Minireview Article

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

ABSTRACT

Disease attack is one of the obstacles in fish farming activities, therefore fish health management through health improvement needs to be done so that fish are protected from disease attacks. The use of natural ingredients that contain antibacterial, immunostimulant is one way that can be used to improve fish health, which is safe and relatively inexpensive. Chitosan is a natural ingredient which is mostly sourced from shrimp shells that have gone through a de-acetylation process that can be used for fish health management. Based on the results of research and tests from several researchers, chitosan has the potential to be used in the management of fish health, because it has an antibacterial, immunostimulating effect, is safe to use, because it does not have toxic effects and has a positive impact on the growth of several types of fish.

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, making it 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 by 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 as a natural polymer has a high molecular weight, 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]. Dietary formula feed processed with chitosan is safe for fish and beneficial for human health [6]. The purpose of this study was to describe the role of chitosan as a natural ingredient to manage the health of aquacultured fish.

2. THE CONTENT AND BENEFITS OF KITOSAN

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 de-acetylation of Chitin (poly(ß-(1-4)-N-acetyl-D-glucose-amine) [7, 8]. 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 [9, 10, 11]. Other benefits include water purification, removal of metal ions and ecological polymers. and reduce odor in water treatment [12]. Chitosan has biological effects, namely as an immunostimulant and adjuvant in cultured fish [13, 14, 15], antibacterial [16], wound healing [17], analgesic [18], anti-oxidant [19], anti-tumor [20, 21]. 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 [22, 23]. Fish feed formulations added with chitosan can increase protein content, reduce lipid and water content of fish meat [24, 25]. Chitosan affects mineral content in humans [26].

3. ANTIBACTERIAL ACTIVITY

Several studies have proven that chitosan has antibacterial activity. 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(1 g kg-1 feed), T2 (2 g kg^{-1} feed) and T3 (3g kg^{-1} feed). The dose of 1 g kg^{-1}/feed can inhibit the growth of the four largest bacteria. The density of *Aeromonas veronii* B551 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 Aero*11 in the control treatment was $(2.9\pm0.026)\times10^7$ (CFU ml-1) and after being treated with chitosan it became $(1.78\pm0.06)\times10^3$ 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±0.09)×103 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 [6]. Chitosan provides antibacterial activity against several types of bacteria, including *Escherichea coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* B [27]. As the results of research conducted by Kurniasi and Kartika (2009) [28] chitosan can inhibit the growth of *Staphylococcus aureus*. The chitosan used was first dissolved in acetic acid with various concentrations of 1,4; 1.0; 0.6; 0.4; 0.2; 0.1 and 0.05% (w/v). while the density of *S. aureus* bacteria used was 10^8 CFU/mL. Based on the results of the study, it was shown that the greater the concentration of the chitosan solution, the lower the antibacterial activity. Chitosan can inhibit the growth of other pathogenic bacteria that attack fish, namely *Aeromonas hydrophila*, *Vibrio parahaemolyticus* and *Pseudomonas fluorescens*, respectively at minimum concentrations of 1000, 150 and 250 ppm [29]. The results of other studies show that chitosan can inhibit the growth of several other types of pathogenic bacteria of freshwater fish, namely *Aeromonas hydrophila*, *Edwardsiella ictaluri* and *Flavobacterium columnare*. Chitosan concentration of 0.8% can inhibit the growth of *A. hydrophila*, while bacteria *E ictaluri* and *F. columnare* showed higher sensitivity to chitosan, at 0.4% chitosan concentration the growth of these two bacteria could be inhibited within 24 hours of incubation at temperature 28^oC. At a

lower concentration of chitosan (0.4%) and a longer incubation time the growth of *A. hydrophila* bacteria was inhibited, while for bacteria *E ictaluri* and *F. columnare* it occurred at a concentration of 0.2% [30]. 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 which has an antibacterial functional group is an amine with a positive charge, while the surface of bacteria has a negative charge, then there is a change in the permeability of the bacterial cell membrane resulting in a change in the permeability of the bacterial cell membrane. causes the osmotic pressure inside the cell to be imbalanced 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, which results 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. [31, 28].

4. IMMUNOSTIMULANT ACTIVITY

Several studies have proven that chitosan can be used as an immunostimulant that can increase the body's resistance to several types of cultured fish against pathogenic bacterial attacks and can also avoid bacterial resistance. Suhenda et al. (2008) [32] conducted a study using chitosan doses of 0 g/g (control), 2 g/g, 4 g/g and 6 g/g given to catfish (*Clarias* sp.) via intramuscular injection. . The results showed that catfish fed with chitosan gave a better non-specific immune response than the control. Indicated by an increase in hematological parameters, namely the number of erythrocytes, leukocytes. hematocrit, hemoglobin and phagocytic index levels. As according to Salam (2021) [6] hematological parameters help to interpret the health status of fish. Feeding added with chitosan caused a significant increase in the number of erythrocytes and white blood cells, which might help in increasing nonspecific immunity through neutrophils and macrophages [33]. An increase in the number of erythrocytes has a positive impact on increasing the amount of oxygen for respiration and high metabolic activity [34, 35]. After 7 days of administration of chitosan, the fish were challenged with *Aeromonas hydrophila* bacteria 10^5 CFU/mL. On day 6 (end of observation) after the challenge test, fish given chitosan produced higher survival rates than the control. Catfish treated with chitosan doses of 2 g/g, 4 g/g, and 6 g/g resulted in 80%, 83.33% and 93.33% survival, respectively, while control fish survival was 53.33%. High survival rate in the treatment of 2 g/g, 4 g/g, and 6 g/g, due to the role of chitosan in increasing specific immune responses, including leukocytes. As according to Capkin and Altinok (2009) [36] leukocytes can increase fish immunity, so it can prevent fish diseases. Catfish treated with 6 g/g chitosan in addition to producing high survival rates, also produced a better percentage of lymphocytes, neutrophils, monocytes and platelets compared to other treated fish and controls. Recovery of appetite after the challenge test, catfish given 6 g/g chitosan was also faster, namely on the 3rd day there was a recovery in response to eating. Rozi et al. (2018) [37] have conducted research on the administration of chitosan through feed to increase the immunity of tilapia which is indicated by an increase in the number of leukocytes. In this study, the weight of tilapia used was 11.53±2.81g/ind, while the dose of chitosan used was P0: without

chitosan (0 ppt), P1: 10 ppt, P2: 50 ppt, P3: 100 ppt. The results showed that the highest number of erythrocytes in tilapia occurred in the treatment of chitosan P1 (10 ppt) of 2.87 x 10^6 ± 5.25 cells/mm3, while the lowest occurred in control fish P0 (0 ppt), which was 2.15 x 10^6 ± 7.76 cells/mm^3. Meanwhile, the highest number of leukocytes in the P3 treatment (100 ppt) was 7.97 x 10^4 ± 2.39 cells/mm3 and the control fish were 1.10 x 10^4 ± 1.42 cells/mm^3. Here it is seen that chitosan can increase fish immunity. Giving chitosan to seeds (average weight of 15 ± 2 g) of asian seabass (*Lates calcarifer*) through feed for 60 days with different doses (control/fish were fed normal diet without chitosan, 5 g kg^{-1}, 10 g kg^{-1} and 20 g kg^{-1} feed) resulted in different haematological conditions. The fish group that was given chitosan significantly increased the total erythrocyte count (TEC) and the total leukocyte count (TLC) which was higher than the control group from day 30 to day 45, then decreased. Fish that were given a dose of chitosan 10 g kg^{-1} on day 45 had a significantly higher increase (P 0.05) TEC (3.68 ± 0.10 x 10^6 cells/mm3) and TLC (3.30 ± 0 ,20 x 10^4 cells/mm3) compared with other treatments. The total serum protein, albumin and globulin content increased significantly in chitosan-treated fish until day 45, after which they decreased. On day 15, the albumin-globulin ratio was significantly higher (P 0.05) in fish fed chitosan with a dose of 20 g kg^{-1} of feed compared to other treatments, which was (0.77 g dL-1 ± 0, 05). The results of observations of cellular immune parameters, namely respiratory explosive activity and phagocytosis ratio increased significantly in fish fed with additional chitosan compared to the control fish group. This increase occurred until the -45th day and then decreased. The exhalation activity and phagocytic ratio in the 10 g kg-1 treatment on day 45 were significantly (P 0.05) higher than the other treatments, 0.56 ± 0.03 and 57.11±2.53 respectively. Humoral immune parameters, namely serum lysozyme and serum bactericidal activity increased after feeding with chitosan added. The highest increase occurred in fish whose feed was given additional chitosan at a dose of 10 g kg^{-1} of feed. After being challenged with *Vibrio anguillarum* by intraperitoneal injection with the bacterial suspension of 0.1 ml (1 x 10^6 CFU mL^{-1}) after 60 days of feeding trial, the fish were given chitosan (5 g kg^{-1}, 10 g kg^{-1} and 20 g kg^{-1} feed) resulted in higher survival, 57.78 ± 5.88%, 75.56 ± 4.44% and 68.89 ± 5.88% respectively, while the survival of control fish was 46.67 ± 3.85%. Among the treatments, fish treated with chitosan at a dose of 10 g kg^{-1} significantly (P 0.05) resulted in the highest survival [38]. Research on the diet of chitosan in crayfish on immune reactions and survival has been carried out. The results showed that crayfish (*Procambarus clarkii*) which were fed with chitosan 10 mg/g feed for 4 weeks produced significantly higher THC (Total Hemocyte Count), proPO (prophenoloxidase) and SOD (Superoxide Dismutase) activities ($P < 0.05$) compared to control and other treatments, each of which was 17.93 ± 0.30 (×10^6 cells/mL), 0.31 ± 0.02 units, 0.33 ± 0.01 units, while the controls were 11.97 ± 0.20 (×10^6 cells/mL), 0.21 ± 0.02 units, and 0.22 ± 0.02 units respectively. After being treated with chitosan, then challenged with white spot syndrome virus (WSSV) showed that shrimp fed additional feed with 10 mg/g chitosan showed a significantly higher RPS (relative percent survival) value ($P < 0.05$) compared to control and other treatments, namely 40 ± 5%, while the control crayfish experienced a mortality of 100% [39]. From the description above. chitosan has the potential to be used as an effective immunostimulant against pathogens in shrimp. Another study regarding the administration of chitosan added to the diet of carp (*Cyprinus carpio*) for 60 days, showed an increase in immunity, which was indicated by an increase in lysozyme levels (at a concentration of 1%) and

serum bactericidal activity (at a concentration of 0.5% and 1%). After being challenged with *Aeromonas hydrophila* bacteria with a density of $2.1x10^7$ CFU through intraperitoneal injection, fish that were added with 0.5 and 1% chitosan significantly ($p<0.05$) resulted in lower mortality compared to other treatments and the lowest occurred in fish given 1% chitosan, which was 60% ± 4.78, while the mortality value of control fish was 76.7% ± 6.7 [8]. Besides being able to increase the resistance of fish to attack by pathogenic diseases, chitosan can also be used to eliminate the toxicity of heavy metals exposed in the fish body. As the results of research conducted by Salaah (2001) [40] showed that chitosan diet was able to eliminate exposure to heavy metal Pb in tilapia (*Oreochromis niloticus*). Tilapia used in the study averaged 35.32±2.4 g, reared at a density of 5g/L. The treatment used was G1: negative control/fish given basic feed); G2: positive control/fish given basic feed and 15 mg/L Pb (positive control); G3: fish were given basic feed containing 0.3% chitosan and 15 mg/L Pb; G4: fish were fed a basic diet containing 0.5% chitosan and 15 mg/L Pb; G5: fish were given basic feed containing 1% chitosan and 15 mg/L Pb. The results showed that the antioxidant enzymatic activity, namely superoxide dismutase (SOD) of liver, gill and muscle organs in the G5 (1% chitosan) treatment were 160.75±7.9, 118.11±7.5 and 66.32±4.4 U/g, respectively, these values were higher compared with liver, gill and muscle organs in G2, G3 and G4 treatments. Likewise, the total antioxidant activities (T-AOC) in the G5 (1% chitosan) treatment were significantly (P 0.05) higher than the G2, G3 and G4 treatments. The total antioxidant activities of fish liver, gill and muscle organs in the G5 treatment were 4.86±0.5, 4.4±0.5 and 3.01±0.6 mM/L, respectively. Innate immune response of Nile tilapia (*O. niloticus*), namely Phagocytosis and lysozyme was significantly (P 0.05) higher in G5 treatment than other treatments (G2, G3 and G4), 17.82±2.1% and 816.44±17.3 U/mL respectively. The results of histopathological examination, the gills of control fish (G1) showed normal filament and lamella structure, while the gill structure of fish in G2 and G3 treatments. Fish gills in G2 treatment were damaged in the form of epithelial cell hyperplasia, many telangiectatic lesions characterized by severe lamellae thrombosis. On the other hand, the fragmentation of the platelet nucleus in the swollen capillaries and the presence of blockages in the blood vessels of the gill filaments. In addition, many lamellae are severely damaged due to sloughing and hypertrophy of the mucosal cells. The gill epithelial cells in G3 treated fish showed little hyperplasia and adhesions. In contrast, fish fed high chitosan (G4 and G5) showed gill structures mostly similar to those of the control group (G1). 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 tissues of tilapia exposed to heavy metal Pb. The optimal level of addition of chitosan feed is 1%, at this concentration can protect fish against the cytotoxic effects of Pb and increase the innate immune response against high invading microbes.

5. 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/tail, while the dose of chitosan used was P0: without chitosan (0 ppt), P1: 10 ppt, P2: 50 ppt, P3: 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 in the P3 treatment (100 ppt) also produced the highest FCR, AGP, SGR and EEP values, respectively 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 % (EEP) [37]. Caspian kutum (*Rutilus frisii kutum*) with an average weight of 1.7 ± 0.15 g fed on feed containing chitosan at different levels (0, 0.25, 0.5, 1 and 2 g kg^{-1}) for 60 days showed different results on growth performance. The results showed that the feed conversion ratio (FCR) was significantly lower in fish whose feed contained 1 g kg (-1) chitosan, compared to other groups ($P < 0.05$), but there was no significant difference ($P > 0, 05$) between treatments with specific growth rates and condition factors [41]. Juvenile *Barbonymus gonionotus* after being given chitosan added to the feed at doses of T0 (without chitosan/control), T1(1 g kg^{-1} feed), T2 (2 g kg^{-1} feed) and T3 (3g kg^{-1} feed) during 60 days resulted in different weight gain, 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 resulted in a significantly higher weight gain ($P < 0.05$) than the fish fry in the control treatment. Fish seeds that were given chitosan 1 g kg^{-1} feed (T1) significantly ($P < 0.05$), resulted 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 given chitosan 1 g kg^{-1} feed produced the highest SGR value, which was 1.80±0.08%/day. While the control fish, treatment T2 and T3 SGR values resulted in SGR values of 1.39±0.04, 1.68±0.53 and 1.53±0.014 %/day, respectively. Here it can be seen that the higher the dose of chitosan given the weight gain and the lower SGR of fish fry. The results of observations on the FCR of fish fry showed that the T1 treatment significantly produced the lowest value, namely 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. From these data, it can be seen that the FCR value increased significantly with increasing the dose of chitosan diet and the dose of chitosan 1 g kg^{-1}feed resulted in the best growth performance [6]. 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 [42].

6. 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.

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