *Panax ginseng* aqueous extract prevents pneumococcal sepsis *in vivo* by potentiating cell survival and diminishing inflammation, Phytomedicine. 22 (2015) 1055–1061. doi:10.1016/j.phymed.2015.07.005.

[38] S.J. Kim, S.J. Yoon, Y.M. Kim, S.W. Hong, S.H. Yeon, K.I. Choe, S.M. Lee, HS-23, *Lonicera japonica* extract, attenuates septic injury by suppressing toll-like receptor 4 signaling, J. Ethnopharmacol. 155 (2014) 256–266. doi:10.1016/j.jep.2014.05.021.

[39] A. Ghochikyan, A. Pichugin, A. Bagaev, A. Davtyan, A. Hovakimyan, A. Tukhvatulin, H. Davtyan, D. Shcheblyakov, D. Logunov, M. Chulkina, A. Savilova, D. Trofimov, E.L. Nelson, M.G. Agadjanyan, R.I. Ataullakhanov, Targeting TLR-4 with a novel pharmaceutical grade plant derived agonist, Immunomax®, as a therapeutic strategy for metastatic breast cancer, J. Transl. Med. 12 (2014) 322. doi:10.1186/s12967-014-0322-y.

[40] C.J. Lin, H.J. Lin, T.H. Chen, Y.A. Hsu, C.S. Liu, G.Y. Hwang, L. Wan, *Polygonum cuspidatum* and its active components inhibit replication of the influenza virus through Toll-like receptor 9-induced interferon beta expression, PLoS One. 10 (2015) 1–13. doi:10.1371/journal.pone.0117602.

[41] S.J. Ryu, H.S. Choi, K.Y. Yoon, O.H. Lee, K.J. Kim, B.Y. Lee, Oleuropein suppresses LPS-induced inflammatory responses in RAW 264.7 Cell and zebrafish, J. Agric. Food Chem. 63 (2015) 2098–2105. doi:10.1021/jf505894b.

[42] A. Rhule, B. Rase, J.R. Smith, D.M. Shepherd, Toll-like receptor ligand-induced activation of murine DC2.4 cells is attenuated by *Panax notoginseng*, J. Ethnopharmacol. 116 (2008) 179–186. doi:10.1016/j.jep.2007.11.019.

[43] M. Pannacci, V. Lucini, F. Colleoni, C. Martucci, S. Grosso, P. Sacerdote, F. Scaglione, *Panax ginseng* C.A. Mayer G115 modulates pro-inflammatory cytokine production in mice throughout the increase of macrophage toll-like receptor 4 expression during physical stress, Brain. Behav. Immun. 20 (2006) 546–551. doi:10.1016/j.bbi.2005.11.007.

[44] J.Y. Ahn, J.Y. Song, Y.S. Yun, G. Jeong, I.S. Choi, Protection of *Staphylococcus aureus*-infected septic mice by suppression of early acute inflammation and enhanced antimicrobial activity by ginsan, FEMS Immunol. Med. Microbiol. 46 (2006) 187–197. doi:10.1111/j.1574-695X.2005.00021.x.

[45] J.Y. Ahn, I.S. Choi, J.Y. Shim, E.K. Yun, Y.S. Yun, G. Jeong, J.Y. Song, The immunomodulator ginsan induces resistance to experimental sepsis by inhibiting Toll-like receptor-mediated inflammatory signals, Eur. J. Immunol. 36 (2006) 37–45. doi:10.1002/eji.200535138.

[46] T.A. Nakaya, M. Kita, H. Kuriyama, Y. Iwakura, J. Imanishi, *Panax ginseng* induces production of proinflammatory cytokines via toll-like receptor, J Interf. Cytokine Res. 24 (2004) 93–100. doi:10.1089/107999004322813336.

[47] B.S. Park, J.-O. Lee, Recognition of lipopolysaccharide pattern by TLR4 complexes, Exp. Mol. Med. 45 (2013) e66. doi:10.1038/emm.2013.97.

[48] P. Swain, S.K. Nayak, P.K. Nanda, S. Dash, Biological effects of bacterial lipopolysaccharide (endotoxin) in fish: A review, Fish Shellfish Immunol. 25 (2008) 191–201. doi:10.1016/j.fsi.2008.04.009.

[49] D.B. Iliev, J.C. Roach, S. Mackenzie, J. V. Planas, F.W. Goetz, Endotoxin recognition: In fish or not in fish?, FEBS Lett. 579 (2005) 6519–6528. doi:10.1016/j.surg.2006.10.010.Use.

[50] M.P. Sepulcre, F. Alcaraz-Pérez, A. López-Muñoz, F.J. Roca, J. Meseguer, M.L. Cayuela, V. Mulero, Evolution of lipopolysaccharide (LPS) recognition and signaling: fish TLR4 does not recognize LPS and negatively regulates NF-kappaB activation., J. Immunol. 182 (2009) 1836–1845. doi:10.4049/jimmunol.0801755.

[51] R. Schwandner, D. Roman, W. Holger, M. Rothe, K. Carsten J, Peptidoglycan-and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2, J. Biol. Chem. 274 (1999) 17406–17409. doi:10.1074/jbc.274.25.17406.

[52] S.E. Girardin, I.G. Boneca, L. a M. Carneiro, A. Antignac, M. Jéhanno, J. Viala, K. Tedin, M.-K. Taha, A. Labigne, U. Zähringer, A.J. Coyle, P.S. DiStefano, J. Bertin, P.J. Sansonetti, D.J. Philpott, Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan., Science (80-.). 300 (2003) 1584–1587. doi:10.1126/science.1084677.

[53] S. Boltaña, F. Reyes-Lopez, D. Morera, F. Goetz, S.A. MacKenzie, Divergent responses to peptidoglycans derived from different E. coli serotypes influence inflammatory outcome in trout, Oncorhynchus mykiss, macrophages., BMC Genomics. 12 (2011) 34.

[54] R.A. Dalmo, J. Bøgwald, Beta-glucans as conductors of immune symphonies, Fish Shellfish Immunol. 25 (2008) 384–96. doi:10.1016/j.fsi.2008.04.008.

[55] G.D. Brown, S. Gordon, Fungal beta-glucans and mammalian immunity, Immunity. 19 (2003) 311–315. doi:http://dx.doi.org/10.1016/S1074-7613(03)00233-4.

[56] D. Pietretti, N.I. Vera-Jimenez, D. Hoole, G.F. Wiegertjes, Oxidative burst and nitric oxide responses in carp macrophages induced by zymosan, MacroGard and selective dectin-1 agonists suggest recognition by multiple pattern recognition receptors, Fish Shellfish Immunol. 35 (2013) 847–857. doi:10.1016/j.fsi.2013.06.022.

[57] M. Heidarieh, M. Soltani, A. Tamimi, M. Toluei, Comparative effect of raw fiber (Vitacel) and alginic acid (Ergosan) on growth performance, immunocompe- tent cell population and plasma lysozyme content of giant sturgeon (*Huso huso*), Turkish J. Fish. Aquat. Sci. 11 (2011) 445–450. doi:10.4194/1303-2712-v11.

[58] P. Yarahmadi, H. Kolangi Miandare, H. Farahmand, A. Mirvaghefi, S.H. Hoseinifar, Dietary fermentable fiber upregulated immune related genes expression, increased innate immune response and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas hydrophila*, Fish Shellfish Immunol. 41 (2014) 326–31. doi:10.1016/j.fsi.2014.09.007.

[59] T.J. Yoon, Y.C. Yoo, T.B. Kang, S.K. Song, K.B. Lee, E. Her, K.S. Song, J.B. Kim, Antitumor activity of the korean mistletoe lectin is attributed to activation of macrophages and NK cells, Arch. Pharm. Res. 26 (2003) 861–867.

[60] T.J. Yoon, Y.C. Yoo, T.B. Kang, K. Shimazaki, S.K. Song, K.H. Lee, S.H. Kim, C.H. Park, I. Azuma, J.B. Kim, Lectins isolated from Korean mistletoe (*Viscum album coloratum*) induce apoptosis in tumor cells, Cancer Lett. 136 (1999) 33–40.

[61] G. Kuttan, D.M. Vasudevan, R. Kuttan, Tumour reducing activity of an isolated active ingredient from mistletoe extract and its possible mechanism of action, J. Exp. Clin. Cancer Res. 11 (1992) 7–12.

[62] D.N. Männel, H. Becken, A. Gundt, A. Kist, H. Franz, Induction of tumor necrosis factor expression by a lectin from *Viscum album*, Cancer Immunol. Immunother. 33 (1991) 177–182.

[63] G. Metzner, H. Franz, A. Kindt, B. Fahlbusch, J. Süss, The *in vitro* activity of lectin I from mistletoe (ML I) and its isolated A and B chains on functions of macrophages and polymorphonuclear cells, Immunobiology. 169 (1985) 461–71. http://www.sciencedirect.com/science/article/pii/S0171298585800024 (accessed January 25, 2016).

[64] E.A. Mueller, F.A. Anderer, Synergistic action of a plant rhamnogalacturonan enhancing antitumor cytotoxicity of human natural killer and lymphokine-activated killer cells: chemical specificity of target cell recognition., Cancer Res. 50 (1990) 3646–51.

[65] T. Hajto, Immunomodulatory effect of Iscador: a *Viscum album* preparation, Oncology. 43 (1986) 51–61.

[66] S.H. Choi, K.H. Park, T.J. Yoon, J.B. Kim, Y.S. Jang, C.H. Choe, Dietary korean mistletoe enhances cellular non-specific immune responses and survival of Japanese eel (*Anguilla japonica*), Fish Shellfish Immunol.

24 (2008) 67–73. doi:10.1016/j.fsi.2007.08.007.

[67] X. Kong, Y. Hu, D. Song, Research progress of immunopharmacology of *Astragalus* polysaccharide, J Chin Vet. 3 (2003) 34–37 [China].

[68] H. Liu, Research status of Chinese herbal immunostimulants and their application in aquaculture, J Fish. 15 (2002) 91–94 [China].

[69] G. Yin, L. Ardó, K.D. Thompson, A. Adams, Z. Jeney, G. Jeney, Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*, Fish Shellfish Immunol. 26 (2009) 140–145. doi:10.1016/j.fsi.2008.08.015.

[70] J. Zhang, Q. Tang, M. Zimmerman-Kordmann, W. Reutter, H. Fan, Activation of B lymphocytes by GLIS, a bioactive proteoglycan from *Ganoderma lucidum*, Life Sci. 71 (2002) 623–638. doi:10.1016/S0024-3205(02)01690-9.

[71] Y.-H. You, Z.-B. Lin, Protective effects of *Ganoderma lucidum* polysaccharides peptide on injury of macrophages induced by reactive oxygen species, Acta Pharmacol. Sin. 23 (2002) 787–791.

[72] M. Jin, K. Zhao, Q. Huang, C. Xu, P. Shang, Isolation, structure and bioactivities of the polysaccharides from *Angelica sinensis* (Oliv.) Diels: A review, Carbohydr. Polym. 89 (2012) 713–722. doi:10.1016/j.carbpol.2012.04.049.

[73] J.H. Kim, Y.J. Mun, S.J. Im, J.H. Han, H.S. Lee, W.H. Woo, Effects of the aqueous extract of Epimedii Herba on the antibody responses in mice, Int. Immunopharmacol. 1 (2001) 935–944.

[74] J.C.J. Chao, S.W. Chiang, C.C. Wang, Y.H. Tsai, M.S. Wu, Hot water-extracted *Lycium barbarum* and *Rehmannia glutinosa* inhibit proliferation and induce apoptosis of hepatocellular carcinoma cells, World J. Gastroenterol. 12 (2006) 4478–4484.

[75] H.H. Yu, S.J. Seo, Y.H. Kim, H.Y. Lee, R.K. Park, H.S. So, S.L. Jang, Y.O. You, Protective effect of *Rehmannia glutinosa* on the cisplatin-induced damage of HEI-OC1 auditory cells through scavenging free radicals, J. Ethnopharmacol. 107 (2006) 383–388. doi:10.1016/j.jep.2006.03.024.

[76] G.-H. Baek, Y.-S. Jang, S.-I. Jeong, J. Cha, M. Joo, S.-W. Shin, K.-T. Ha, H.-S. Jeong, *Rehmannia glutinosa* suppresses inflammatory responses elicited by advanced glycation end products, Inflammation. 35 (2012) 1232–1241. doi:10.1007/s10753-012-9433-x.

[77] J.-L. Wang, X. Meng, R. Lu, C. Wu, Y.-T. Luo, X. Yan, X.-J. Li, X.-H. Kong, G.-X. Nie, Effects of *Rehmannia glutinosa* on growth performance, immunological parameters and disease resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio* L.), Aquaculture. 435 (2015) 293–300. doi:10.1016/j.aquaculture.2014.10.004.

[78] G. Zhang, S. Gong, D. Yu, H. Yuan, Propolis and Herba Epimedii extracts enhance the non-specific immune response and disease resistance of Chinese sucker, *Myxocyprinus asiaticus*, Fish Shellfish Immunol. 26 (2009) 467–472. doi:10.1016/j.fsi.2009.01.011.

[79] J. Jian, Z. Wu, Influences of traditional Chinese medicine on non-specific immunity of Jian Carp (*Cyprinus carpio* var. Jian), Fish Shellfish Immunol. 16 (2004) 185–191. doi:10.1016/S1050-4648(03)00062-7.

[80] X. Yang, J.L. Guo, J.Y. Ye, Y.X. Zhang, W. Wang, The effects of *Ficus carica* polysaccharide on immune response and expression of some immune-related genes in grass carp, *Ctenopharyngodon idella*, Fish Shellfish Immunol. 42 (2015) 132–7. doi:10.1016/j.fsi.2014.10.037.

[81] C. Wiart, S. Mogana, S. Khalifah, M. Mahan, S. Ismail, M. Buckle, a. K. Narayana, M. Sulaiman, Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia, Fitoterapia. 75 (2004) 68–73. doi:10.1016/j.fitote.2003.07.013.

[82] L.K. a M. Leal, a. a G. Ferreira, G. a. Bezerra, F.J. a Matos, G.S.B. Viana, Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: A comparative study, J. Ethnopharmacol. 70 (2000) 151–159. doi:10.1016/S0378-8741(99)00165-8.

[83] D. Christybapita, M. Divyagnaneswari, R. Dinakaran Michael, Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus*, Fish Shellfish Immunol. 23 (2007) 840–852. doi:10.1016/j.fsi.2007.03.010.

[84] R. Das, R. Prakash Raman, H. Saha, R. Singh, Effect of *Ocimum sanctum* Linn. (Tulsi) extract on the immunity and survival of *Labeo rohita* (Hamilton) infected with *Aeromonas hydrophila*, Aquac. Res. (2013) 1111–1121. doi:10.1111/are.12264.

[85] K.S. Mistry, Z. Sanghvi, G. Parmar, S. Shah, The antimicrobial activity of *Azadirachta indica*, *Mimusops elengi*, *Tinospora cardifolia*, *Ocimum sanctum* and 2% chlorhexidine gluconate on common endodontic pathogens: An *in vitro* study, Eur. J. Dent. 8 (2014) 172–177. doi:10.4103/1305-7456.130591.

[86] S. Kumar, R.P. Raman, P.K. Pandey, S. Mohanty, A. Kumar, K. Kumar, Effect of orally administered azadirachtin on non-specific immune parameters of goldfish *Carassius auratus* (Linn. 1758) and resistance against *Aeromonas hydrophila*, Fish Shellfish Immunol. 34 (2013) 564–73. doi:10.1016/j.fsi.2012.11.038.

[87] X. Gao, W. Wang, S. Wei, W. Li, Antioxidant, antioedema and analgesic activities of *Andrographis paniculata* extracts and their active constituent andrographolide, Phyther. Res. 23 (2009) 958–964. doi:10.1002/ptr.

[88] Y. Xu, R.L. Marshall, T.K.S. Mukkur, An investigation on the antimicrobial activity of *Andrographis paniculata* extracts and andrographolide *in vitro*, Asian J. Plant Sci. 5 (2006) 527–530. doi:10.3923/ajps.2006.527.530.

[89] W.W. Chao, Y.H. Kuo, B.I.F. Lin, Anti-inflammatory activity of new compounds from *Andrographis paniculata* by NF-κB transactivation inhibition, J. Agric. Food Chem. 58 (2010) 2505–2512. doi:10.1021/jf903629j.

[90] J. Levita, A. Nawawi, A. Mutholib, S. Ibrahim, Andrographolide inhibits COX-2 expression in human fibroblast cells due to its interaction with arginine and histidine in cyclooxygenase site, J. Appl. Sci. 10 (2010) 1481–1484.

[91] K.A. Basha, R.P. Raman, K.P. Prasad, K. Kumar, E. Nilavan, S. Kumar, Effect of dietary supplemented andrographolide on growth, non-specific immune parameters and resistance against *Aeromonas hydrophila* in *Labeo rohita* (Hamilton), Fish Shellfish Immunol. 35 (2013) 1433–41. doi:10.1016/j.fsi.2013.08.005.

[92] G. Yogeshwari, C. Jagruthi, S.M. Anbazahan, L.S.S. Mari, J. Selvanathan, J. Arockiaraj, N.B. Dhayanithi, T.T. Ajithkumar, C. Balasundaram, H. Ramasamy, Herbal supplementation diet on immune response in *Labeo rohita* against *Aphanomyces invadans*, Aquaculture. 437 (2015) 351–359. doi:10.1016/j.aquaculture.2014.12.024.

[93] A.M. Metwally, A.A. Omar, F.M. Harraz, S.M. El Sohafy, Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves, Pharmacogn Mag. 6 (2010) 212–218. doi:10.4103/0973-1296.66939.

[94] H.Y. Chen, G.C. Yen, Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves, Food Chem. 101 (2007) 686–694. doi:10.1016/j.foodchem.2006.02.047.

[95] S.S. Giri, S.S. Sen, C. Chi, H.J. Kim, S. Yun, S.C. Park, V. Sukumaran, Effect of guava leaves on the growth performance and cytokine gene expression of *Labeo rohita* and its susceptibility to *Aeromonas hydrophila* infection, Fish Shellfish Immunol. 46 (2015) 217–224. doi:10.1016/j.fsi.2015.05.051.

[96] A. Haug, S. Myklestad, B. Larsen, O. Smidsrød, G. Eriksson, R. Blinc, S. Paušak, L. Ehrenberg, J. Dumanović, Correlation between chemical structure and physical properties of alginates, Acta Chem. Scand. 21 (1967) 768–778. doi:10.3891/acta.chem.scand.21-0768.

[97] D. Vollstad, J. Bøgwald, O. Gåserød, R. a. Dalmo, Influence of high-M alginate on the growth and survival of Atlantic cod (*Gadus morhua* L.) and spotted wolffish (*Anarhichas minor* Olafsen) fry, Fish Shellfish Immunol. 20 (2006) 548–561. doi:10.1016/j.fsi.2005.07.004.

[98] R.T. Lorenz, G.R. Cysewski, Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin, Trends Biotechnol. 18 (2000) 160–167. doi:10.1016/S0167-7799(00)01433-5.

[99] M. Guerin, M.E. Huntley, M. Olaizola, *Haematococcus* astaxanthin: Applications for human health and nutrition, Trends Biotechnol. 21 (2003) 210–216. doi:10.1016/S0167-7799(03)00078-7.

[100] I. Higuera-Ciapara, L. Félix-Valenzuela, F.M. Goycoolea, Astaxanthin: a review of its chemistry and applications, Crit. Rev. Food Sci. Nutr. 46 (2006) 185–196. doi:10.1080/10408690590957188.

[101] C. Jagruthi, G. Yogeshwari, S.M. Anbazahan, L.S.S. Mari, J. Arockiaraj, P. Mariappan, G.R.L. Sudhakar, C. Balasundaram, R. Harikrishnan, Effect of dietary astaxanthin against *Aeromonas hydrophila* infection in common carp, *Cyprinus carpio*, Fish Shellfish Immunol. 41 (2014) 674–80. doi:10.1016/j.fsi.2014.10.010.

[102] D.L. McKay, J.B. Blumberg, A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.), Phyther. Res. 20 (2006) 619–633. doi:10.1002/ptr.

[103] M. Adel, A. Abedian Amiri, J. Zorriehzahra, A. Nematolahi, M.Á. Esteban, Effects of dietary peppermint (Mentha piperita) on growth performance, chemical body composition and hematological and immune parameters of fry Caspian white fish (*Rutilus frisii kutum*), Fish Shellfish Immunol. 45 (2015) 841–847. doi:10.1016/j.fsi.2015.06.010.

[104] I. Gülçin, Ö. I. Küfrevioğlu, M. Oktay, M.E. Büyükokuroğlu, Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.), J. Ethnopharmacol. 90 (2004) 205–215. doi:10.1016/j.jep.2003.09.028.

[105] R.E. Uncini Manganelli, L. Zaccaro, P.E. Tomei, Antiviral activity *in vitro* of *Urtica dioica* L., *Parietaria diffusa* M. et K. and *Sambucus nigra* L., J. Ethnopharmacol. 98 (2005) 323–327. doi:10.1016/j.jep.2005.01.021.

[106] I. Hadizadeh, B. Peivastegan, H. Hamzehzarghani, Antifungal activity of essential oils from some medicinal plants of Iran against *Alternaria alternate*, Am. J. Appl. Sci. 6 (2009) 857–861.

[107] C.C. Ngugi, E. Oyoo-Okoth, J. Mugo-Bundi, P.S. Orina, E.J. Chemoiwa, P.A. Aloo, Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with <i>Aeromonas hyd, Fish Shellfish Immunol. 44 (2015) 533–41. doi:10.1016/j.fsi.2015.03.025.

[108] L. Tort, Stress and immune modulation in fish, Dev. Comp. Immunol. 35 (2011) 1366–75. doi:10.1016/j.dci.2011.07.002.

[109] L.M. Lacorte, F.R.F. Seiva, J.C. Rinaldi, F.K. Delella, A. Moroz, C. Sarobo, A.F. Godinho, W.J. Fávaro, A. a H. Fernandes, S.L. Felisbino, Caffeine reduces cadmium accumulation in the organism and enhances the levels of antioxidant protein expression in the epididymis, Reprod. Toxicol. 35 (2013) 137–143. doi:10.1016/j.reprotox.2012.10.009.

[110] M. Abdel-Tawwab, K.M. Sharafeldin, M.N.M. Mosaad, N.E.M. Ismaiel, Coffee bean in common carp, *Cyprinus carpio* L. diets: Effect on growth performance, biochemical status, and resistance to waterborne zinc toxicity, Aquaculture. 448 (2015) 207–213. doi:10.1016/j.aquaculture.2015.06.010.

[111] S. Chatzifotis, F. Kokou, K. Ampatzis, I.E. Papadakis, P. Divanach, C.R. Dermon, Effects of dietary caffeine on growth, body composition, somatic indexes, and cerebral distribution of acetyl-cholinesterase and nitric oxide synthase in gilthead sea bream (*Sparus aurata*), reared in winter temperature, Aquac. Nutr. 14 (2008) 405–415. doi:10.1111/j.1365-2095.2007.00541.x.

[112] J. Jian, Z. Wu, Effects of traditional Chinese medicine on nonspecific immunity and disease resistance of large yellow croaker, *Pseudosciaena crocea* (Richardson), Aquaculture. 218 (2003) 1–9. doi:10.1016/S0044-8486(02)00192-8.

[113] J. Tang, J. Cai, R. Liu, J. Wang, Y. Lu, Z. Wu, J. Jian, Immunostimulatory effects of artificial feed supplemented with a Chinese herbal mixture on *Oreochromis niloticus* against *Aeromonas hydrophila*, Fish Shellfish Immunol. 39 (2014) 401–6. doi:10.1016/j.fsi.2014.05.028.

[114] D. See, N. Broumand, L. Sahl, J. Tilles, *In vitro* effects of echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients, Immunopharmacology. 35 (1997) 229–235.

[115] S.M. Aly, M.F. Mohamed, *Echinacea purpurea* and *Allium sativum* as immunostimulants in fish culture using Nile tilapia (*Oreochromis niloticus*), J. Anim. Physiol. Anim. Nutr. (Berl). 94 (2010). doi:10.1111/j.1439-0396.2009.00971.x.

[116] U. Chandur, S. Shashidhar, S. Chandrasekar, M.N. Rao, Studies of preliminary phytochemical and anti-arthritic activity of heart wood of *Cedrus deodar* (Roxb.), Res. J. Pharm. Biol. Chem. Sci. 2 (2011) 654–660.

[117] E. Awad, A.S. Awaad, M.A. Esteban, Effects of dihydroquercetin obtained from deodar (*Cedrus deodara*) on immune status of gilthead seabream (*Sparus aurata* L.), Fish Shellfish Immunol. 43 (2015) 43–50. doi:10.1016/j.fsi.2014.12.009.

[118] W.M. Bandaranayake, Bioactivities, bioactive compounds and chemical constituents of mangrove plants, Wetl. Ecol. Manag. 10 (2002) 421–452. doi:10.1023/A:1021397624349.

[119] M. Premanathan, R. Arakaki, H. Izumi, K. Kathiresan, M. Nakano, N. Yamamoto, H. Nakashima, Antiviral properties of a mangrove plant, *Rhizophora apiculata* Blume, against human immunodeficiency virus, Antiviral Res. 44 (1999) 113–122. doi:10.1016/S0166-3542(99)00058-3.

[120] N.B. Dhayanithi, T.T. Ajithkumar, J. Arockiaraj, C. Balasundaram, H. Ramasamy, Immune protection by *Rhizophora apiculata* in clownfish against *Vibrio alginolyticus*, Aquaculture. 446 (2015) 1–6. doi:10.1016/j.aquaculture.2015.04.013.

[121] N.B. Dhayanithi, T.T. Ajith Kumar, J. Arockiaraj, C. Balasundaram, R. Harikrishnan, Dietary supplementation of *Avicennia marina* extract on immune protection and disease resistance in *Amphiprion sebae* against *Vibrio alginolyticus*, Fish Shellfish Immunol. 45 (2015) 52–8. doi:10.1016/j.fsi.2015.02.018.

[122] I. Khafagi, A. Gab-Alla, Waleed SalamaMoustafa Fouda3, Biological activities and phytochemical constituents of the gray mangrove Avicennia marina (Forssk.) Vierh, J. Coast. …. 5 (2008) 62–69.

[123] M.P. Lincy, K. Paulpriya, V.R. Mohan, *In vitro* antioxidant activity of *Avicennia marina* (Forssk) Vierh pneumatophore (Avicenniaceae), Sci. Res. Report. 3 (2013) 106–114.

[124] P. Abeysinghe, Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenica bacteria, Indian J. Pharm. Sci. 72 (2010) 167–172.

[125] H.Y. Chee, H. Kim, M.H. Lee, *In vitro* antifungal activity of Limonene against *Trichophyton rubrum*, Mycobiology. 37 (2009) 243–6. doi:10.4489/MYCO.2009.37.3.243.

[126] N. Sharma, A. Tripathi, Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem, Microbiol. Res. 163 (2008) 337–344. doi:10.1016/j.micres.2006.06.009.

[127] Ü. Acar, O.S. Kesbiç, S. Yılmaz, N. Gültepe, A. Türker, Evaluation of the effects of essential oil extracted from sweet orange peel (*Citrus sinensis*) on growth rate of tilapia (*Oreochromis mossambicus*) and possible disease resistance against *Streptococcus iniae*, Aquaculture. 437 (2015) 282–286. doi:10.1016/j.aquaculture.2014.12.015.

[128] K.H. Kim, Y.J. Hwang, S.C. Bai, Resistance to *Vibrio alginolyticus* in juvenile rockfish (*Sebastes schlegeli*) fed diets containing different doses of aloe, Aquaculture. 180 (1999) 13–21. doi:10.1016/S0044-8486(99)00143-X.

[129] B. Vázquez, G. Avila, D. Segura, B. Escalante, Antiinflammatory activity of extracts from *Aloe vera* gel, J. Ethnopharmacol. 55 (1996) 69–75.

[130] G. Dotta, J.I.A. de Andrade, E.L. Tavares Gonçalves, A. Brum, J.J. Mattos, M. Maraschin, M.L. Martins, Leukocyte phagocytosis and lysozyme activity in Nile tilapia fed supplemented diet with natural

extracts of propolis and *Aloe barbadensis*, Fish Shellfish Immunol. 39 (2014) 280–4. doi:10.1016/j.fsi.2014.05.020.

[131] N.N. Gabriel, J. Qiang, J. He, X.Y. Ma, M.D. Kpundeh, P. Xu, Dietary *Aloe vera* supplementation on growth performance, some haemato-biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT), Fish Shellfish Immunol. 44 (2015) 504–14. doi:10.1016/j.fsi.2015.03.002.

[132] A.M.M. Abd-El-Rhman, Antagonism of *Aeromonas hydrophila* by propolis and its effect on the performance of Nile tilapia, Oreochromis niloticus, Fish Shellfish Immunol. 27 (2009) 454–9. doi:10.1016/j.fsi.2009.06.015.

[133] E. Isogai, H. Isogai, K. Hirose, S. Hayashi, K. Oguma, *In vivo* synergy between green tea extract and levofloxacin against enterohemorrhagic *Escherichia coli* O157 infection, Curr. Microbiol. 42 (2001) 248–251. doi:10.1007/s002840010212.

[134] J.M. Weber, A. Ruzindana-Umunyana, L. Imbeault, S. Sircar, Inhibition of adenovirus infection and adenain by green tea catechins, Antiviral Res. 58 (2003) 167–173. doi:10.1016/S0166-3542(02)00212-7.

[135] V. Crespy, G. Williamson, A review of the health effects of green tea catechins in *in vivo* animal models, J. Nutr. 134 (2004) 3431S–3440S.

[136] M. Abdel-Tawwab, M.H. Ahmad, M.E. a Seden, S.F.M. Sakr, Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against *Aeromonas hydrophila* infection, J. World Aquac. Soc. 41 (2010) 203–213. doi:10.1111/j.1749-7345.2010.00360.x.

[137] N. Ishihara, T. Araki, Y. Tamaru, A. Nishimura, N. Aoi, D. Chu, L.R. Juneja, T. Morishita, M.P. Science, Influence of green tea polyphenols on feed performance, growth performance, and fish body component in yellowtail (*Seriola quinqueradiata*), Jpn. J. Food Chem. 9 (2002) 7–14.

[138] V. Patil, T. Källqvist, E. Olsen, G. Vogt, H.R. Gislerød, Fatty acid composition of 12 microalgae for possible use in aquaculture feed, Aquac. Int. 15 (2007) 1–9. doi:10.1007/s10499-006-9060-3.

[139] M. Reyes-Becerril, F. Guardiola, M. Rojas, F. Ascencio-Valle, M.Á. Esteban, Dietary administration of microalgae *Navicula* sp. affects immune status and gene expression of gilthead seabream (*Sparus aurata*)., Fish Shellfish Immunol. 35 (2013) 883–9. doi:10.1016/j.fsi.2013.06.026.

[140] M. Reyes-Becerril, C. Angulo, N. Estrada, Y. Murillo, F. Ascencio-Valle, Dietary administration of microalgae alone or supplemented with *Lactobacillus sakei* affects immune response and intestinal morphology of Pacific red snapper (*Lutjanus peru*), Fish Shellfish Immunol. 40 (2014) 208–16. doi:10.1016/j.fsi.2014.06.032.

[141] P. Makridis, C. Moreira, R. Alves Costa, P. Rodrigues, M.T. Dinis, Use of microalgae bioencapsulated in Artemia during the weaning of Senegalese sole (Solea senegalensis Kaup), Aquaculture. 292 (2009) 153–157. doi:10.1016/j.aquaculture.2009.04.016.

[142] J. Skjermo, Ø. Bergh, High-M alginate immunostimulation of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae using Artemia for delivery, increases resistance against vibriosis, Aquaculture. 238 (2004) 107–113. doi:10.1016/j.aquaculture.2004.05.038.

[143] J. Skjermo, T. Defoort, M. Dehasque, T. Espevik, Y. Olsen, G. Skjak-brfek, P. Sorgeloos, O. Vadstein, Immunostimulation of juvenile turbot (*Scophthalmus maximus* L.) using an alginate with high mannuronic acid content administered via the live food organism Artemia, Fish Shellfish Immunol. 5 (1995) 531–534. doi:10.1016/S1050-4648(95)80053-0.

[144] L.E.C. Conceição, J. Skjermo, G. Skjåk-Bræk, J. a J. Verreth, Effect of an immunostimulating alginate on protein turnover of turbot (*Scophthalmus maximus* L.) larvae, Fish Physiol. Biochem. 24 (2001) 207–212.

[145] P. Díaz-Rosales, M. Chabrillón, R.T. Abdala, F.L. Figueroa, M.C. Balebona, M. a. Moriñigo, Effect of dietary administration of *Porphyridium cruentum* on the respiratory burst activity of sole, *Solea senegalensis* (Kaup), phagocytes, J. Fish Dis. 31 (2008) 489–495. doi:10.1111/j.1365-2761.2008.00923.x.

[146] T. Espevik, M. Otterlei, G. Skjåk-Braek, L. Ryan, S.D. Wright, A. Sundan, The involvement of CD14 in stimulation of cytokine production by uronic acid polymers, Eur. J. Immunol. 23 (1993) 255–261. doi:10.1002/eji.1830230140.

[147] M. Otterlei, K. Ostgaard, G. Skjåk-Braek, O. Smidsrød, P. Soon-Shiong, T. Espevik, Induction of cytokine production from human monocytes stimulated with alginate, J. Immunother. 10 (1991) 286–291. doi:10.1097/00002371-199108000-00007.

[148] T.H. Flo, L. Ryan, E. Latz, O. Takeuchi, B.G. Monks, E. Lien, O. Halaas, S. Akira, G. Skjåk-Bræk, D.T. Golenbock, T. Espevik, Involvement of Toll-like receptor (TLR) 2 and TLR4 in cell activation by mannuronic acid polymers, J. Biol. Chem. 277 (2002) 35489–35495. doi:10.1074/jbc.M201366200.

[149] M. Otterlei, a. Sundan, G. Skjak-Braek, L. Ryan, O. Smidsrod, T. Espevik, Similar mechanisms of action of defined polysaccharides and lipopolysaccharides: Characterization of binding and tumor necrosis factor alpha induction, Infect. Immun. 61 (1993) 1917–1925.

[150] S. Chung, C.J. Secombes, Activation of rainbow trout macrophages, J. Fish Biol. 31 (1987) 51–56.

[151] E.J. Nya, B. Austin, Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum), J. Fish Dis. 32 (2009) 971–977. doi:10.1111/j.1365-2761.2009.01101.x.

[152] S. Chrubasik, M.H. Pittler, B.D. Roufogalis, Zingiberis rhizoma: A comprehensive review on the ginger effect and efficacy profiles, Phytomedicine. 12 (2005) 684–701. doi:10.1016/j.phymed.2004.07.009.

[153] M. Agarwal, S. Walia, S. Dhingra, B.P.S. Khambay, Insect growth inhibition, antifeedant and antifungal activity of compounds isolated/ derived from *Zingiber officinale* Roscoe (ginger) rhizomes, Pest Manag. Sci. 57 (2001) 289–300. doi:10.1002/ps.263.

[154] K. Endo, E. Kanno, Y. Oshima, Structures of antifungal diarylheptenones, gingerenones A, B, C and isogingerenone B, isolated from the rhizomes of *Zingiber officinale*, Phytochemistry. 29 (1990) 797–799. doi:10.1016/0031-9422(90)80021-8.

[155] J.-K. Kim, Y. Kim, K.-M. Na, Y.-J. Surh, T.-Y. Kim, [6]-Gingerol prevents UVB-induced ROS production and COX-2 expression *in vitro* and *in vivo*, Free Radic. Res. 41 (2007) 603–614. doi:10.1080/10715760701209896.

[156] R. Grzanna, L. Lindmark, C.G. Frondoza, Ginger-an herbal medicinal product with broad anti-inflammatory actions, J. Med. Food. 8 (2005) 125–132. doi:10.1089/jmf.2005.8.125.

[157] E.J. Nya, B. Austin, Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic, Fish Shellfish Immunol. 30 (2011) 845–850. doi:10.1016/j.fsi.2011.01.008.

[158] S. Zedlitz, R. Kaufmann, W.-H. Boehncke, Allergic contact dermatitis from black cumin (*Nigella sativa*) oil-containing ointment, Contact Dermatitis. 46 (2002) 188. doi:10.1034/j.1600-0536.2002.460318.x.

[159] S.C. El-Saleh, O. a. Al-Sagair, M.I. Al-Khalaf, Thymoquinone and *Nigella sativa* oil protection against methionine-induced hyperhomocysteinemia in rats, Int. J. Cardiol. 93 (2004) 19–23. doi:10.1016/S0167-5273(03)00108-6.

[160] M.S. Hanafy, M.E. Hatem, Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin), J. Ethnopharmacol. 34 (1991) 275–278.

[161] E. Awad, D. Austin, A.R. Lyndon, Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum), Aquaculture. 388-391 (2013) 193–197. doi:10.1016/j.aquaculture.2013.01.008.

[162] B. Dülger, N. Hacıoglu, S. Bilen, Antimicrobial activity of *Cotinus coggyria* from Turkey, Asian J. Chem. 21 (2009) 4139–4140.

[163] S. Bilen, M. Bulut, A.M. Bilen, Immunostimulant effects of *Cotinus coggyria* on rainbow trout

(*Oncorhynchus mykiss*), Fish Shellfish Immunol. 30 (2011) 451–5. doi:10.1016/j.fsi.2010.12.013.

[164] F.S. Zanuzzo, E.C. Urbinati, M.L. Rise, J.R. Hall, G.W. Nash, A.K. Gamperl, *Aeromonas salmonicida* induced immune gene expression in *Aloe vera* fed steelhead trout,*Oncorhynchus mykiss* (Walbaum), Aquaculture. 435 (2015) 1–9. doi:10.1016/j.aquaculture.2014.09.010.

[165] N. Sheikhzadeh, K. Nofouzi, A. Delazar, A.K. Oushani, Immunomodulatory effects of decaffeinated green tea (*Camellia sinensis*) on the immune system of rainbow trout (*Oncorhynchus mykiss*), Fish Shellfish Immunol. 31 (2011) 1268–9. doi:10.1016/j.fsi.2011.09.010.

[166] J. Thawonsuwan, V. Kiron, S. Satoh, A. Panigrahi, V. Verlhac, Epigallocatechin-3-gallate (EGCG) affects the antioxidant and immune defense of the rainbow trout, *Oncorhynchus mykiss*, Fish Physiol. Biochem. 36 (2010) 687–697. doi:10.1007/s10695-009-9344-4.

[167] H. Watanuki, K. Ota, A.C.M. a R. Tassakka, T. Kato, M. Sakai, Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*, Aquaculture. 258 (2006) 157–163. doi:10.1016/j.aquaculture.2006.05.003.

[168] S. Yeganeh, M. Teimouri, A.K. Amirkolaie, Dietary effects of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*), Res. Vet. Sci. 101 (2015) 84–88. doi:10.1016/j.rvsc.2015.06.002.

[169] P.D. Karkos, S.C. Leong, C.D. Karkos, N. Sivaji, D. a. Assimakopoulos, *Spirulina* in clinical practice: Evidence-based human applications, Evidence-Based Complement. Altern. Med. 2011 (2008) 27–31. doi:10.1093/ecam/nen058.

[170] E. Baba, G. Uluköy, C. Öntaş, Effects of feed supplemented with *Lentinula edodes* mushroom extract on the immune response of rainbow trout, *Oncorhynchus mykiss*, and disease resistance against *Lactococcus garvieae*, Aquaculture. 448 (2015) 476–482. doi:10.1016/j.aquaculture.2015.04.031.

[171] C.A. Janeway, R. Medzhitov, Innate immune recognition, Annu. Rev. Immunol. 20 (2002) 197–216. doi:10.1146/annurev.immunol.20.083001.084359.

[172] S. Boltaña, N. Roher, F.W. Goetz, S.A. Mackenzie, PAMPs, PRRs and the genomics of gram negative bacterial recognition in fish, Dev. Comp. Immunol. 35 (2011) 1195–203. doi:10.1016/j.dci.2011.02.010.

[173] A. Falco, P. Frost, J. Miest, N. Pionnier, I. Irnazarow, D. Hoole, Reduced inflammatory response to *Aeromonas salmonicida* infection in common carp (*Cyprinus carpio* L.) fed with β-glucan supplements, Fish Shellfish Immunol. 32 (2012) 1051–7. doi:10.1016/j.fsi.2012.02.028.

[174] A. Falco, J.J. Miest, N. Pionnier, D. Pietretti, M. Forlenza, G.F. Wiegertjes, D. Hoole, 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. 36 (2014) 494–502. doi:10.1016/j.fsi.2013.12.005.

[175] N. Pionnier, A. Falco, J.J. Miest, A.K. Shrive, D. Hoole, Feeding common carp *Cyprinus carpio* with β-glucan supplemented diet stimulates C-reactive protein and complement immune acute phase responses following PAMPs injection., Fish Shellfish Immunol. 39 (2014) 285–95. doi:10.1016/j.fsi.2014.05.008.

[176] V. Selvaraj, K. Sampath, V. Sekar, 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. 19 (2005) 293–306. doi:10.1016/j.fsi.2005.01.001.

[177] C. Misra, B. Das, S. Mukherjee, P. Pattnaik, Effect of long term administration of dietary beta-glucan on immunity, growth and survival of *Labeo rohita* fingerlings, Aquaculture. 255 (2006) 82–94. http://www.sciencedirect.com/science/article/pii/S0044848605007696 (accessed February 21, 2013).

[178] P.K. Sahoo, S.C. Mukherjee, Effect of dietary beta-1,3 glucan on immune responses and disease resistance of healthy and aflatoxin B1-induced immunocompromised rohu (*Labeo rohita* Hamilton), Fish Shellfish Immunol. 11 (2001) 683–95. doi:10.1006/fsim.2001.0345.

[179] V. Selvaraj, K. Sampath, V. Sekar, 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. 114 (2006) 15–24. doi:10.1016/j.vetimm.2006.06.011.

[180] V. Selvaraj, K. Sampath, V. Sekar, Administration of lipopolysaccharide increases specific and non-specific immune parameters and survival in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*, Aquaculture. 286 (2009) 176–183. doi:10.1016/j.aquaculture.2008.09.017.

[181] G. Biswas, H. Korenaga, H. Takayama, T. Kono, H. Shimokawa, M. Sakai, Cytokine responses in the common carp, *Cyprinus carpio* L. treated with baker's yeast extract, Aquaculture. 356-357 (2012) 169–175. doi:10.1016/j.aquaculture.2012.05.019.

[182] S.K. Gupta, a K. Pal, N.P. Sahu, R. Dalvi, V. Kumar, S.C. Mukherjee, Microbial levan in the diet of *Labeo rohita* Hamilton juveniles: effect on non-specific immunity and histopathological changes after challenge with *Aeromonas hydrophila*, J. Fish Dis. 31 (2008) 649–57. doi:10.1111/j.1365-2761.2008.00939.x.

[183] A. Rodríguez, A. Cuesta, J. Ortuño, M.. Esteban, J. Meseguer, Immunostimulant properties of a cell wall-modified whole *Saccharomyces cerevisiae* strain administered by diet to seabream (*Sparus aurata* L.), Vet. Immunol. Immunopathol. 96 (2003) 183–192. doi:10.1016/S0165-2427(03)00164-8.

[184] J. Ortuño, A. Cuesta, A. Rodríguez, M.A. Esteban, J. Meseguer, Oral administration of yeast, *Saccharomyces cerevisiae*, enhances the cellular innate immune response of gilthead seabream (*Sparus aurata* L.), Vet. Immunol. Immunopathol. 85 (2002) 41–50. http://www.ncbi.nlm.nih.gov/pubmed/11867166.

[185] A. Cuesta, J. Meseguer, M.A. Esteban, Total serum immunoglobulin M levels are affected by immunomodulators in seabream (*Sparus aurata* L.), Vet. Immunol. Immunopathol. 101 (2004) 203–10. doi:10.1016/j.vetimm.2004.04.021.

[186] S. He, Z. Zhou, Y. Liu, P. Shi, B. Yao, E. Ringø, I. Yoon, Effects of dietary *Saccharomyces cerevisiae* fermentation product (DVAQUA®) on growth performance, intestinal autochthonous bacterial community and non-specific immunity of hybrid tilapia (*Oreochromis niloticus* ♀×*O. aureus* ♂) cultured , Aquaculture. 294 (2009) 99–107. doi:10.1016/j.aquaculture.2009.04.043.

[187] H.H. Yu, F. Han, M. Xue, J. Wang, P. Tacon, Y.H. Zheng, X.F. Wu, Y.J. Zhang, Efficacy and tolerance of yeast cell wall as an immunostimulant in the diet of Japanese seabass (*Lateolabrax japonicus*), Aquaculture. 432 (2014) 217–224. doi:10.1016/j.aquaculture.2014.04.043.

[188] S. Torrecillas, A. Makol, M.J. Caballero, D. Montero, L. Robaina, F. Real, J. Sweetman, L. Tort, M.S. Izquierdo, Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides, Fish Shellfish Immunol. 23 (2007) 969–81. doi:10.1016/j.fsi.2007.03.007.

[189] M. a Esteban, A. Cuesta, J. Ortuno, J. Meseguer, J. Ortuño, J. Meseguer, J. Ortuno, J. Meseguer, Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system, Fish Shellfish Immunol. 11 (2001) 303–315. doi:10.1006/fsim.2000.0315.

[190] M.E. El-Boshy, A.M. El-Ashram, F.M. Abdelhamid, H. a Gadalla, Immunomodulatory effect of dietary *Saccharomyces cerevisiae*, beta-glucan and laminaran in mercuric chloride treated Nile tilapia (*Oreochromis niloticus*) and experimentally infected with *Aeromonas hydrophila*, Fish Shellfish Immunol. 28 (2010) 802–8. doi:10.1016/j.fsi.2010.01.017.

[191] Q. Ai, K. Mai, L. Zhang, B. Tan, W. Zhang, W. Xu, H. Li, Effects of dietary beta-1, 3 glucan on innate immune response of large yellow croaker, *Pseudosciaena crocea*, Fish Shellfish Immunol. 22 (2007) 394–402. doi:10.1016/j.fsi.2006.06.011.

[192] N. Couso, R. Castro, B. Magariños, A. Obach, J. Lamas, Effect of oral administration of glucans on the resistance of gilthead seabream to pasteurellosis, Aquaculture. 219 (2003) 99–109. doi:10.1016/S0044-8486(03)00019-X.

[193] N.T. Kirchhoff, T. D'Antignana, M.J. Leef, C.J. Hayward, R.J. Wilkinson, B.F. Nowak, Effects of immunostimulants on ranched southern bluefin tuna *Thunnus maccoyii*: immune response, health and performance, J. Fish Biol. 79 (2011) 331–55. doi:10.1111/j.1095-8649.2011.03019.x.

[194] M.T. Cook, P.J. Hayball, W. Hutchinson, B.F. Nowak, J.D. Hayball, Administration of a commercial immunostimulant preparation, EcoActiva (TM) as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Pagrus auratus*, Sparidae (Bloch and Schneider)) in winter, Fish Shellfish Immunol. 14 (2003) 333–345. doi:10.1006/fsim.2002.0441.

[195] M. Bagni, L. Archetti, M. Amadori, G. Marino, Effect of long-term oral administration of an immunostimulant diet on innate immunity in sea bass (*Dicentrarchus labrax*), J. Vet. Med. B. Infect. Dis. Vet. Public Health. 47 (2000) 745–51. http://www.ncbi.nlm.nih.gov/pubmed/11204129.

[196] M. Bagni, N. Romano, M.G. Finoia, L. Abelli, G. Scapigliati, P.G. Tiscar, M. Sarti, G. Marino, Short- and long-term effects of a dietary yeast beta-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*), Fish Shellfish Immunol. 18 (2005) 311–25. doi:10.1016/j.fsi.2004.08.003.

[197] a K. Siwicki, D.P. Anderson, G.L. Rumsey, Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis., Vet. Immunol. Immunopathol. 41 (1994) 125–39. http://www.ncbi.nlm.nih.gov/pubmed/8066989.

[198] H.M.T. Kunttu, E.T. Valtonen, L.-R. Suomalainen, J. Vielma, I.E. Jokinen, The efficacy of two immunostimulants against *Flavobacterium columnare* infection in juvenile rainbow trout (*Oncorhynchus mykiss*), Fish Shellfish Immunol. 26 (2009) 850–7. doi:10.1016/j.fsi.2009.03.013.

[199] W.M. Sealey, F.T. Barrows, A. Hang, K. a. Johansen, K. Overturf, S.E. LaPatra, R.W. Hardy, Evaluation of the ability of barley genotypes containing different amounts of β-glucan to alter growth and disease resistance of rainbow trout *Oncorhynchus mykiss*, Anim. Feed Sci. Technol. 141 (2008) 115–128. doi:10.1016/j.anifeedsci.2007.05.022.

[200] G. Ghaedi, S. Keyvanshokooh, H. Mohammadi Azarm, M. Akhlaghi, Effects of dietary β-glucan on maternal immunity and fry quality of rainbow trout (*Oncorhynchus mykiss*), Aquaculture. 441 (2015) 78–83. doi:10.1016/j.aquaculture.2015.02.023.

[201] E. Casadei, S. Bird, J.L.G. Vecino, S. Wadsworth, C.J. Secombes, The effect of peptidoglycan enriched diets on antimicrobial peptide gene expression in rainbow trout (*Oncorhynchus mykiss*), Fish Shellfish Immunol. 34 (2013) 529–37. doi:10.1016/j.fsi.2012.11.027.

[202] E. Casadei, S. Bird, S. Wadsworth, J.L. González Vecino, C.J. Secombes, The longevity of the antimicrobial response in rainbow trout (*Oncorhynchus mykiss*) fed a peptidoglycan (PG) supplemented diet, Fish Shellfish Immunol. 44 (2015) 316–20. doi:10.1016/j.fsi.2015.02.039.

[203] A. Skalli, M. Castillo, K.B. Andree, L. Tort, D. Furones, E. Gisbert, The LPS derived from the cell walls of the Gram-negative bacteria *Pantoea agglomerans* stimulates growth and immune status of rainbow trout (*Oncorhynchus mykiss*) juveniles, Aquaculture. 416-417 (2013) 272–279. doi:10.1016/j.aquaculture.2013.09.037.

[204] A. Guttvik, B. Paulsen, R.A. Dalmo, S. Espelid, V. Lund, J. Bøgwald, Oral administration of lipopolysaccharide to Atlantic salmon (*Salmo salar* L.) fry. Uptake, distribution, influence on growth and immune stimulation, Aquaculture. 214 (2002) 35–53. doi:10.1016/S0044-8486(02)00358-7.

[205] J. Kumari, P.K. Sahoo, Non-specific immune response of healthy and immunocompromised Asian catfish (*Clarias batrachus*) to several immunostimulants, Aquaculture. 255 (2006) 133–141. doi:10.1016/j.aquaculture.2005.12.012.

TABLE I: Effect of different plant and algal extracts used as immunostimulant diets in the immune response in fish.

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Acipenseriformes						
Huso huso	100-110	Ergosan and Vitacel	1.3% Vitacel	90d	Serum non-specific parameters: lysozyme (↑15,90dpt), lymphocytes count (↓15,90dpt), neutrophils (↓15dpt, ↑90dpt), eosinophils (↑15,90dpt)	Heidarieh et al., 2011
			0.5% Ergosan		Serum non-specific parameters: (↑15,90dpt), lymphocytes count (↑15dpt, ↓90dpt), neutrophils (↓15dpt), eosinophils (↑15,90dpt)	
			1.3% Vitacel + 0.5% Ergosan		Serum non-specific parameters: lysozyme (↑15,90dpt), lymphocytes count (↓90dpt), neutrophils (↓15dpt, ↑90dpt), eosinophils (↑15,90dpt)	
Anguilliformes						
Anguilla japonica	200	Korean mistletoe (*Viscum album* Coloratum)	0,10%	14d	Lysozyme activity (↑14d), phagocytic activity (↑14d)	Choi et al., 2008
			0,50%		Respiratory burst activity (↑14d), lysozyme activity (↑14d), phagocytic activity (↑14d)	
			1%		Respiratory burst activity (↑14d), lysozyme activity (↑14d), phagocytic activity (↑14d)	
Cypriniformes						
Cyprinus carpio	10,2	Roasted coffee powder (RCP; Coffea arabica)	0.5 g/kg diet	10wks	NSD	Abdel-Tawwab et al., 2015
			1.0 g/kg diet		Plasmatic nitroblue tetrazolium (NBT) (↑10wks)	
			2.0 g/kg diet		Plasmatic nitroblue tetrazolium (NBT) (↑10wks)	
			5.0 g/kg diet		Plasmatic nitroblue tetrazolium (NBT) (↑10wks)	
Cyprinus carpio	7,49	Dried Rehmannia root powder (DP)	LDP (2%)	80d	Gene expression: in kidney TGF-β (↓80d), gut TGF-β(↓80d)	Wang et al., 2015
			HDP (4%)		Gene expression: in gut TGF-β (↓80d)	
		Prepared Rehmannia root powder (PP)	LPP (2%)		Phagocytic rate (↑80d), phagocytic index (↑80d); Gene expression in spleen iNOS (↑80d); gut IL-1β (↑80d), iNOS (↑80d), TNF-α (↑80d), ↓TGF-β (↓80d)	
			HPP (4%)		Lysozyme activity (↑80d), phagocytic rate (↑80d), phagocytic index (↑80d); Gene expression in kidney IL-1β (↑80d), iNOS (↑80d), TNF-α (↑80d); spleen IL-1β (↑80d), TNF-α (↑80d), and gut IL-1β (↑80d), TGF-β (↓80d)	
		Dried Rehmannia root extract (DE)	LDE (0.5%)		Gene expression in spleen iNOS (↑80d), IL-10 (↓80d), TGF-β (↓80d), and gut TNF-α (↑80d), TGF-β (↓80d)	
			HDE (1%)		Phagocytic rate (↑80d), phagocytic index (↑80d); Gene expression in kidney IL-10 (↓80d), spleen IL-1β (↑80d), IL-10 (↓80d), gut IL-10 (↓80d), TGF-β (↓80d)	
		Prepared Rehmannia root extract (PE)	LPE (0.5%)		Gene expression in kidney IL-10 (↓80d), TGF-β (↓80d); spleen TNF-α (↑80d), gut TGF-β (↓80d)	
			HPE (1%)		Gene expression in kidney IL-10 (↓80d), TGF-β (↓80d); spleen TNF-α (↑80d); gut TGF-β (↓80d)	
Cyprinus carpio	62,8	Chinese herb: *Astragalus radix* (plant) and *Ganoderma lucidum* (mushroom)	1% Astragalus	5wks	Respiratory burst activity (↑3waf), phagocytic activity of isolated blood cells (↑3waf,4waf,5waf), plasma lysozyme activity (↑2waf,3waf,4waf)	Yin et al., 2009
			1% Ganoderma		Respiratory burst activity (↑1waf), phagocytic activity of isolated blood cells (↑2waf,3waf,4waf), plasma lysozyme activity (↑2waf,4waf)	
			0.5% Astragalus + 0.5% Ganoderma		Phagocytic activity of isolated blood cells (↑3, 4waf), plasma lysozyme activity (↑2waf,3waf)	
		Vaccinated against *A. hydrophila*/*A. salmonicida*	1% Astragalus		Respiratory burst activity (↑2waf,5waf,↓3waf), phagocytic activity of isolated blood cells (↓3waf,↑5waf), plasma lysozyme activity (↑3waf)	
			1% Ganoderma		Respiratory burst activity (↓3waf), phagocytic activity of isolated blood cells (↑1waf,5waf, ↓2waf,3waf), plasma lysozyme activity (↑2waf)	
			0.5% Astragalus + 0.5% Ganoderma		Respiratory burst activity (↑5waf), phagocytic activity of isolated blood cells (↑1waf,5waf, ↓2waf), plasma lysozyme activity (↑5waf)	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Cyprinus carpio var. Jian	101	Astragalus Root (*Radix astragalin seu Hedysari*) and Chinese Angelica Root (*R. Angelicae Sinensis*)	1% (w/w)	30d	Serum non-specific immune parameters: NBT-positive cells (↑20,30daf), lysozyme activity (↑20,30daf), complement activity (↑20,30daf)	Jian and Wu, 2004
			1.5% (w/w)		Serum non-specific immune parameters: NBT-positive cells (↑20,30daf), lysozyme activity (↑20,30daf), complement activity (↑20,30daf)	
Carassius auratus	20,33	Azadirachtin EC 25%	0.1%	28d	Total erythrocyte count (↑14,28d), Total leukocyte count (↑14,28d), Haemoglobin levels (↓14d), Mean corpuscular volume level (↓14,28d), Mean corpuscular haemoglobin level (↓14,28d), Total protein (↑14,28d), globulin (↑14,28d), Albumin:globulin (↓14,28d), NBT level (↑14,28d), Myeloperoxidase activity (↑14,28d), lysozyme activity (↑14,28d), Total immunoglobulin content (↑14,28d), SGOT activity (↑14,28d), glucose level(↑14,28d), phagocytic and SGPT activity (↑14d)	Kumar et al., 2013
			0.2%		Total erythrocyte count (↑14,28d), Total leukocyte count (↑14,28d), Mean corpuscular volume level (↓14,28d), Mean corpuscular haemoglobin level (↓14,28d), Mean corpuscular haemoglobin concentration levels (↑14d), Total protein (↑14,28d), Albumin (↑28d), globulin (↑14,28d), Albumin:globulin (↑14,28d), NBT level (↑14,28d), Myeloperoxidase activity (↑14,28d), lysozyme activity (↑14,28d), Total immunoglobulin content (↑14,28d), Phagocytic (↑14,28d), SGOT and SGPT activity (↑14,28d), glucose level (↑28d)	
			0.4%		Total erythrocyte count (↑14,28d), Total leukocyte count (↑14,28d), Mean corpuscular volume level (↓14,28d), Mean corpuscular haemoglobin level (↓14,28d), Mean corpuscular haemoglobin concentration levels (↑14d), Mean corpuscular haemoglobin concentration levels (↓28d), Total protein (↑14,28d), Albumin (↑14,28d), globulin (↑14,28d), Albumin:globulin (↓28d), NBT level (↑14,28d), Myeloperoxidase activity (↑14,28d), lysozyme activity (↑14,28d), Total immunoglobulin content (↑14,28d), Phagocytic activity (↑14,28d), SGOT activity (↑14,28d), SGPT activity (↑14,28d), glucose level (↑14,28d)	
			0.8%		Total erythrocyte count (↑14,28d), Total leukocyte count (↑14,28d), Mean corpuscular volume level (↓14,28d), Mean corpuscular haemoglobin level (↓14,28d), Mean corpuscular haemoglobin concentration levels (↑14d), Total protein (↑14,28d), Albumin (↑14d), globulin (↑14,28d), Albumin:globulin (↓28d), NBT level (↑14,28d), Myeloperoxidase activity (↑14,28d), lysozyme activity (↑14,28d), Total immunoglobulin content (↑14,28d), Phagocytic activity (↑14,28d), SGOT activity (↑14,28d), SGPT activity (↑14,28d), glucose level (↑14,28d)	
			1.6%		Total erythrocyte count (↑14,28d), Total leukocyte count (↑14,28d), Haemoglobin levels (↓14,28d), Mean corpuscular volume level (↓14,28d), Mean corpuscular haemoglobin level (↓14,28d), Mean corpuscular haemoglobin concentration levels (↓28d), Total protein (↑14,28d), Albumin (↑28d), globulin (↓28d), Albumin:globulin (↑14,28d), NBT level (↑14,28d), Myeloperoxidase activity (↑14,28d), lysozyme activity (↑14,28d), Total immunoglobulin content (↑14,28d), Phagocytic activity (↑14,28d), SGOT activity (↑14,28d), SGPT activity (↑14,28d), glucose level (↑14d)	
Ctenopharyngodon idella	80	*Ficus carica* polysaccharide (FCP)	0.1% w/w	21d	Serum lysozyme (↑7d,14,21d) , complement C3 (↑14,21d), bactericidal activity (↑7d,14d), total protein (↑7,14d), albumin (↑7,14d), globulin (↑7,14d), gene expression HSP70 (↓14,21d)	Yang et al., 2015
			0.5% w/w		Serum lysozyme (↑7,14,21d) , complement C3 (↑14,21d), bactericidal activity (↑7d, 14d), total protein (↑7,14d), albumin (↑7,14d), globulin (↑7,14d), immune-related gene expression (IL-1β (↑14, 21d); TNF-α (↑14,21d); HSP70 (↓14,21d)	
			1.0% w/w		Serum lysozyme (↑7,14,21d) , complement C3 (↑14,21d), bactericidal activity (↑7,14d), total protein (↑7,14d), albumin (↑7,14d), globulin (↑7,14d), immune-related gene expression (IL-1β (↑14,21d); TNF-α (↑14,21d); HSP70 (↓14,21d)	
Myxocyprinus asiaticus	58,2	Propolis and Herba Epimedii (3:1 (w/w))	0,10%	5wks	lysozyme activity (↑4waf,5waf)	Zhang et al., 2009
			0,50%		Respiratory burst activity of phagocytic cells (↑3waf,4waf), phagocytic activity (↑4waf,5waf), lisozyme activity (↑2waf,4waf)	
			1,00%		Phagocytic activity (↑3waf), lysozyme activity (↑1waf)	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Labeo rohita	11,1	*Psidium guajava* L.	0.1%	60d	Lysozyme activity (↑60d), alternative complement pathway (↑60d)), gene expression in head kidney: TNF-α (↑60d); intestine: TNF-α (↑60d)	Giri et al., 2015
			0.5%		Lysozyme activity (↑60d), alternative complement pathway (↑60d), phagocytic activity (↑60d); gene expression in head kidney: TNF-α (↑60d, IL-1β (↑60d), IL-10 (↓60d), TGF-β (↓60d); intestine TNF-α (↑60d), IL-1β (↑60d), TGF-β (↓60d), COX-2 (↓60d); hepatopancreas TNF-α (↑60d), IL-1β (↑60d), ↓IL-10 (↓60d), TGF-β (↓60d), NF-κB (↓60d), iNOS (↓60d), COX-2 (↓60d)	
			1.0%		Lysozyme activity (↑60d), alternative complement pathway (↑60d), phagocytic activity (↑60d); Gene expression in head kidney: TNF-α (↑60d), IL-1β (↑60d), ↓IL-10 (↓60d), TGF-β (↓60d), NF-κB (↓60d), iNOS (↓60d); intestine TNF-α (↑60d), IL-1β (↑60d), IL-10 (↓60d), TGF-β (↓60d), iNOS (↓60d), COX-2 (↓60d); hepatopancreas: TNF-α (↑60d), IL-1β (↑60d), IL-10 (↓60d), TGF-β (↓60d), NF-κB (↓60d), iNOS (↓60d), COX-2 (↓60d)	
			1.5%		Lysozyme activity (↑60d), alternative complement pathway (↑60d), phagocytic activity (↑60d), IgM (↑60d); gene expression in head kidney: IL-10 (↓60d), NF-κB (↓60d), iNOS (↓60d), COX-2 (↓60d); intestine: TNF-α (↑60d), IL-10 (↓60d), NF-κB (↓60d), iNOS (↓60d), COX-2 (↓60d); hepatopancreas: TNF-α (↑60d), IL-1β (↑60d), TGF-β (↓60d), NF-κB (↓60d), iNOS (↓60d), COX-2 (↓60d)	
Labeo rohita	6,6	*Ocimum sanctum* Linn. (Tulsi) extract	0.05% (T1)	42d	Superoxide anion production (↑42d), lysozyme activity (↑42d), hemato-immune parameters: RBC (↑42d), WBC (↑42d), total protein (↑42d), albumin (↑42d), hemoglobin (↑42d)	Das et al., 2013
			0.1%(T2)		Superoxide anion production (↑42d), lysozyme activity (↑42d), total Ig (↑42d), hemato-immune parameters: albumin/globulin ratio (↓42d), RBC (↑42d), WBC (↑42d), total protein (↑42d), globulin (↑42d), hemoglobin (↑42d)	
			0.2% (T3)		Superoxide anion production (↑42d), lysozyme activity (↑42d), total Ig (↑42d), hemato-immune parameters: albumin/globulin ratio (↓42d), RBC (↑42d), WBC (↑42d), total protein (↑42d), albumin (↑42d), globulin (↑42d), hemoglobin (↑42d)	
			0.5% (T4) 1% (T5)		Hemato-immune parameters: RBC (↑42d), total Ig (↑42d) NSD	
Labeo rohita	9,5	Andrographo-lide	T1 (0.05%)	42d	NBT (↑14,28,42d), myeloperoxidase activity (↑14,42d), serum lysozyme activity (↑14,28,42d), antiprotease activity (↑14,28,42d), phagocytic activity (↑14,28,42d)	Basha et al., 2013
			T2 (0.10%)		NBT (↑14,28,42d), myeloperoxidase activity (↑14,28,42d), serum lysozyme activity (↑14,28,42d), antiprotease activity (↑14,28,42d), phagocytic activity (↑14,28,42d)	
			T3 (0.20%)		NBT (↑14,28,42d), myeloperoxidase activity (↑14,28,42d), serum lysozyme activity (↑14,28,42d), antiprotease activity (↑14,28,42d), antiprotease activity (↑28,42d), phagocytic activity (↑14,28,42d)	
			T4 (0.40%)		NBT (↑14,28,42d), myeloperoxidase activity (↓14d,↑42d), serum lysozyme activity (↑14,28,42d), antiprotease activity (↑14,28,42d), phagocytic activity (↑14,28,42d)	
			T5 (0.80%)		NBT (↑14,28,42d), myeloperoxidase activity (↑14,28,42d), serum lysozyme activity (↑14,28d), antiprotease activity (↑14,28,42d), phagocytic activity (↑14,28,42d)	
Rutilus frisii kutum	1,12	*Mentha piperita*	1%	8wks	Skin mucus: bacterial growth inhibition to *L. Monocytogenes* (↑8wks); protein level (↑8wks), alkaline phosphatase activity (↑8wks); serum total protein (↑8wks), serum respiratory burst activity (↑8wks); WBC (↑8wks)	Adel et al., 2015
			2%		Skin mucus: bacterial growth inhibition to *S. iniae, Y. ruckeri, L. monocytogenes, E. coli* (↑8wks), protein level (↑8wks), alkaline phosphatase activity (↑8wks); serum lysozyme activity (↑8wks), serum respiratory burst activity (↑8wks); WBC (↑8wks), Hb (↑8wks), neutrophils(↑8wks)	
			3%		Skin mucus: bacterial growth inhibition to *S. iniae, Y. ruckeri, L. monocytogenes, E. coli* (↑8wks), protein level (↑8wks), alkaline phosphatase activity (↑8wks); serum total protein (↑8wks), serum lysozyme activity (↑8wks), serum IgM (↑8wks), serum respiratory burst activity (↑8wks); RBC (↑8wks), WBC (↑8wks), Hct (↑8wks), Hb (↑8wks), lymphocytes (↑8wks), neutrophils (↑8wks)	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Perciformes						
Oreochromis niloticus	0,8	Echinacea (*Echinacea purpurea*) extract	1.0 ppt (begins in summer)	E1 2 mo control diet + 1 mo Echinacea	Total leucocytes count (↑3mo), lymphocytes (↑3mo), monocytes (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7 mo)	Aly & Mohamed, 2010
				E2 1 mo control diet + 2 mo Echinacea	Neutrophil adherence (↑3mo), Ht (↑3mo), total leucocytes count (↑3mo), lymphocytes (↑3mo), monocytes (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
				E3 3 mo Echinacea	Neutrophil adherence (↑3mo), Ht (↑3mo), neutrophils (↑3mo), monocytes (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
		Garlic	1.0 ppt (begins in summer)	G1 2 mo control diet + 1 mo garlic	Neutrophils (↑3mo), monocytes (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
				G2 1 mo control diet + 2 mo garlic	Neutrophil adherence (↑3mo), neutrophils (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
				G3 3 month garlic	Neutrophil adherence (↑3mo), Ht (↑3mo), body gain (↑3mo), SGR (↑3mo), survival rate (↑3mo,7mo)	
Oreochromis niloticus	54,3	Propolis and *Aloe barbadensis*	0.5% propolis and Aloe extract (1:1)	15d	NSD	Dotta et al., 2014
			1.0% propolis and Aloe extract (1:1)		NSD	
			2.0% propolis and Aloe extract (1:1)		NSD	
Oreochromis niloticus GIFT strain	20	Chinese herbal mixture (CHM)	0.5 % CHM (w/w)	4wks	Serum lysozyme activity (↑4wks), serum malondialdehyde content (↓3,4wks)	Tang et al., 2014
			1.0 % CHM (w/w)		Serum lysozyme activity (↑4wks), serum SOD activity (↑4wks), total peroxidase activity (↑4wks), malondialdehyde content (↓2,3,4wks); relative expression of TNF-α (↑1wk in spleen, head	
			1.5 % CHM (w/w)		Serum lysozyme activity (↑4wks), serum SOD activity (↑4w), total peroxidase activity (↑2,3,4wks), malondialdehyde content (↓2,3,4wks); relative expression of TNF-α (↑1wk in spleen, head kidney), IL-1β (↑1wk in spleen, head kidney)	
			2.0 % CHM (w/w)		Serum lysozyme activity (↑1,3,4wks), serum SOD activity (↑4wks), total peroxidase activity (↑1,2,3,4wks), malondialdehyde content (↓2,3,4wks); relative expression of TNF-α (↑1wk in spleen, head kidney), IL-1β (↑1wk in head kidney)	
Oreochromis niloticus	1.5–2.0	Green tea (*Camellia sinensis L*)	0.125 g/kg diet	12wks	Monocytes count (↓12wks), granulocytes count (↓12wks), glucose (↑12wks), globulin (↑12wks), NBT (↑12wks)	Abdel-Tawwab et al., 2010
			0.25 g/kg diet		Monocytes count (↓12wks), granulocytes count (↓12wks), glucose (↑12wks), globulin (↑12wks), NBT (↑12wks)	
			0.50 g/kg diet		RBC count (↑12wks), WBC count (↑12wks), lymphocytes count (↑12wks), monocytes (↓12wks), granulocytes (↓12wks), glucose (↑12wks), total lipids (↑12wks), total proteins (↑12wks), albumin (↑12wks), globulin (↑12wks), NBT (↑12wks)	
			1.0 g/kg diet		RBC (↑12wks), WBC (↑12wks), lymphocytes (↑12wks), monocytes (↓12wks), granulocytes (↓12wks), glucose (↑12wks), total lipids (↑12wks), total proteins (↑12wks), albumin (↑12wks), globulin (↑12wks), NBT (↑12wks)	
			2.0 g/kg diet		RBC count (↑12wks), WBC count (↑12wks), lymphocytes count (↑12wks), monocytes (↓12wks), granulocytes (↓12wks), glucose (↑12wks), total lipids (↑12wks), total proteins (↑12wks), albumin (↑12wks), globulin (↑12wks), NBT (↑12wks)	
Oreochromis niloticus	8	Crude propolis and its ethanolic-extract	1% propolis-ethanolic-extract	28d	Mean weight (↓0d,↑7d,14d,28d), average daily gain (↑28d), SGR (↑28d), FCR (↓28d), FER (↑28d), HCV (↑28d), small lymphocytes (↑28d), monocytes (↑28d), neutrophils (↓28d), serum lysozyme content (↑28d), serum bactericidal activity (↓28d)	Azza M.M. Abd-El-Rhman, 2009
			1% ethanol containing crude propolis		Mean weight (↑28d), average daily gain (↑28d), SGR (↑28d), FCR (↓28d), FER (↑28d), HCV (↑28d), small lymphocytes (↑28d), serum lysozyme content (↑28d), serum bactericidal activity (↓28d)	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Oreochromis mossambicus	0,91	Essential oil sweet orange peel (*Citrus sinensis*)	CEO1 (1 g kg−1)	90d	Serum biochemical and hemato-immune parameters (glucose (↓90d), triglyceride (↓90d), cholesterol (↓90d), albumin (↓90d), mean cell volume (↓90d), mean cell hemoglobin (↓90d), mean cell hemoglobin concentration (↓90d), hemoglobin (↑90d), RBC (↑90d), lysozyme activity (↑90d), myeloperoxidase activity (↑90d)	Acar et al., 2015
			CEO3 (3 g kg−1)		Serum biochemical and hemato-immune parameters: glucose (↓90d), cholesterol (↓90d), mean cell volume (↓90d), mean cell hemoglobin (↓90d), total protein (↑90d), hematocrit (↑90d), RBC (↑90d), mean cell hemoglobin concentration (↑90d), lysozyme activity (↑90d), myeloperoxidase activity (↑90d)	
			CEO5 (5 g kg−1)		Serum biochemical and hemato-immune parameters: glucose (↓90d), cholesterol (↓90d), mean cell volume (↓90d), mean cell hemoglobin (↓90d), mean cell hemoglobin concentration (↓90d), total protein (↑90d), globin (↑90d), hematocrit (↑90d), RBC (↑90d), mean cell hemoglobin concentration (↑90d), lysozyme activity (↑90d), myeloperoxidase activity (↑90d)	
Oreochromis mossambicus	25, 50	*Eclipta alba* aqueous extract	0,01%	3wks	Serum lysozyme activity (↑1waf,2waf,3waf), serum natural haemolytic complement activity (↑1waf), serum antiprotease activity (↑2waf,3waf), leukocytes myeloperoxidase content (↑1waf), reactive oxygen species production by peripheral blood leucocytes (↑1waf,2waf)	Christybapita et al., 2007
			0,10%		Serum lysozyme activity (↑1waf,2waf,3waf), serum natural haemolytic complement activity (↑2waf), serum antiprotease activity (↑2waf,3waf), leukocytes myeloperoxidase content (↑1waf), reactive oxygen species production by peripheral blood leucocytes (↑1waf,2waf), reactive nitrogen species production by peripheral blood leucocytes (↑2waf)	
			1%		Serum lysozyme activity (↑1waf,2waf,3waf), serum antiprotease activity (↑2waf,3waf), leukocytes myeloperoxidase content (↑1waf), reactive oxygen species production by peripheral blood leucocytes (↑1waf), reactive nitrogen species production by peripheral blood leucocytes (↑2waf)	
Lutjanus peru	250	Microalgae Navicula sp	Navicula + L. sakei	8wks	Myeloperoxidase activity (↑4wks), lysozyme (↑8wks), trypsin inhibition (↑4,8wks), total IgM (↑4,8wks); enzymatic activity of phosphatase alkaline, esterase (↓4wks,↑8wks), protease (↑4wks, ↓8wks), superoxide dismutase (↓4wks, ↑8wks), catalase (↑4,8wks); intestine histological scores of microvilli height (↑4wks), enterocyte vacuolization (↓4,8wks), melanomacrophages-like (↓4,8wks), area goblet cells (↑4wks), total goblet cells (↑4wks), goblet cells with acid mucins (↑4wks), goblet cells with mixed, neutral+acid, mucins (↑4wks)	Reyes-Becerril et al., 2014
			Navicula		Average body weight (↑8wks), total protein (↑4wks), myeloperoxidase activity (↑4wks, ↓8wks), lysozyme (↑8wks), trypsin inhibition (↑8wks), total IgM (↑4,8wks); enzymatic activity of phosphatase alkaline, esterase (↑8wks), protease (↑4wks), superoxide dismutase (↓4wks), catalase (↑4,8wks); intestine histological scores of microvilli height (↑4wks, ↓8wks), enterocyte vacuolization (↓4,8wks), intraepithelial leucocytes (↑4,8wks), melanomacrophages-like (↓4wks), total goblet cells (↑4wks), goblet cells with neutral mucins (↓4,8wks), goblet cells with acid mucins (↑4wks), goblet cells with mixed, neutral+acid, mucins (↑4wks)	
			L. sakei		Average body weight (↑8wks), hemoglobin (↑8wks), myeloperoxidase activity (↑4wks, ↓8wks), lysozyme (↑4,8wks), trypsin inhibition (↑4,8wks), total IgM (↑4,8wks); enzymatic activity of phosphatase alkaline (↑4,8wks), esterase (↑4,8wks), protease, superoxide dismutase (↑4,8wks), catalase (↑4,8wks); intestine histological scores of microvilli height (↑4wks), enterocyte vacuolization (↑4,8wks), intraepithelial leucocytes (↓4,8wks), melanomacrophages-like (↓4,8wks), area goblet cells (↑4wks), total goblet cells (↑4wks), goblet cells with neutral mucins (↓8wks), goblet cells with mixed, neutral+acid, mucins (↑4,8wks)	
Pseudosciaena crocea	120	Astragalus Root (*Radix astragalin seu Hedysari*) and Chinese Angelica Root (*Radix angelicae sinensis*)	0.5% (w/w)	30d	NSD	Jian and Wu, 2003
			1.0% (w/w)		Non-specific immune parameters such as NBT-positive cells (↑20,25,30daf), lysozyme activity (↑20,25,30daf), complement haemolytic activity (↑15,20,25,30daf)	
			1.5% (w/w)		Non-specific immune parameters such as NBT-positive cells (↑20,25,30daf), lysozyme activity (↑20,25,30daf), complement haemolytic activity (↑15,20,25,30daf)	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Sparus aurata	8	Dihydroquercetin from deodar (*Cedrus deodara*)	0.1 g 100 g-1	14d	Phagocytosis activity (↑14d), respiratory burst activity(↑14d), complement activity (↑14d), total protein (↑14d), antiprotease activity (↑14d), total IgM (↑14d), bactericidal activity (↓14d)	Awad et al., 2015
			0.5 g 100 g-1		NSD	
			1 g (1%) 100 g-1		Respiratory burst activity (↑14d), total protein (↑14d), antiprotease activity (↑14d)	
Sparus aurata	80	Microalgae Navicula sp	SM (silage microalgae Navicula + *L sakei*)	4wks	Leucocytes peroxidase activity (↑2wks), % phagocytic cells (↑4wks), ACH50 (↑2wks); expression of β-defensin in HK (↑4wks); expression of trypsin (↑4wks)	Reyes-Becerril et al., 2013
			LM (lyophilized microalgae)		% phagocytic cells (↑4wks); expression of IL-8 (↑2wks in HK; ↑4wks in intestine); transferrin (↑2wks, 4wks), IL-8 (↑4wks), IL-1β (↑4wks), COX-2 (↑2wks); α-amylase (↑2wks), trypsin (↑2wks), alkaline phosphatase (↑2wks)	
Pleuronectiformes						
Hippoglossus hippoglossus L.	larvae	High-M alginate (*Durvillaea antarctica*)	50-150 ng per larva/day	7-9d, 20-22d, 41-43d, 85-87d	NSD (dry weight in larvae)	Skjermo & Bergh, 2004
Solea senegalensis	80	Red algae (*Porphyridium cruentum*) lyophilized cells	1%	4wks	NSD	Diaz-Rosales et al., 2008
Scophthalmus maximus L.	larvae	FMI (*Ascophyllum nodosum*)	0.5 g FMI wet weight capsules/l	2-13dph	Protein synthesis (↑13dph), protein degradation (↑13dph), efficiency of retention of synthetised protein (↓13dph)	Conciecao et al., 2001
Salmoniformes						
Onchorynchus mykiss	20	Lentinula edodes	1%	6wks	Hematolo-immune parameters: total leucocyte (↑1,2,3,4,5,6wks), monocytes (↑1,2,3,4,5,6wks), neutrophils (↑1,2,3,4,5,6wks), lymphocytes (↓1,2,3,4,5,6wks), phagocytic activity (↑1,2,3,4,5,6wks), phagocytic index (↑1,2,3,4,5,6wks), serum lysozyme activity (↑1,2,3,4,5,6wks), myeloperoxidase activity (↑1,2,3,4,5,6wks)	Baba et al., 2015
			2%		Hemato-immune parameters: total leucocyte (↑1,2,3,4,5,6wks), monocytes (↑1,2,3,4,5,6wks), neutrophils (↑1,2,3,4,5,6wks), lymphocytes (↓1,2,3,4,5,6wks), phagocytic activity (↑2,3,4,5,6wks), phagocytic index (↑1,2,3,4,5,6wks), serum lysozyme activity (↑1,2,3,4,5,6wks), myeloperoxidase activity (↑1,2,3,4,5,6wks)	
Onchorynchus mykiss	70–110	*Aloe vera*	5g kg^{-1} A. vera (0.5%)	6wks	NSD	Zanuzzo et al., 2015
Onchorynchus mykiss	101	*Spirulina platensis*	2,50%	10wks	Serum glucose (↓10wks)	Yeganeh et al., 2015
			5%		WBC (↑10wks); LDL-cholesterol (↓10wks); serum albumin (↑10wks), serum glucose (↓10wks)	
			7,50%		WBC (↑10wks), hemoglobin (↑10wks), LDL-cholesterol (↓10wks); serum albumin (↑10wks), serum cortisol (↓10wks), serum glucose (↓10wks)	
			10%		RBC (↑10wks), WBC (↑10wks), hemoglobin (↑10wks); HDL-cholesterol (↑10wks), LDL-cholesterol (↓10wks); serum albumin (↑10wks), serum total protein (↑10wks), serum cortisol (↓10wks), serum glucose (↓10wks)	
Onchorynchus mykiss	81,65	Vitacel	10 g kg^{-1}	45d	Expression of immune-related genes in head kidney: lysozyme (↑15,45d), TNF-α (↑15,45d), HSP70 (↓45d), hematological parameters: WBC (↑45d), hematocrit (↑45d); serum non-specific immune parameters: complement activity (↑15,45d), bactericidal activity (↑15,45d), lysozyme (↑45d), agglutination antibody titer (↑45d)	Yarahmadi et al., 2014
Onchorynchus mykiss	18	Black cumin seed oil (*N. sativa*) nettle extract (Quercetin)	1% *N. sativa*	14 days	Myeloperoxidase activity (↑14d), total serum IgM (↑14d)	Awad et al., 2013
			2% *N. sativa*		Lysozyme activity (↑14d), myeloperoxidase activity (↑14d), antiprotease activity (↑14d), total serum IgM (↑14d)	
			3% *N. sativa*		Lysozyme activity (↑14d), total protein (↑14d), myeloperoxidase activity (↑14d), bactericidal activity (↓14d), antiprotease activity (↑14d)	
			0.1% Quercetin		Total serum IgM (↑14d)	
			0.5% Quercetin		Lysozyme activity (↑14d), myeloperoxidase activity (↑14d), total serum IgM (↑14d)	
			1% Quercetin		Lysozyme activity (↑14d), total protein (↑14d), myeloperoxidase activity (↑14d), bactericidal activity (↓14d), antiprotease activity (↑14d)	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Onchorynchus mykiss	18	Nettle extract (Quercetin)	0,10%	14d	Total serum IgM (↑14d)	Elham Awad., 2013
			0,50%		Lysozyme activity (↑14d), Myeloperoxidase (↑14d), Total serum IgM (↑14d)	
			1%		Lysozyme activity (↑14d), Myeloperoxidase (↑14d), total protein (↑14d), bactericidal activity (↑14d), antiproteases activity (↑14d)	
		N. sativa oil	1%	14days	Myeloperoxidase (↑14d), Total serum IgM (↑14d)	
			2%		Lysozyme activity (↑14d), Myeloperoxidase (↑14d), antiproteases activity (↑14d), Total serum IgM (↑14d)	
			3%		Lysozyme activity (↑14d), Myeloperoxidase (↑14d), total protein (↑14d), bactericidal activity (↑14d), antiproteases activity (↑14d)	
Onchorynchus mykiss	35	Decaffeinated green tea (*Camellia sinensis*)	20 mg (T1)per kg feed	30d	Bactericidal assay (↑14d), α1-antiprotease (↑14d), peroxidase content (↑14d)	Sheikhzadeh et al., 2011
			100 mg (T2) per kg feed		Lysozyme activity (↑14d), α1-antiprotease (↑14d), peroxidase content (↑14d)	
			500 mg (T3) per kg feed		Peroxidase content (↑14d)	
Onchorynchus mykiss	14	Garlic	0,50%	14d	Haematological parameters [RBC (↑21daf), WBC (↓28daf), monocytes (↑14,28daf, ↓21daf), lymphocytes (↑21daf), neutrophils (↓28daf), thrombocytes (↑21daf)], electrolyte indices [Calcium (↑14daf)], respiratory burst of blood leucocytes (↑14,21,28daf), lysozyme activity (↑14,21daf)	Nya & Austin, 2011
			1,00%		Haematological parameters [RBC (↑14daf), WBC (↑14,28daf), monocytes (↑14,28daf), lymphocytes (↑21daf), neutrophils (↓21,28daf)], electrolyte indices [Calcium (↑14,21daf)], respiratory burst of blood leucocytes (↑21,28daf), lysozyme activity (↑14,21daf)	
Onchorynchus mykiss	89,2	Tetra (*Cotinus coggyria*)	0,50%	3wks	Non-specific immune parameters [extracellular superoxide anion production (↑6,9wks), intracellular superoxide anion production (↑6,9wks), phagocytic activity (↑6,9wks), lysozyme activity (↑6,9wks), total protein level (↑6,9wks)]	Bilen et al., 2011
			1,00%		Non-specific immune parameters [extracellular superoxide anion production (↑6,9wks), intracellular superoxide anion production (↑6,9wks), phagocytic activity (↑6,9wks), lysozyme activity (↑6,9wks), total protein level (↑6,9wks)]	
Onchorynchus mykiss	89,25	Tetra (*Cotinus coggyria*)	0.5%	3wks + ISD 6wks	Extracellular and intracellular respiratory burst activities, phagocytic activity, lysozyme activity, total protein level (↑at the end of the 3rd, 6th and 9th weeks)	Bilen et al., 2011
			1.0%		Extracellular and intracellular respiratory burst activities, phagocytic activity, lysozyme activity, total protein level (↑at the end of the 3rd, 6th and 9th weeks)	
Onchorynchus mykiss	35	Decaffeinated green tea (*C. sinensis*)	20 mg/kg feed (T1)	30d	α1 antiprotease (↑30d) ; peroxidase content (↑30d)	Sheikhzadeh et al., 2011
			100 mg/kg feed (T2)		Lysozyme activity (↑30d); α1 antiprotease (↑30d) ; peroxidase content (↑30d); antigen-specific antibody response (↑30d)	
			500 mg/kg feed (T3)		Peroxidase content (↑30d)	
Onchorynchus mykiss	145	Epigallocatechin-3-gallate (EGCG)	20 mg kg-1 EGCG	8wks	NSD	Thawonsuwan et al., 2010
			100 mg kg-1 EGCG		Serum vitamin E level (↑8wks)	
			100 mg kg-1 vitamin E		Serum vitamin E level (↑8wks)	

TABLE I: (*...continuation*)

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Onchorynchus mykiss	14	Ginger (*Z. officinale* Roscoe)	0,05%	14d	Average haematological data (RBC↑, WBC↑, Hct↑, lymphocytes↑, monocytes↑, and neutrophils proportion↑ at 14d), phagocytic ratio (↑14d), Blood leucocytes superoxide anion production (↑14d), lysozyme activity(↑15,30,60 min), serum bactericidal activity(↓14d), anti-protease activity (↑14d), serum alternative haemolytic complement activity (↑14d), biochemical indices (globulin (↑14d))	Nya & Austin, 2009
			0,10%		Average haematological data (RBC↑, WBC↑, Hct↑, lymphocytes↑, monocytes↑, and neutrophils proportion↑ at 14d), phagocytic ratio (↑14d), superoxide anion production by blood leucocytes (↑14d), lysozyme activity (↑15,30,60min), serum bactericidal activity (↑14d), anti-protease activity (↑14d), serum alternative haemolytic complement activity (↑14d), biochemical indices (total protein (↑14d), globulin (↑14d))	
			0,50%		Average haematological data (RBC↑, WBC↑, Hct↑, lymphocytes↑, monocytes↑, and neutrophils proportion↑ at 14d)), phagocytic ratio(↑14d), superoxide anion production by blood leucocytes (↑14d), lysozyme activity (↑15,30,60min), serum bactericidal activity (↑14d), anti-protease activity (↑14d), serum alternative haemolytic complement activity (↑14d), biochemical indices (total protein (↑14d), globulin (↑14d))	
			1,00%		Average haematological data (RBC↑, WBC↑, Hct↑, lymphocytes↑, monocytes↓, and neutrophils proportion↑ at 14d)), phagocytic ratio (↑14d), superoxide anion production by blood leucocytes (↑14d), lysozyme activity (↑15,30,60min), serum bactericidal activity (↑14d), anti-protease activity (↑14d), serum alternative haemolytic complement activity (↑14d), biochemical indices (total protein (↑14d), globulin (↑14d))	
Onchorynchus mykiss	41	Mistletoe (*V. album*)	0,10%	3wks	Plasma protein concentration (↑3wk)	Dugenci et al., 2003
			1,00%		Plasma protein concentration (↑3wk)	
		Nettle (*U. dioica*)	0,10%		Plasma protein concentration (↑3wk)	
			1,00%		Plasma protein concentration (↑3wk)	
		Ginger (*Z. officinale*)	0,10%		NSD	
			1,00%		Extracellular oxidative radical production (↑3wk), phagocytosis of blood leukocytes (↑3wk), plasma protein concentration (↑3wk)	
Gadiformes						
Gadus morhua L	0.5-1	High-M alginate (*D. antarctica*)	0,01%	59d (sampling every 10th d	SGR (↑)	Vollstad et al., 2006
			0,06%		SGR (↑)	
			0,10%		SGR (↑)	

AAD: After administration diet, **AEC:** After each cycle, **AGR:** Absolute growth rate, **BSD:** Basal diet, **BWG:** Body weight gain, **CFU:** Colony forming units, **d:** Days, **dac:** Days after challenge, **daf:** Days after feeding, **dai:** Days after immunization, **dot:** Days of treatment, **dpc:** Days post challenge, **dph:** Days post-hatching, **dpi:** Days post injection, **dpt:** Days post-trial, **FCR:** Feed conversion ratio, **FER:** Feed efficiency ratio, **FWG:** Final weight gain, **Hct:** Hematocrit, **HCV:** Hematocrit value, **HK:** Head-kidney, **IS:** Immunostimulant, **ISD:** Immunestimulant diet, **LDL:** Low density lipoprotein, **MG:** Mid-gut, **mo:** Months, **NBT:** Nitroblue tetrazolium, **NS:** No specified, **NSD:** No significant differences, **PER:** Protein efficiency ratio, **PWG:** Percent weight gain, **RBC:** Red blood cells count, **RPS:** Relative percent survival, **SGR:** Specific growth rate, **wac:** Weeks after challenge, **waf:** Week after feeding, **WBC:** White blood cells count, **WG:** Weight gain, **wks:** Weeks

TABLE II: Effect of different plant and algal extracts used as immunostimulant diets in the immune response different fish species challenged with a pathogen.

Spp.	BW (g)	IS	IS dose	Administration	Challenge						Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation	Effect	
Anguilliformes											
Anguilla japonica	200	Korean mistletoe (*Viscum album* Coloratum)	0,10%	14d	*A. hydrophila* (ATCC 49140)	IP injection	$3x10^5$ CFU	14d	0-14dpi	33.3% total survival rates	Choi et al., 2008
			0,50%							66.6% total survival rates	
			1%							80% total survival rates	
Cypriniformes											
Cyprinus carpio	7,49	Dried Rehmannia root powder (DP)	LDP (2%)	80d	*A. hydrophila*	IP injection	$4×10^7$ CFU/mL	80d	48hpi	NSD	Wang et al., 2015
			HDP (4%)							↑Survival rate	
		Prepared Rehmannia root powder (PP)	LPP (2%)							↑Survival rate	
			HPP (4%)							↑Survival rate	
		Dried Rehmannia root extract (DE)	LDE (0.5%)							NSD	
			HDE (1%)							↑Survival rate	
		Prepared Rehmannia root extract (PE)	LPE (0.5%)							NSD	
			HPE (1%)							NSD	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	IS dose	Administration	Challenge					Effect	Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation		
Cyprinus carpio	26,3	Astaxanthin	0 mg kg-1	30d	*A. hydrophila*	IP injection	$3.1x10^7$ cfu/ml	30d	1,2,4wpi	85% mortality	Jagruthi et al., 2014
			25 mg kg-1							35% mortality (RPS: 58.8%); Phagocytic index (↑4wks), lysozyme activity (↑1,2,4wks), serum bactericidal activity (↓1,2,4wks); RBC (↑2,4wks), WBC (↑2,4wks), total protein (↑4wks)	
			50 mg kg-1							10% mortality (RPS: 88.2%); Phagocytic ratio (↑2,4wks), phagocytic index (↑1,2,4wks), superoxide anion production by blood leucocytes (↑1,2,4wks), lysozyme activity (↑1,2,4wks), serum bactericidal activity (↓1,2,4wks), serum anti-protease activity (↑2,4wks); RBC (↑1,2,4wks), WBC (↑1,2,4wks), hematocrit (↑1,2,4wks), hemoglobin (↑1,2,4wks), total protein (↑1,2,4wks), albumin (↑1,2,4wks), globulin (↑1wks)	
			100 mg kg-1							20% mortality (RPS: 76.5%); Phagocytic ratio (↑2,4wks), phagocytic index (↑2,4wks), superoxide anion production by blood leucocytes (↑2,4wks), lysozyme activity (↑1,2,4wks), serum bactericidal activity (↓1,2,4wks), serum anti-protease activity (↑2,4wks); RBC (↑1,2,4wks), WBC (↑1,2,4wks), hematocrit (↑4wks), hemoglobin (↑2,4wks), total protein (↑1,2wks), albumin (↑1,2wks)	
Cyprinus carpio	45,9	*Aegle marmelos*	5 g/kg feed	50d	*A. hydrophila*	IP injection	$1.5x10^4$ cells/ml	50d	20dpi	RBC count (↑5,10,15,20dpi), WBC count (↑), haemoglobin content (↑)	Pratheepa et al., 2010
			10 g/kg feed							RBC count (↑5,10,15,20dpi), WBC count (↑)	
			20 g/kg feed							RBC count (↑5,10,15,20dpi), WBC count (↑)	
			25 g/kg feed							WBC count (↑)	
			50 g/kg feed							WBC count (↑)	
Cyprinus carpio	62,8	*A. radix*(A)	1% A	5wks	*A. hydrophila* strain OB 212	IP injection	$1x10^6$ cells/fish	5wks	0-6dpi	↓cumulative mortality (60%)	Yin et al., 2009
		G. lucidum (G)	1% G							↓cumulative mortality (58%)	
			0.5%A+0.5%G							↓cumulative mortality (60%)	
		Vaccinated against	1% A							↓cumulative mortality compared with control group (% NS)	
			1% G							↓cumulative mortality compared with control group (% NS)	
		A. hydrophila/ A. salmonicida	0.5%A+0.5%G							↓cumulative mortality (38%) compared with control group	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	IS dose	Administration	Challenge						Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation	Effect	
Myxocyprinus asiaticus	58,2	Propolis+ Herba Epimedii 3:1 w/w	0,10%	5wks	*A. hydrophila*	IP injection	$5x10^7$ bacteria	5wks	1wpi	NSD	Zhang et al., 2009
			0,50%							Cumulative mortality (↓0.5% TCM)	
			1,00%							NSD	
Labeo rohita	44,6	*Rauvolfia tetraphylla*	1 g kg-1	4wks	*A. invadans*	IM Injection	$2.6x10^4$ spores/ml	NS	4wks	NSD	Yogeshwari et al., 2015
			5 g kg-1							WBC (↑2,3,4wks), total protein (↑3,4wks), total serum albumin (↑2,3,4wks), total serum globulin (↑2,3,4wks), phagocytic activity (↑2,3,4wks), respiratory burst activity (↑3,4wks), myeloperoxidase activity (↑2,3,4wks), serum lysozyme activity (↑2,3,4wks), antiprotease activity (↑2,3,4wks)	
			10 g kg-1							total protein (↑3,4wks), total serum globulin (↑4wks), respiratory burst activity (↑3,4wks), myeloperoxidase activity (↑3,4wks), antiprotease activity (↑2,3,4wks)	
Labeo rohita	11,1	*Psidium guajava L.*	0.1%	60d	*A. hydrophila*	IP injection	$1x10^7$	60d	15d	40% post-challenge survival	Giri et al., 2015
			0.5%							66.66% post-challenge survival	
			1.0%							53.33% post-challenge survival	
			1.5%							46.66% post-challenge survival	
Labeo rohita	6,6	*Ocimum sanctum* Linn. (Tulsi) extract	0.05% (T1)	42d	*A. hydrophila*	IP injection	$1x10^6$ cells/mL	42d	18d	Non-specific immune parameters (↑superoxide anion production, ↑lysozyme activity), hemato-immune parameters (↑total protein, albumin, hemoglobin), RPS (↑56.66)	Das et al., 2013
			0.1%(T2)							Non-specific immune parameters (↑superoxide anion production, ↑lysozyme activity, ↑total Ig), hemato-immune parameters (↑albumin/globulin ratio, ↑RBC, WBC, total protein, globulin, hemoglobin), RPS (↑63.33)	
			0.2% (T3)							Non-specific immune parameters (↑superoxide anion production, ↑lysozyme activity, ↑total Ig), hemato-immune parameters (↑albumin/globulin ratio, ↑RBC, WBC, total protein, albumin, globulin, hemoglobin), RPS (↑70.00)	
			0.5% (T4)							hemato-immune parameters (↑RBC	
			1% (T5)							NSD	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	IS dose	Administration	Challenge						Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation	Effect	
Labeo rohita	9,5	andrographolide	T1 (0.05%)	42d	*A. hydrophila*	IP injection	$1.8x10^6$ cfu/ml	42d	14dpi	↑relative percentage survival (RPS, second)	Basha et al., 2013
			T2 (0.10%)							↑RPS (first)	
			T3 (0.20%)							↑RPS (third)	
			T4 (0.40%)							↑RPS (forth)	
			T5 (0.80%)							NSD regarding to control	
Carassius auratus	20,33	Azadirachtin EC 25%	0.1%	28d	*A. hydrophila*	IP injection	$2x10^6$ cells	28d	14d	Significant increased of survival	Kumar et al., 2013
			0.2%							Significant increased of survival	
			0.4%							Significant increased of survival	
			0.8%							Significant increased of survival	
			1.6%							Significant increased of survival	
Ctenopharyngodon idella	80	*Ficus carica* polysaccharide (FCP)	0.1%	21d	*F. columnare*	IP injection	$5x10^7$ (no detailed units)	21d	15d	45% RPS	Yang et al., 2015
			0.5%							50% RPS	
			1.0%							No detailed	
Catla catla	16	*Aegle marmelos*	5 g/kg feed	30d	*P. aeruginosa*	water exposure	$19.5x10^4$ cells/ml	30d	5,10,15dpc	Phagocytic ratio (↑5,10,15dpc)	Pratheepa et al., 2011
			15 g/kg feed							Phagocytic ratio (↑5,10,15dpc)	
			20 g/kg feed							Phagocytic ratio (↑5,10,15dpc)	
			25 g/kg feed							Phagocytic ratio (↑5,10,15dpc)	
			30 g/kg feed							Phagocytic ratio (↑5,10,15dpc)	
Catla catla	150	*Achyranthes aspera* seed	0.5 g	4wks	Chicken red blood cells (c-RBC)	IP injection	500µl of cRBC 20%(v/v)	4wks	7dpi	Antigen-specific antibody response (↑), α1-antiprotease inhibitors level (↑), total protease inhibitors level (↑), RNA/DNA ratio of spleen (↑)	Rao Y et al., 2005
									14dpi	α1-antiprotease inhibitors level (↑), RNA/DNA ratio of spleen (↑)	
									21dpi	Antigen-specific antibody response (↑), globulin level (↑), α1-antiprotease inhibitors level (↑), total protease inhibitors level (↑), RNA/DNA ratio of kidney (↑)	
									28dpi	α1-antiprotease inhibitors level (↑) total protease inhibitors level (↑), RNA/DNA ratio of kidney (↑)	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	IS dose	Administration	Challenge: Pathogen	Route	Dose	Time (dpd)	Time of evaluation	Effect	Ref
Perciformes											
Oreochromis niloticus GIFT strain	4,83	*Aloe vera*	0,50%	60d	*S. iniae*	IP injection	7.7 x10^6 CFU/mL (0.6mL per 100g body weight)	60d	96hpi	Hematological parameters: RBC (↑48hpi), hemoglobin (↑48hpi), hematocrit (↑48hpi), mean cell hemoglobin concentration (↑0hpi); immune cell counts of WBC (↑48,96hpi); serum total protein (↑0hpi)	Gabriel et al., 2015
			1,00%							Hematological parameters: mean cell hemoglobin concentration (↑0hpi); serum total protein (↑0hpi)	
			2,00%							Hematological parameters: mean cell hemoglobin concentration (↑0hpi); serum parameters total protein (↑0hpi), serum glucose (↑0hpi)	
			4,00%							Hematological parameters: RBC (↓48,96hpi), hemoglobin (↓48,96hpi), hematocrit (↓48,96hpi), mean cell hemoglobin (↓48hpi); immune cell counts of WBC (↓48hpi); serum total protein (↓48,96hpi), serum glucose (↑0hpi)	
Oreochromis niloticus GIFT strain	20	Chinese herbal mixture (CHM)	0.5 % CHM (w/v	4wks	*A. hydrophila*	IP injection	0.2 ml of a 1.0x10^8 CFU/ ml	4wks	2wks	↓Cumulative mortality	Tang et al., 2014
			1.0 % CHM (w/w)							↓Cumulative mortality	
			1.5 % CHM (w/w)							↓Cumulative mortality	
			2.0 % CHM (w/w)							↓Cumulative mortality	
Oreochromis niloticus	0,8	Echinacea (*E. purpurea*) extract	1.0 ppt (begins in summer)	E1 (2mo BSD + 1mo with Echinacea)	*A. hydrophila*	IP injection	1x10^8 bacteria/ml	3mo, 7mo	7dpi	70% mortality (RLP=26.32) 3mo. 65% mortality (RLP=27.78) 7mo	Aly & Mohamed, 2010
				E2 (1mo with BSD + 2mo with Echinacea)						65% mortality (RLP=31.58) 3mo. 85% mortality (RLP=5.56) 7mo	
				E3 (3mo with Echinacea)						50% mortality (RLP=47.37) 3mo. 50% mortality (RLP=44.44) 7mo	
		Garlic	1.0 ppt (begins in summer)	G1 (2mo with BSD + 1mo with garlic)						80% mortality (RLP=15.79) 3mo. 65% mortality (RLP=27.78) 7mo	
				G2 (1mo with BSD + 2mo with garlic)						65% mortality (RLP=31.58) 3mo. 75% mortality (RLP=16.67) 7mo	
				G3 (3mo with garlic)						60% mortality (RLP=36.84) 3mo. 50% mortality (RLP=44.44) 7mo.	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	IS dose	Administration	Challenge					Effect	Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation		
Oreochromis niloticus	1.5–2.	green tea (*C. sinensis L*)	0.125 g/kg diet	12wks	*A. hydrophila*	IP injection	0.1 mL of 5×10^5 cells/mL	12wks	10d	↓Cumulative fish mortality	Abdel-Tawwab et al., 2010
			0.25 g/kg diet							↓Cumulative fish mortality, ↓*Aeromonas hydrophila* counts	
			0.50 g/kg diet							↓Cumulative fish mortality, ↓*Aeromonas hydrophila* counts	
			1.0 g/kg diet							↓Cumulative fish mortality, ↓*Aeromonas hydrophila* counts	
			2.0 g/kg diet							↓Cumulative fish mortality, ↓*Aeromonas hydrophila* counts	
Oreochromis mossambicus	25	*Eclipta alba* aqueous extract	0,0001	3wks	*A. hydrophila* (AHO21)	Injected	$1x10^8$ cells/fish	1wk	0-15dpi	Percentage mortality (↓)	Christybapita et al., 2007
			0,001							Percentage mortality (↓)	
			0,01							Percentage mortality (↓)	
							$1.4x10^7$ CFU/ml	90d	0-15dac	NSD	
Amphiprion sebae	21,4	*Rhizophora apiculata*	0% (infected untreated group)	2wks	*V. alginolyticus*	IM Injection	0.1 ml of 1.5×10^7 cells/ml	2wks	4wks	10% survival rate	Dhayanithi et al., 2015
			1%							Non-specific immune parameters: superoxide anion production by blood leucocytes (↑3,4wks), serum antiprotease activity (↑4wks); 65% survival rate	
			5%							Non-specific immune parameters: lysozyme activity (↑3,4wks), phagocytic index (↑3,4wks), respiratory burst activity (↑3,4wks), alternative complement activity (↑3,4wks), superoxide anion production by blood leucocytes (↑3,4wks), serum antiprotease activity (↑3,4wks); 85% survival rate	
			10%							Non-specific immune parameters: lysozyme activity (↑3,4wks), phagocytic index (↑3,4wks), respiratory burst activity (↑3,4wks), alternative complement activity (↑3,4wks), superoxide anion production by blood leucocytes (↑3,4wks), serum antiprotease activity (↑3,4wks); 80% survival rate	

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	IS dose	Administration	Challenge						Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation	Effect	
Amphiprion sebae	20,2	*Avicennia marina*	0% (infected untreated group)	2wks	*V. alginolyticus*	IM Injection	0.1 ml of 1.0×10^7 cells/ml	2wks	8wks	10% survival rate	Dhayanithi et al., 2015
			1%							Serum lysozyme activity (↑8wks), alternative complement activity (↑6,8wks); 70% survival rate	
			4%							Serum lysozyme activity (↑6,8wks), respiratory burst activity (↑6,8wks), alternative complement activity (↑6,8wks), phagocytic activity (↑6,8wks); 85% survival rate	
			8%							Respiratory burst activity (↑6,8wks); 80% survival rate	
Pseudosciaena crocea	120	Astragalus Root and Chinese Angelica Root	0.5% (w/w)	30d	*V. alginolyticus*	immersion bath	10^8 cells/ml	20d	5wks		Jian and Wu, 2003
			1.0% (w/w)							Cumulative mortality (↓6.7%)	
			1.5% (w/w)							Cumulative mortality (↓6.7%)	
Pleuronectiformes											
Solea senegalensis	80	red algae (*Porphyridium cruentum*)	0,01	4wks	*P. damselae* subsp. piscicida strain Lgh41/01	IP injection	$6x10^8$ bacteria/ml	2wks	3,4wks	respiratory burst activity of phagocytes from head kidney (↑4wk)	Diaz-Rosales et al., 2008
Scophthalmus maximus L.	Fish larvae	alginate with high mannuronic acid (*Ascophyllum nodosum*)	NS	1d	*V. anguillarum*	exposure (30 min)	$1x10^5$ cells/ml	2d	1wpc	Reduction in the mortality of 39%	Skjermo et al., 1995
Salmoniformes											
Onchorynchus mykiss	20	*Lentinula edodes*	1% L. edodes	6wks	*L. garvieae*	IP injection	0.1 mL of 1.0×10^8 CFU/mL	45d	15d	40% RPS	Baba et al., 2015
			2% L. edodes							55% RPS	
Onchorynchus mykiss	70–11(	*Aloe vera*	0,50%	6wks	formalin-killed atypical *A. salmonicida* (ASAL).	IP injection	1 µL/g of ASAL OD600=1	6wks	24hpi	↑ Spleen-somatic index	Zanuzzo et al., 2015

TABLE II: (*...continuation)*

Spp.	BW (g)	IS	IS dose	Administration	Challenge						Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation	Effect	
Onchorynchus mykiss	81,65	Vitacel	10 g kg^{-1} Vitacel	45d	*A. hydrophila*	IP injection	0.1ml of $3.5x10^7$ CFU	45d	10d	47.78% RPS	Yarahmadi et al., 2014
Onchorynchus mykiss	14	Garlic	0,005	14d	*A. hydrophila* AE 57	IP injection	$1x10^6$ cells/ml	24h AAD	0-14dpi	14 days (RPS=86%), 21 days (RPS=75%), and 28 days (RPS=68%)	Nya & Austin, 2011
			0,01							14 days (RPS=80%), 21 days (RPS=55%), and 28 days (RPS=46%)	
Onchorynchus mykiss	35	decaffeinated green tea *(Camellia sinensis*)	20 mg (T1)per kg feed	30d	chicken red blood cell (C-RBC)	IP injection	0.5ml of suspension (2%)	30d	5,15d ADD	NSD	Sheikhzadeh et al., 2011
			100 mg (T2) per kg feed							↑Hemagglutination titre	
			500 mg (T3) per kg feed							NSD	
Onchorynchus mykiss	14	ginger (*Zingiber officinale* Roscoe)	0,0005	14d	*A.hydrophila* AE 57	IP injection	$1x10^7$ cells/ml	14d	0-14dpi	4% mortalities (RPS=94%)	Nya & Austin, 2009
			0,001							NS	
			0,005							0% mortalities (RPS=100%)	
			0,01							16% mortalities (RPS=75%)	

AAD: After administration diet, **AEC:** After each cycle, **AGR:** Absolute growth rate, **BSD:** Basal diet, **BWG:** Body weight gain, **CFU:** Colony forming units, **d:** Days, **dac:** Days after challenge, **daf:** Days after feeding, **dai:** Days after immunization, **dot:** Days of treatment, **dpc:** Days post challenge, **dph:** Days post-hatching, **dpi:** Days post injection, **dpt:** Days post-trial, **FCR:** Feed conversion ratio, **FER:** Feed efficiency ratio, **FWG:** Final weight gain, **Hct:** Hematocrit, **HCV:** Hematocrit value, **HK:** Head-kidney, **IS:** Immunostimulant, **ISD:** Immunestimulant diet, **LDL:** Low density lipoprotein, **MG:** Mid-gut, **mo:** Months, **NBT:** Nitroblue tetrazolium, **NS:** No specified, **NSD:** No significant differences, **PER:** Protein efficiency ratio, **PWG:** Percent weight gain, **RBC:** Red blood cells count, **RLP**: Relative level of protection, **RPS:** Relative percent survival, **SGR:** Specific growth rate, **wac:** Weeks after challenge, **waf:** Week after feeding, **WBC:** White blood cells count, **WG:** Weight gain, **wks:** Weeks

TABLE III: Effect of different PAMPs immunostimulant diets in the immune response in fish.

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Cypriniformes						
Cyprinus carpio	40	β-glucan MacroGard (S. cerevisiae)	10 mg/Kg of BW	25d	Expression of il1β (↓25d in MG); il10 (↓25d in spleen, HK and MG); tnfα1 (↓25d in MG); tnfα2 (↓25d in MG); cxca (↓25d in spleen and HK); mx (↑25d in liver and MG)	Falco et al., 2014
			MacroGard + PBS inyection AAD		Expression of mx (↑25+1dpi in liver and HK)	
			MacroGard + poly(I:C) inyection AAD		Expression of mx (↑25+1dpi in liver, HK, spleen and MG)	
Cyprinus carpio	40	β-glucan MacroGard (S. cerevisiae)	10 mg/Kg of BW	25d	Expression of crp1 (↓7d,25d in liver and HK, ↓25d in MG); crp2 (↓7d in liver, HK and MG, ↑25d in MG); c1rs (↓7d,25d in liver); bf/c2 (↓7d in liver, ↑7d in MG); c3 (↓7d,25d in liver, ↓7d in HK, ↑7d,25d in MG); masp2 (↓25d in liver, ↑25d in HK, ↑7d in MG)	Pionnier et al., 2014
			C + 4mg/kg LPS inyection AAD	25d (sampling 1,3,7dpi)	Expression of crp2 (↑7d, in liver, ↓1d in HK); c1rs (↓1d in liver, ↑7d in HK and MG); bf/c2 (↑7d in HK); c3 (↑1d in liver, ↑3d in liver and MG); masp2 (↑7d in HK and MG)	
			C + 5 mg/kg poly(I:C) inyection AAD		Expression of crp1 (↓1d in liver, ↑1d in HK and MG); crp2 (↑1d,7d in liver and HK); c1rs (↓1d in liver, ↑3d in liver, ↑7d in HK and MG); c3 (↑1d in MG, ↑3d in liver, ↑7d in HK); masp2 (↓1d in liver, ↑3d in liver, ↑7d in HK)	
			10 mg/Kg MacroGard + PBS inyection AAD		Expression of crp1 (↑1d,3d,7d in liver, ↓1d ↑3d in HK, ↑3d in MG); crp2(↑1d in liver and MG, ↓1d in HK, ↓3d in liver and HK, ↑7d in HK and MG); c1rs (↑7d in liver, ↓1d,3d in HK); bf/c2 (↓1d,3d in liver, ↑1d,7d, ↓3d in HK, ↑7d in MG); masp2 (↓1d in liver, HK and MG, ↓3d in liver and MG, ↑7d in liver and HK)	
			10 mg/Kg MacroGard + 4mg/kg LPS inyection AAD		Expression of crp1 (↑1d in liver, ↑3d in liver and HK); crp2(↓3d in liver, ↑1d,7d in HK); c1rs (↓3d in HK); bf/c2 (↑1d in HK and MG, ↓3d in HK and MG, ↑7d in liver and HK); c3 (↑3d in liver, ↓3d in MG, ↑7d in HK); masp2 (↓3d in HK and MG, ↑7d in HK)	
			10 mg/Kg MacroGard + 5 mg/kg poly(I:C) inyection AAD		Expression of crp1 (↑1d in liver, ↑3d in liver and HK); crp2 (↑1d in liver, HK and MG, ↓3d in liver and HK, ↑7d in HK); c1rs (↑1d,7d in liver, ↓3d in liver); bf/c2 (↑1d,7d in liver, HK and MG, ↓3d in liver, HK and MG); c3 (↑1d in liver, ↓3d in MG, ↑7d in HK); masp2 (↑1d in liver and HK, ↑7d in liver, HK and MG, ↓3d in liver, HK and MG)	
Cyprinus carpio	100	baker´s yeast extract CW-I (TableMark)	5mg/Fish	3d (sampling 1,3,5,7,10d AAD)	Expression of il1β (↑1d, ↓3d,10d in HK); TNF-α (↑1d, ↓3d,5d,7d,10d in HK); Il-12p35 (↑1d, ↓3d,5d,7d,10d in HK); Il-12p40 (↑1d, ↓3d,5d,7d,10d in HK); CXC-chemokine (↑1d,5d,7d in HK); IFN-γ2 (↑1d,5d in HK); IL-10 (↓1d,3d,5d,7d,10d in HK). Superoxide anion (↑3d in phagocytic cells); Phagocytic activity (↑1d,3d in kidney cells); Phagocytic index (↑3d in kidney cells)	Biswas et al., 2012
Cyprinus carpio	78,4	MacroGard	6 mg/Kg of BW	14d	Expression of tnfα2 (↓14d in gut and HK); il10 (↓14d in gut)	Falco et al., 2012
Cyprinus carpio	28	LPS *(A. hydrophila)*	1mg	1,7,14d	NE	Selvaraj et al., 2009
			2.5mg		NE	
			5mg		NE	
Cyprinus carpio	28	LPS (*A. hydrophila)*	0.1% β-glucan + 0.025% LPS	1,7,14d	HK-macrophage bactericial activity (↑16d)	Selvaraj et al., 2006
		β-glucan (*S. cerevisiae*)	0.5% β-glucan + 0.125% LPS		HK-macrophage bactericial activity (↑16d); HK-macrophage oxigen burst activity (↑16d)	
			1% β-glucan + 0.25% LPS		HK-macrophage bactericial activity (↑16d); HK-macrophage oxigen burst activity (↑16d)	
Cyprinus carpio	28	β-glucan (*S. cerevisiae*)	0,01	1,3,5d	NE	Selvaraj et al., 2005
			0,02		NE	
			0,04		NE	

TABLE III: (*...continuation)*

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Labeo rohita	4.5	microbial levan	0.25%	60d	Albumin/Globulin ratio (↓60d)	Gupta et al., 2008
			0.50%		Serum lyzozyme activity (↑60d)	
			0.75%		Albumin/Globulin ratio (↓60d); Serum lyzozyme activity (↑60d)	
			1.00%		Haemoglobin content (↑60d); Serum total protein content (↑60d); Albumin/Globulin ratio (↓60d); Serum lyzozyme activity (↑60d); Blood phagocytes respiratory burst activity (↑60d)	
			1.25%		Haemoglobin content (↑60d); Serum total protein content (↑60d); Albumin/Globulin ratio (↓60d); Serum lyzozyme activity (↑60d); Blood phagocytes respiratory burst activity (↑60d)	
Labeo rohita	35	β-glucan Sigma (*S. cerevisiae*)	100mg/Kg feed	56d	Total serum protein content (↑42d); WBC count (↑42d); Blood leucocytes cells respiratory burst (↑42,56d); Blood leucocytes-phagocytic ratio (↑14d,28d,42d,56d); Blood leucocytes-phagocytic index (↑14d,42d,56d); Serum lysozyme activity (↑42d,56d); Haemolytic complement activity (↑14d,28d,42d,56d); Serum bactericidal activity (↑14d,28d,42d,56d)	Misra et al., 2006
			250mg/Kg feed		Total serum protein content (↑28d,42d); WBC count (↑42d); Blood leucocytes cells respiratory burst (↑14d,28d,42d,56d); Blood leucocytes-phagocytic ratio (↑14d,28d,42d,56d); Blood leucocytes-phagocytic index (↑14d,28d,42d, ↓56d); Blood leucocytes-lymphokine production index (↑14d,42d); Serum lysozyme activity (↑28d,42d,56d); Haemolytic complement activity (↑14d,28d,42d,56d); Serum bactericidal activity (↑14d,28d,42d,56d)	
			500mg/Kg feed		Total serum protein content (↑28d,42d); WBC count (↓14d,56d, ↑28d,42d); Blood leucocytes-respiratory burst(↑14d,28d,42d); Blood leucocytes-phagocytic ratio (↑14d,28d,42d,56d); Blood leucocytes-phagocytic index (↑14d,28d,42d,56d,); Blood leucocytes-lymphokine production index (↓42d); Serum lysozyme activity (↑28d,42d,56d); Haemolytic complement activity (↑14d,28d,42d); Serum bactericidal activity (↑14d,28d,42d,56d)	
Labeo rohita	39	β-glucan yeast (Sigma)	0.1%	53-60d	Bacterial agglutination titre (↑60d); Haemagglutination titre (↑60d); Haemolysin titre (↑60d); Bactericidal activity (↑60d); Serum phagocytic ratio (↑60d); Serum phagocytic index (↑60d); Serum leucocrit (↑60d)	Sahoo and Mukherjee, 2001
Sparus aurata	100-200	chitin (sigma)	25g/Kg	6wks	Total serum IgM content (↑6wks)	Cuesta et al., 2004
			50g/Kg		Total serum IgM content (↑2,4,6wks)	
			100g/Kg		Total serum IgM content (↑6wks)	
		Yeast cells (*S. cerevisiae)*	1g/Kg	4wks	Total serum IgM content (↑2wks)	
			5g/Kg		Total serum IgM content (↑2wks)	
			10g/Kg		Total serum IgM content (↑2wks)	
		levamisole synthetic (Sigma)	0.075g/Kg	10d	Total serum IgM content (↑3wks)	
			0.15g/Kg		Total serum IgM content (↑2,3,4wks)	
			0.3g/Kg		Total serum IgM content (↑2wks)	
Sparus aurata	0.3, 5, 10	β-glucan MacroGard (S. cerevisiae)	1g/kg feed	2wks	HK-Macrophage phagocytic activity (↑1wks)	Couso et al., 2003
			10g/kg feed		Spleen-Macrophage respiratory burst activity (↑1,2wks); Spleen-Macrophage phagocytic activity (↑1wks)	
		β-glucan Fibosel *(S. cerevisiae)*	1g/kg feed		Spleen-Macrophage respiratory burst activity (↑2wks); Spleen-Macrophage phagocytic activity (↑1wks)	
			10g/kg feed		Spleen-Macrophage respiratory burst activity (↑1,2wks)	
		β-glucan VitaStim *(S. cerevisiae)*	1g/kg feed		Spleen-Macrophage respiratory burst activity (↑2wks)	
			10g/kg feed		NSD	

TABLE III: (*...continuation)*

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Sparus aurata	175	Whole yeast *(S. cerevisiae)* Whole yeast (*S. cerevisiae)* fks-1	10g/kg feed	6wks	HK-Leucocyte respiratory burst activity (↑4wks); HK-Leucocyte Natural cytotoxic activity (↑4,6wks) Natural complement activity (↓6wks); Serum peroxidase content (↓6 wks); Serum lysozyme activity (↑2,4wks); HK-Leucocyte respiratory burst activity (↑4wks); HK-leucocyte natural cytotoxic activity (↑4,6wks); Leucocyte phagocytic ability (↑2,4,6wks); Leucocyte phagocytic capacity (↑4wks)	Rodríguez et al., 2003
Sparus aurata	150	whole yeast *(S. cerevisiae)*	1g/kg feed	4wks	HK-leucocyte natural cytotoxic activity (↑4wks)	Ortuño et al., 2002
			5g/kg feed		HK-leucocyte phagocytic ability (↑4wks); Leucocyte phagocytic capacity (↑4wks)	
			10g/kg feed		HK-Leucocyte respiratory burst activity (↑2wks); Leucocyte phagocytic ability (↑4wks); HK-leucocyte phagocytic capacity (↑4wks); Leucocyte myoloperoxidase content (↑2wks)	
Sparus aurata	125	chitin (Sigma)	25mg/Kg	6wks	Natural haemolytic complement activity (↑2wks); HK-leucocyte respiratory burst activity (↑4wks); HK-leucocyte natural cytotoxic activity (↑2wks)	Esteban et al., 2001
			50mg/Kg		Natural haemolytic complement activity (↑2wks); HK-leucocyte respiratory burst activity (↑4wks); HK-leucocyte natural cytotoxic activity (↑2wks)	
			100mg/Kg		Natural haemolytic complement activity (↑2wks); HK-leucocyte respiratory burst activity (↑4wks); HK-leucocyte natural cytotoxic activity (↑2wks)	
Perciformes						
Dicentrarchus labrax	34	MOS	0,02	9wks	NE	Torrecillas et al., 2007
			0,04		HK-macrophages phagocytic activity (↑9wks)	
Dicentrarchus labrax	80	β-glucan MacroGard (S. cerevisiae)	0.1%	15d (sampling 15,30,45d AAD)	Serum complement activity (↑15d); Serum lysozyme activity (↑30d); gills-HSP70 content (↑30d); liver-HSP70 content (↑30d)	Bagni et al., 2005
			0.1%	4 cycles: 15 d every 60d (sampling 45d AEC I, II, III,	NE	
		β-glucan Ergosan (*S. cerevisiae*)	0.5%	15d (sampling 15,30,45d AAD)	Serum complement activity (↑15d,30d); Serum lysozyme activity (↑30d); gills-HSP70 content (↑30d); liver-HSP70 content (↑30d)	
			0.5%	4 cycles: 15 d every 60d (sampling 45d AEC I, II, III,	Serum lysozyme activity (↑45d/IV)	
Dicentrarchus labrax	414	β-glucan MacroGard (S. cerevisiae)	0,02	2wks every 3mo	Serum complement activity (↑40wks); Plasma lysozyme activity (↑40wks)	Bagni et al., 2000
Lateolabrax japonicus	18	Yeast cell walls (*S. cerevisiae)*	250 mg/Kg	72d	NSD	Yu et al., 2014
			500 mg/Kg		NSD	
			1000 mg/Kg		NSD	
			2000 mg/Kg		NSD	
			20000 mg/Kg		NSD	
Oreochromis niloticus	80-100	β-glucan Biosaf (*S. cerevisiae)*	10g/kg feed	21d	Serum bactericidal activity (↑21d); Serum nitric oxide (↑21d); Serum lysozyme activity (↑21d); HK-macrophage respiratory burst index (↑21d); HK-macrophage phagocytic activity (↑21d)	El-Boshy et al., 2010
		β-glucan (extracted) *(S. cerevisiae)*	0.1%		Serum bactericidal activity (↑21d); Serum nitric oxide (↑21d); Serum lysozyme activity (↑21d); HK-macrophage respiratory burst index (↑21d); HK-macrophage phagocytic activity (↑21d); Lymphocyte transformation index (↑21d)	
		laminaria (*L. japonica)*	0.1%		Serum bactericidal activity (↑21d); Serum nitric oxide (↑21d); Serum lysozyme activity (↑21d); HK-macrophage respiratory burst index (↑21d); HK-macrophage phagocytic activity (↑21d)	

TABLE III: (*...continuation)*

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Oreochromis niloticus ♀ *x Oreochromis aureus* ♂	51	DVAQUA	0.125g/Kd diet	8wks	Serum lysozyme activity (↑8wks); serum C3 content (↑8wks); serum C4 content (↑8wks); HK-macrophage phagocytic activity (↑8wks); HK-macrophage respiratory burst activity (↑8wks)	He et al., 2009
			0.25g/Kd diet		Serum lysozyme activity (↑8wks); serum C3 content (↑8wks); serum C4 content (↑8wks); HK-macrophage phagocytic activity (↑8wks); HK-macrophage respiratory burst activity (↑8wks)	
			0.50g/Kd diet		Serum lysozyme activity (↑8wks); serum C3 content (↑8wks); serum C4 content (↑8wks); HK-macrophage phagocytic activity (↑8wks)	
			1.00g/Kd diet		Serum lysozyme activity (↑8wks); serum C3 content (↑8wks); serum C4 content (↑8wks); HK-macrophage phagocytic activity (↑8wks); HK-macrophage respiratory burst activity (↑8wks)	
			2.00g/Kd diet		Serum lysozyme activity (↑8wks); serum C3 content (↑8wks); serum C4 content (↑8wks); HK-macrophage phagocytic activity (↑8wks)	
Pagrus auratus	180	β-glucan EcoActiva *(S. cerevisiae)*	winter 0.1% v/w	84d	HK-Macrophage respiratory burst activity (↑3d,7d,14d,28d,56d)	Cook et al., 2003
			summer 0.1% v/w		HK-Macrophage respiratory burst activity (↑28d)	
Pseudosciaena crocea	10	β-glucan (*S. cerevisiae*)	0.09%	8wks	Serum lysozyme content (↑8wks); HK-macrophages phagocytosis activity (↑8wks); HK-macrophages respiratory burst activity (↑8wks)	Ai et al., 2007
			0.18%		Serum lysozyme content (↑8wks)	
Thunnus maccoyii	18.6 K	β-glucan Sanictum (*S. cerevisiae*)	5.2mg/Kg feed at 35% FR Inyected in baitfishes	every 2nd day for 12wks	Serum lysozyme content (↑8wks)	Kirchhoff et al., 2011
Salmoniformes						
Onchorynchus mykiss	100	peptidoglican (PG)	10 mg/Kg feed	28d	Expression of omDB-3 (↑21d,28d); omDB-4 (↑21d,28d); omCATH-1 (↑21d,28d); omCATH-2 (↑21d,28d); omLEAP-2a (↑21d,28d)	Casadei et al., 2015
			14d PG + 7-14d control diet	14d + 7-14d control diet	Expression of omDB-3 (↑21d,28d); omDB-4 (↑21d,28d); omCATH-2 (↑21d); omLEAP-2a (↑21d,↓28d)	
Onchorynchus mykiss	4 Kg	β-glucan MacroGard (S. cerevisiae)	0,001	3mo	Total serum Ig content (↑3mo); Serum IgM content (↑3mo)	Ghaedi et al., 2015
	0.18		0,002		ACH50 (↑3mo); Lysozyme (↑3mo); Total Ig (↑3mo); IgM (↑3mo)	
			C+C (L1)	2mo	NSD	
			C + 0,1% (L2)		ACH50 (↑2mo)	
			C + 0,2% (L3)		ACH50 (↑3mo); Lysozyme (↑3mo); Total Ig (↑3mo); IgM (↑3mo)	
			0,1% + C (L4)		NSD	
			0,1% + 0,1% (L5)		NSD	
			0,2% + C (L6)		NSD	
			0,2% + 0,2% (L7)		ACH50 (↑3mo); Lysozyme (↑3mo); Total Ig (↑3mo); IgM (↑3mo)	
Onchorynchus mykiss	4,2	IP-PA1 (lipopolysaccharide *P. agglomerans*)	10 µg LPSs /Kg of body weight	93d	NSD	Skalli et al., 2013
			20 µg LPSs /Kg of body weight		Blood bactericidal activity (↑93d); Blood lysozyme activity (↑93d); Blood hemolytic activity (↑93d); NBT (↑93d)	

TABLE III: (*...continuation)*

Ssp.	BW (g)	IS	Dose	Time Administration	Immunological effects	Ref
Onchorynchus mykiss	150	Peptidoglican (PG)	5 mg PG/Kg	14d	Expression of omDB-1 (↑1d,7d in skin, ↓7d in liver); omDB-2 (↑7d in gills, ↓1d in gut); omDB-3 (↑1d,7d,14d in skin, ↑7d, ↓14d in gills, ↑1d in gut); omDB-4 (↑14d in skin, ↓7d in gills, ↑1d in gut, ↓14d in gut and liver); CATH-1 (↑1d in gut, ↑7d in skin, ↑14d in skin, gut and liver, ↑14d in gills); CATH-2 (↑1d in skin, gills and liver, ↑7d in skin, ↑14d in skin, gills and gut); Hepcidin (↑1d in liver, ↑7d in gills); LEAP-2a (↑14d in skin and gut, ↓14d in gills and liver)	Casadei et al., 2013
			10 mg PG/Kg		Expression of omDB-1 (↑14d in skin); omDB-2 (↑7d in gills and gut); omDB-3 (↑7d,14d in skin, ↑1d in gills, ↓1d in gut, ↓14d in gills, gut and liver); omDB-4 (↑14d in skin, ↑7d in liver, ↓14d in gut and liver); CATH-1 (↑1d in liver, ↑14d in skin and gut, ↓14d in gills); CATH-2 (↑7d in skin, ↑14d in skin, gills, gut and liver); Hepcidin (↑7d in gills, ↑1d,7d,14d in liver); LEAP-2a (↑1d in liver, ↑7 in skin, ↑14d in skin and gut, ↓14d in gills and liver)	
			50 mg PG/Kg		Expression of omDB-1 (↑7d,14d in skin); omDB-2 (↑1d in skin, ↑7d in gills, gut and liver); omDB-3 (↑1d,7d,14d in skin, ↑1d,7d in gills, ↓14d in gills, gut and liver); omDB-4 (↑1d,7d,14d in skin, ↑7d in gills and liver, ↓14d in gut and liver); CATH-1 (↑1d in skin, gills and gut, ↑7d in skin, ↑14d in skin, gut and liver, ↓14d in gills); CATH-2 (↑1d in skin and gills, ↑7d in skin, ↑14d in skin, gills and gut); Hepcidin (↑1d in skin, ↑7d in gills and liver, ↑14d in liver); LEAP-2a (↑1d,7d in skin, ↑14d in skin and gut, ↓14d in gills and liver)	
			100 mg PG/Kg		Expression of omDB-1 (↑1d,14d in skin); omDB-2 (↑7d in gills and gut); omDB-3 (↑1d skin, gills, gut and liver, ↑7d in skin and gills, ↑14d in skin, ↓14d in gills and liver); omDB-4 (↑7d in gills and liver, ↑14d in skin, ↓14d in gut and liver); CATH-1 (↑1d in gut and liver, ↑7d in skin and gills, ↓14d in gills, ↑14d in skin, gut and liver); CATH-2 (↑7d, in skin, gills and liver, ↑1d,↑14d in skin, gills and gut); Hepcidin (↑7d in gills, ↑1d,14d in liver); LEAP-2a (↑1d in skin, ↑7d in skin and gills, ↓14d in gills and liver, ↑14d in skin and gut)	
Onchorynchus mykiss	8,8	β-glucan MacroGard (S. cerevisiae)	0.2%	21d	NSD	Kunttu et al., 2009
			0.6%		Blood-respiratory burst activity (↑21d)	
			1.8%		Blood-respiratory burst activity (↑21d)	
Onchorynchus mykiss	14.3	β-glucan barley (*H. vulgare*)	12.2g/Kg feed	9wks	NSD	Sealey et al., 2008
			16.7g/Kg feed		NSD	
			26.4g/Kg feed		NSD	
		Wheat diet (control) + 2g/Kg MacroGard	4.5g/Kg feed		NSD	
Salmo salar	fry	LPS (*A. salmonicida)*	0.1%	62d	NSD	Guttvik et al., 2002
Siluriformes						
Clarias batrachus	49	Lactoferrin (bovine)	100mg/Kg feed	7d	Blood phagocytes-respiratory burst activity (↑31d); Leucocyte phagocytic activity (↑31d); Leucocyte myeloperoxidase content (↑31d)	Kumari and Sahoo, 2006
		β-glucan yeast (sigma)	0.1% in feed	7d	Blood-phagocytes respiratory burst activity (↑31d); Leucocyte myeloperoxidase content (↑31d)	
		levamisole synthetic (sigma)	50mg/Kg feed	10d	Blood-phagocytes respiratory burst activity (↑31d); Leucocyte myeloperoxidase content (↑31d)	
		vitamin C CRNA Roche	500mg/Kf feed	30d	Leucocyte phagocytic activity (↑31d); Leucocyte myeloperoxidase content (↑31d)	

AAD: After administration diet, **AEC:** After each cycle, **AGR:** Absolute growth rate, **BSD:** Basal diet, **BWG:** Body weight gain, **CFU:** Colony forming units, **d:** Days, **dac:** Days after challenge, **daf:** Days after feeding, **dai:** Days after immunization, **dot:** Days of treatment, **dpc:** Days post challenge, **dph:** Days post-hatching, **dpi:** Days post injection, **dpt:** Days post-trial, **FCR:** Feed conversion

ratio, **FER:** Feed efficiency ratio, **FWG:** Final weight gain, **Hct:** Hematocrit, **HCV:** Hematocrit value, **HK:** Head-kidney, **IS:** Immunostimulant, **ISD:** Immunestimulant diet, **LDL:** Low density lipoprotein, **MG:** Mid-gut, **mo:** Months, **NBT:** Nitroblue tetrazolium, **NS:** No specified, **NSD:** No significant differences, **PER:** Protein efficiency ratio, **PWG:** Percent weight gain, **RBC:** Red blood cells count, **RLP**: Relative level of protection, **RPS:** Relative percent survival, **SGR:** Specific growth rate, **wac:** Weeks after challenge, **waf:** Week after feeding, **WBC:** White blood cells count, **WG:** Weight gain, **wks:** Weeks

TABLE IV: Effect of different PAMPs used as immunostimulant diets in the immune response of different fish species challenged with a pathogen.

Spp.	BW (g)	IS	IS dose	Administration	Challenge					Effect	Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation		
Cypriniformes											
Cyprinus carpio	100	baker´s yeast extract CW-I (TableMark)	5mg/Fish	3d	A. hydrophila	IP injection	$3.4x10^9$ cfu/mL	1d ADD	2,6,12,24hpi	Decrease in viable pathogen cell counts at 6 hours post injection.	Biswas et al., 2012
Cyprinus carpio	78	MacroGard	6 mg/Kg of BW	14d	*A. salmonicida*	IP injection	250µL ($4x10^8$/mL)	14d	0,6,12hpi, 1,3,5dpi	Expression of tnfα1 (↓3d in gut, ↑6h in head kidney); tnfα2 (↓6h, ↑3d in gut, ↑6h in head kidney); il1β (↓6h in gut); il6fam (↓6h,1d in gut, ↑3d in gut); il10 (↓6h in head kidney)	Falco et al., 2012
Cyprinus carpio	28	LPS (*A. hydrophila)*	1mg	1,7,14d	*A. hydrophila*	IP injection	$2.94x10^7$	16d	14dpi	Not significant change in survival rate compared with control. NSD on antibody titre	Selvaraj et al., 2009
			2.5mg							Not significant change in survival rate compared with control. NSD on antibody titre	
			5mg							Not significant change in survival rate compared with control. NSD on antibody titre	
Cyprinus carpio	28	LPS (*A. hydrophila)* + β-glucan (*S. cerevisiae*)	0.1% β-glucan + 0.025% LPS	1,7,14d	*A. hydrophila*	IP injection	$2.94x10^7$	16d	14dpi	↓ survival 28.5% (Control 50%). NSD on antibody titre	Selvaraj et al., 2006
			0.5% β-glucan + 0.125% LPS							survival 50.0% (Control 50%). NSD on antibody titre	
			1% β-glucan + 0.25% LPS							↑ survival 71.4% (Control 50%). NSD on antibody titre	
Cyprinus carpio	28	β-glucan (*S. cerevisiae*)	1%	1,3,5d	*A. hydrophila*	IP injection	LD_{50}	7d	0-7dpi	NSD in survival rate compared with control Slightly increased	Selvaraj et al., 2005
			2%							NSD in survival rate compared with control. NSD on antibody titre	
			4%							NSD in survival rate compared with control. NSD on antibody titre	
Labeo rohita	4.5	Microbial levan	0.25%	60d	*A. hydrophila*	IP injection	0.2ml ($1.8x10^8$ cfu/ml)	60d	10dpi	Reduced mortality rate compared with control group	Gupta et al., 2008
			0.50%							Reduced mortality rate compared with control group	
			0.75%							Reduced mortality rate compared with control group	
			1.00%							Reduced mortality rate compared with control group	
			1.25%							Reduced mortality rate compared with control group	

TABLE IV: (*...continuation*)

Spp.	BW (g)	IS	IS dose	Administration	Challenge						Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation	Effect	
Labeo rohita	35	β-glucan Sigma (*S. cerevisiae)*	100mg/Kg feed	56d	*A. hydrophila* *E. tarda*	IP injection	$1.6x10^8$; $1.3x10^8$ cfu per fish	56d	28dpi	Significant reduced mortality rate compared with control group. NSD between *A. hydrophila* and *E. tarda.*	Misra et al., 2006
			250mg/Kg feed							Significant reduced mortality rate compared with control group. NSD between *A. hydrophila* and *E. tarda.*	
			500mg/Kg feed							Significant reduced mortality rate compared with control group. NSD between *A. hydrophila* and *E. tarda.*	
Labeo rohita	39	β-glucan yeast Sigma	0.1%	53-60d	*A. hydrophila*	IP injection	$1x10^5$	60d	10dpi	Significant increase of the survival relative to the control group	Sahoo and Mukherjee, 2001
Sparus aurata	0.3	β-glucan Macrogard *(S. cerevisiae)*	1g/kg feed	2wk	*P. damselae* subsp. *Piscicida*	5 min bath	LD50	21d	10dpi	Significant reduced mortality rate compared with control group	Couso et al., 2003
			10g/kg feed							Significant reduced mortality rate compared with control group	
		β-glucan Fibosel *(S. cerevisiae)*	1g/kg feed	2wk						Significant reduced mortality rate compared with control group	
			10g/kg feed							Significant reduced mortality rate compared with control group	
		β-glucan VitaStim *(S. cerevisiae)*	1g/kg feed	2wk						Significant reduced mortality rate compared with control group	
			10g/kg feed							Significant reduced mortality rate compared with control group	
		β-glucan Macrogard *(S. cerevisiae)*	1g/kg feed	1,2,4,5wk				43d	10dpi	Significant reduced mortality rate compared with control group	
			5g/kg feed							Significant reduced mortality rate compared with control group	
			10g/kg feed							Significant reduced mortality rate compared with control group	

TABLE IV: (*...continuation*)

Spp.	BW (g)	IS	IS dose	Administration	Challenge						Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation	Effect	
Perciformes											
Dicentrarchus labrax	34	MOS	2%	12wk	*V. alginolyticus*	cohabitation (3:1) IP injection	$2.4x10^8$ cfu/mL per fish	at end of 9th wk	3wkpi	Reduction of cohabitation infected fish	Torrecillas et al., 2007
			4%							No cohabitation infected fish	
			2%	10wk		anal canalisation	$2.4x10^8$ cfu/mL per fish	at end of 9th wk	24,48hpi, 1wkpi	Decrease of infected fish at 48 hpi	
			4%							Decrease of infected fish at 48 hpi	
Lateolabrax japonicus	18	Yeast cell walls (*S. cerevisiae*)	250 mg/Kg	72d	*A. veronii*	IM injection	$8x10^4$ cells/100 g BW	72d	7dpi	NSD	Yu et al., 2014
			500 mg/Kg	72d						higher cumulative survival rate	
			1000 mg/Kg	72d						NSD	
			2000 mg/Kg	72d						NSD	
			20000 mg/Kg	72d						higher cumulative survival rate	
Oreochromis niloticus	30	β-glucan GC-Goldcell-Biorigin (*S. cerevisiae*)	0,1% B glucan+600mg/ kg Vit C	7,15,30,45d	*A. hydrophila*	IP injection	$1x10^5$ CFU/ml	7,15,30,45d	at the end of every period for each diet	NSD	Barros et al., 2014
Oreochromis niloticus	80-100	β-glucan Biosaf (*S. cerevisiae)*	10g/kg feed	21d	*A. hydrophila*	IP injection	$0.4x10^7$	22d	10dpi	Significant reduced mortality rate compared eith control group	El-Boshy et al., 2010
		β-glucan (extracted) *(S. cerevisiae)*	0.1%							Significant reduced mortality rate compared with control group	
		laminaria (*L. japonica)*	0.1%							Significant reduced mortality rate compared with control group	
Pseudosciaena crocea	10	β-glucan (*S. cerevisiae*)	0.09%	8wk	*V. harveyi*	IP injection	$1.6x10^8$ cfu per fish	at end of 8th wk	6dpi	Significant reduced mortality rate compared with control group	Ai et al., 2007
			0.18%							No reduced mortality rate compared with control group	

TABLE IV: (*...continuation*)

Spp.	BW (g)	IS	IS dose	Administration	Challenge					Effect	Ref
					Pathogen	Route	Dose	Time (dpd)	Time of evaluation		
Salmoniformes											
Onchorynchus mykiss	4 Kg	β-glucan MacroGard (S. cerevisiae)	0,10%	3mo	*Y. ruckeri*	3 min bath challenge	$2x10^8$ cells/mL	NS	25dpi	NSD	Ghaedi et al., 2015
			0,20%	3mo						NSD	
	0.18		C+C (L1)	2mo						NSD	
			C + 0,1% (L2)	2mo						NSD	
			C + 0,2% (L3)	2mo						Significant increased in survival	
			0,1% + C (L4)	2mo						NSD	
			0,1% + 0,1% (L5)	2mo						Significant increased in survival	
			0,2% + C (L6)	2mo						NSD	
			0,2% + 0,2% (L7)	2mo						Significant increased in survival	
Onchorynchus mykiss	8,8	β-glucan MacroGard (S. cerevisiae)	0.2%	21d	*F. columnare*	2hr bath	$6.5x10^6$	22d	8dpi	Mortality was higher than that of fish fed ordinary feed	Kunttu et al., 2009
			0.6%							Mortality was higher than that of fish fed ordinary feed	
			1.8%							Mortality was higher than that of fish fed ordinary feed	
Onchorynchus mykiss	14.3	β-glucan barley (*H. vulgare*)	12.2g/Kg feed	9wk	Immunized fish (IHNV 039-82) Challenged 4wpi with IHNV 220-90	IP injection	$1.0x10^6$ PFU	14wk	18dpi	No reduced mortality rate compared with control group	Sealey et al., 2008
			16.7g/Kg feed							Significant reduced mortality rate compared with control group	
			26.4g/Kg feed							Significant reduced mortality rate compared with control group	
		Wheat diet (control) + 2g/Kg MacroGard	4.5g/Kg feed							Significant reduced mortality rate compared with control group	
Salmo salar	fry	LPS (*A. salmonicida)* + basal diet Skretting	0.1%	62d	*A. salmonicida* subsp. *salmonicida*	1 hr bath	$9.3x10^4$ cfu/ml	NS	42dpi	Mortality was significant higher than that of fish fed control feed	Guttvik et al., 2002
		LPS (*A. salmonicida)* + basal diet Supra G1 and G2 NorAqua	0.01%	64d	*A. salmonicida; V. angillarum*	1 hr bath; 45 min bath	$1.0x10^7$ cfu/ml; $1.0x10^7$ cfu/ml	64d	53dpi	Not significant diferences in survival rate compared with control.	
			0.03%						31dpi	Not significant diferences in survival rate compared with control.	

AAD: After administration diet, **AEC:** After each cycle, **AGR:** Absolute growth rate, **BSD:** Basal diet, **BWG:** Body weight gain, **CFU:** Colony forming units, **d:** Days, **dac:** Days after challenge, **daf:** Days after feeding, **dai:** Days after immunization, **dot:** Days of treatment, **dpc:** Days post challenge, **dph:** Days post-hatching, **dpi:** Days post injection, **dpt:** Days post-trial, **FCR:** Feed conversion ratio, **FER:** Feed efficiency ratio, **FWG:** Final weight gain, **Hct:** Hematocrit, **HCV:** Hematocrit value, **HK:** Head-kidney, **IS:** Immunostimulant, **ISD:** Immunostimulant diet, **LDL:** Low density lipoprotein, **MG:** Mid-gut, **mo:** Months, **NBT:** Nitroblue tetrazolium, **NS:** No specified, **NSD:** No significant differences, **PER:** Protein efficiency ratio, **PWG:** Percent weight gain, **RBC:** Red blood cells count, **RLP**: Relative level of protection, **RPS:** Relative percent survival, **SGR:** Specific growth rate, **wac:** Weeks after challenge, **waf:** Week after feeding, **WBC:** White blood cells count, **WG:** Weight gain, **wks:** Weeks, **wpi**: weeks post immunization.