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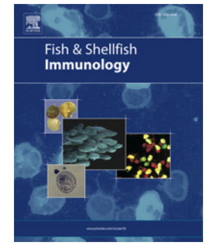

REVIEW

ß-glucans as conductors of immune symphonies

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Abstract The use of immunostimulants has received increased attention due to the discovery of Toll-like receptors (TLR) or/and pattern recognition receptors (PRR). These receptors have been found to bind molecules from a range of pathogens including self-molecules. When cell damage has occurred many of the released molecular structures act as so-called ''danger'' signals possessing pathogen-associated molecular patterns (PAMP). These danger signals often consist of repeating molecular moieties yielding high molecular weight compounds. Examples are ß-glucans and CpG containing DNA, but some danger signals possess low molecular weight structures. It has been found that the PRR bind unit structures of PAMP, and that PAMP-binding involves several other humoral and cell membrane proteins, exemplified by the more or less simultaneous LPS recognition displayed by MD-2, CD-14 and TLR4 on the cell membrane. Also, the binding of ß-glucans has been shown to include several different cell membrane receptors. Several immunostimulants are commercially exploited in aquaculture as feed additives. This applies to ß-glucans, alginates and nucleotides. Despite their use as feed additives no targeted approach has been conducted to include PAMP as adjuvants in fish vaccines. Interestingly, most of the PAMP studied activate antigen-presenting cells together with naïve T cells into dendritic cells and Th1 or Th2 cells [1]. In turn, this may activate Th1 and Th2 immune responses with production of Th1 or Th2 signature molecules such as IFN-gamma and IL-4, respectively [2—4]. This review will mainly focus on binding characteristics of ß-glucans, their effects on T helper cell differentiation, effects on functional levels, gene expression profiles and application of the commonly used ß-glucan in the aquaculture sector. In addition, ß-glucans show promises in shrimp aquaculture by inducing disease resistance, this review will also highlight the use and the effects of ß-glucans in experimental models.

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Introduction

A number of immunostimulants has molecular architecture possessing repeating units of a certain moiety such as glucose in ß-glucans and (deoxy)riboses in DNA/RNA, fatty acid chains in bacterial lipopolysaccharides (LPS) and certain lipoproteins. Such patterns are abundant in microbial communities of prokaryotes, and can be termed pathogen-associated molecular patterns, PAMP, if they initiate inflammatory responses. Interestingly, most of the PAMP studied activate antigen-presenting cells together with naive T cells into dendritic cells and T helper cells [1–4]. During microbial breakdown/degradation, a number of PAMP may be released that would initiate inflammatory responses upon receptor binding and intracellular activation of signal transducers and transcription factors. The exact composition of released danger signals would be decisive for the out-put gene transcription leading to synthesis and release of pro-inflammatory cytokines. Immunostimulants have been used as feed additives for many years in aquaculture, and yeast ß-glucan may be the one with the longest track record. Homo-polysaccharides, consisting of one sugar, are described by use of the suffix ''an''. Thus a poly-glucose (poly-glucopyranose) is termed ''glucan''. Other carbohydrates may be hetero-polysaccharides that may contain several different constituent residues. ß(1,3)-D-glucans (ß-glucans) are the most commonly used term for homopolysaccharides that has ß(1,3)-D-linkages in the backbone, and may also possess ß-D-glucosidic linkages at position 6 in different, often repeating units (branches). In nature, ß-glucans are widespread and are found in plants, algae, bacteria, yeast and mushrooms. ß-glucans possess differences in molecular weights and degree of branching. Yeast ß-glucan is a particulate carbohydrate that consists of glucose and mannose and is a major constituent of the cell membrane. Of the major fish feed producers, Skretting and Ewos include yeast ß-glucan in their fish feed assortments, while BioMar includes bioactive alginate.

Growth performance

Besides the information presented in comprehensive review articles on immunostimulants [5], several new reports have described the effects of ß-glucans on growth performance in fish. It has been found that ß-glucans such as yeast glucans obtained from *Saccharomyces cerevisiae* incorporated in fish feed increase the growth rate of certain species of fish at certain environmental conditions [6–9]. However, in other scientific experiments no significant growth increase has been found after feeding fish with different ß-glucans [10–12]. It appears that a growth increase is dependent on the amount of ß-glucan incorporated in the diet, duration of feeding, environmental temperature and the species under study. To obtain increased growth performance, feeding strategies should be developed for each fish species with respect to ß-glucan dose and duration. In addition, soluble or particulate ß-glucans may confer different responses since the intestine only absorb soluble ß-glucans. There are no indications that particulate ß-glucans are taken up by the fish intestine or digested by ß-glucan degrading enzymes thereby being nutritious. Feed additives such as bioactive alginates, enriched with mannuronic acid residues, have also been reported both beneficial and detrimental with respect to weight increase and protein turnover in fish larvae and juveniles [13–15]. Dietary nucleotides may induce weight gain in fish although it seems that it is likely that species and dose dependencies occur [11,16,17]. Following on, the weight of salmon given formulated feed with 0.01% and 0.03% w/w *Aeromonas salmonicida* LPS for 62 days exhibited statistically higher weight compared to control fish. At the same time, the weight of fish that received 0.1% LPS did not differ from controls [18]. To date, there are no plausible explanations for the growth enhancing effects displayed by diets containing immunostimulants. One hypothesis may include a local intestinal inflammatory response, after administration that induces resistance against pathogens - that otherwise would result in decreased weight gain and maybe disease. Evidently, to assess the growth enhancing effects of immunostimulants more sophisticated analysis must be companioned. This should be highly feasible due to the advanced genomic tools available for gene expression analysis.

Immune responses after oral administration

Sea bass (*Dicentrarchus labrax*) were fed a diet supplemented with 2% ß-1,3/ß-1,6-glucan over a 2-week period every 3 months [19]. The plasma complement activity was elevated in immunostimulated fish compared with controls. In another trial, alternative pathway of complement activation and lysozyme activity were both significantly enhanced in day 15 samples obtained from fish fed ß-glucan (MacroGard™) and alginic acid (Ergosan) after the first out of four 2-week feeding cycles. The immunostimulants did not confer any immune modulation measured as elevated lysozyme levels, increased complement activity, and modulated T and B cell numbers in peripheral blood after the fish were fed for 35 weeks [11]. The dietary effect of β-1,3-glucan on innate immune responses of large yellow croaker (*Pseudosciaena crocea*) was studied [8]. The diet was supplemented with 0.09% and 0.18% β-1,3-glucan and the feeding trial lasted for 8 weeks. Low concentration of glucan (0.09%) significantly enhanced the respiratory burst and phagocytic activity in head kidney macrophages while the high concentration (0.18%) did not. The serum lysozyme activity in fish fed diets with both glucan concentrations were significantly higher than the control, and the 0.18% glucan diet significantly stimulated the lysozyme activity compared to 0.09%. No significant differences were seen in alternative complement pathway activity with any of the ß-glucan concentrations (see overview in Table 1). A commercial ß-glucan (EcoActiva) was administered as a feed supplement to the snapper, *Pagrus auratus* [6]. The immunostimulant increased macrophage oxygen radical production during the wintertime, but not during the summer time. In contrast to macrophage activity EcoActiva did not potentiate neither the classical nor the alternative complement activity. The results of this study suggested that it may be favourable to include ß-glucan in the feed to snapper during the wintertime both to increase resistance against diseases and to increase growth rates. Immunomodulation by a yeast ß-1,3/ß-1,6-glucan in