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Incorporation of baker's yeast cells as immunostimulant in feed enhance resistance of nile tilapia to *Aeromonas hydrophila*

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Abstract: The purpose of research was to evaluate the effect of baker's yeast cell as immunostimulant on the resistance of nile tilapia (*Oreochromis niloticus*) challenged with *A. hydrophila*. Juveniles were obtained from Aquaculture Development and Training Board (BP3I) Tateli, Fisheries and Marine Office of North Sulawesi and transported to Faculty of Fisheries and Marine Science Sam Ratulangi University. Fish was then distributed into five outdoor concrete tanks (tank A, B, C, D, and E) at a density of 45. After acclimatizationfor one week, fish was fed diet supplemented with baker's yeast cells as treatment. Fish in tank A received dietwithout supplementation with baker's yeast while fish in tanks B, C, D, and E received diet(treatment) supplemented with 5, 10, 15, 20 g yeast cells/kg feed, respectively. After feeding for four weeks with treatment diets, the fish was captured and placed into glass aquarium with density of 10 fish/aquarium. Each treatment diet had three replication. Fish was then intraperitoneally injected with 0.2 mL of *A. hydrophila* suspension containing $5x10^6$ cfu. Mortality of fish was observed for 14 days. During the observation, fish was fed with diet without supplementation of yeast cells at 5% body weight per day, twice daily at 08.00 and 16.00. Research result showed that supplementation of baker's yeast cells into fish diet had significant effect on fish resistance ($p<0.05$). The highest resistance (73.3%) was observed in fish fed diet containing 5 g yeast cells per kg of diet while control fish was only 50%. As conclusion, incorporation of baker's yeast cells into feed improved the resistance of nile tilapia to bacterial pathogen.
Keywords: baker's yeast, immunostimulant, resistance, Oreochromis niloticus, A. hydrophila.

Introduction

Disease problem in aquaculture has led to significant economic losses, continued to occur and probably has become the most limiting factor in tilapia aquaculture. Therefore, increasing health status and resistance of fish to various pathogens are needed to be considered. Methods for control fish diseases included the use of antibiotic,vaccine, and immunostimulants. The prevention of fish disease outbreaks in aquaculture system are conventionally using antibiotics or disinfectant chemicals. However the use of antibiotics had created several problems including toxicity, cost, and government restriction[1]. Antibiotic resistance canbe transferred to the aquaculture environment and to human pathogens and Antibiotic residue could be accumulated in the fish body and dangerous for human health.[2] Moreover, the control of diseases that occur in intensive aquaculture has resulted in the increase of antibiotic-resistant pathogen, bioaccumulation, pollution, and immunosuppression[3,4].

Vaccine is highly effective in preventing the occurrence of diseases in aquaculture. However, vaccine works only on specificpathogen thus its efficacy limited. In addition, though vaccination is the method of choice over antibiotic treatments for the control of many fish diseases, vaccines for other diseases are unavailable or, at the best, in the early stages of their development[5].

Recently in aquaculture industries, increasing consideration has been given to the use of immunostimulant as adjuvants to vaccine and as potential alternative to the use of antibiotics[5].Immunostimulant are valuable to control fish diseases and may be useful in fish culture. The immunostimulatory effects of glucan,, chitin, lactoferrin and levamisole, nutritional factors such as Vitamin B and C, growth hormone and prolactin for fish and shrimp had been reported[6],but their mode of action remain unclear[7]. Immonostimulant increase resistance to infection disease by enhancing non-specific defence mechanisms[8].In fish larval aquaculture, the use of immunostimulants as dietary supplements can improve the innate defense of fish and thus providing resistance to pathogens during period of hight stress such as grading, reproduction, sea transfer and vaccination[9]. The immunomodulation of larval fish has been proposed as a potential method for improving larval survival by increasing the innate response of the developing animal until its adaptive immune renponse is sufficiently develop to mount an effective response to the pathogen. Immunostimulant did not leave any residue in fish body and environment and not harmful for human health. The use of immunostimulants offer an alternative to antibiotic or chemicals and now has attracted more attention from researchers[10].

More environment-friendly disease control strategiesare urgently needed to promote sustainable aquaculture production. Currently numerous whole organisms and natural products have been used as immunostimulant sources to prevent and control fish diseases such as herbals [11-14], yeast [4,15-18], seaweed[19]. Herbs are currently used incommercial aquaculture as growth-promoting substances,antimicrobial agents, nutrients as well as manyother applications. Their potential to prevent and controlfish diseases is also being studied. Modulation of theimmune response using medicinal plant products as apossible therapeutic measure has become the focus ofextensive scientific investigation[10].

β-1-3 glucan of certain fungi and yeasts have been successfully used as immunostimulants to enhance resistance of fishes and shellfishes against bacterial and viral infections[16].Baker's yeast (*Saccharomyces cereviciae*) is a natural product from the brewing industry that contains various immunostimulating compounds such as β-1-3 glucan and chitin and can enhance immune responses and disease resistance of various fish spacies[18].Yeast products are frequently used as feed ingredients in aquaculture because of their nutritional value, which include proteins, lipids, vitamins and minerals[4]. 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. This research aimed to evaluate the potential of baker's yeast as immunostimulant to enhance resistance of nile tilapia to *Aeromonas hydrophila*.

Material and method

Fish used

Nile tilapia juvenile (mean weight 28.78 g)were obtained from Aquaculture Development and Training Board (BP3I) Tateli, Fisheries and Marine Office of North Sulawesi. Fish were transported to the Faculty of Fisheries and Marine Science and then stocked in five outdoor concrete tanks (tank A, B, C, D and E) measuring 2x1x1 m^3each. The density of fish in each tank was 45 individuals.Each tank was equipped with one inlet pipe, out let, and aerator. Acclimatization was conducted for one week and during this process, fish was fed commercial pelletat 5% of body weight per day, twice a day at 08.00 am and 16.00 pm.

Yeast

Baker's yeast (*Saccharomyces cereviciae*)used as immunostimulant was obtained from department store while feed used was commercial fish pellet containing 30% protein, 6%lipid, 5%fiber, 10%ash and 12%water.

Feed preparation

Baker's yeast cells used as immunostimulant was diluted in water and supplemented in the pellet at 5, 10, 15 and 20 g/kg of feed while the control pelletwas not supplemented with baker's yeast cells.The mixtures

were air-dried at room temperature. After dry, feed was placed in plastic bag and stored at refrigerator at 4^{o}C until use.

Research procedure and data collection

After acclimatization, fish was fed pellet supplemented with different doses of baker's yeast cells . Fish in tank A received pellet without supplementation of yeast cells while fish in tanks B, C, D, and E received pellet supplemented with 5, 10, 15, 20 g yeast cells/kg pellet respectively. Fish was fed with experimental diets forfour consecutive weeks at 5% of body weight per day, twice daily at 08.00 am and 16.00 pm. Water quality during the experiment was kept stable by regular monitoring. To maintained the optimal level of water, water exchange as much as one-third was conducted once every three days.

At the end of feeding period, the fish from each tank was captured and restocked in glass aquarium, each with three replications (A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3, E1, E2, E3). Fish was then injected interperitoneally with 0.2 mL of *A. hydrophila* suspension containing $5x10^6$ cfu. Mortality of fish was observed daily for 14 days. After challenge test, fish was fed standard diet without supplementation of yeast cell at 3% bw/d, twice daily at 08.00 and 16.00. Fish resistance was measured based on the formula as follows:

SR (%) = Nt/ No x 100
Where: SR = survival rate (%)
Nt = number of life fish at the end of experiment
No = initial number of fish

Statistical analysis

Data obtained were was analyzed by one-way analysis of variance (ANOVA). The difference effect between means was analyzed by Duncan Test. Significant level was set at 0.05

Result and Discussion

Supplementation of baker's yeast cells into fish pellet displayed significant effect on fish survival ($p<0.05$). At 14 days after challenge test with *A. hydrophila*, mean survival of fish fed pellet supplemented with 5 g yeast cells per kg of pellet was different significantly as compared to that of control fish. As the doses increased, the mean survival tend to decreased.In control fish, mortality started to occur at one day post challenge. In fish treated with 5 g yeast cells/kg of diet, mortality was observed from day 3 to day 9 after challenge. At higher doses, mortality began to occur at day 2 until day 12 post challenge test.

Table 1. Mean survival of nile tilapia at 14 days post challenge with *A. hydrophila*

Yeast cells (g/kg feed)	Survival (%)
0	50^{a}
5	73.3^{c}
10	63.3^{bc}
15	60^{ab}
20	53.3^{ab}

Different super scribes in the same column were significantly different

It was observed that mean survival of fish fed pellet containing 20 g of yeast cells/kg feed were low and almost similar with that of control fish. This finding indicated that in applying an immunostimulant in aquaculture, the dose and duration of administration time should be taken into account because long-term administrationand overdoses of immunostimulants might 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. Dose and frequency of administration of immunostimulants are essential in health management[20].

In this research, the highest survival was achieved in fish treated with 5 g yeast cells per kg of feed. In our previous study, the use of baker yeast as immunostimulant improved nonspecific immune response[21]. This might be contributed to the increase of resistance of fish to bacterial pathogen. Similar report showed an increase in nonspecific immune response and resistance of nile tilapia to *A. hydrophila* was observed in fish fed

diet supplemented with 5 g yeast cells per kg of feed[22]. Striped bass (mean weight 25,3 g) fed diet containing 2-4% yeast cells for nine weeks and challenged with *S. iniae*had no mortality while control fish had 20% mortality[23].

Baker's yeast cell contains immunostimulating compounds such as β-glucan, nucleotides, mannan, oligosaccharides and chitin[3,17,23]. These compounds have the capability to enhance immune responsesof 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[9]. It leads to an increase in various components of immunity such as phagocytic activity, complement activity, lysozyme and serum Ig level and disease resistance as well [24]. In large yellow croaker(*Pseudosciaena crocea*), fish treated with β-glucan 0.09% and challenged with *Vibrio harveyi*had lower cumulative mortality than control fish treated with 0.18% β-glucan[25].There was no significant differences between fish treated with 0.18% β-glucan and control fish. In carp, interperitoneal injection of 500 µg of β-glucan significantly increased survival rate of fish at day seventh after challenge test with *A. hydrophila*[26]. Another research also reported that 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[27]. In nile tilapia, supplementation of β-glucan extracted from yeast *S. cereviciae* significantly enhanced nonspecific immune response and resistance to *A. hydrophila*[28]. Another research found that mortality of nile tilapia treated with *Saccharomyces* (10 $g.kg^{-1}$ feed), β-glucan (0,1%) and laminarian (0,1%) significantly lower than control fish after 21 days of application[29]. Supplementation of β-glucan to the koi for 56 days showed considerable improvement in the immune response, growth, and survival of koi[30].

In kuruma shrimp, directly enhanced resistance of shrimp to bacterial pathogen with survival rate achieved 66.6% while control shrimp was only 8.3% [31]. In *Labeo rohita* (Ham.), supplementationof 5% baker's yeast cells into feed and applied orally for oral administration of feed for eight weeks increased resistance of fish after challenged with *A. hydophila*[11].Survival rate of treated fish was 96.66% while control fish was only 26%.

Yeast cell contains 0.9% purine and pyrimidine while yeast extract contains 2.3% [32]. In *S. cereviciae*, 12-20% of the total nitrogen can be composed of RNA nitrogen, mainly in the purine and pyrimidine bases of nucleoprotein[33].Nucleotides play important role in essential physiological and biochemical functions including encoding and decipheringgenetic information, mediating energy metabolismand cell signaling as well as serving as components ofcoenzymes, allosteric effectors and cellular agonists[32]. Supplementation of nucleotides might improve cellular and humoral immuneresponses of various fish as well as shrimp. Rainbow trout fed diet containing nucleotides extracted from *S. cereviciae*daily for 3 days had higher survival rate than control fish after challenged with *V. anguillarum*[5]. In grouper (*Epinephelus malabaricus*), fish fed diet supplemented with nucleotides for eigth weeks had better growth and immune responses compared to control fish[34]. In shrimp*Litopenaeus vannamei*, supplementation of 400 mg nucleotides in one kg of feed significantly enhanced immunity and resistance to*V. harveyi*[35].

Conclusion

The present study showed that incorporation of baker's yeast cells into feed improved the resistance of nile tilapia to bacterial pathogen. The best effect was observed at low dose, but at high dose, the resistance declined as the doses increased.

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