



EFFECT OF HERBS SOLUTION ADDITION UPON FEED TOWARDS SURVIVAL RATE OF BONYLIP BARB (*OSTEOCHILUS HASSELTII*) INFECTED BY *AEROMONAS HYDROPHILA*

Muhammad Fathi Dhiaulhaq¹, Rosidah¹, Kiki Haetami¹, Roffi Grandiosa¹

¹Departemen of Fisheries, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Bandung – Sumedang Highway Km 21, Jatinangor 45363, Indonesia
Author correspondent: dhiaulhaqfathi@gmail.com

KeyWords

Aeromonas hydrophila, Bonylip barb, herbs solution, survival rate

ABSTRACT

The purpose of this study is to determine whether addition of herbal solutions can increase immune response seen from the survival rate of bonylip barb. The research method used was a completely randomized design (CRD) with five treatments and three replications. The treatments included the addition of herbs solution to the feed at concentrations of 0 mL/kg (A), 100 mL/kg (B), 200 mL/kg (C), 300 mL/kg (D), and 400 mL/kg (E). The treatments were maintained for 21 days. Following that, fish were challenged with 10^8 CFU/ml *Aeromonas hydrophila*. After that, survival was observed for 14 days. The parameters of this study were mortality, survival rate, and water quality. The data of survival rate were analyzed using ANOVA and followed by Duncan's Multiple Range Test. Following that, a regression analysis was performed with the addition of herbs solution with survival rate. Results showed that the highest SR were on treatment 38,33% (C), 31,67% (B), 6,67% (E), 5,00% (A), 3,33% (D). The correlation value obtained from the regression analysis are $R = 0,6167$ or 61,67%. Addition of fish herbs at 200 mL/kg was effective in maintaining the body resistance of bonylip barb that infected by *A. hydrophila* seen from survival rate of 38,33%.

The first page should be used only for Title/ Keyword/ Abstract section. The main paper will start from second page.

PREFACE

Bonylip barb (*Osteochilus hasseltii*) is one of the endemic fish of Indonesia, especially in West Java area, which lives in freshwaters. Bonylip barb has characteristics that are almost like goldfish because these fish are still in the same family as goldfish (Syamsuri et al. 2017)¹. Viewed from the aspect of environmental sustainability, commodities of fish can act as a dirt cleaner because it is included in the group that eats plankton, periphyton, and aquatic plants (Subagja et al. 2007)².

Development and sustainability of aquaculture often face obstacles, especially when there is an attack of disease, both infectious and non-infectious. Many pathogenic attacks or infections cause disease caused by bacteria named *Aeromonas hydrophila*. This bacterium is very influential in freshwater fish farming and often causes disease outbreaks with a high mortality rate (80 – 100%) in a short time (1 – 2 weeks) (Christy et al. 2019)³. *Aeromonas hydrophila* is a pathogenic bacterium that causes Motile Aeromonas Septicemia (MAS), especially for freshwater fish species such as catfish, gourami, catfish, tilapia, and goldfish which can cause disease outbreaks (Sukandar 2018)⁴.

Disease control in fish can be done by giving ingredients that can increase the fish's body resistance by giving antibacterial or antibiotic substances. Continuous use of antibiotics will have an unfavorable impact, which can cause pathogen resistance to antibiotics. In addition, it can cause residues in fish so that it can be harmful for humans who consume (Wahjuningrum et al. 2010)⁵.

Several studies have been directed at the use of natural ingredients, both from plant and animal extracts, as well as microorgan-

ism derivatives, to replace the role of chemicals used as immunostimulants (Kurniawan 2012)⁶. The use of natural ingredients that are environmentally friendly, easy to obtain, cheaper, and have minimal side effects, among others, by adding polyherbal in the form of herbs solution to the feed. Herbs solution or herbal supplements are a mixture of several herbal ingredients (Puspitasari 2017)⁷. The composition in the herbs solution includes turmeric, ginger, javanese ginger, greter galingale, noni fruit, tropical almond leaves, betel leaves and stems, molasses, and yeast. Based on research on the use of herbal ingredients that make up herbs solution, it is known to have a good impact on the fish's immune system with active substances in the form of flavonoids, saponins, and triterpenoids (Harikrishnan et al. 2011)⁸.

This study aims to analyze and determine the optimal concentration of addition herbs solution on feed to increase the body's resistance of fish which is indicated by the survival rate.

Methodology

Research method used in present study were Completely Randomized Design (CRD) with 5 treatments and 3 replications. Treatment A (control), treatment B (100 mL/kg), treatment C (200 mL/kg), treatment D (300 mL/kg) and E (400 mL/kg). Ingredients used in the present study were herbs solution with the composition: turmeric, ginger, javanese ginger, greter galingale, noni, tropical almond leaves, betel leaves and stems, molasses and yeast. Materials used for culture of *Aeromonas hydrophila* are Nutrient Agar, Nutrient Brooth, *Aeromonas hydrophila* isolate, distilled water and 70% alcohol. The tools used for bacterial culture are ose needle, bunsen, petri dish, 250 ml Erlenmeyer, hot plate, incubator shaker, test tube, falcon tube, laminar flow and magnetic stirrer. The tools used in this research are aquarium, heater, aerator, pH meter, DO meter, 1 ml syringe. Herbs solution given treatment period is 21 days, after which it was tested challenged with *Aeromonas hydrophila* at a density of 10⁸ CFU / ml and the observed survival for 14 days.

Test fish used bonylip barb with a length of 5-7 cm. Each aquarium contains 20 fish. Preparation of the test feed was carried out by weighing 3% of total fish weight, then mixed with herbs solution using a disposable syringe according to the treatment, then allowed to stand for 12 hours. Feeding was carried out twice a day (08.00 and 16.00)

Research Parameters are :

- Survival Rate (Effendie 1997)⁹

$$SR = \frac{Nt}{No} \times 100\%$$

Description:

SR = Fish Survival Rate (%)

Nt = Number of Fish that Live at the End of the Study

No = Number of Fish that Lived at the Beginning of the Study

- Regression Analysis

Quadratic regression analysis with the equation model, as follows:

$$Y = a + bx + cx^2$$

Values of a, b and c are obtained using determination method with the equation model:

$$\sum Y = an + b\sum X + c\sum X^3$$

$$\sum XY = a\sum X^2 + c\sum X^3$$

$$\sum X^2Y = a\sum X^3 + c\sum X^4$$

Optimum value obtained by the equation:

$$\frac{dy}{dx} = \frac{-b}{2a}$$

- Paramaters of water quality are as follow:

No	Parameter	Satuan	Alat Ukur
1	Suhu	°C	Termometer
2	Dissolved Oxygen	mgL ⁻¹	DO Meter
3	pH	-	pH Meter

Data of survival rate was analyzed for variance (F test) ANOVA at the 95% confidence level, if a significant difference was obtained (P<0.05), the Duncan test was performed to determine differences between treatments. After that, the survival rate data with the provision of fish herbal medicine were analyzed by regression to determine the correlation value. Water quality data were analyzed descriptively.

Result and Discussion

- Survival Rate

Observations of fish survival rate tests were conducted for 14 days after being challenged with *A. hydrophila* (Figure 1).

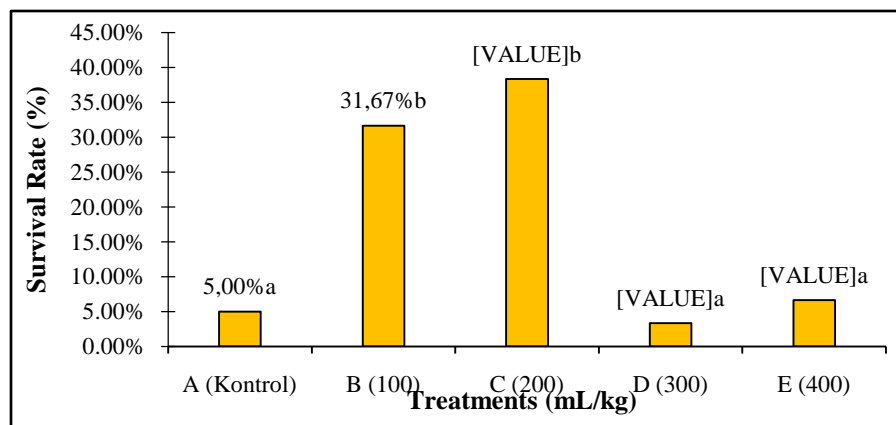


Figure 1. Survival Rate After Challenged Tests

Based on Figure 1 shows treatment A (control), D (300 mL/kg) and E (400 mL/kg) resulted in low survival, respectively 5.00%, 3.33% and 6.67%. This shows that treatment A that is not induced by immunostimulants in the form of herbal fish has a low survival rate. According to Hardi et al. (2017)¹⁰, tilapia that was not induced by immunostimulants had a high mortality rate against *Pseudomonas* sp. (80%) and *A. hydrophila* (60%). However, treatments D and E that were given herbs solution showed a low survival rate. The low survival rate is thought to be the effect of higher saponin content compared to treatments B and C. The herbs solution contains *Terminalia cattapa* L leaves which have secondary metabolites, one of which is saponins. According to Su and Chen (2008)¹¹, the application of saponins at a dose of 1 mg/L and 2 mg/L can increase the survival rate of vannamei shrimp, on the other hand, excessive saponin administration causes stress symptoms and is followed by death. In addition, according to Ardu-lanisa (2017)¹², the decrease in survival rate along with the increase in the dose of noni fruit extract can be caused by the content of saponins that have the potential to be toxic as well as antibacterial in Java barb.

Based on the results of Duncan's test at a significance level of 5%, treatments A, D and E were not significantly different, but significantly different from treatments B and C, while treatments B and C were not significantly different. This can be seen from treatments B (100 mL/kg) and C (200 mL/kg) which showed high survival compared to other treatments, namely 31.67% and 38.33%, respectively. This could be due to the administration of herbs solution as an immunostimulant that worked more effectively than other treatments. The active ingredients contained in the herbs solution have compounds that function as antibacterial and stimulants for the fish's immune system. It is known that the turmeric contained in the herbs solution has an active ingredient in the form of curcumin. according to Yonar et al. (2019)¹³, feeding with 2% curcumin to rainbow trout can increase the survival rate by 76.67% after being infected with bacteria *A. salmonicida*. Low mortality rate on fish herbal medicine was caused by the active compound curcumin producing cellular and humoral immune responses that allowed to reduce the mortality rate and defend fish against infection of *A. salmonicida*. In addition to turmeric, there is ginger which is also contained in herbs solution which show similar results. according to Talpur (2013)¹⁴, the administration of ginger at doses of 5 and 10 g/kg in the feed had a high survival rate against infection *Vibrio harveyi* of 86.6% in white snapper (*Lates calcarifer*). In addition, Monteiro et al. (2021)¹⁵ explained that feed added with ginger was able to increase several non-specific immunities such as increasing total neutrophils in leukocytes and phagocytic ability which play a role in protection against invading pathogens that attack the fish body.

From the description above, good feed utilization was shown by giving herbs solution with a concentration of 200 mL/kg which had the highest survival value from other treatments, which was 38.33%. Treatment D (300 mL/kg) and E (400 mL/kg) showed a decrease in survival rates. This is due to changes in the characteristics of the feed because of the addition of herbs solution, such as clumping of feed that occurs. The feed clumps because the herbs solution in large quantities can soften and make the feed stick to each other. This causes the feed to be difficult for fish to consume and tends to be on the edge of the rearing container.

Treatments B and C had a high survival rate compared to other treatments, but the value did not reach 50%. This can be caused by the presence of secondary metabolites that have the potential to be toxic if in high and dilute concentrations. One of them is the saponins contained in the leaves of ketapang and noni. According to Muharrama et al. (2015)¹⁶, saponins are compounds that are toxic to fish, especially in very dilute solutions and have a hemolytic activity that can damage red blood cells and can cause the fish's respiratory process to be hampered. In addition, according to Rosmawaty (2016)¹⁷, high levels of saponin and alkaloid concentrations cause high mortality in fish.

- Regression Analysis of Herbs Solution on Survival Rate

Regression analysis of the addition of herbs solution on survival was carried out to see the correlation value of the addition of herbs solution to survival. In addition, regression analysis can also determine the optimal concentration of herbs solution to increase the body's resistance to bonylip barb which is indicated by survival. The graph of the relationship between the addition of herbs solution to the survival of the test fish can be seen in Figure 2.

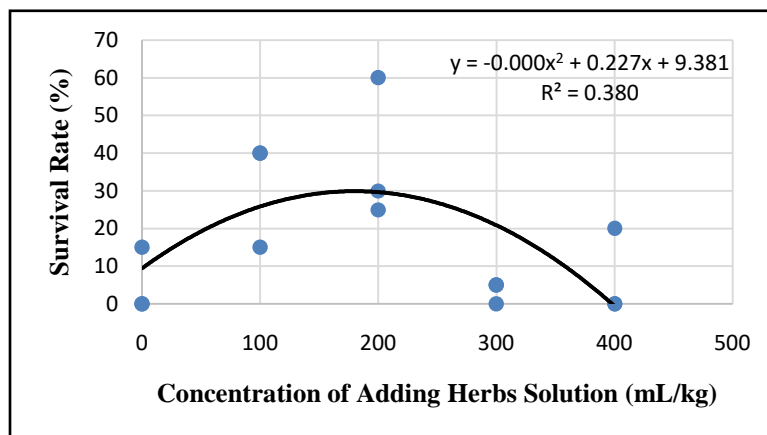


Figure 2. Correlation of Addition Herbs Solution on Feed to Survival Rate of Bonylip Barb

Based on the results of regression analysis, the relationship between two treatments shows a quadratic relationship with the equation $Y = -0.0006x^2 + 0.2274x + 9.381$. Also obtained the value of the relationship of determination (R^2) = 0.3803 (Figure 2), after it obtained the value of correlation (R) = 0.6167, meaning that 61.67% of survival after challenge test is influenced by the addition of herbs fish in feed and 38.33% caused by other factors. Based on these equations obtained optimum concentration value addition of fish on the feed herbs can improve the survival of the infected fish nilem *A. hydrophila* that is equal to 189.5 mL/kg.

- Water Quality Measurement

Observations of water quality were carried out for 21 days during the maintenance period of giving herbs solution in feed. The results of observations of water quality during the maintenance period are presented in the following table.

Treatments (mL/kg)	Parameters of Water Quality		
	DO (mg/L)	pH	Temp (°C)
A (Control)	6,3-7,7	8,18-8,53	25,4-29,1
B (100)	5-7,9	8,22-8,45	25,3-28,5
C (200)	5,7-8	8,04-8,41	25,2-27,1
D (300)	5-7,9	8,18-8,47	25,5-29,3
E (400)	5,4-8	8,1-8,51	26,9-30
Quality Standard*(Zoneveld 1991)	≥ 5 mg/L	6,0 – 9,0	18 – 30°C

Based on Table, the measurement results of dissolved oxygen have an average value in the range of 5 – 8 mg/L, the average pH is in the range of 8.04 – 8.53 and the average temperature range is 25.2 – 30 °C. Based on the results of measurements and observations of water quality during the study, giving herbs solution to feed did not adversely affect water quality. It can be seen from the value of the range of water quality without giving herbs solution not much different from the average range. Water quality during the maintenance period was in the optimal range and following with the quality standards based on Zoneveld (1991)¹⁸ for Cyprinidae fish culture.

Conclusion

Based on the research, it can be concluded that:

1. Herbs solution at a concentration of 200 mL/kg of feed resulted in the highest survival (38.33%) after being infected with *Aeromonas hydrophila*.
2. Based on regression analysis, 61.67% survival was influenced by the addition of herbs solution in the feed. The optimum value of the addition of herbs solution that can increase the survival of bonylip barb is 189.5 mL/kg.

References

- [1] Syamsuri, AI, Alfian, MW, Muharta, VP, Mukti, AT, Kismiyati, Satyantini, WH 2017. Technique of Enlarging Nilem (*Osteochilus hasselti*) at the Cen-

- ter for Development and Promotion of Carp and Nilem Fish Stocks (Bppsign) Tasikmalaya, West Java. *Journal of Aquaculture and Fish Health* Vol. 7 No.2.
- [2] Subagja, J., R. Gustiano., and Winarlin. 2007. Reproductive Technology of Nilem (*Osteochilus hasselti* CV): Gonad Maturation, Egg Handling and Provision of Prospective Parent. XXVII World Food Day National Seminar. case 187 - 194.
 - [3] Christy G, Kusdarwati R, Handijatno D. 2019. Determination of the aerolysin gene in *Aeromonas hydrophila* using the polymerase chain reaction (pcr) technique. IOP Conf. Series: Earth and Environmental Science 236 (2019) 012097
 - [4] Sukandar, A. 2018. Overview of The Blood Picture Of Hard-Lipped Barb (*Osteochilus hasselti* Cv) That Infected By *Aeromonas hydrophila* bacteria ABSTRACT. 9, 78–85.
 - [5] Wahjuningrum, D., E. H. Solikhah., T. Budiardi and M. Setiawati. 2010. Control of infection in *Aeromonas hydrophila* Dumbo Catfish (*Clarias* sp) with a mixture of meniran (*Phyllanthus niruri*) and Garlic (*Allium sativum*) in feed. *Indonesian Journal of Aquaculture.*, 9 (2): 93-103
 - [6] Kurniawan, A. 2012. *Aquatic Diseases*. UBB Press: Bangka Belitung. p.49 – 54
 - [7] Puspitasari D. 2017. Effectiveness of Herbal Supplements on Growth and Survival of Catfish Seeds (*Clarias* sp.). *Ilman Journal*, Vol 5 (1) :53-59.
 - [8] Hari Krishnan, R., Balasundaram, C., MS Heo. 2011. Impact Of Plant Products On Innate and Adaptive Immune System Of Cultured Finfish And Shellfish. *Aquaculture* 317. 1-15.
 - [9] Effendie MI 1979. *Fisheries Biology Methods*. Dewi Sri Foundation. Bogor. Indonesia. 112 pp.
 - [10] Hardi, Esti H., Saptiani, G., Kusuma, IW, Suwinarti, W., & Nugroho, RA 2017. Immunomodulatory and antibacterial effects of *Boesenbergia pandurata*, *Solanum rostratum*, and *Zingiber zerumbet* on tilapia, *Oreochromis niloticus*. *AAFL Bioflux*, 10(2), 182–190.
 - [11] Su, BK, & Chen, JC 2008. Effect of Saponin Immersion on Enhancement of The Immune Response of White Shrimp *Litopenaeus vannamei* and Its Resistance Against *Vibrio alginolyticus*. *Fish and Shellfish Immunology*, 24, 74–81.
 - [12] Ardulanisa, R., Prayitno, SB, & Haditomo, AHC (2017). The Effect of Soaking Noni Fruit Extract (*Morinda citrifolia*) on the Survival of Java Barb (*Barbonymus gonionotus*) Infected by *Aeromonas hydrophila*. *Journal of Aquaculture Management and Technology*, 6 (3), 258–265.
 - [13] Yonar, ME, Mişe Yonar, S., spir, & Ural, M. 2019. Effects of curcumin on hematological values, immunity, antioxidant status and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas salmonicida* subsp. chromogenes. *Fish and Shellfish Immunology*. Vol. 89. 83 – 90.
 - [14] Talpur, AD, Ikhwanuddin, M., & Ambok Bolong, AM 2013. Nutritional effects of ginger (*Zingiber officinale* Roscoe) on the immune response of Asian sea bass, *Lates calcarifer* (Bloch) and disease resistance against *Vibrio harveyi*. *Aquaculture*, Vol400.: 46-52.
 - [15] Monteiro, PC, Brandão, FR, Farias, CFS, de Alexandre Sebastião, F., Majolo, C., Dairiki, JK, de Oliveira, MR, Chaves, FCM, de Almeida O'Sullivan, FL, Martins, ML, & Chagas, EC 2021. Dietary supplementation with essential oils of *Lippia sidoides*, *Ocimum gratissimum* and *Zingiber officinale* on the growth and hemato-immunological parameters of *Colossoma macropomum* challenged with *Aeromonas hydrophila*. *Aquaculture Reports*, 19: 1 - 8.
 - [16] Muharrama, ARW, Syawal, H., & Lukistyowati, I. 2015. Sensitivity of Noni Fruit Extract (*Morinda citrifolia* L.) Against *Streptococcus agalactiae*. *UNRI Student Online Journal*, 2(1), 107–111.
 - [17] Rosmawaty, R., Rosidah, & Liviawaty, E. 2016. Utilization of Jengkol Skin Extract in Fish Feed to Increase Immunity of Juvenile Gouramy (*Osphronemus gouramy*) Against Bacterial Infections of *Aeromonas hydrophila*. *Journal of Fisheries and Marine Affairs*, VII (1), 14–22.
 - [18] Zonneveld, N., Huisman, E., & Boon, J. 1991. *Principles of Fish Farming*. Jakarta: Gramedia Pustaka Utama. 318 p.