Oral administration of probiotic improve survival of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila* infection

[1]Henky Manoppo, [2]Reiny A. Tumbol, [3]Jeane Sumaraw

[1] Aquaculture Study Program, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, Indonesia; [2] Aquatic Science Study Program, Graduate School of Sam Ratulangi University, Manado, Indonesia; [3] Board of Fish Quarantine, Quality Control and Safety of Fisheries Product, Manado, Indonesia. Corresponding author: H. Manoppo, hmanoppo@yahoo.com

Abstract. This research was conducted to evaluate the effect of probiotic originated from carp on survival of fish against *Aeromonas hydrophila* infection. Fish measuring 5-8 cm was purchased from fish farmers in Tatelu Village, North Minahasa Regency, North Sulawesi Province. After adaptation for one week, the fish were fed pellet already supplemented with probiotic at different concentrations. The control pellet contained no probiotics but coated with 2% yellow egg as in the treatment pellets. Fish were feed probiotic pellet for four weeks as much as 5% body weight^{-1} day^{-1}, with feeding frequency of twice a day at 08.00 and 16.00. At the end of feeding, the fish were challenged with *A. hydrophila* through cohabitation with infected fish. The result showed that survival rate of fish treated with probiotic at concentrations between $1x10^7$ and $1x10^8$ cfu mL^{-1} was higher than of control. Thus, administration of probiotic originated from carp intestine has potential to improve survival of the same species against the tested bacterial pathogen.
Key Words: aquaculture, immune response, disease resistance, challenge test.

Introduction. The rapid development of aquaculture along with the increasing demand for fish has encouraged aquaculture to be carried out more intensively (Reverter et al 2014). On the other hand, intensification has increased stress levels in fish, increased the risk of disease outbreaks, damaged water quality and economic losses to the aquaculture industry (Fečkaninová et al 2017; Wang et al 2008).

Prevention and treatment of disease in aquaculture generally use antibiotics and chemicals. However, the use of antibiotics in fish and shrimp farming has caused various problems such as the emergence of antibiotic-resistant pathogens and the accumulation of antibiotic residues in body tissues (Kong et al 2020; Wang et al 2018; Zokaeifar et al 2012). In addition, the intensive and repeated use of antibiotics or chemicals can cause various negative effects such as bioaccumulation, pollution, damage to environmental microbes and suppress the immune system of fish (Wang & Xu 2014; Biswas et al 2012; Karthik et al 2014). Citarasu (2010) stated that although antibiotics and chemicals can have a positive effect on fish and shrimp, their use cannot be recommended because of the residues that accumulate in fish and shrimp meat. Therefore, research on the use of feed with ingredients that can improve health status and stimulate the growth of cultivated species is needed in an effort to develop environmentally friendly aquaculture (Hoseinifar et al 2015). One of the ingredients that have the potential and is increasingly being applied is probiotics.

Probiotics have recently been widely applied in aquaculture as an alternative to antibiotics in preventing disease. Probiotics are living micro-organisms that can improve host health through the provision of nutrients and protection against pathogens and can be used as a substitute for antibiotics/chemicals (Perez-Sanchez et al 2014; Hoseinifar et al 2018). Probiotics can be in the form of live or dead organisms or microalgae or yeast that are given through feed or into pond water to improve disease resistance and growth performance as well (Banu et al 2020; Prabhurajeshwar & Candrakanth 2019).

Research reports showed that the use of probiotics in aquaculture could improve immune system, fish resistance to pathogens as well as improve growth, feed digestibility, feed efficiency, and appetite (Ling et al 2018; Lin et al 2019; Kong et al 2020; Kavitha et al 2018). In addition, probiotics could inhibit the development of pathogens, maintain the micro flora balance of the digestive tract, improve digestive function and improve water quality (Wang et al 2018; Sornplang & Piyadeatsoontorn 2016). Application of probiotics in aquatic organisms is also potential to increase growth and feed intake by influencing the processes of digestive enzymes such as amylases, proteases and alginate lyases (Chowdhury & Roy 2020; Zokaeifar et al 2012). Hai (2015) also stated that probiotics effectively participate in the digestive process by producing extracellular enzymes such as proteases, lipases and carbohydrolases produce growth factors. In our previous study (Manoppo et al 2019), it was found that probiotic *Lactobacillus* sp. isolated from intestine of carp was potential to promote growth performance and feed efficiency of the same species. The aim of the present study was to evaluate the effect of the same probiotic on survival of fish against *Aeromonas hydrophila* infection.

Material and Method

Experimental fish. The research was conducted from July to mid November 2020. About 250 healthy carp juveniles measuring 5-8 cm (average weight of 4.29±0.16 g) were purchased from fish farmers in Tatelu Village, North Minahasa Regency, Province of North Sulawesi. Fish infected with *A. hydrophila* were also collected from ponds around Tatelu Village.

Probiotics and feed preparation. Probiotic bacteria (*Lactobacillus* sp.) was obtained from the stock available at the Laboratory of Fish Health, Environment and Toxicology, Faculty of Fisheries and Marine Science. The bacteria were cultured and propagated using MRS agar. The feed used was commercial pellets having the composition of 31% protein, 5% lipid, 5% crude fibre, 13% ash, and 12% water. The treatments contained probiotics at $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$ cfu mL^{-1}. Probiotic density was prepared by suspending bacterial colonies into 0.85 NaCl and comparing it with McFarland's standard solution to obtain a density of $1x10^9$ cfu mL^{-1}. Furthermore, the concentration of bacteria was diluted by mixing 1 mL of solution containing $1x10^9$ cfu mL^{-1} into 9 mL NaCl to obtain a concentration of $1x10^8$ cfu mL^{-1}, and so on until $1x10^6$ cfu mL^{-1} was obtained. Each treatment was added into the pellets by spraying evenly then dried. After dried, the pellets were coated with 2% egg yolk and then dried again. Control diet was not supplemented with probiotic but coated with 2% egg yolk too. The treatment feed was then placed in plastic box and stored in the refrigerator until used.

Research procedure. The fish seeds obtained were spread into five 60 L-buckets. Before starting the experiment, the fish were acclimatized for one week. During adaptation period, fish were fed with standard pellet without probiotic addition at a dose of 5% body $weight^{-1}$ day^{-1}, twice a day at 08.00 and 16.00. Each bucket was aerated continuously using an aerator. A water pump (capacity 1500 L/H) was also provided for water recirculation.

After adaptation process, the fish were fed pellet already supplemented with probiotic at different concentrations for four weeks. The density of the stocked fish was 25 individuals per bucket. The feed dosage was the same as in the adaptation period, namely 5% body $weight^{-1}$ day^{-1}, twice a day at 08.00 and 16.00. About two-third of water volume was exchanged every two days while uneaten pellet and fish waste were removed every day by syphoning.

Challenge test. At the end of feeding, the fish were challenged with *A. hydrophila* through cohabitation with infected fish. This was done by adding five infected live fish in each treatment. Clinical sign and mortality were observed continuously for two weeks after challenge test.

Survival rate. Survival rate of fish were calculated following the formula applied by Kong et al (2020):

$$\text{Survival rate (\%)} = (\text{final number of fish/initial number of fish}) \times 100$$

Result and Discussion. Survival of fish observed at 14 days after challenged with *A. hydrophila* bacteria is presented in Figure 1. The highest survival rate was achieved with pellets supplemented with probiotic at concentration of 1×10^7 cfu mL^{-1} (treatment C), followed by 1×10^8 cfu mL^{-1} (treatment D). The lowest survival rate was observed in treatment A and B (Figure 1).

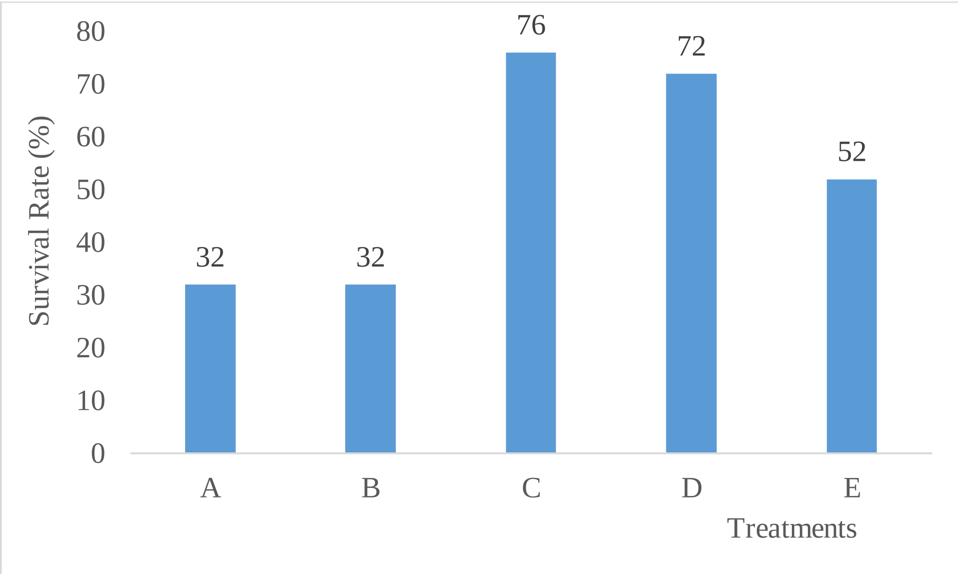

Figure 1. Survival of *Cyprinus carpio* 14 days post-challenge with *Aeromonas hydrophila* (A = Control; B = probiotic 1×10^6 cfu mL^{-1}, C = probiotic 1×10^7 cfu mL^{-1}, D = probiotic 1×10^8 cfu mL^{-1}, E = probiotic 1×10^9 cfu mL^{-1}).

The result of the present research showed the best survival rate of fish obtained in fish fed pellet added with probiotic at 1×10^7 - 1×10^8 cfu mL^{-1}. This result was in line with the statement of Salinas et al (2008) in which the addition of probiotic at 10^7 cfu mL^{-1} generated the best stimulatory effects resulted from the increase in cellular innate immune response of fish.

Several reports stated that probiotics given to fish could increase immune response, resistance to pathogens, survival as well as growth, digestibility, feed efficiency, and appetite (Cavalcante et al 2020; Kong et al 2020; Mulyasari et al 2016; Kesarcodi-Watson et al 2008; Merrifield et al 2010). In the research of Undi et al (2020), carp seeds fed with pellet supplemented with probiotic *Lactobacillus* sp. at 1×10^7 - 1×10^8 cfu mL^{-1} for six weeks showed a significant increase in total leukocytes and phagocytic activity. Pangalila et al (2020) also reported that addition of probiotic *Lactobacillus* sp. originating from catfish intestine at concentration of 1×10^8 cfu mL^{-1} significantly increased the total leukocytes and phagocytic activity of carp seeds after being given for four weeks. Mohammadian et al (2019) found that *C. carpio* (65±5 g) treated with combination of 5×10^7 cfu *L. casei*, 1% β-glucan and mannan oligosaccharide and challenged with *A. hydrophila* had lower mortality compared to control. Furthermore, Kong et al (2020) reported that administering the probiotic *L. lactis* isolated from the intestine of snakehead fish (*Channa argus*) alone or in combination with *Enterococcus faecalis* for 56 days significantly increased survival rate of fish compared to control with a survival rate of 63.3%. This was resulted from the increase of humoral immunity as indicated by an increase in IgM, ACP, AKP, LZM levels, and C3 and C4 activity in serum, which in turn effectively increases the humoral immunity of fish.

Harikrishnan et al (2012) found that the addition of Lactobacillus probiotics in feed enhanced the nonspecific immune response and resistance of Olive Flounder (*Paralichthys olivaceus*). Nguyen et al (2017) confirmed *L. lactis* probiotics isolated from wild marine fish and given to fish with an average size of 80.84 g for 8 weeks improved the nonspecific immune response of *P. olivaceus*. Furthermore, Jang et al (2019) also reported that *P. olivaceus* fed with *Lactobacillus plantarum* probiotic for 8 weeks had better immunity as compared to control; the mortality rate of fish after being challenged with *Streptococcus iniae* was 12.5%, while the control fish reached 100%. In a study conducted by Dawood et al (2016), red sea bream (*Pagrus major*) fed diet with the addition of probiotics *L. rhamnosus* and *L. lactis* for 56 days showed a significant increase in fish immune response as indicated by increased hematocrit, total plasma protein, bactericidal activity, peroxidase, so that the two types of probiotics are concluded to stimulate an increase in fish health status if added to feed. Ullah et al (2018) had also reported that commercial probiotics given to fish for 90 days increased the nonspecific immune response of mori fish (*Cirrhinus mrigala*) which was marked by an increase in total leukocytes, lysozyme activity, total plasma protein and immunoglobulin compared to control fish. In *Labeo rohita*, application of *L. plantarum* probiotics was potential to increase fish resistance to disease attacks and increase growth performance (Giri et al 2013).

Lactobacillus can also be applied in shrimp farming. In a study conducted by Karthik et al (2014), tiger shrimp treated with *Lactobacillus* sp. and challenged with *Vibrio harveyi* had a mortality of 6% while untreated shrimp had a mortality of 80%. In vanname shrimp, the mortality of shrimp treated with *Lactobacillus* sp. was 12%, while the mortality of shrimp that was not treated was 100%. Through meta-analysis, Toledo et al (2019) found that shrimps treated with probiotic had higher survival than control. Another report by Wang et al (2019) showed that the probiotics *L. pentosus*, *L. fermentum*, *Bacillus subtilis* and *Saccharomyces cerevisiae* in mixed form given to white shrimp *Litopenaeus vannamei* at a concentration of 10^7 - 10^9 cfu (kg feed)$^{-1}$ for 56 days significantly improved the health status of the shrimp as indicated by increased survival after challenged with the bacterium *Vibrio alginolyticus*. *L. plantarum* originated from wild shrimp was reported to increase survival of *L. vannamei* (Kongnum & Hongpattarakere 2012). Xie et al (2019) also reported that giving a mixture of probiotics Lactobacillus, *B. subtilis* and *B. licheniformis* was able to increase nonspecific immune response and growth of white shrimp.

The present research proved that probiotic originated from carp can enhance the resistance of the same species against bacterial pathogen. The best response was achieved in fish fed pellet with the addition of probiotic at concentration of 10^7 – 10^8 cfu mL^{-1} (survival 72-76%) while control only 32%. The increase in survival was probably resulted from the increase in immune response of fish. In addition, Hagi & Hashino (2009) stated that Lactobacillus isolated from carp intestines consisting of *L. lactis* and *Lactobacillus raffinolactis* were found to have high anti-bacterial activity against fish pathogens (*Aeromonas* sp). At the higher probiotic concentration (treatment E), survival tended to decrease up to 44%. Hai (2015) stated that the effectiveness of probiotic is influenced by dosage and time duration in which overdosage can induce suppression on fish immune response. Further research is needed to be conducted to prove whether the response is similar, better or worse if the probiotic applies to other different fish species.

According to Bermudez-Brito et al (2012), there are four different ways probiotics fight pathogens in fish gut. First, probiotics will compete to get the same nutrients required by pathogens so that pathogens will experience a high nutritional deficiency because they are more widely used by probiotic bacteria. Second, probiotics prevent the attachment of pathogens in the intestine to the intestinal wall by reducing the surface area available for pathogen colonization. Third, probiotics will send signals to immune cells and then immune cells will produce cytokines to further stimulate the production of leukocytes to destroy pathogens. Fourth, probiotics attack pathogens by releasing bacteriocytes that kill pathogens directly.

Conclusions. Oral administration of probiotic originated from carp intestine showed potential to improve survival of the same species against *A. hydrophila* challenge. The best survival rate was achieved in fish fed pellet supplemented with probiotic at concentration of $1x10^7 - 1x10^8$ cfu mL^{-1}.

Acknowledgements. The authors wish to thank the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for funding the research through Research and Community Servive Fund (DRPM) fiscal year of 2020.

References

Biswas G., Korenaga H., Takayama H., Kono T., Shimokawa H., Sakai M., 2012 Cytokine responses in the common carp, *Cyprinus Carpio* L. treated with Baker's yeast extract. Aquaculture 356-357:169-175.

Banu M. R., Akter S., Islamb M. R., Mondola M. N., Hossainc M. A., 2020 Probiotic yeast enhanced growth performance and disease resistance in freshwater catfish gulsa tengra, *Mystus cavasius*. Aquaculture Reports 16:100237, https://doi.org/10.1016/j.aqrep.2019.100237

Bermudez-Brito M., Plaza-Diaz J., Munoz-Quezada S., Gomez-Llorente C., Gil A., 2012 Probiotic mechanisms of action. Annals of Nutrition & Metabolism 61:160-174.

Cavalcante R. B., Telli G. S., Tachibana L., Dias D. C., Oshiro E., Natori M. M., da Silva W. F., Ranzani-Paiva M. J., 2020 Probiotics, prebiotics and synbiotics for Nile tilapia: Growth performance and protection against *Aeromonas hydrophila* infection. Aquaculture Reports 17:100343.

Chowdhury M. A., Roy N. C., 2020 Probiotic supplementation for enhanced growth of striped catfish (*Pangasianodon hypophthalmus*) in cages. Aquaculture Report 18:100504.

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

Dawood M. A. O., Koshio S., Ishikawa M., Yokoyama S., Basuini E. E. M., Hossain S. M., Nhu H. T., Dossou S., Moss S. A., 2016 Effect of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*. Fish & Shellfish Immunology 49:275-285.

Fečkaninová A., Koščová J., Mudroňová D., Popelka P., Toropilová J., 2017 The use of probiotic bacteria against Aeromonas infections in salmonid aquaculture. Aquaculture 469:1-8.

Giri S. S., Sukumaran V., Oviya M., 2013 Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish. Fish and Shellfish Immunity 34:1134-1140.

Hagi T., Hoshino T., 2009 Screening and characterization of potential probiotic lactic acid bacteria from cultured common carp intestine. Bioscience, Biotechnology, and Biochemistry 73(7):1479-1483.

Hai N. V., 2015 The use of probiotics in aquaculture. Journal of Applied Microbiology 119:917-935.

Harikrishnan R., Balasundaramb C., Soo M. H., 2012 Effect of probiotic enriched diet on *Paralichthys olivaceus* affected with Lympocyctis disease virus (LCDV). Fish and Shellfish Immunology 29:868-874.

Hoseinifar S. H., Esteban M. A., Cuesta A., Sun Y. Z., 2015 Prebiotics and fish immune response: A review of current knowledge and future perspectives. Reviews in Fisheries Science & Aquaculture 23:315–328

Hoseinifar S. H., Sun Y. Z., Wang A., Zhou Z., 2018 Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Frontiers in Microbiology 9:2429.

Jang W. J., Leeb J. M., Hasana M. T., Leed B. J., Lim S. G., Konga I. S., 2019 Effects of probiotic supplementation of a plant-based protein diet on intestinal microbial

diversity, digestive enzyme activity, intestinal structure, and immunity in olive flounder (*Paralichthys olivaceus*). Fish & Shellfish Immunology 92:719-727.
Karthik R., Hussain A. J., Muthezhilan R., 2014 Effectiveness of *Lactobacillus* sp. (AMET1506) as probiotic against Vibriosis in *Penaeus monodon* and *Litopenaeus vannamei* shrimp aquaculture. Bioscience Biotechnology Research Asia 11:297-305.
Kavitha M., Raja M., Perumal P., 2018 Evaluation of probiotic potential of *Bacillus* spp. isolated from the digestive tract of freshwater fish *Labeo calbasu* (Hamilton, 1822). Aquaculture Report 11:59-69.
Kesarcodi-watson A., Kaspar H. Lategan, Gibson M. J., 2008 Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. Aquaculture 274:1-14.
Kong Y., Gao C., Dub X., Zhao J., Lia M., Shana X., Wang G., 2020 Effects of single or conjoint administration of lactic acid bacteria as potential probiotics on growth, immune response and disease resistance of snakehead fish *(Channa argus*). Fish & Shellfish Immunology 102:412-421.
Kongnum K., Hongpattarakere T., 2012 Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. Fish & Shellfish Immunology 32:170-177.
Ling Y., Zang R., Ke C., Hong G., 2018 Effects of dietary supplementation of probiotics on growth, immune responses, and gut microbiome of the abalone *Haliotis disvericolor.* Aquaculture 493:289-295.
Lin T., Liu X., Xiao D., Zhang D., Cai Y., Zhu X., 2019 *Lactobacillus* spp. as probiotics for prevention and treatment on enteritis in the lined seahorse (*Hippocampus erectus*) juveniles. Aquaculture 503:16-25.
Manoppo H., Tumbol R. A., Sinjal H. J., Sumaraw J., 2019 Growth and feed efficiency enhancement by probiotic originating from intestine of carp, *Cyprinus carpio*. AACL Bioflux 12(6):2188-2194.
Merrifield D. L., Bradley G., Baker R. T. M., Davies S. J., 2010 Probiotic applications for rainbow trout (*Oncorhychus mykiss* Walbaum). II. Effect on growth performance, feed utilization, intestinal microbiota and related health criteria postantibiotic treatment. Aquaculture Nurtition 16:496-503.
Mohammadian T., Nasirpour M., Tabandeh M. R., Mesbah M., 2019 Synbiotic effects of B-glucan, mannan oligosaccharide and *Lactobacillus casei* on growth performance, intestine enzymes activities, immune-hematological parameters and immune-related gene expression in common carp, *Cyprinus carpio*: an experimental infection with *Aeromonas hydrophila*. Aquaculture 511:634197.
Mulyasari, Widanarni, Suprayadi A., Zairin Jr., Sunarno M. T. D., 2016 Screening of probiotics from the digestive tract of gouramy (*Osphronemus gouramy*) and their potency to enchance the growth of tilapia (*Oreochromis niloticus*). AACL Bioflux 9(5):1121-1132.
Nguyen T. L., Park I. C., Kim D. H., 2017 Improved growth rate and disease resistance in olive flounder, *Paralichthys olivaceus*, by probiotic *Lactococcus lactis* WFLU12 isolated from wild marine fish. Fish & Shellfish Immunology 471:113-120.
Pangalila N., Manoppo H., Tumbol R. A., Lumenta C., Kreckhoff R. L., Warouw V., 2020 [Immune response of carp *Cyprinus carpio* fed diet supplemented with *Lactobacillus* sp. with different concentration]. Ejournal Budidaya Perairan 8(1):38-47. [In Indonesian].
Perez-Sanchez T., Ruiz-Zarzuela I., de Blas I., Balcazar J. L., 2014 Probiotics in aquaculture: a current assessment. Reviews in Aquaculture 6:133-146.
Prabhurajeshwar C., Chandrakanth K., 2019 Evaluation of antimicrobial properties and their substances against pathogenic bacteria in-vitro by probiotic Lactobaccillus strains isolated from commercial yogurt. Clinical Nutrition Experimental 23:97-115.
Reverter M., Bontemps N., Lecchini D., Banaigs B., Sasal P., 2014 Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture 433:50-61.
Salinas I., Abelli L., Bertoni F., Picchietti S., Roque A., Furones D., Cuesta A., Meseguer J., 2008 Monospecies and multispecies probiotic formulations produce different

systemic and local immunostimulatory effects in the gilthead seabream (*Sparus aurata* L.). Fish & Shellfish Immunology 25:114–123.
Sornplang P., Piyadeatsoontorn S., 2016 Probiotic isolates from Unconventional Sources: A review. Journal of Animal Science and Technology 58:26, doi: 10.1186/s40781-016-0108-2.
Toledo A., Frizo L., Signorini M., Bossier P., Arenal A., 2019 Impact of probiotics on growth performance and shrimp survival: A meta-analysis. Aquaculture 500:196-205.
Ullah A., Zuberi A., Ahmad M., Shaha A. B., Younus N., Ullah S., Khattak M. N. K., 2018 Dietary administration of the commercially available probiotics enhanced the survival, growth, and innate immune responses in Mori (*Cirrhinus mrigala*) in a natural earthen polyculture system. Fish & Shellfish Immunology 72:266-272.
Undi C. S., Manoppo H., Kreckhoff R. L., Tumbol R. A., Pangkey H., 2020 [Use of probiotic to enhance non specific immune response of carp (*Cyprinus carpio*)]. Ejournal Budidaya Perairan 8(2):42-50. [In Indonesian].
Wang Y. B., Li J. R., Lin J., 2008 Probiotics in aquaculture: Challenges and outlook. Aquaculture 281:1-4.
Wang H., Wang C., Tang Y., Sun B., Huang J., Song X., 2018 Pseudoalteromonas probiotics as potential biocontrol agents improve the survival of *Penaeus vannamei* challenged with acute hepatopancreatic necrosis disease (AHPND)-causing *Vibrio parahaemolyticus*. Aquaculture 494:30-36.
Wang Y. B., Xu Z. R., 2014 Probiotics treatment as method of biocontrol in Aquaculture. Feed Research 12:42-45.
Wang Y. C., Hu Y. S., Chiu S. C., Liu H. C., 2019 Multiple-strain probiotics appear to be more effective in improving the growth performance and health status of white shrimp, *Litopenaeus vannamei*, than single probiotic trains. Fish & Shellfish Immunology 84:1050-1058.
Xie J. J., Liu Q. Q., Liao S., Fang H. H., Yin P., Xie W. S., Tian X. L., Liu J. Y., Niu J., 2019 Effects of dietary mixed probiotics on growth, non-specific immunity, intestinal morphology and microbiota of juvenile pacific white shrimp, *Litopenaeus vannamei.* Fish & Shellfish Immunology 90:456-465.
Zokaeifar H., Balcázar J. L., Saad C. R., Kamarudin M. S., Sijam K., Arshad A., Nejat N., 2012 Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. Fish & Shellfish Immunology 33:683-689.

Received: 01 October 2020. Accepted: 02 November 2020. Published online: 12 November 2020.
Authors:
Henky Manoppo, Sam Ratulangi University, Faculty of Fisheries and Marine Science, Aquaculture Study Program, Indonesia, 95115 Manado, e-mail: hmanoppo@yahoo.com
Reiny Antonetha Tumbol, Graduate School of Sam Ratulangi University, Aquatic Science Study Program, Indonesia, 95115 Manado, e-mail: reinytumbol@yahoo.com
Jeane Sumaraw, Board of Fish Quarantine, Quality Control and Safety of Fisheries Product, Indonesia, 95258 Manado, email: jeanesu1104@gmail.com

How to cite this article:
Manoppo H., Tumbol R. A., Sumaraw J., 2020 Oral administration of probiotic improve survival of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila* infection. AACL Bioflux 13(6):3377-3383.