Dietary ginger (*Zingiber officinale*) enhance resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*

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Abstract. The objective of research was to evaluate the effect of ginger (*Zingiber officinale*) extract on the resistance of Nile tilapia (*Oreochromis niloticus*) challenged with *Aeromonas hydrophila*. Tilapia fingerlings measuring 10-12 cm in length were gathered from Board of Aquaculture Development and Training of Fish Farmer in Tateli Village, put in oxygenated plastic bags and transported to the Laboratory of Aquaculture Technology, Faculty of Fisheries and Marine Science, Sam Ratulangi University. The fish was first acclimatized for one week and then distributed into 15 aquaria with a density of 10 individuals per aquarium. Furthermore, the fish was fed treatment pellets supplemented with 0, 1.25, 2.5, 5, and 10 g of *Z. officinale* powder/kg of pellet for 30 days as much as 3%/body weight/day. The commercial pellet had a composition of 30% protein, 6% lipid, 5% fiber, 10% ash and 12% water. At day 31th, the fish was injected intraperitoneally with 0.2 mL of *A. hydrophila* suspension containing $5x10^7$ cfu/mL. Survival rate of fish was observed up to 8 days after challenge. The result showed that supplementation of *Z. officinale* extract significantly influenced the resistance of fish against *A. hydrophila* infection as compared to that of control treatment. The highest survival rate was observed in fish fed pellet containing 2.5 g *Z. officinale* extract.
Key Words: aquaculture, fish disease, immunostimulant, medicinal plant, intraperitoneal injection.

Introduction. Disease is one of the major problems encountered in aquaculture activity whether caused by bacteria, virus, parasites and fungi. The occurrence of disease usually is not caused by a single factor, but by a complete interaction between fish, environment and pathogen (Post 1987). Due to disease attack, many fish farmers had suffered significant economic losses, and even many of them had left the activity.

Many attempts have been implemented to increase the resistance of fish against various pathogen and to improve fish growth (Talpur & Ikhwanuddin 2013). Citarasu (2010) reported that hormone, antibiotic, vitamin and several chemical substances were used in aquaculture to control diseases. Even though these substances gave positive effect but they cannot be recommended because of their residual and other side effect. Babu et al (2013) reported that antibiotics and chemicals used in aquaculture may create various problems such as bioaccumulation, pollution, antibiotic-resistant pathogen, immunosuppression and high expenditure. Excessive use of antibiotic will be accumulated in fish body and is dangerous for human that consume the fish (Wu et al 2013).

In attempt to control the occurrence of diseases in aquaculture, scientists have focused on the use of immunostimulant to replace antibiotics (Galina et al 2009). A variety of immunostimulants that had been proved for its use including dietary nucleotide (Burrels et al 2001), baker's yeast (Abdel-Tawwab et al 2008; Tewary & Patra 2011; Manoppo et al 2015), life yeast *Hanseniaspora opuntiae* C21 (Ma et al 2013). Probiotics and biofloc had also been use in aquaculture to enhance immunity and growth of fish and shrimp (de la Banda et al 2010; Crab et al 2012).

In the past few years, medicinal plants began to be developed as an alternative for antibiotics and chemicals used in aquaculture (Fazlolahzadeh et al 2011). The alternative herbal bio-medicinal products in aquaculture operations, that have the characteristics of growth promoting ability and tonic to improve the immune system, act as appetite stimulators. They increase consumption, induce maturation, and have antimicrobial capability and also anti-stress characteristic that will be of immense use in the culture of shrimps and other fin fishes without any environmental and hazardous problems. Herbal compounds such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectines and polypeptides have been shown to be very effective alternative to antibiotics and other synthetic compounds. Ginger (*Zingiber officinale*) is one of the potential medicinal plants to improve disease resistance against pathogens. It contains many medicinal properties for treating diseases (Jabran et al 2015). This research aimed to evaluate the effect of *Z. officinale* extract on resistance of Nile tilapia (*Oreochromis niloticus*) challenged with *Aeromonas hydrophila*. The research was conducted in Laboratory of Aquaculture Technology, Faculty of Fisheries and Marine Science, Sam Ratulangi University from February to April 2017.

Material and Method

Experimental fish. The research used *O. niloticus* fingerlings measuring 10-12 cm in length. The fish was gathered from Board of Development and Training of Fish Farmer in Tateli Village, placed in oxygenated plastic bags and transported to Laboratory of Aquaculture Technology, Faculty of Fisheries and Marine Science, Sam Ratulangi University.

Z. officinale extract. Fresh *Z. officinale* was bought from a supermarket and brought to the laboratory. *Z. officinale* was then washed, peeled, cut in small pieces and then dried at room temperature for three days. After dried, ginger was ground using a grinder and sieved with a sifter (1/16' wire size). *Z. officinale* powder was weighted according to the dose required namely 0, 2.5, 5, 10 g, put in 250 mL conical flask containing 100 mL of distilled water and left over night.

Feed preparation. After overnight maceration, the extract was filtered, placed into a plastic sprayer and spread thoroughly into commercial pellet having a composition of 30% protein, 6% lipid, 5% fiber, 10% ash and 12% moisture. Pellet was dried at room temperature for 24 hours, and after dried, it was packed in plastic bags and stored in refrigerator until use.

Experimental design. The research used Complete Randomized Design with five different doses of *Z. officinale* as treatments, each with three replications. The treatment doses consisted of 0, 1.25, 2.5, 5, and 10 g *Z. officinale*/kg feed.

Experimental procedure. Before running the experiment, the fish was acclimatized for one week. During the acclimatization, fish was feed commercial pellet without supplementation of *Z. officinale* extract at 3%/body weight/day, twice a day at 09:00 AM and 16:00 PM. Furthermore the fish was moved into 15 aquaria (measuring 60 x 40 x 40 cm^3 each) with a density of 10 individuals per aquarium. Each aquarium was equipped with an aerator and one submersible water pump for water recirculating. Fish was fed treatment pellet previously supplemented with *Z. officinale* extract for 30 days as much as 3%/body weight/day at 09:00 AM and 16:00 PM. Water exchange was done every three days as much as 30% to keep a good water quality. During the experiment, the average of water temperature was 28°C and pH 6.5-7.5.

At the end of feeding the fish was challenged with *A. hydrophila* suspension through intraperitoneal injection. Each fish was injected with 0.2 mL bacterial suspension containing $5x10^7$ cfu/mL. Fish mortality was observed up to 8 days post-challenged. Resistance of fish was calculated by formula:

$$SR (\%) = (Nt / No) \times 100$$

Where:

SR - Survival rate;

Nt - number of live fish at 8th day after challenge test;

No - number of live fish at the beginning of challenge test.

Statistical analysis. Data obtained were expressed as mean±SD. One-way analysis of variance (ANOVA) was used to evaluate the effect of *Z. officinale* on *O. niloticus* resistance while the difference effect between means was analyzed by Duncan Test using SPSS 22 for windows. Significant level was set at 0.05.

Results and Discussion. Resistance of *O. niloticus* fed pellet supplemented with different doses of *Z. officinale* extracts for 30 days and challenged with *A. hydrophila* is presented in Table 1.

Table 1

Resistance of *Oreochromis niloticus* after challenged test with *Aeromonas hydrophila*

Ginger extracts (g/kg of pellet)	*Resistance (%)**
0	43.3±11.5[a]
1.25	56.6±5.7[a]
2.5	76.6±5.7[b]
5	43.3±5.7[a]
10	53.3±5.7[a]

*Different superscripts in the same column indicate significant differences.

The highest cumulative survival was achieved in fish fed with the addition of 2.5 g *Z. officinale* extract per kg of feed, followed by fish in the treatment of 1.25 g/kg of feed. At higher doses, survival appeared to decrease, and even had the same value as that of control fish. This result indicated that if the fish are fed with the addition of *Z. officinale* extract with a concentration that is too high, then the survival decrease. In this case, the excessive doses will have an immunosuppression effect that suppresses the immune system of the fish (Sakai 1999).

Resistance of fish was expressed as survival rate achieved after challenge test with *A. hydrophila* suspension containing $5x10^7$ cfu/mL. Mortality was observed up to 8 days post-challenge. In all treatment, mortality began to occur on day two post challenged and continued until day seven. Cumulative survival of fish is presented in Figure 1.

The result of variance analysis showed that the addition of *Z. officinale* extract in feed had significant effect on the resistance of *O. niloticus* against *A. hydrophila* ($p<0.05$). The highest survival rate was observed in fish fed pellet with addition of *Z. officinale* extract of 2.5 g/kg of feed which reached 76.6% while in the untreated fish the survival rate was 43.3%. Based on Duncan's Test, survival rate on treatment of 2.5 g/kg treated feed was significantly different from control fish ($p<0.05$). There was no significant differences observed between fish fed pellet supplemented with *Z. officinale* extract at 1.25, 5, 10 g/kg feed and control. In the report by Nya & Austin (2009), rainbow trout (*Oncorhynchus mykiss*) fed with the addition of 5 g of *Z. officinale* flour/kg of feed for 14 days had no mortality after challenge test with *A. hydrophila* while the control fish had a mortality of 64%. These results were supported by an increase in the number of neutrophils, macrophages and lymphocytes, and enhanced phagocytic, respiratory burst, lysozyme, bactericidal and anti-protease activities compared with the control. In addition, growth, feed conversion and protein efficiency increased significantly. In our previous study, the addition of *Z. officinale* in *Z. officinale* feed (mean weight 27.31 g) for 28 days was able to increase total leucocyte and phagocytic activity (Payung & Manoppo 2015).

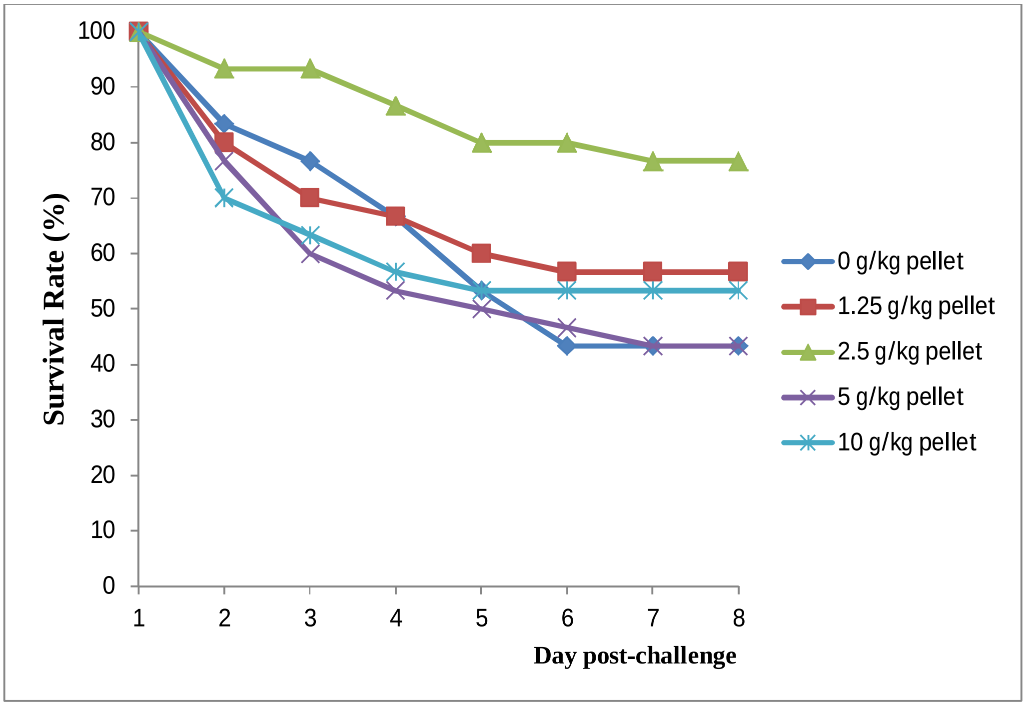

Figure 1. Cumulative survival of *Oreochromis niloticus* after challenge test with *A. Hydrophila.*

In catfish (*Clarias gariepinus*), the use of *Z. officinale* was safe and effective to treat the ectoparasitic protozoa *Trichodina* and *Epistylis spp*. at dose of 20 mg/L (Abo-Esa 2008). The study also found that the use of *Z. officinale* in aquaculture activities will improve water quality especially total ammonia. The research of Kusumawardani et al (2008) showed that red ginger extract have antibacterial activity against *A. hydrophila*. This pathogen can be killed by extract of red ginger at MIC (0.08 g/mL) and MBC (0.32 g/mL). Sari et al (2013) reported that fresh extract of red ginger (*Z. officinale* var. *Rubrum*) gave the highest inhibition to the growth of *S. aureus* (15.83 mm) and *E. coli* (15.33 mm) while elephant ginger (*Z. officinale* var. *Roscoe*) gave the highest inhibition growth of *C. albicans* (10.7 mm). In animal, *Z. officinale* extract was able to induce immune response of chicken (Wiryawan et al 2005) and reduce inflammation signs of erythema in rat with second degree burns (Susila et al 2014).

Recently, various medicinal plants are widely used in aquaculture as an antimicrobial agent, immunostimulant, growth-promoting substances and nutrients. Marentek et al (2013) reported that *O. niloticus* (mean weight 10.4 g) fed diet supplemented with garlic displayed higher total leucocyte count and phagocytosis activity, and better growth as compared to that of control fish. Aly et al (2008) found survival rate of *O. niloticus* fed diet supplemented with garlic was significantly higher compared to control fish. Garlic induced immunity and health status and consequently improved the growth performance. In rainbow trout *O. mykiss* (Walbaum), Nya & Austin (2009) found that fish fed diets containing 0.5 and 1.0 g garlic per 100 g of feed and infected with *A. hydrophila* had a reduction in mortalities to 4% compared with the controls (88%). There was stimulation of the number of erythrocytes and leucocytes, a significantly higher hematocrit, enhancement of phagocytic activity, respiratory burst, lysozyme, anti-protease and bactericidal activities following feeding with garlic. There was also a significant increase in growth, feed conversion and protein efficiency. In carp (*Cyprinus carpio*), Manoppo et al (2016) reported that fish fed garlic-supplemented diet had better weight gain than control fish. Punitha et al (2008) reported that addition of extracts of *Cynodon dactylon*, *Piper longum*, *Phyllanthus niruri*, *Tridax procumbens,* and *Z. officinalis* to fish feed for 60 days significantly improved the survival of *Epinephelus tauvina* against *Vibrio harveyi* infection. The results also found that herbal diets were significantly increased growth and immune responses by improving immune parameters such as phagocytic activity and albumin-globulin ratio as compared to the control group. Another report by Yin et al (2006) stated that feeding *O. niloticus* with 0.1 and 0.5% *Astragalus radix* for one week enhanced lysozyme activity and for 3 weeks stimulated phagocytosis by phagocytic blood cells, however, respiratory burst activity of phagocytic cells was not increased. Ji et al (2007) reported that red seabream *Pagrus major* fed diets supplemented with either *Massa medicata, Crataegi fructus, Artemisia capillaries, Cnidium officinale*, or a mixture of all the herbs had higher survival, specific growth rate, feed efficiency, condition factor and hemoglobin levels than fish given the control diet without herbs. Abasali & Mohamad (2010) added ethanol extract of *Ocimum basilium, Cinnamomum zeylanicum, Juglans regia* and *Mentha piperita* into fish feed. The result showed that treated fish had enhanced bactericidal activity, serum lysozyme, respiratory burst activity, WBC, RBC, hemoglobin, total serum protein, albumin and globulin, higher and survival rate compared to the control group.

This research found that the addition of *Z. officinale* extract in fish feed was able to increase the resistance of fish to pathogens because this material contains ingredients that can improve the immune system. According to Tan & Vanitha (2004), *Z. officinale* contains gingerrol which can stimulate the secretion of IL-1 and IL-6. Galina et al (2009) reported that *Z. officinale* extract effectively increased phagocytosis and respiratory burst activity of leukocyte cells. Increased fish immunity will result in increased fish resistance to pathogens.

Conclusions. The present study found that supplementation of *Z. officinale* into fish feed improved resistance of fish against *A. hydrophila* infection. The highest cumulative survival was achieved in fish fed with the addition of *Z. officinale* extract of 2.5 g/kg of feed while at higher doses, survival appeared to decrease. Excessive doses will have an immunosuppression effect, thus reduced the resistance of fish to pathogen.

References

Abasali H., Mohamad S., 2010 Immune response of common carp (*Cyprinus carpio*) fed with herbal immunostimulants diets. Journal of Animal and Veterinary Advances 9(13):1839-1847.

Abdel-Tawwab M., Abddel-Rahman A. M., Ismael N. E. M., 2008 Evaluation of commercial live baker's yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Tilapia *Oreochromis niloticus* (L) challenged *in situ* with *Aeromonas hydrophila*. Aquaculture 280:185-189.

Abo-Esa J. F. K., 2008 Study in some ectoparasitic diseases of catfish, *Clarias gariepinus* with their control by ginger, *Zingiber officinale*. Mediterranean Aquaculture Journal 1(1):1-9.

Aly S. M., Atti N. M. A., Mohamed M. F., 2008 Effect of garlic on the survival, growth, resistance and quality of *Oreochromis niloticus*. 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, Proceedings of the ISTA 8, pp. 277-296.

Babu D. T., Antony S. P., Jospeph S. P., Bright A. R., Philip R., 2013 Marine yeast *Candida aquaetextoris* S527 as a potential immunostimulant in black tiger shrimp *Penaeus monodon*. Journal of Invertebrate Pathology 122:243-252.

Burrels C., Williams P. D., Soutthgate P. J., Wadsworth S. L., 2001 Dietary nucleotide: A novel supplement in fish feed. 2. Effects on vaccination, salt water transfer, growth rates and physiologyof Atlantic salmon (*Salmo salar* L.). Aquaculture 199:171-184.

Citarasu T., 2010 Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International 18:403-414.

Crab R., Defoirdt T., Bossier P., Verstraete W., 2012 Biofloc technology in aquaculture: beneficial effect and future challenges. Aquaculture 356-357:351-356.

de La Banda I. G., Lobo C., Leon-Robio J. M., Tapia-Paniagua S., Balebona M. C., Morinigo M. A., Moreno-Ventas X., Lucas L. M., Linares F., Ariji S., 2010 Influence of two closely related probiotics on juvenile Senegalese sole (*Solea sanegalensis,* Kaup 1858) performance and protection against *Photobacterium damselae* subsp. *Piscicida*. Aquaculture 306:281-288.

Fazlolahzadeh F., Keramati K., Nazifi S., Shirian S., Seifi S., 2011 Effect of garlic (*Allium sativum*) on hematological parameters and plasma activities on ALT and AST of rainbow trout in temperature stress. Australian Journal of Basic and Applied Sciences 5(9):84-90.

Galina J., Yin G., Ardo L., Jeney Z., 2009 The use of immunostimulating herbs in fish. An overview of research. Fish Physiology and Biochemistry 35:669-676.

Jabran A. G. M., Soeharto S., Sujuti H., 2015 The effect of ginger (*Zingiber officinale* Roscoe) extract on liver histopathology and alanine aminotransferase serum level in carbofuran-induced rats. International Jurnal of PharmTech Research 8(5):889-897.

Ji S. C., Takaoka O., Jeong G. S., Lee S. W., Ishimaru K., Seoka M., Takii M., 2007 Dietary medicinal herbs improve growth and some non-specific immunity of red sea bream *Pagrus major*. Fisheries Science 73:63-69.

Kusumawardani I. R., Kusdarwati R., Hadijatno D., 2008 Daya anti bakteri ekstrak jahe merah (*Zingiber officinale Rosc.*) dengan konsentrasi yang berbeda terhadap pertumbuhan *Aeromonas hydrophila* secara in vitro. Berkala Ilmiah Perikanan 3(1):75-82.

Ma Y., Liu Z., Yang Z., Li M., Liu J., Song J., 2013 Effect of dietary life Yeast *Hanseniaspora opuntiae* C21 on the immune and disease resistance Against *Vibrio splendidus* infections in juvenile sea cucumber *Apostichopus japonicas.* Fish and Shellfish Immunology 34:66-73.

Manoppo H., Kolopita M. E. F., Malatunduh R., 2016 Growth promoter effect of garlic (*Allium sativum*) on carp (*Cyprinus carpio* L). International Journal of PharmTech Research 9(4):283-288.

Manoppo H., Manurung U. N., Tumbol R. A., 2015 Efficacy of baker's yeast as immunostimmulant in tilapia (*Oreochromis niloticus*). International Journal of ChemTech Research 8(2):559-565.

Marentek G. A., Manoppo H., Longdong S. N. J., 2013 Kajian penggunaan bawang putih (*Allium sativum*) dalam meningkatkan respon imun non-spesifik dan pertumbuhan ikan nila (*Oreochromis niloticus*). Jurnal Budidaya Perairan 1(1):1-7.

Nya E. J., Austin B., 2009 Use of ginger *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Disease 32:971-977.

Nya E. J., Austin B., 2009 Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Disease 32:963–970.

Payung C. N., Manoppo H., 2015 Peningkatan respon kebal non-spesifik dan pertumbuhan ikan nila (*Orechromis niloticus*) Melalui pemberian jahe, *Zingiber officinale*. Jurnal Budidaya Perairan 3(1):11-18.

Post G., 1987 The fish health. T.F.H Publication Inc. For Revised and Expended Edition. New Jersey.

Punitha S. M. J., Babu M. M., Sivaram V., Shankar V. S., Dhas S. A., Mahesh T. C., Immanuel G., Citarasu T., 2008 Immunostimulating influence of herbal biomedicines on nonspecific immunity in Grouper *Epinepheleus tauvina* juvenile against *Vibrio harveyi* infection. Aquaculture International 16:511-523.

Sakai M., 1999 Current research status of fish imunostimulants. Aquaculture 172:63-92.

Sari K. I. P., Periadnadi, Nasir N., 2013 Uji Antimikroba ekstrak segar jahe-jahean (Zingiberaceae) terhadap *Staphylococcus aureus*, *Escherichia coli* dan *Candida albicans*. Jurnal Biologi Universitas Andalas (J. Bio. UA) 2(1):20-24.

Susila H. A., Sumarno, Dina D., 2014 Efek ekstrak jahe (*Zingiber officinale Rosc.*) terhadap penurunan tanda inflamasi eritema pada tikus putih (*Rattus novergicus*) galur wistar dengan luka bakar derajat II. Majalah Kesehatan FKUB 1(4):214-222.

Talpur A. D., Ikhwanuddin M., 2013 Azadirachta indica (neem) leaf dietary effect on the immunity response and disease resistance of Asian sea bass, Lates challenged with Vibrio harveyi. Fish & Shellfish Immunology 34(1):254-264.

Tan B. K. H., Vanitha J., 2004 Immunomodulatory and antimicrobial effect of some traditional chinese medicinal herbs: a review. Current Medicinal Chemistry 11(11):1423-1430.

Tewary A., Patra B. C., 2011 Oral administration of baker's yeast (*Saccharomyces cerevisiae*) acts as a growth promoter and immunomodulator in *Labeo rohita* (Ham). Journal of Aquaculture Research and Development 2(1):1-7.

Wiryawan K. G., Suharti S., Bintang M., 2005 Kajian antibakteri temulawak, jahe dan bawang putih terhadap performans dan respon imun ayam pedaging. Media Peternakan 28(2):52-62.

Wu Y. R., Gong Q. F., Fang H., Liang W. W., Chen M., He R. J., 2013 Effect of *Sophora flavescent* on non-specific immune response of tilapia GIFT (*Oreochromis niloticus*) and disease resistance against *Sterptococcus agalactiae*. Fish & Shellfish immunology 34:220-227.

Yin G., Jeney G., Racs T., Xu P., Jun X., Jeney Z., 2006 Effect of two chinese herbs (*Astragalus radix* and *Scutellara radix*) on nonspecific immune system of tilapia, *Oreochromis niloticus*. Aquaculture 253:39-47.

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