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Efficacy of baker's yeast as immunostimulant in nile tilapia (*Oreochromis niloticus*)

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Abstract: This study was conducted to evaluate the effect of baker's yeast cell as immunostimulant on nile tilapia (*Oreochromis niloticus*) immune responses. Fish was obtained from Aquaculture Development and Training Board (BP3I) Tateli, Fisheries and Marine Office of North Sulawesi and distributed into five outdoor concrete tanks at a density of 45 fish. After acclimatization for one week, fish was graded (mean weight 28.78 g) and set at 30 fish per tank. Fish in tank A received pellet not supplemented with baker's yeast while fish in tanks B, C, D, and E received pellet supplemented with 5, 10, 15, 20 g yeast cells/kg feed respectively. Feeding was applied four consecutive weeks at 5% of body weight per day, twice daily at 08.00 am and 16.00 pm. Immune parameters including total leucocytes count and phagocytosis activity were measured at week two and week four. After feeding for four weeks, administration of yeast cell exhibited significant effect on the total leucocyte count and phagocytosis activity ($p<0.01$). The highest value of these two parameters was observed in fish treated with 5 g yeast cells. The result also showed that at high dose, the immune parameters declined as the administration time elongated. In conclusion, incorporation of baker's yeast cells into feed improved immunity of nile tilapia cultured outdoor by increasing total leucocyte count and phagocytosis activity of phagocyte cells.
Keywords : Saccharomyces cereviciae, immunostimulant, nonspecific immune response, total leucocyte count, phagocytosis activity, Oreochromis niloticus.

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the important freshwater aquaculture species in Indonesia. This species grows relative fast and has high tolerance to environmental changes. Rapid intensification of aquaculture has raised the occurrence of infectious diseases weather caused by virus, bacteria, fungi and parasites. Disease problem has led to significant economic losses, continued to occur and probably has become the most limiting factor in tilapia aquaculture.

Improving fish performance and disease resistance of culture species to various pathogens are major challenges faced by fish culturist[1]. Several attempts have been implemented to control the disease including the use of antibiotic and vaccine. However, the intensive use of chemicals or antibiotics in intensive aquaculture has brought about unwanted developments such as bioaccumulation, pollution, antibiotic-resistant pathogens, immunosuppression, and high expenditure[2,3]. Antibiotic residue could be accumulated in the fish body and dangerous for human health. Antibiotic resistance can be transferred to the aquaculture environment and to human pathogens[4]. Vaccine has been proved to be effective in preventing the occurrence of diseases in aquaculture. However, the efficacy of vaccines was limited only on specific pathogen.

The use of immunostimulants offer an alternative to antibiotic or chemicals currently being used to combat disease in aquaculture has attracted more attention from researchers[5]. In contrast to vaccines, immunostimulant works by enhancing the non-specific immune response of fish and crustacean[6,7]. Immunostimulant did not leave any residue in fish body and environment and not harmful for human health.

More environment-friendly disease control strategies are urgently needed to promote sustainable aquaculture production. Currently numerous natural products have been used as immunostimulant sources to control fish diseases such as herbals[8-13], yeast[3,14-17], seaweed[18]. Herbs are currently used in commercial aquaculture as growth-promoting substances, antimicrobial agents, nutrients as well as many other applications. Their potential to prevent and control fish diseases is also being studied. Modulation of the immune response using medicinal plant products as a possible therapeutic measure has become the focus of extensive scientific investigation[5].

Yeast products are frequently used as feed ingredients in aquaculture because of their nutritional value, which include proteins, lipids, vitamins and minerals. The major component of yeast cell wall is β-1-3 glucan (50–60%) capable of stimulating the non-specific immune function of fish and crustaceans[3,17]. This research was carried out in October – December 2014 aiming to evaluate the effect of baker's yeast as immunostimulant on nile tilapia immune responses.

Experimental

Fish used

Juveniles of nile tilapia with an average body weight of 28.78 gwere derived from Aquaculture Development and Training Board (BP3I) Tateli, Fisheries and Marine Office of North Sulawesi. Fish were put in oxygenated-plastic bag, transported to the Faculty of Fisheries and Marine Science and then distributed into five 2x1x1 m^3outdoor concrete tanks (tank A, B, C, D and E) at a density of 45 individuals per tank. Each tank was equipped with one inlet pipe, out let, and aerator. Fish were acclimatized for one week and fed standard pellet twice a day at 08.00 am and 16.00 pm at a rate of 5% of body weight per day.

Yeast

The research used baker's yeast (*Saccharomyces cereviciae*) as immunostimulant bought from department store while feed used was commercial fish pellet.

Feed preparation

Standard pellet was supplemented with baker's yeast cells as immunostimulant. The doses of yeast were 5, 10, 15 and 20 g/kg of pellet while the control food was not supplemented with yeast cells. After being weighted, yeast cells were suspended in some pure water (1:10 v/w) and mixed thoroughly into pelletand air-dried at room temperature. After dry, feed was placed in plastic bag and stored at refrigerator at 4^oC until use.

Research procedure and data collection

After acclimatization, fish was graded and set at 30 individuals per tank. Fish in tank A received pellet not supplemented with baker's yeast while fish in tanks B, C, D, and E received pellet supplemented with 5, 10, 15, 20 g yeast cells/kg feed respectively. Feeding was applied four consecutive weeks at 5% of body weight per day, twice daily at 08.00 am and 16.00 pm. During the experiment, water quality was kept stable by regular monitoring. To maintained at optimal level of water, water exchange as much as one-third was conducted once every three days.

Immune parameters including total leucocytes count and phagocytosis activity were measured at two weeks interval. Fish blood was withdrawn from vena caudalis according to the procedure of Stolen [19]. Blood sample was collected from three fishes of each treatment using a 1 mL syringe previously rinsed with anti-coagulant (EDTA) and transferred to sterile eppendorf. Measurement of immune parameters were conducted at day 14 and 28.

Total Leucocyte Count

The fresh blood samples were used for the calculation of total leucocytes and phagocytosis activity of phagocytes cells. To estimate the total number of leucocytes, 50 µL of blood was placed in an appendorf and then mixed with 450 µL of Turk's solution (1:10). The mixture was homogenized by gentle swing and

incubated for five minutes at room temperature. Total leucocyte count was calculated using haemacytometer under light microscope.

Phagocytosis activity was estimated by mixing 0.1 mL of blood with an equal volume of zymosan as stimulator. The mixture of blood and yeast cells was homogenized by gentle movement and then incubated at room temperature for 20 minutes. Furthermore, the blood was smeared on glass slide and stained with Giemsa. Phagocytosis activity (%) was expressed as:

$$\frac{\textit{Number of leucocytes engulfing zymosan}}{\textit{Number of leucocytes observed}} \times 100$$

Statistical analysis

Data obtained were expressed as mean±Stdv and analyzed by one-way analysis of variance (ANOVA). The difference effect between means was determined and compared by Duncan Test. Significant level was set at 0.05

Result and Discussion

Total Leukocyte

Oral administration of baker's yeast for two weeks exhibited non-significanteffect on the total leucocyte count ($p=0.25$). Total leucocytes of fish between different treatment were almost similar. However, administration of baker's yeast showed significant effect ($p<0.01$) after being fed for four weeks (Table 1). The highest total leucocyte was achieved on fish fed feed supplemented with yeast cells at 5 g/kg feed, followed by 10 g/kg feed.

Table 1. Total leucocyte count of fish (x 10^7 cells\mL) fed diets supplemented with different doses of yeast cells

Yeast cells (g/kg feed)	Week-2	Week-4
0	4.62^{a}	4.89^{a}
5	5.55^{a}	6.62^{b}
10	6.09^{a}	6.57^{b}
15	5.82^{a}	5.91^{b}
20	5.68^{a}	4.97^{a}

Different super scribes in the same column were significantly different

Phagocytosis Activity

Supplementation of yeast cells in feed for two weeks showed significant effect on phagocytosis activity of phagocytes cells of fish ($p=0.04$). This effect was sustained until weeks four of feeding ($p<0.01$). The highest phagocytosis activity of phagocytes cells was observed in fish fed feed with 5 g yeast cells per kg feed followed by 10 g/kg feed (Table 2).

Table 2. Mean phagocytosis activity (%) of nile tilapia fed on feed supplemented with baker's yeast cells

Yeast cells (g/kg feed)	Week- 2	Week- 4
0	27.97^{a}	32.37^{a}
5	35.98^{ab}	48.63^{c}
10	40.39^{b}	46.41^{bc}
15	41.08^{b}	42.66^{b}
20	45.01^{b}	34.72^{a}

Different super scribes in the same column were significantly different

Research results displayed that application of baker's yeast cells into feed could stimulate the increase of total leucocytes and phagocytosis activity of nile tilapia phagocytes. It had been also reported that the use of yeast cell walls was able to enhance the innate immunity of Rohu (*Labeo rohita* Ham) and had a positive correlation with growth parameters[20]. Through the absorption of yeast wall particle the immune function of fish was stimulated. In laboratory research, total leucocyte and phagocytosis activity of nile tilapia (mean weight 10.57 g) increased significantly after feeding for four weeks with pellet supplemented with 10 g yeast cells per kg of pellet[21]. In common carp (*Cyprinus carpio* L), significantly increased phagocytic activity and superoxide anion production in kidney cells,and resistance to a bacterial pathogen, were observed in the yeast extract-treated fish compared to non-treatedfish[2]. In shrimp (*Marsupenaeus japonicus*), immune system of shrimp fed with yeast extract-supplemented diet increased significantly compared with shrimp fed control diet[22]. Supplementation of live bakers' yeast cells into fry nile tilapia diet induced growth performance, feed utilization and immunity, and is promising as an alternative method to antibiotics for disease prevention in tilapia aquaculture[1].Yeast-by product from baker's yeast industry may be used as feed supplement and has positive effect on nonspecific immune response and growth of several fish species [23].

Baker's yeast is a particularly important natural bio-product sinceit contains immunostimulating compounds such as β-glucan, nucleotides, mannan, oligosaccharides and chitin[2,14,24,25]. Such compounds have the capability to enhance immune response sof various fish species[6]. Immunostimulants may directly initiate activation of the innate immune defense mechanisms acting on receptors and triggering intracellular gene activation that may results in production of antimicrobial molecules[26]. It leads to an increase in various components of immunity such as phagocytic activity, complement activity, lysozyme and disease resistance as well as serum Ig level [12].

Nucleotides play important role in essential physiological and biochemical functions including encoding and deciphering genetic information, mediating energy metabolism and cell signaling as well as serving as components of coenzymes, allosteric effectors and cellular agonists[25]. Nucleotides are synthesized de novo in most of the tissues, but some immune and intestinal cells lack this process and thus depend on exogenous supply. Supplementation of nucleotides might improve cellular and humoral immune responses of various fish as well as shrimp. Oral administration of nucleotides to fish daily for 3 days resulted in enhanced responses of phagocytic and nitro blue tetrazolium (NBT) activities in kidney phagocytic cells[27]. This activation of kidney cells was observed for at least 10 days post-treatment. The serum complement and lysozyme activities also increased in fish treated with nucleotides. Furthermore, the number of *A. hydrophila* in nucleotide-treated fish significantly decreased in the blood, kidney and liver after intraperitoneal injection. Thus yeast nucleotides appear to enhance nonspecific immune responses in fish. Nucleotides added to fish diet will optimize the function of cell replication including immune cells[28]. Nucleotides increased immune responses and resistance of fish a number of pathogen simultaneously[29]. Furthermore, supplementation of nucleotides also increased efficacy of vaccination, fish growth, larval quality and tolerance to stress. In grouper (*Epinephelus malabaricus*), fish fed diet supplemented with nucleotides for eigth weeks had better growth and immune responsescompared to control fish[30]. In shrimp, supplementation of nucleotides in feed significantly enhanced immunity and resistance of *Litopenaeus vannamei*[31].

β-glucan presents at the cell wall of yeast S. cereviciae have a potent stimulatory effect on immune system of fish, crustacean and mammals [6,32]. β-1,3/1,6-glucans bind specifically to a "receptor molecule" on the surface of phagocytes. After binding, the cells become more active in engulfing, killing and digesting bacteria and at the same time they secrete signal molecules (*cytokine*s) which stimulate the formation of new white blood cells. And also activate antibody-producing white blood cells (B- and T-cells)[7,33]. Supplementation of baker's yeast also increased feed and protein digestibility and thus resulted in better growth and feed efficiency [34].

Nile tilapia fed with pellet supplemented with β-glucan extracted from yeast *S. cereviciae* significantly enhanced nonspecific immune response and resistance to *A. hydrophila*[35]. In common carp (*Cyprinus carpio*), fish treated with β-glucan 0,1% had higher total leucocyte count and resistance to bacterial pathogen as compared to untreated fish[36]. *Labeo rohita* (meanweight 35 ± 5 g) fed diet with β-glucan supplementation for 28 days had total leucocyte count higher than that of control fish[37]. Another research found total leucocytes, lymphocytes and eunosinophyl of nile tilapia treated with *Saccharomyces* (10 $g.kg^{-1}$ feed), β-glucan (0,1%) and laminarian (0,1%) significantly decreased as the survival of fish decreases after opposed to mercury as stressor[38].Application of β-glucan by intraperitoneal injection on carp significantly enhanced immune responses

and survival rate after challenged with *A. hydrophila*[39]. Supplementation of β-glucan to the koi for 56 days showed considerable improvement in the immune response, growth, and survival of koi[32].

In this research, there was no significant effect of yeast on total leucocytes and phagocytosis activity two weeks after feeding. As the doses of yeast increased the immune parameters increased too. But after feeding for four weeks, total leucocytes and phagocytosis activity of fish tended to decrease as the doses of yeast cells increased. At high doses, both total leucocytes and phagocytosis activity of fish treated with pellet contained 20 g of yeast cells/kg feed were low and almost similar with that of fish treated with control pellet. The best effect was observed in fish treated with low dose (5 g/kg of feed). This finding explained that the dose and administration time should be taken into account in applying an immunostimulant in aquaculture. The efficacy of immunostimulant by oral method decreases with long-term administration and overdoses of immunostimulants induce immunosuppression in fish[6]. Thus for the effective use of immunostimulants, dosages, method of administration, administration time and the physiological condition of fish need to be considered. In health management, dose and frequency of administration of immunostimulants are essential [17].

Conclusion

Based on the results, it could be concluded that incorporation of baker's yeast cells into feed improved immunity of nile tilapia cultured outdoor by increasing total leucocyte count and phagocytosis activity of phagocyte cells. The best effect was observed at low dose, but at high dose, the immune parameters declined as the administration time elongated. Thus research to established the optimal administration time is necessary to be conducted.

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