ABSTRACT.- Di Domenico J., Canova R., Soveral L.F., Nied C.O., Costa M.M., Frandoloso R. & Kreutz L.C. 2017. Immuno-
modulatory effects of dietary β-glucan in silver catfish (Rhamdia quelen). Pesquisa Veterinária Brasileira 37(1):73-78.
Laboratório de Microbiologia e Imunologia Aplicada, Programa de Pós-Graduação em Bioexperimentação, Universidade
de Passo Fundo, Campus I, Bairro São José, BR-282 Km 171, Passo Fundo, RS 99052-900, Brazil. E-mail:lckreutz@upf.br

The immunomodulatory effects of dietary β-glucan were evaluated in silver catfish. β-glucan was added to the diet (0.01%
and 0.1%) and fed to the fish for 21 days, to evaluate effects on blood and some innate immune parameter, or fed for 42
days, to evaluate growth rate and resistance to challenge with pathogenic Aeromonas hydrophila. We found that
adding β-glucan to the diet had no effect on fish growth and no effect on blood cells, or serum bacterial agglutination
and serum myeloperoxidase activity. However, fish that received β-glucan in the diet had the natural hemolytic activity of
complement significantly higher compared to control fish. Furthermore, fish fed with β-glucan and challenged with
A. hydrophila had fewer bacteria in blood and presented a significantly higher survival rate compared to control fish.
Thus, we concluded that β-glucan might be explored as feed additive aiming to improve silver catfish innate immunity
and resistance to specific pathogen.

INDEX TERMS: Silver catfish, Rhamdia quelen, fish, immunostimulants, β-glucan, Aeromonas hydrophila.

INTRODUCTION
The occurrence of infectious diseases is one of the major causes of losses in modern aquaculture (Sitjà-Bobadilla
Although antibiotics have been widely used for disease treatment, mainly in fries and fingerlings (Brudese-th et al. 2013), the surge of antibiotics-resistant bacteria, and antibiotics residues in water and meat, raised major concerns towards this management procedure. In this sce-
nario, strengthening the fish defense mechanisms by vacci-
nation to specific pathogens or by adding immune modu-
lating molecules in the diet has been increasingly explored as an economically viable procedure to prevent disease out-
breaks (Bricknell & Dalmo 2005, Sommerset et al. 2005, Plant & Laptra 2011, Bairwa et al. 2012).

Immunomodulating molecules interact with immunolog-
cal cells and are widely used to improve defense mech-
anisms. Medicinal herbs and plants are major source of such molecules (Galina et al. 2009, Van Hai 2015); pre- and pro-
biotics (Nayak 2010), vitamins (Ortuño et al. 2001, Azad et al. 2007, Kiron 2012) and synthetic molecules (Maqsood et al. 2009) have also been evaluated for their effects on the immune system. Amongst immune modulating mole-
cules, β-glucan, a linear polysaccharide extracted from the cell wall of yeast, algae and fungi (Dalmo & Bøgwald 2008) stands as a model of pathogen associated molecular pat-
terms, and as such has been widely exploi-
ted as an economically viable procedure to prevent disease ou-
tbreaks (Bricknell & Dalmo 2005, Sommerset et al. 2005, Plant & Laptra 2011, Bairwa et al. 2012).

We aimed to evaluate the effects of β-glucan-enriched diet on innate immunity and resistance to challenge by Aeromo-

nas hydrophila infection.

**MATERIALS AND METHODS**

**Fishes.** All fishes used in these experiment were produced and obtained from our experimental unit (centro de pesquisa agrope-

cuária – Cepagro) and were free of specific infections. During the acclimatization period (7 days) and up to the end of the experi-

ments, fish were kept in self-cleaning tanks containing 1000L of continuously running water, protected from direct sun light, and fed a commercial pelleted feed containing 42% protein. Water conditions were within the expected values, as previously report-
ted (Kreutz et al. 2014). For inoculations and blood sampling, fish were anesthetized with clove oil (50mg/L - Sigma, Brazil). The ex-

periments were approved by the Ethics Committee for the Care and Use of Experimental Animals (CEUA, protocol 011/2012) of the Universidade de Passo Fundo. Prior to and after the experiments all fish were weighed and measured to evaluate relative weight gain (WG) and specific growth rate (SGR): WG = 100 x (final weight - initial weight)/initial weight and SGR = 100 x [ln final weight - ln initial weight]/days of the experiment (Lugert et al. 2014).

**Evaluation of β-glucan as feed additive aiming to modu-
late the immune system.** Two experiments were carried out to evaluate the effect of mixing β-glucan (MacroGard®, Biorigin, Brazil) on blood cells and innate immune parameters, WG, SGR and survival to challenge with A. hydrophila. In the first trial, silver catfish juveniles (70-90 g) were allocated into three groups: one group received pelleted food added with 0.01% of β-glucan; a second group had 0.1% of β-glucan added to the food, and a third group had no β-glucan on the food (control group). All fish were fed at libitum twice a day. β-glucan was mixed to the food pellets as recommended by the manufacturer and fed to fish for 28 days. Each group consisted of 15-17 fishes and the experiment was carried out in duplicates. At the end of the feeding trial, all fish were captured and anesthetized for blood sampling at the caudal vein. One blood aliquot was dropped in EDTA-containing tubes aiming hematological analysis. A second aliquot was allowed to clot at 4º and centrifuged at 1500 x g to separate the serum, which was stored at -20º C and used to evaluate innate immune parameter. One week later, all fish were captured again, anesthetized and immunized with BSA (200 µg/fish) mixed to montanide (20% v/v) aiming to evaluate the effect of the feeding trial in the production of antibodies to BSA. During this time β-glucan was removed from the diet and all fish received the same pelleted food. Then, after 28 days of vaccination with BSA (at day 63 of the experimental trial) all fish were captured and blood samples were collected without anticoagulant, and processed as described above to evaluate anti-BSA antibodies by ELISA.

In the second feeding trial, 150 fish were allocated equally to three feeding groups in duplicates: no β-glucan, 0.01% and 0.1% of β-glucan in the food, and fed at libitum twice a day. All fish were weighted and measured prior to the feeding trial and at 42 days to evaluate weight gain and SGR. Then, at the forty second day, immediately after measurements, all fish were intraperitoneally challenged with Aeromonas hydrophila (2x10 ⁹ Colony Forming Units – CFU /fish) as previously described (Kreutz et al. 2010). After 24h, 10 fish from each group were captured for blood sampling aiming to detect bacteremia.

**Hematological, innate immune parameters analysis and bacteremia detection.** For hematological evaluation, blood smear were made with EDTA-containing blood all quots, air-dried and stained with Wrigth-giemsa. Hematocrit, hemoglobin and erythrocyte counts were determined on whole blood within 2h after sampling, as previously described (Barcellos et al. 2004a). Innate immune
parameters (total serum myeloperoxidase, serum bacteria agglutination activity and complement natural hemolytic activity) were performed as previously described (Kreutz et al. 2011).

The presence of bacteremia in fish was assessed by seeding 100 μl of whole blood on Brain Heart Infusion (BHI) plates. After seeding, plates were incubated at 37°C for 24 h and the number of CFU was annotated. The remaining fish were observed daily for seven days to evaluate clinical signs, skin lesions and mortality aiming to determine the survival rate following challenge.

**Enzyme-linked immunosorbent assay to measure anti-BSA antibodies in fish serum.** The ELISA assay was performed as recently described (Kreutz et al. 2016). Briefly, 96-well ELISA plates were coated overnight (4°C) with BSA (5μg/well) diluted in carbonate-bicarbonate buffer (pH 9.6) and then blocked with PBS containing 0.05% Tween 20 (PBST) and 3% skin milk (Sigma) (PBST-Sk3%). Fish serum samples diluted 1:100 in PBST-SK1% were added in duplicates to the wells. After 1h incubation at 23°C and washing with PBST, rabbit anti-silver catfish IgM antibodies diluted 1:400 in PBST-SK1% was added to the wells. The plates were incubated and washed as described above. Horseradish peroxidase conjugated goat anti-rabbit IgG was added to the plates (diluted 1:20.000 in PBST-SK1%) and incubated 1h at 23°C. After washing, color development was performed using O-phenyldiamine (OPD - 0.067%; Sigma). Plates were read at 492 nm with an Anthos 2010 ELISA plate reader.

**Statistical analysis.** The results obtained were analyzed by the Shapiro-Wilk’s test and were found to have normal distribution. Differences amongst treatments were analyzed by t-test or ANOVA followed by Bonferroni’s multiple comparisons test, and plotted using GraphPad Prism Software v.5 (GraphPad Software, Inc., USA). P-values of 0.05 or smaller were considered significant. Results are expressed as the mean ± standard error of the mean (SEM).

**RESULTS**

The effect of feeding β-glucan enriched diet on blood cell and innate immune parameters

Blood cell parameters observed in the current study (Table 1) were within the range reported previously for silver catfish (Barcellos et al. 2004a, Kreutz et al. 2011). In all groups, at the end of the experiment, erythrocyte counts were lower (p<0.05) compared to the counts obtained prior to the feeding trial. Monocytes (control and 0.01% β-glucan groups) and thrombocytes (0.01% β-glucan group) were also reduced by the end of the experiment.

The inclusion of β-glucan in the diet had no effect on total serum myeloperoxidase activity or in the capacity of serum to agglutinate inactivated *A. hydrophila* (data not shown). In addition, the β-glucan enriched diet had no effect on the fish capacity to produce antibodies to BSA (data not shown). However, the addition of β-glucan to feed had a significant (p<0.05) effect on the complement natural hemolytic activity upon sheep red blood cells (Fig.1).

The effect of feeding β-glucan enriched diet on the resistance of fish to challenge with *Aeromonas hydrophila*

The inclusion of β-glucan on the diet had no effect on weight gain and SGR (Table 2). Resistance to *A. hydrophila* infection was evaluated by measuring bacteremia, at 24 h p.i., and survival rate up to 7 days p.i. The number of CFU in the blood of fish fed diets containing β-glucan was significantly lower (p<0.05) compared to fish from the control group (Fig.2). In addition, the survival rate of fish fed β-glucan was significantly higher (p<0.05) than the survival rate of the fish from the control (Fig.3). In the control group, mortality was observed 24h p.i. and continued up to 96h p.i. In the group of fish fed a diet containing 0.01% β-glucan, fish mortality was observed at the third and fourth day. No mortality was observed in the group of fish fed with 0.1% β-glucan.

**DISCUSSION**

Outbreaks of infectious diseases on cultivated fish species are difficult to control and represent a major cause of reduced productivity. In addition, the occurrence of specific infections might raise sanitary barrier to fish products. In this scenario, controlling disease outbreaks by vaccination and the use of immune modulator enriched diets represents a major achievement for aquaculture species. Although fish vaccination is still crawling, it has been successfully used to control important fish diseases (Sommerset et al. 2005, Secombes 2008, Van Muiswinkel 2008, Plant & Lapatra 2011). And, more recently, vaccination commingled with the inclusion of immune-modulator molecules in the diet offered and additional strategy to overcome major pathogen (Newaj-Fyzul & Austin 2015). However, because an efficient immune response to inoculated antigen still relies on intraperitoneal injections, the hurdles and cost of individual vaccination hinders application to low commercial value species. Thus, for this species, feed additives with immune modulating capability might become widely used.

The mechanisms of β-glucan effect on fish innate immune system are not clear but might involve improved phagocytic activity and increased expression of cytokines in macrophage, neutrophils and dendritic cell. Indeed, in vitro

| Parameter       | Control Day 0 | Control Day 28 | β-glucan 0.01% Day 0 | β-glucan 0.01% Day 28 | β-glucan 0.1% Day 0 | β-glucan 0.1% Day 28 |
|-----------------|---------------|----------------|---------------------|----------------------|-------------------|---------------------|
| Hematocrit (%)  | 40.0 ± 2.0    | 37.0 ± 2.0     | 40.0 ± 1.0          | 49.0 ± 0.9           | 40.0 ± 1.0        | 41.0 ± 0.9          |
| Hemoglobin (g/l)| 11.0 ± 0.7    | 10.0 ± 0.5     | 10.0 ± 0.4          | 10.0 ± 0.53          | 11.0 ± 0.4        | 11.0 ± 0.3          |
| Erythrocytes (106/μl) | 1.6 ± 0.08 | 1.1 ± 0.06     | 1.4 ± 0.07          | 1.0 ± 0.05*          | 1.6 ± 0.05        | 1.2 ± 0.05*         |
| Leucocytes (μl) | 265.3 ± 19.0  | 191.6 ± 18.1   | 311.0 ± 23.0        | 167.0 ± 10.6         | 270.0 ± 26.0      | 234.0 ± 21.0        |
| Neutrophils (μl) | 10.924 ± 2.9 | 6.800 ± 1.4    | 10.367 ± 2.1        | 7.293 ± 1.3          | 9.169 ± 2.5       | 8.943 ± 2.7         |
| Monocytes (μl)  | 2.270 ± 870   | 696 ± 232*     | 1.885 ± 190         | 420 ± 140            | 4.100 ± 1.9       | 1.321 ± 385         |
| Lymphocytes (μl) | 106.235 ± 14.0 | 94.175 ± 14.2 | 104.613 ± 22.0      | 75.350 ± 7.2         | 144.507 ± 22.0    | 113.742 ± 13.85     |
| Thrombocytes (μl) | 148.500 ± 16.000 | 93.140 ± 8.470 | 203.779 ± 22.166    | 82.770 ± 5.543*     | 116.945 ± 11.358  | 113.815 ± 9.831     |
studies indicated that β-glucan triggered proinflammatory cytokines in exposed macrophage meliorating phagocytic activity, respiratory burst, serum lysozyme, myeloperoxidase, serum bactericidal activity and complement natural hemolytic activity (Hawlisch & Köhl 2006).

In our work, although serum levels of bacterial agglutinins and myeloperoxidase were not affected by adding β-glucan on the diet, the natural complement hemolytic activity was significantly improved in fish fed β-glucan. The complement cascade in teleost fish is one of the major natural defense mechanism toward parasites, fungi, virus and bacteria (Magnadóttir 2006, Alvarez-Pellitero 2008). The activation of complement, either the classical or alternative pathway, leads to the production of several soluble compo-

Inclusions of β-glucan into the diet of carps (Labeo rohita and Cyprinus carpio) also improved complement activity (Misra et al. 2006) and heightened expression of complement genes in several tissues (Pionnier et al. 2013). Furthermore, the activity of the alternative complement cascade might also be improved by adding Saccharomyces cerevisiae to the diet of Epinephelus coioides (Chiu et al. 2010). These indicate that complement per se might be capable of controlling initial infection by specific pathogens.

In contrast, the production of anti-BSA antibodies in fish fed β-glucan and vaccinated with BSA+montanide was similar to fish from the control groups (data not shown). Indeed, feeding β-glucan to fish hardly improves the production of total or specific immunoglobulins, as observed in Nile tilapia, Oreochromis niloticus (Whittington et al. 2005), common carp (Selvaraj et al. 2006), rainbow trout (Skov et al. 2012, Ghaedi et al. 2015) and gilthead sea bream, Sparus aurata (Guzmán-Villanueva et al. 2014).

The effect of β-glucan on blood cells is controversial. We found that monocytes and thrombocytes were reduced by the end of the feeding trial in the control group and in the group fed with 0.01% β-glucan. High variation found within

Table 2. Weight gain in silver catfish fed with a diet containing β-glucan. All fish were weighted and measured prior to and after the end of the experiment (42 days) to evaluate weight gain (%) and specific growth rate (SGR). Values represent the mean ± S.E.M (n=50)

| Parameter            | Control       | β-glucan 0.01% | β-glucan 0.1% |
|----------------------|---------------|---------------|---------------|
| Initial weight (g)   | 20.3±0.3      | 20.2±0.4      | 20.3±0.3      |
| Final weight (g)     | 67±1.8        | 66±2          | 72±1.6        |
| Weight gain (%)      | 230±8.8       | 227±10        | 252±7.9       |
| SGR (%/day⁻¹)        | 1.2±0.03      | 1.2±0.03      | 1.3±0.02      |

Fig.1. Increased natural complement hemolytic activity in silver catfish fed a diet containing β-glucan. The results are expressed as the mean ± SEM (n=30). Significant differences from the control group (p<0.05) are indicated by asterisk.

Fig.2. Number of colony forming units (CFU) in the blood of silver catfish challenged with Aeromonas hydrophila (2x10⁸ CFU/fish). Blood samples were collected aseptically 24h after challenging and cultured in BHI plates (0.1ml blood/plate). Data are represented as the mean ± SEM (n=10) of the natural logarithm of the number of colonies observed in each plate. Significant differences from the control group (p<0.05) are indicated by asterisk.
Fish fed with β-glucan (0.01% and 0.1%) had hydrophilia by challenging fish with an intraperitoneal injection of β-glucan would prevent the stress effect on blood cells. However, we are aware at the end of the experiment and it would be tempting to number of monocytes and thrombocytes were not altered after a two week feeding trial with β-glucan (500mg/Kg of feed)(Misra et al. 2006). In fish fed with 0.1% β-glucan, the liceation of immune cells due to cortisol release (Gabriel et al. 2011). In addition, at least one study indicated that Indian carps (Labeo rohita) had reduced total leukocytes counts after a two week feeding trial with β-glucan (500mg/Kg of feed)(Misra et al. 2006). In fish fed with 0.1% β-glucan, the number of monocytes and thrombocytes were not altered at the end of the experiment and it would be tempting to attribute this effect to β-glucan. However, we are aware that a larger number of fish should be used to assume that β-glucan would prevent the stress effect on blood cells.

Nonetheless, the beneficial effect of adding β-glucan to silver catfish diet has been unequivocally demonstrated by challenging fish with an intraperitoneal injection of A. hydrophila. Fish fed with β-glucan (0.01% and 0.1%) had significantly less bacteria in blood at 24 h p.i. and a significantly higher survival rate. In fact, silver catfish fed 0.1% β-glucan had a survival rate of 100%. The beneficial effect on resistance to challenge with specific pathogen has been reported in several fish species (Misra et al. 2006, Welker et al. 2007, Garcia & Villarroel 2009, Pionnier et al. 2013), β-glucan is thought to stimulate the complement cascade, phagocytosis, serum lysozyme and bactericidal activity. Combined, these mechanisms suffice the control of inoculated pathogens and prevent a widespread dissemination that could cause organs failure. In our work, indeed, fish fed β-glucan had a significantly lower bacteremia that corresponded to lower clinical signs, lesions and mortality.

Several studies indicated that β-glucan improved fish weight gain (Whittington et al. 2005, Welker et al. 2007, Garcia & Villarroel 2009, Chiu et al. 2010, Guzmán-Villanueva et al. 2013, Ghaedi et al. 2015). However, in our study, the addition of β-glucan to the diet had no effect on SGR. Differences in the feeding regimen and fish species should account for this observation. In at least one study, β-glucan improved the secretion of digestive enzymes and this could be related to improved growth (Guzmán-Villanueva et al. 2013).

In summary, although we could not demonstrate a beneficial effect on blood cells and some innate immune parameters, the addition of β-glucan to the diet improved natural complement hemolytic activity, reduced bacteremia levels and, most importantly, increased fish resistance to challenge with A. hydrophila. Taken together, we would strongly recommend the use of β-glucan on silver catfish diet aiming to improve overall health.

Acknowledgements.- This study was carried out using financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant # 476317/2012-6), Brazil, and from the Secretaria de Desenvolvimento Econômico, Ciência e Tecnologia (SDECT, grant # 481-2500/13-2). Raissa Canova is a Master student with a CAPES fellowship (01589073029) and Cristian O. Nied and Lucas Soeveral was an undergraduate student with a CNPq fellowship (125852/2013-4) nd FAPERGS fellowship.

Conflict of interest.- The authors have no competing interests.

REFERENCES
Alvarez-Pellitero P. 2008. Fish immunity and parasite infections: from innate immunity to immunophylactic prospects. Vet. Immunol. Immunopathol. 126:171-196.
Aoki T, Takano T, Santos M.D. & Kondo H. 2008. Molecular In innate Immunity in Teleost Fish: review and future perspectives. p.263-276. In: Tuskamoto K, Kawamura T, Takeuchi T, Beard Jr TD. & Kaiser MJ. (Eds). Fisherys for Global Welfare and Environment. 5th World Fisheries Congress TERRAPUB, Tokyo, Japan.
Azad IS, Dayal J.S., Poornima M. & Ali S.A. 2007. Supra dietary levels of vitamins C and E enhance antibody production and immune memory in juvenile milkfish, Chanos chanos (Forsskal) to formalin-killed Vibrio vulnificus. Fish Shellfish Immunol. 23:154-63.
Bairwa M.K., Jakhar K., Satyanarayana Y. & Reddy D. 2012. Animal and plant originated immunostimulants used in aquaculture. J. Nat. Prod. Plant Resour. 2(3):397-400.
Barcellos L.J.G., Kreutz L.C., Rodrigues L.B., Ruschel L., Costa A., Ritter F., Bedin A.C. & Bolognesi L. 2008. Aeromonas hydrophila in Rhamdia quelen : aspects macro e microscópico das lesões e perfil de resistência a antimicrobianos. Bolm Inst. Pesca, São Paulo, 34 (3):355-363.
Bricknell L & Dalmo R.A. 2005. The use of immunostimulants in fish larval aquaculture. Fish Shellfish Immunol. 19:457-472.

Brudeseth B.E., Wiulsrød R., Fredriksen B.N., Lindmo K., Lakling K.E., Bordevik M., Steine N., Klevan A. & Gravningen K. 2013. Status and future perspectives of vaccines for industrialised fin-fish farming. Fish Shellfish Immunol. 35:1759-1768.

Chiu C.-H., Cheng C.-H., Gua W.-R., Guu Y.-K. & Cheng W. 2010. Dietary administration of the probiotic, Saccharomyces cerevisiae P13, enhanced the growth, innate immune responses, and disease resistance of the grouper, Epinephelus coioides. Fish Shellfish Immunol. 29:1053-1059.

Dalmo R.A. & Bagwald J. 2008. Beta-gucans as conductors of immune symphonies. Fish Shellfish Immunol. 25:384-396.

Das B.K., Debnath C., Patnaik P., Swain D.K., Kumar K. & Misra C.K. 2006. Effect of dietary β-glucan on immunity and survival of early stage of Anabas testudineus (Bloch). Fish Shellfish Immunol. 27:679-683.

Gabriel U.U., Akinrotimi O.A. & Eseimokumo F. 2011. Haematological responses of wild Nile tilapia Oreochromis niloticus after acclimation to captivity. Jordan J. Biol. Sci. 4:225-230.

Galina J., Yin G., Arld L. & Jeney Z. 2009. Effect of feed type and feeding frequency on macrophage functions in tilapia (Oreochromis niloticus L.). Fish Shellfish Immunol. 27:325-329.

Ghaedi G., Keyvanshokooh S., Azarm H.M. & Akhlaghi M. 2015. Effects of a fish-based probiotic on hepatic antioxidant and digestive enzyme activities of Pacific red snapper (Oncorhynchus mykiss). Aquaculture 441:78-83.

Gomes L.D.C., Golombieski J.L., Gomes A.R.C. & Baldisserotto B. 2000. Biologia do jundiá Rhamdia quei (Teleostei, Pimelodidae). Ciência Rural 30:179-185.

Guzmán-Villanueva L.T., Ascencio-Valle F., Macías-Rodriguez M.E. & Tovar-Ghaedi G., Keyvanshokooh S., Azarm H.M. & Akhlaghi M. 2015. Effects of dietary β-glucan on maternal immunity and fry quality of rainbow trout (Oncorhynchus mykiss). Aquaculture 441:78-83.

Magnadóttir B. 2006. Innate immunity of fish (overview). Fish Shellfish Immunol. 20:137-151.

Magnadóttir B., Lange S., Gudmundsdottir S., Bagwald J. & Dalmo R.A. 2005. Ontogeny of humoral immune parameters in fish. Fish Shellfish Immunol. 19:429-439.

Maqsood S., Samoon M.H. & Singh P. 2009. Immunomodulatory and growth promoting effect of dietary levamisole in cyprinids fingerlings against the challenge of Aeromonas hydrophila. Turk. J. Fish. Aquat. Sci. 9:111-120.

Martins M.L., Xu D.H., Shoemaker C.A. & Klesius P.H. 2011. Temperature effects on immune response and hematological parameters of channel catfish Ictalurus punctatus vaccinated with live threons of Ichthyophthirius multifiliis. Fish Shellfish Immunol. 31:774-780.

Miest J.J., Falco A., Pionnier N.P.M., Frost P., Irnazarow I., Williams G.T. & Hoole D. 2012. The influence of dietary β-glucan, PAMP exposure and Aeromonas salmonicida on apoptosis modulation in common carp (Cyprinus carpio). Fish Shellfish Immunol. 33:846-856.

Misra C.K., Das B.K., Mukherjee S.C. & Pattnaik P. 2006. Effect of long term administration of dietary β-glucan on immunity, growth and survival of Labeo rohita fingerlings. Aquaculture 255:82-94.

Nayak S.K. 2010. Probiotics and immunity: a fish perspective. Fish Shellfish Immunol. 29:2-14.

Niewaj-Fyzul A. & Austin B. 2015. Probiotics, immunostimulants, plant products and oral vaccines, and their role as feed supplements in the control of bacterial fish diseases. J. Fish Dis. 38:937-955.

Ortúñio J., Cuesta A., Angeles Esteban M. & Meseguer J. 2001. Effect of oral administration of high vitamin C and E dosages on the gilthead seabream (Sparus aurata L.) innate immune system. Vet. Immunol. Immunopathol. 79:167-180.

Pionnier N., Falco A., Miest J., Frost P., Irnazarow I., Shrive A. & Hoole D. 2013. Dietary β-glucan stimulate complement and C-reactive protein acute phase responses in common carp (Cyprinus carpio) during an Aeromonas salmonicida infection. Fish Shellfish Immunol. 34:819-831.

Plant K.P. & Lapatra S.E. 2011. Advances in fish vaccine delivery. Dev. Comp. Immunol. 35:1256-262.

Sehara T., Sathish K. & Sekar V. 2006. Adjuvant and immunostimulatory effects of β-glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with Aeromonas hydrophila. Vet. Immunol. Immunopathol. 114:15-124.

Sitja-Bobadilla A. 2008. Living off a fish: a trade-off between parasites and the immune system. Fish Shellfish Immunol. 25:409-416.

Skov J., Kania P.W., Holten-Andersen L., Foug P. & Buchmann K. 2012. Immunomodulatory effects of dietary β-1,3/1,6-glucan on the growth, innate immune responses and resistance to stress and infectious disease in Oreochromis niloticus. Fish Shellfish Immunol. 33:2-14.

Sommerset I., Krossøy B., Biering E. & Frost P. 2005. Vaccines for fish in aquaculture: a review. Aquaculture 248:217-225.