Minireview Article

A mini-Review : The Role of Chitosan in Aquaculture Fish Health Management

ABSTRACT

Sustainable fish farming activities are a hope for all cultivators. Suitable and stable environmental conditions are important factors for the sustainability of fish to grow, reproduce, avoid stress and disease. Providing a balanced diet is one of the environmental conditions that must be considered. Diets equipped with additives have been in great demand by researchers in the field of aquaculture, one of which is chitosan. Chitosan (β-(1-4)-N-acetyl-D-glucosamine) is a natural polymer derived from the exoskeleton of insects, crustaceans, and fungal cell walls, through the process of deacetylation of chitin base. The biological properties of chitosan include immunostimulant, antibacterial, wound healing, analgesic, anti-oxidant, anti-tumor and intestinal microbial control. In addition, chitosan is also a safe additive. Based on some of the biological properties of chitosan, it is possible to apply it in cultivation. Therefore, it is of great interest to researchers in the field of aquaculture. The purpose of this article is to inform the effect of chitosan on fish which includes antibacterial activity against several types of bacteria that attack fish, immunostimulant activity and growth in fish. Based on the results of research and tests from several researchers described above, chitosan has the potential to be used in the management of fish health, because it has antibacterial, immunostimulating effects, is safe to use for sustainable aquaculture because it does not have toxic effects and has a positive impact on the growth of various fish species, and can eliminate heavy metals in the fish body. Chitosan can be given through feed or injection. Chitosan can be applied effectively for the management of fish health, if the concentration used is appropriate, adjusted for the type of fish and the type of disease.

Keywords: chitosan; disease; antibacterial; immunostimulant.

1. INTRODUCTION

Fish health management is a wise step in fish farming activities to produce healthy fish that can grow optimally. Disease control is part of fish health management. Disease prevention measures are the right steps in disease control, because they are relatively easier and cheaper than treatment measures when fish are already sick. Efforts to treat diseases in cultured fish are still using antibiotics. However, the use of antibiotics has been met with increasing opposition because of their negative long-term effects on the environment and potential harm to human consumers [1]. In addition, the price of antibiotics is relatively expensive, if the use of antibiotics to treat sick fish on a pond scale causes high costs, so it is less efficient [2]. To avoid the negative impact of the use of antibiotics, efforts to control disease in the aquaculture system can be carried out with preventive measures using natural ingredients that are relatively effective, cheaper and safer for fish and humans as well as environmentally friendly. Chitosan is a fishery waste that comes from crustacean skins after undergoing demineralization, deproteination, and deacetylation. The basic ingredients of chitosan are easy to obtain, available in large quantities,

and have not been used optimally. Chitosan is a natural polymer that has a high molecular weight, is biocompatible, non-toxic, eco-friendly, and biodegradable (3, 4). Besides being able to be used for disease prevention (immunostimulation), because it can induce the immune system, it can also be used as a medicine, because it is antibacterial and accelerates wound healing [5 , 6]. Dietary formula feed treated with chitosan is safe for fish and beneficial for human health [7]. The purpose of this study was to describe the role of chitosan as a natural ingredient to manage the health of aquacultured fish.

2. ARTICLE WRITING METHOD

In writing this scientific article, it is done through a literature search from various sources. The main library source search is done through the website. All references are references from primary sources. The literature search used the keywords 'chitosan', 'immunostimulant', 'disease', 'antibacterial'.

3. RESULTS AND DISCUSSION

3.1 The Content and Benefits of Chitosan

Chitosan is a natural compound with many benefits, obtained from the exoskeleton of crustaceans, insect cuticles and cell walls of some microbes, containing (1-4)-linked 2-acetamido-2-deoxy-β-D-glucose (N-acetyl glucosamine) which is a product of base deacetylation of Chitin (poly(ß-(1-4)-N-acetyl-D-glucose-amine) [8, 9]. Chitosan is an important polymer, used in various fields. In industry and agriculture, used to protect and stimulate plant growth, as a preservative, thickener, and stabilizer in the manufacture of sauces, fruit coatings, seeds and frost protection [10, 11, 12]. severe (14).Chitosan has biological effects, namely as an immunostimulant and adjuvant in cultured fish [15, 16], antibacterial [17], wound healing [18], analgesic [19], anti-oxidant [20], anti-tumor [21, 22]. For land animals, chitosan can as an additive added to feed has the effect of increasing growth and endurance, lowering cholesterol levels and controlling intestinal microbes [23, 24]. Fish feed formulations added with chitosan can increase protein content, reduce lipid and water content of fish meat [25, 26]. Chitosan affects mineral content in humans [27]. From the description above, it can be seen that the exoskeleton of crustaceans and insect cuticles cannot be used directly before becoming chitosan. Chitin contained in the exoskeleton of crustaceans and insect cuticles is a precursor in the manufacture of chitosan. Chitin is obtained through acid processing (demineralization), then followed by alkaline processing (deproteination), and finally the decolorization stage. Meanwhile, chitosan is obtained by deacetylating the formed chitin. To obtain chitosan, it is necessary to have N-acetylated levels lower than 50%, depending on the origin of the polymer. Therefore, chitosan is a chitin derivative with deacetylated base [28]. The polycationic nature (with a glucosamine group) possessed by chitosan causes chitosan to have many benefits, because it can chelate anything with negative mutants, such as fat (useful as a dietary supplement), cancer (as anticancer), would healing dressing, drug delivery, water purification, etc. So the application is quite wide for food and non-food [29].

3. 2 Antibacterial Activity

Several studies have proven that chitosan has antibacterial activity, including bacteria that usually attack freshwater fish, which is the focus of this article. In vitro chitosan was able to inhibit the growth of four strains of pathogenic bacteria, namely *Aeromonas veronii* B55, *A. veronii* Aero1, *Enterococcus faecalis* F1B1, and *E. Faecalis* PS6. The doses of chitosan used were T0 (without chitosan/control), T1(0.1%), T2 (0.2%) and T3 (0.3%). A dose of 0.1% can inhibit the growth of the four bacteria which is quite large. The density of *Aeromonas veronii* B55 in the control treatment was $(2.9\pm0.047)\times10^{7}$ CFU ml-1 and after being treated with chitosan it became $(1.5\pm0.12)\times10^{4}$ CFU ml^{-1}. The density of *A. veronii* Aero11 in the control treatment was $(2.9\pm0.026)\times10^{7}$ (CFU ml^{-1}) and after being treated with chitosan it became (1.78±0.06)×103 CFU ml^{-1}, the bacterial density of *Enterococcus faecalis* F1B1 in the control treatment was ($3.0\pm0.08)\times10^{5}$ CFU ml^{-1} and after being treated with chitosan it became $(1.83\pm0.09)\times10^{3}$ CFU ml^{-1}, and the density of E. faecalis PS61 bacteria in the control treatment was $(2.93\pm0.04)\times10^{5}$ CFU ml^{-1} after being treated with chitosan it became $(1.53\pm0.25)\times10^{3}$ CFU ml^{-1} [7] (Table 1).

Table 1. Effects of chitosan on the in vitro growth of fish pathogenic bacterial strain

Fish pathogenic bacteria	Bacterial density (CFU ml-1) at dose	
	T0	T1
Aeromonas veronii B55	$(2.9\pm0.047)\times10^{7}$	$(1.5\pm0.12)\times10^{4}$
A. veronii Aero1	$(2.9\pm0.026)\times10^{7}$	$(1.78\pm0.06)\times10^{3}$
Enterococcus faecalis F1B1	$(3.0\pm0.08)\times10^{5}$	$(1.83\pm0.09)\times10^{3}$
E. Faecalis PS6.	$(2.93\pm0.04)\times10^{5}$	$(1.53\pm0.25)\times10^{3}$

In the table above, it can be seen that the inhibition of pathogenic bacteria by chitosan was greatest in the A. veronii Aero1 strain compared to other bacterial strains. This shows that although the dose of chitosan used is the same, the inhibitory response is different for each type of bacteria. Other types of fish pathogenic bacteria that can be inhibited by chitosan are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* [30]. In addition to the type of bacteria, the dose of chitosan given also affects the inhibition of bacterial growth. As the results of research conducted by Kurniasih and Kartika (2009) showed that the greater the concentration of the chitosan solution, the lower the antibacterial activity. The chitosan dose of 1.4% had the smallest inhibition on the growth of *Staphylococcus aureus* bacteria (density 10^{8} CFU/mL) compared to the 1.0 dose; 0.6; 0.4; 0.2; 0.1 and 0.05%, but the most optimum dose is 0.6%. The dose of chitosan and different types of bacteria also affect the inhibition of bacterial growth. Research conducted by Goy (2009) showed different results, the bacteria *Aeromonas hydrophila*, *Vibrio parahaemolyticus* and *Pseudomonas fluorescens* gave different inhibitory effects to the minimum concentration of chitosan given, each of which was 1000, 150 and 250 ppm, respectively. In addition to the dose and type of bacteria, incubation time also affects the process of inhibiting bacteria. In addition to the dose and type of bacteria, incubation time also affects the process of inhibiting bacteria. Chitosan concentration of 0.8% can inhibit the growth of *Aeromonas hydrophila* bacteria, while *Edwardsiella ictaluri* and *Flavobacterium columnare* bacteria can be inhibited at a lower concentration of chitosan, ie 0.4% with the same incubation time. When the concentrations were reduced by 0.4% (*A. hydrophila*) and 0.2% (*E. ictaluri* and

F.columnare, respectively), a longer incubation time was required to produce maximum inhibition [33]. From the description above, it can be seen that chitosan can act as an antibacterial to inhibit the growth of both gram-positive and gram-negative bacteria. The mechanism of inhibition of bacteria by chitosan can occur through several possibilities, first because there is a difference in charge that interacts electrostatically, where chitosan has an antibacterial functional group, namely an amine that has a positive charge, while the surface of bacteria has a negative charge, so there is a change in the permeability of the membrane. bacterial cells causing an imbalance of osmotic pressure inside the cells which hinders the growth of microbes. In addition, there are changes in the composition of bacteria, including protein, amino acids and glucose, in other words, chitosan inhibits the metabolism of microorganisms which results in cell death. In the cell also occurs hydrolysis events in the cell wall which causes the release of cell electrolytes, which causes the death of a cell. The second possibility is that the positive charge of chitosan interacts with bacterial DNA, resulting in inhibition of RNA and protein synthesis. In this mechanism, chitosan must have a small molecular weight in order to enter the cell of microorganisms. [34 , 31].

3.3 Immunostimulant Activity

Several studies have proven that chitosan can be used as an immunostimulant that can increase non-specific body resistance in several types of cultured fish, which is indicated by an increase in hematological parameters, including the number of leukocytes, erythrocytes, lysozyme, and phagocytosis levels. Chitosan is applied to fish as a supplement that is added to the feed. Tilapia weighing 11.53±2.81g/head after being fed with chitosan added at a dose of 10 ppt, 50 ppt and 100 ppt experienced an increase in leukocyte levels, with the best dose of 100 ppt [35]. In carp the increase in body resistance after being given chitosan for 60 days was based on several parameters, namely serum lysozyme activity, serum bactericidal activity, nitroblue-tetrazolium (NBT) reduction, total serum protein, albumin and globulin, white blood cell count (WBC). The results showed lysozyme activity in all groups of carp treated with chitosan with various levels (1%, 0.5% and 2.5%) and the control did not show a significant difference ($p = 0.087$), as well as serum bactericidal activity did not show significant difference. However, on the 60th day of observation, lysozyme levels (at a concentration of 1%) and serum bactericidal activity (at concentrations of 0.5% and 1%) showed slightly higher values. NBT activity as a respiratory indicator burst activity at concentrations of 1% and 0.5% experienced a significant increase ($p=0.035$) compared to control, as well as the total serum protein and globulin slightly increased at these concentrations ($p=0.085$). All treatments WBC value and blood leukocyte ratio showed no significant difference ($p>0.05$). Although some parameters did not show significant differences, after being challenged with *Aeromonas hydrophila* (density $2.1|x10^7$ CFU) carp treated with 1% chitosan significantly ($p<0.05$) resulted in the lowest mortality (60%± 4,78) and the highest was in the control treatment (76,7% ± 6,7) [9]. In contrast to carp, asian seabass (*Lates calcarifer*) at a dose of 1% chitosan on the 30th and 45th days of observation, several parameters experienced a significant increase (P 0.05) higher than the control and other treatments. These parameters were total erythrocyte count (TEC), total leukocyte count (TLC), serum lysozyme, serum bactericidal activity, respiratory blast activity and phagocytic ratio. Meanwhile, the albumin-globulin ratio parameter on day 15 at a dose of 2% was significantly

(P≤0.05) higher than control and other treatments. After a challenge test with *Vibrio anguillarum* bacteria by intraperitoneal injection with the bacterial suspension of 0.1 ml (1 x 10^6 cfu mL-1) after 60 days of feeding trial, fish were given chitosan with a dose of 1% significantly (P≤0.05) resulted in the highest survival [36]. The provision of chitosan as an immunostimulant to fish, apart from being given through feed, as described above, can also be given by injection into the fish's body. As has been done in catfish, administration of chitosan at various dosage levels (2 g/g, 4 g/g and 6 g/g body weight), then challenged with Aeromonas hydrophila bacteria 10^5 CFU/ml can provide a non-specific immune response. better than the control. The specific immune response observed included the total number of erythrocytes and leukocytes as well as the levels of hematocrit, hemoglobin and phagocytic index. Another observed immune response was the percentage of lymphocytes, neutrophils, monocytes and platelets, which were also better in chitosan-treated fish than control fish. with the highest percentage in the group of fish given 6 g/g chitosan. In addition, this concentration also resulted in the highest survival (93.33%) compared to control and other treatments [37]. From the results of the research described above, it can be seen that the activity of chitosan as an immunostimulant can be indicated especially from an increase in non-specific immune responses which include hematological parameters, namely the amount of serum lysozyme activity, serum bactericidal activity, total number of erythrocytes and leukocytes, hematocrit levels, hemoglobin and phagocytic index and the percentage of lymphocytes, neutrophils, monocytes and trombocytes. As according to Salam (2021) hematological parameters help to interpret the health status of fish. This was proven in several types of fish after being given chitosan, then challenged with pathogenic bacteria resulted in higher survival than control. Capkin and Altinok (2009) suggest almost the same thing, an increase in leukocytes which is part of the hematology parameter indicates an increase in fish immunity, so that fish can avoid disease attacks. The same opinion was expressed by Irianto (2002), that chitosan can cause a significant increase in the number of white blood cells and erythrocytes in fish, so that it can help in increasing non-specific immunity through the work of neutrophils and macrophages which are part of white blood cells in phagocytosing pathogenic bacteria, so that fish can be protected from disease. While the increase in the number of erythrocytes has a positive impact on increasing the amount of oxygen for respiration and metabolic activity of fish [40]. herefore, NBT activity, respiratory burst activity, total serum protein and globulins were used as parameters in observing the immune activity of chitosan. Immune activity of chitosan also occurred in shrimp, where crayfish (*Procambarus clarkii*) which were fed additional feed with chitosan 10 mg/g of feed for 4 weeks produced THC (Total Hemocyte Count), proPO (prophenoloxidase) and SOD (Superoxide Dismutase) activities. which was significantly higher (P < 0.05) than the control, and after being challenged with the white spot syndrome virus (WSSV) the RPS (relative percent survival) value was significantly (P < 0.05) higher than the control and other treatments [41]. Another activity of chitosan is that it can eliminate the toxicity of heavy metals exposed in the body of fish, as evidenced by observing the enzymatic activity of antioxidants, namely superoxide dismutase (SOD) in liver, gill and muscle organs. The study was conducted on four groups of *Oreochromis niloticus* fish (mean weight 35.32±2.4g), each group was given a basic diet of 15 mg/L Pb), each of which was added 0% chitosan (control), 0.3% , 0.5% and 1%. The results showed that the antioxidant enzymatic activity, namely superoxide dismutase (SOD) in the liver, gill and muscle organs in fish given

1% chitosan was significantly higher (P≤0.05) compared to other treatments, each of which was 160.75±7.9, 118.11±7.5 and 66.32±4.4 U/g. Likewise, the total antioxidant activities (T-AOC) in the 1% chitosan treatment were significantly (P≤ 0.05) greater than the other treatments. Histopathological examination results showed the same results, where the gills of tilapia which were given high chitosan (0.5% and 1%) mostly showed relatively normal/healthy gill structure [42]. Here it is seen that chitosan given in the feed can activate the antioxidant system and the innate immune response (phagocytic and lysozyme activity), and protect the tissue in tilapia exposed to heavy metal Pb can protect fish against the cytotoxic effects of Pb and increase the innate immune response against high-invading microbes, especially at a concentration of 1%. From the description of immunostimulant activity, it is shown that chitosan can be used as an immunostimulant that can increase the body's resistance of fish and shrimp to disease attacks, and can also eliminate heavy metals exposed in the fish's body. Each type of fish and each fish disease has a different response to the dose of chitosan given, but the results of the research described above show that the higher the dose of chitosan given, the better the response to the haematological conditions of fish blood, metabolism and in eliminating heavy metals. This shows that chitosan has almost no toxic effect. As the opinion of Gopalakannan and Arul (2006) that chitosan has no toxic effect and does not cause significant morphological changes in fish.

3.4 Growth Effect

Chitosan is not only beneficial for the management of fish health, from several research results chitosan also has a positive impact on the growth of fish and shrimp. Research has been done on the provision of chitosan through feed to increase the growth of tilapia. In this study, the weight of tilapia used was 11.53±2.81g/head, while the doses of chitosan used were 0 ppt, 10 ppt, 50 ppt, and 100 ppt. The results showed that the average final weight of tilapia in P3 treatment was 24.10±6.48 g, significantly (p<0.05) higher than the control (P0) of 17.09±2.81 with the average absolute weight gain was 12.19±3.45 g. The highest total length value was also found in treatment P3 (100 ppt), with a mean length value of 11.79±1.29 cm and an average absolute length value of 2.06±0.19. Tilapia at 100 ppt treatment also produced the highest FCR (feed conversion ratio), ADG (Average Daily Growth), SGR(specific growth rate) and FE (Feed Efficiency) values, each of 3.48±2.25; 0.13±0.03; 0.85±0.84 and 28.70±16.54. While the trend values obtained in the control treatment were 11.44±2.81 (FCR), 0.03±0.01 g/day (ADG), 0.29±0.05 (SGR) and 8, 75±16.65 % (FE) [34]. Caspian *kutum (Rutilus frisii* kutum) with an average weight of 1.7 ± 0.15, g were fed a diet containing chitosan at different levels (0, 0.25, 0.5, 1 and 2 g kg-1) during 60 days showed different results on growth performance. The results showed that the FCR was significantly lower in fish whose feed contained 0.1% chitosan, compared to other groups (P < 0.05), but there was no significant difference (P > 0.05) between treatment with specific growth rate and condition factors [44]. Juvenile fish *Barbonymus gonionotus* after being given chitosan added to the feed at doses of T0 (without chitosan/control), T1(0.1%), T2 (0.2%) and T3 (0.3%) for 60 days in an increase in different weights , respectively 26.11 ± 1.40 g, 39.40 ± 1.47 g, 34.37 ± 1.24 g, and 30.17 ± 0.60 g. From this data, it can be seen that the fish seed treated with chitosan was in a significantly higher weight gain (P < 0.05) than the fish fry in the control treatment. Fish seeds that were given 0.1% chitosan significantly (P < 0.05), in the highest weight gain compared to all treatments at all observation times (days 15, 30,

45, and 60). Similar to the results of SGR (specific growth rate) observations, fish fed with 0.1% chitosan produced the highest SGR value, which was 1.80±0.08%/day. While the control fish, 0.2% and 0.3% SGR values resulted in SGR values of 1.39±0.04, 1.68±0.53 and 1.53±0.014 %/day, respectively. The results of observations on the FCR of fish fry showed that the T1 treatment significantly produced the lowest value, which was 1.20±0.029, while the FCR values for the control treatment, T2 and T3 were 1.45±0.022, 1.30±0.02 and 1.38±0.006, respectively. increased significantly with increasing dietary dose of chitosan and chitosan dose of 0,1% resulted in the best growth performance [7]. The description above shows that in general chitosan has a positive effect on fish growth and FCR, this is because chitosan supplementation in fish can increase the concentration of GH (Growth hormone) in serum and amelioric morphological structures of the small intestine. The small intestine is a major organ in the process of digestion and absorption of nutrients, and plays an important role in the intestinal mucosa [34]. In research Zaki *et al*. (2015), the length of the villus can increase along with the increasing dose of chitosan. In addition, chitosan provides a beneficial environment for enterocyte proliferation, preventing intestinal atrophy [46]. However, from the results of the research described above, it shows that the increase in the dose of chitosan given was not accompanied by an increase in body weight or a decrease in the value of FCR. Fish fed with chitosan-mixed feed, both as an immunostimulant and for safe growth for fish, did not cause toxicity and did not have significant morphological changes in fish [43].

4. CONCLUSION

Based on the results of research and tests from several researchers described above, chitosan has the potential to be used in the management of fish health, because it has an antibacterial, immunostimulating effect, is safe to use for sustainable aquaculture because it does not have toxic effects and has a positive impact on the growth of several types of fish and fish. can also eliminate heavy metals in the fish body. Chitosan can be given through feed or injection. Chitosan can be applied effectively for the management of fish health, if the concentration used is appropriate, adjusted for the type of fish and the type of disease.

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