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Beta Glucan: A valuable nutraceutical for promoting health in aquaculture

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ABSTRACT

Immunostimulants are considered as an effective tool for enhancing immune status of cultured organisms. Among different immunostimulants used in aquaculture practices, β -glucan is one of the promising immunostimulant, which is a homopolysaccharide of glucose molecule linked by the glycoside bond. It forms the major constituents of cell wall of some plants, fungi, bacteria, mushroom, yeast, and seaweeds. Major attention on β-glucan was captivated with the gain in knowledge on its receptors and the mechanism of action. The receptor present inside the animal body recognizes and binds to β-glucan, which in turn renders the animal with high resistance and enhanced immune response. This review highlights β-glucan as an immunostimulant, its effective dosages, and route of administration and furthermore provides an outline on role of β-glucan in enhancing growth, survival, and protection against infectious pathogens pertaining to fishes and shellfishes. Study also summarizes the effect of β -glucan on its receptors, recognition of proteins, immune-related enzymes, immune-related gene expression and their mechanisms of action.

KEYWORDS

β–Glucan;
Prophenoloxidase;
Immunostimulant;
Aquaculture.

INTRODUCTION

Among various immunostimulants, β-Glucans stands apart because its mode of action has been clearly established, when administered into an animal, β-glucan is readily recognized by the animal and this recognition triggers an immune response reaction in the animal. The cells and specific sites on the cells that play a role in this immune response have been studied in detail. It is now well established that cells involved in the immune response by a broad range of animals have specific receptors with which the β-glucan binds leading to a cascade of events that result in an increased immune status of the animal. The receptors have been identified and characterized in the white blood cells of fish and haemocytes of crustaceans. The primary response to β -glucan in animals is the activation of non-specific defence mechanisms that seek to protect the animal against any type of invading pathogen. So β -glucan also improves the efficacy of vaccination as an adjuvant.

β-Glucans are naturally occurring polysaccharides with glucose as structural component, linked by b-glycosidic bonds. In nature, β-glucans are wide spread in the cell wall of many plants (wheat, rye, barley, and oat), baker's and brewer's yeast (*Saccharomyces genus*), and Echinaceae members[1]. Other sources of β -glucan include seaweed like *Laminaria* sp.[2], various species of mushrooms such as Shiitake (*Lentinus edodes*), Maitake (*Grifola frondosa*), Reishi (*Ganoderma lucidum*)[3], Schizophylan (*Schizophyllum commune*), and SSG (*Sclerotinia sclerotiorum*)[4], and certain fungi (*Agaricus subrufesuns*). β -glucans are also the structural constituents of some of the pathogenic fungi, *Pneumocystis carini*[5], *Cryptococcus neoformans*[6], and some bacteria belonging to Rhizobiaceae family[7]. The common sources of β -glucan are derived from the cell wall of baker's yeast *Saccharomyces cerevisiae* and the most important among all are β -1, 3 and 1, 6 glucan. Glucans are heterogeneous group of glucose polymers, consisting of a backbone of β (1, 3)-linked β-D-glucopyranosyl units with β-(1,6)-linked side chains of varying distribution and length. β -glucans derived from different sources have differences in their structure. Oat and barley β -glucans are linear with β (1, 4) and (1, 3) linkages. Mushrooms β-glucans have short β (1, 6)-linked branches from β (1, 3) backbone. Yeast β-glucans have β (1, 6) branches further with additional β (1, 3) regions. These structural differences can trigger difficulties in extraction and differences on their activity. Larger molecular weight glucans activate leukocytes, stimulating their phagocytic, cytotoxic, and antimicrobial activities, and production of reactive oxygen species (ROS). Low molecular weight glucans have less cellular effects, whereas very short glucans are considered as inactive[8]. Studies have shown that insoluble (1, 3/1, 6) β-glucans have greater biological activity than that of its soluble (1, 3/1, 4) counterparts[9]. Basically, glucan molecules are of two types on the basis of glycosidic bonds present in them, that is, α -glucan (dextran with 1,6, starch with α -1,4- and α -1,6-glycosidic bonds) and β -glucan (cellulose with β -1,4, zymosan with β -1,3, laminarin with β -1,3- and β -1,6, lichenin with β -1,3 and β -1,4 glycosidic bond). Because of complex structure in β-glucans, they have superior ability to activate the immune response and act as biological response modifiers[10].

β-glucan also exhibit hypocholesterolemic and anticoagulant properties. Recently, they have been demonstrated to be anti-cytotoxic, antimutagenic and antitumorogenic, making them promising candidate as pharmacological promoters of health.

β -glucan refer to the glucose linkages which are classified as follows;

- 1, 3 glucan linkage
- 1, 4 glucan linkage
- 1, 6 glucan linkage
- 2, 3 glucan linkage
- 3, 6 glucan linkage

Properly manufactured β 1, 3 glucan, more commonly known as β 1, 3-D glucan, has only a small amount of the other β-glucan present in the finished material where as β-glucan derived from oats and barley have a higher percentage of the 1, 4 β-glucan linkage present and have only been shown to provide beneficial effect in cardiovascular health. This may explain the specific health benefits associated with barley and oat derived β-glucan products.

Types of glucans

1 Alpha glucans
2 Beta glucans

α- and β-glycosidic bonds are differentiated depending on whether the substituent groups on the carbons flanking the ring oxygen are pointing in the same or opposite directions in the standard way of drawing sugars molecule.

Alpha glucans

Dextran	-α-1,6-glucan
Glycogen	-α-1,4- and α-1,6-glucan
Pullulan	-α-1,4- and α-1,6-glucan
Starch	-α-1,4- and α-1,6-glucan

Beta glucans

Cellulose	-β-1,4-glucan
Curdlan	-β-1,3-glucan
Laminarin	-β-1,3- and β-1,6-glucan
Chrysolaminarian	-β-1,3-glucan
Lentinan	-a strictly purified β-1,6:β-1,3-glucan
Lichenin	-β-1,3- and β-1,4-glucan
Pleuran	- β-1,3- and β-1,6-glucan
Zymosan	-β-1,3-glucan

Source of β-glucan

1 They commonly occur as cellulose in plant & trees and especially found in cereal grains of family Graminaceae (Oat, wheat, Rice, Barley etc)
2 In the cell wall, they are found in as example-
I Baker's yeast (*Echinacea* and *Saccharomyes*

cerevisiae)

II Medicinal mushrooms (Reishi, Shiitake and Maitake)

III Certain types of fungi (*Agaricus subrufescens)*

IV Bacteria (belonging to Rhizobiaceae etc) & seaweeds

The four most popular sources of β-glucan are:

(1) Yeast

It is one of the most studied considered as "the mother of all β glucan's" for its immune system support results as well as in a wide variety of other areas such as on radiation injuries, tumors and has also been shown cholesterol lowering properties.

(2) Barley

It is a source of dietary fiber and heart health.

(3) Oat

It is a source of dietary fiber and heart health and has shown beneficial hypocholesterolemic effects. Barley & Oat act within the colon and intestinal tract and reduce cholesterol, lowers blood sugar level & insulin.

(4) Mushrooms

Studies show mushroom derived glucan support immune system function however not at the effectiveness of yeast derived glucan. They stimulate tumor necrosis factor; block colon cancer cell progression, protected lymphocyte blood cells from undergoing DNA damage etc.

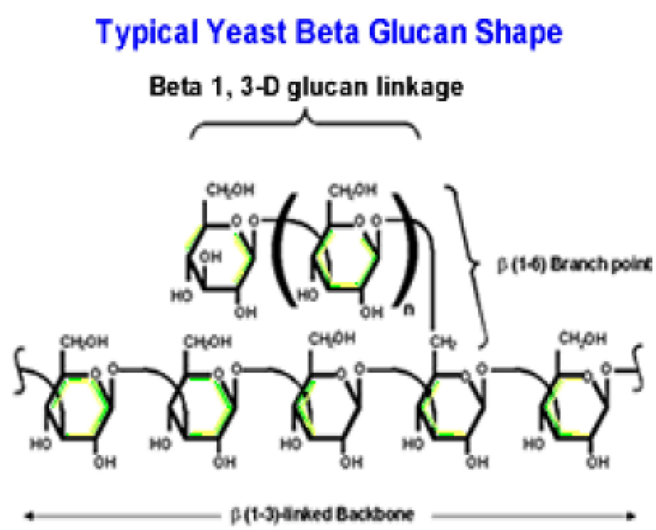
Yeast β glucan

β -glucan is a complex polysaccharide that can be connected in several different ways, including (1,3) β glucan, (1,4) β glucan and (1,6) β glucan (they are mainly a combination of all three). These are medium-chain molecules that can be extracted from Baker's yeast, mushrooms, oats and barley. It isn't completely clear whether or not there are absolute differences between the various derivations of β -glucan. For example, oat and barley β-glucan appear to remain within the colon and intestinal tract and help lower cholesterol and reduce the insulin and blood sugar levels in the body. Mushroom β-glucan, on the other hand, appears from available research to have some anti-tumor and immune system effects.

Research on yeast β-glucan is a bit different and a great deal more detailed than the research performed on the other sources of β-glucan. In fact, a great deal of detailed research has been done on yeast β-glucan showing it to have a substantial effect on the immune system, on injuries due to radiation and on tumor regression. The research is so precise that exact receptor sites on immune cells have been discovered that give β-glucan to immune supporting and tumor fighting effects.

In one research effort, it was found that yeast (1,3) β-glucan binds directly to the Dectin-1 receptor on macrophages, giving us our first line of defense against dangerous bacteria and other health challenges. Dectin-1 is believed to be how β -glucan is taken up into the body in the intestinal tract, as well as how it activates the macrophage to do its job as a molecular scavenger.

β -glucan has binding sites to similar receptors on human monocytes, activating them as part of the immune system. Immune cells, such as neutrophils, re-



lease leukotrienes which are active in the infection-fighting process. Beta glucan is known to be a part of this activation.

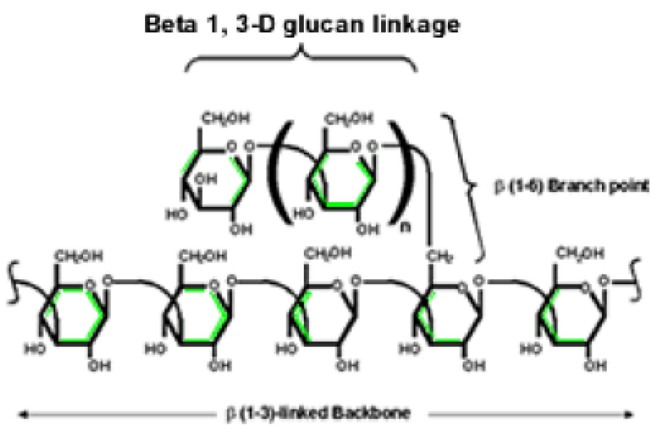
Yeast Beta glucan has a potential role in the prevention of sepsis following surgery, in radiation injury, in cancer irradiation and in the prevention of pathogenic infections. The data is very strong and has been determined down to the biochemical. The greatest problem with yeast derived β -glucan is the quality issue. Much controversy surrounds glucan products these days. This controversy centers on the quality issue(s) of purity level and molecular 1, 3-d linkage. In order to be effective the material must contain the proper 1, 3-d linkage.

Mushroom beta glucan

A ubiquitous polysaccharide, β-glucan is a bioactive molecule found in many different substances. When it comes to our health, the main sources of β-glucan include barley, oats, yeast and mushroom because β-glucan is a long-chain polysaccharide, the different sugars can be linked in several different ways, which allows for different appearances and functions to the different forms of β-glucan. Glucose (glucan) linkages of (1-3), (1-4) and (1-6) link types are known to occur and are known to have some health benefits. It is not well known if the linkage type makes a difference in how the substance works or if longer chains are better than shorter chains. However, the medical evidence does point in that direction.

Mushroom β-glucan and yeast β-glucan appear to have some overlapping features. The mushroom source of β-glucan has been used in many countries in Asia for health purposes for a long time. β-glucan extracted from different mushrooms now has trade names used for research and health uses, like Lentinan, Schizophyllan, Krestin and PSK. Having these kinds of standardized mushroom β-glucan can make it easier to compare research studies between laboratories.

Typical Mushroom Beta Glucan Shape



β-glucan is derived from the ripe fruiting bodies of various mushrooms. Mushroom β-glucan has shown effectiveness as an anti-tumor defense and as an immune system booster. In one recent study, mushroom β -glucan was able to activate and promote the release of infection-fighting cytokines. However the immune response results of yeast-derived β-glucan have shown to be more effective. It has also been shown to provide positive results on cholesterol and blood sugar challenged individuals.

Beta glucans are commercially available as

Glucasan^{R}

A non wheat yeast derivative is taken as a human food supplement. Now available in the UK.

Leucogard^{R}

A poultry, swine and fish food supplement used as an alternative to antibiotic growth additives.

Glucaferm^{R}

Specially developed for use in the very latest face creams; it firms the skin and reverses the signs of skin ageing.

Health-promoting activities

β -Glucans are believed to have various immunomodulatory properties. Studies in vitro and in vivo reveal that the immunostimulating activity of β-glucan depends on structure, molecular weight and number of branches. β-Glucans act through stimulation of the immune system, exerting a beneficial effect against a variety of bacterial, viral, fungal and parasitic. The immunostimulating effect of β -glucan is probably associated with the activation of cytotoxic macrophages and T-helper and natural killer (NK) cells and with the promotion of T lymphocyte differentiation and activation, for the alternative complement pathway. β-Glucans have also been described as modulators of both humoral and cellular immunity. β (1, 3)-D-Glucans from fungi were shown to be capable of having beneficial effects in pre-inflammatory responses, indicating that β-glucan can be a modulator of the anti-inflammatory response as interleukin mediators. Animal studies indicate that β-glucans, when used as a nutritional supplement, stimulate growth and improve nutrient retention and immune system function, the latter by stimulating CD8 and TCR1 cells. It has been demonstrated that Candida albicans (yeast) b-glucans activate macrophages and induce interleukin (IL-6) and tumor necrosis factor (TNF) in vitro, promoting vascular permeability and stimulating the classic complement pathway. β-Glucans from mushroom mycelium show larger molecular weights than β-glucans from yeasts. However, the two β-glucans demonstrate similar ability in the induction of macrophages and chemotactic factor. When blood cells from hepatitis C patients were exposed to *Agaricus blazei* extract, a β-glucan mediated immunomodulatory effect was observed in monocytes. However, the immunomodulatory activity observed in vitro and in animal models were not observed in vivo in humans, possibly due to the fact that β-glucans are not absorbed well by the intestine. β -Glucan from oats has been demonstrated to have antimicrobial effects against *E. coli* and *B. subtilis*. In a comparison of cationic and native β -glucans, the latter was shown to inhibit the growth of these bacteria by approximately 35% while the cationic one was found to cause 80% inhibition in both microorganisms, indicating that β-glucan amination promotes antimicrobial effects. *Saccharomyces cerevisae* β-glucan extract was shown have antimicrobial activity in mice, against *Staphylococus aureus* resistant to antibiotics, because β-glucan administration helps in the elimination of bacteria and increases the number of monocytes and neutrophils, thereby resulting in antibiotic potential. The combination of an antifungal agent and β-glucan in paracoccidiomycosis treatment was demonstrated to improve therapeutic response, where the patients that received only the antifungal agent had more frequent relapses than the group that received the β-glucan–antifungal combination. The administration of β-glucan to mice infected with *Eimeria vermiformes* showed increased resistance to infection due to immunomodulation, which involved non-specific as well as specific response. β-glucans partially restored T and B cell response to the mitogen in mice infected by Toxocara canis, reducing the larval number found in the muscles of the animals that received β -glucans. Survival increased in mice exposed to Venezuelan equine encephalomyelitis virus, when pre-treated with β-glucan. b-Glucan produced higher resistance in mice to virulent *Francisella tularensis* when pre-treatment was given intravenously in comparison with intranasal administration. In addition, the vaccine against Venezuelan equine encephalomyelitis virus combined with b-glucan was found to be more protective in mice and monkeys. β-glucans are not degraded by human enzymes, which provide them with nutritional fiber properties. The greatest interest in these fibers is due to their demonstrated protective hypocholesterolemic effect, reducing risk of chronic diseases. It is known that β -glucans reduce blood cholesterol levels. The ingestion of β-glucan increases intestinal viscosity and reduces cholesterol absorption, thereby promoting its excretion.

Beta glucan as immune stimulant

1 Glucans function as potent immunostimulants in mammals and fish.

2 Immune-stimulants are chemical compounds that activate WBCs & may render fish and other animals more resistant to infections by viruses, fungi bacteria and parasites.

3 β-1,3-glucans may improve health, growth and general performance of many different animal groups, including farmed shrimp, fish and other land animals.

- In fish, they can activate macrophages, increasing their capacity to kill pathogens[11].
- They have also been shown to reinforce other non-specific immune factors such as lysozyme and complement activities[12].
- When administered orally, glucans have a proven protective effect in several fish species and against a variety of bacterial pathogens including *Aeromonas hydrophila*[13] *Edwardsiella tarda*[14] *& Vibrio salmonicida*[15].

Mode of action

- β-1,3/1,6-Glucans bind specifically to a receptor molecule on the surface of phagocytes. This receptor is found in all animal groups from invertebrates, such as shrimp, to man.
- When the receptor is engaged by β -1,3/1,6-glucan, the cells become more active in engulfing, killing and digesting bacteria and secrete signal molecules (*cytokines*) which stimulate the formation of new WBC.
- Animals with specific immune mechanisms in addition to non-specific defense (fishes and higher up in evolution), the activated phagocytes produce cytokines which also activate antibody-producing white blood cells (B- and T-cells).
- Therefore β -1,3/1,6-glucan enhances also the efficacy of vaccines.

Experimental dosage & effects of beta glucan in fish[16,17]

- An experiment was carried using multiple injections of different dosages of β-glucan from barley.

- To show that it would enhance the immune response and disease resistance against opportunistic pathogens *Aeromonas hydrophila* & *Edwardsiella tarda* in *Labeo rohita fingerlings*.
- They has shown that injections of 10 mg of beta-glucan kg^{-1} body wt. for three times can be advocated to enhance the immune response of fish species under aquaculture after testing with 4 dosages 0, 5, 10, 15 mg kg^{-1} of barley β -glucans in PBS.

Research findings

Effect on *Labeo rohita* fingerling

- Four different Diets with 0, 100, 250 or 500 mg of β-glucan kg^{-1} diet were fed for 56 days to *Labeo rohita* fingerling by Misra *et al*.,[16,17].
- Biochemical and hematological parameters were evaluated at two week intervals.
- After 56 days, the SGR & FCR were calculated and fish were challenge with *A. hydrophila* & *E. tarda* in 2 groups.

Effect against *Vibrio harveyi* infection in large yellow croaker[18]

- Diet supplemented with 0% (control), 0.09% (low) and 0.18% (high) beta-1, 3 glucan was used to investigate the effects on the innate immune response and protection against *Vibrio harveyi* infection in large yellow croaker.
- The results of 8 weeks feeding trial showed that low glucan supplementation significantly enhanced fish growth, whereas high supplementation did not & fish fed the diet with high glucan had significantly higher lysozyme activity compared with low beta-glucan.

Effect in sea bass

- Study on the immunomodulatory activity of yeast derived glucan on innate and specific immunity in sea bass was carried by Bagni *et al*.[19].
- Serum complement, lysozyme, total proteins and heat shock protein (HSP) concentrations were measured at 15, 30 and 45 days. Significant elevation in serum complement activity at 15 days, serum lysozyme, gill and liver HSP concentration at 30 days & no significant change at 45 days was seen.
- They have shows some innate immune responses in sea bass, particularly under conditions of immunodepression related to environmental stress.

Effects of β-glucans on carpet shell clam (*Ruditapes decussatus*) & mediterranean mussel (*Mytilus galloprovincialis)*

- Effects of β-glucans on carpet shell clam (*Ruditapes decussatus*) and Mediterranean mussel (*Mytilus galloprovincialis*) were determined by Costaa *et al*.[20].
- β-glucan treated mussels and clams showed enhanced Nitric oxide.
- In clams, hemolymph treated with several doses of β-glucans limited the growth of the three bacteria, *Vibrio algynolyticus* (strain TA15), *Vibrio splendidus* (strain TA2) and *Escherichia coli*. These results were not shown by mussel.
- They suggested that the immune responses of these animals can be up and down modulated by external stimuli & there behavior to immunity varies.

Effect on juvenile olive flounder

- Effects of dietary supplementation of β-1,3 glucan and a laboratory developed feed stimulant, BAISM, as feed additives for juvenile olive flounder, *Paralichthys olivaceus* were studied by Yoo *et al*.[21].
- Eight experimental diets were formulated.
- These results indicated that the optimum dietary supplementation level of β-1,3 glucan and BAISM could be approximately 0.10% β-1,3 glucan + 0.90% BAISM (G0.1B0.9) of diet.

Future perspectives

β-Glucans have been shown to possess important biological properties regardless of origin TABLE 1. This finding calls for future perspectives into wide production through biotechnology using microorganisms such as *S. cerevisae*, as well as the insertion of specific genes for the production control of a particular b-glucan in foods (food transgenic), depending on its eventual purpose as a pharmaceutical product or functional food, respectively. The identification of foods that produce high levels of these polysaccharides and that can undergo modifications of the b-glucan gene control to improve their absorption and consequently efficacy; can be an approach in the production of functional foods with medicinal properties. Besides, the consumption of food with antimutagenic activity could contribute to a reduction in risk of cancer and of other degenerative diseases. However, further studies are needed involving epidemiologic evaluation of the public's intake of cereals such as oats and barley which are β-glucan-rich foods, for the moment no epidemiological study concerning the incidence of cancer and consumption of β-glucan is available, the only epidemiological study involving β-glucan relates its consumption with the decrease of cholesterol levels. Furthermore, a better future perspective would be the use of β-glucan in supporting the treatment of cancer patients submitted to chemotherapy, to improve immunologic status and reduce untoward effects on normal tissues, although attention must be paid once the reduction of isoenzymes of the CYP450 family could lead to low level of activation of the chemotherapeutic drug, leading to higher presence of the drug in the organism Also use of intravenous solution of b-glucan should be used carefully due to the possibility of granuloma formation and hepatosplenomegaly. β-Glucan may, in some cases, have use as an antitumor agent, but clinical trials in humans are still needed. Studies of β-glucans have shown these polysaccharides to be important and promising substances in the promotion of health in humans, and that further investigation is warranted.

TABLE 1 : Structure, origin and biological activities of β - glucans

Structure	Source	Effects
β (1,3) (1, 6)	*Saccharomyces cerevisae*	Antiparasitic
		Antibacterial
		Antiviral
		Antifungal
		Antimutagenic/antigenotoxic
		Antitumoral
		Hematopoietic stimulator
		Mitogenic
	Candida albicans	Imunostimulating activity
	Poria cocus	Antitumoral
	Agaricus blazei	Cytokine induction
		Antimutagenic/antigenotoxic
	Lentinus edodes	Inhibition of CYP450 isoenzymes
		Antitumor
	Schizophyllum commune	Antitumor
	Coriolus versicolor	Antitumor
β (1,3) (1, 4)	Oat	Antimicrobial
		Antiparasitic
		Hypocholesterolemic
		Anti-thrombotic
	Barley	Antimutagenic

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