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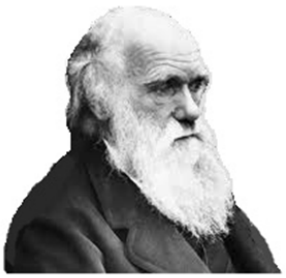

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Effect of Types Isolated Lactic Acid Bacteria on Hematocrit and Differential Leukocytes Fingerling Common Carp (*Cyprinus carpio* L.) Infected with *Aeromonas hydrophila* bacteria

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ABSTRACT

This study aims to see the condition of hematocrit levels and differential leukosit after immersion in LAB isolated and after *in vivo,* using *Aeromonas hydrophila* bacterium. LAB isolated were obtained from the organs of the common carp intestine. The study used a Completely Randomized Design (RAL) consisting of four treatments and three replications. The treatment used was immersion of test fish for 24 hours in several isolates of LAB with a density of 10^8, namely A: without LAB isolate (control), B: isolate CcB7, C: isolate CcB8, D: isolate CcB15. Immersion was done three times, with a frequency of seven days. Parameters observed included hematocrit levels, leukocyte differentiation and survival rate. The results showed that immersion in LAB isolate CcB15 will enhance the differential leukocytes and hematocrit levels that serve as indicators of carp body resistance, which is shown by high levels of white and red blood cells.

***Keywords*:** fingerling of Common carp, Lactic acid bacteria, Hematocrit levels, Differential leukosit, survival rate, immunostimulant, *Aeromonas hydrophila*, *Cyprinus carpio*

1. INTRODUCTION

Common carp (*Cyprinus carpio* L.) is one type of freshwater consumption fish which has important economic value, contains omega 3 and low fatty acids so that it can be used as a

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source of animal protein that is relatively inexpensive to meet human nutritional needs and is safe for health, because it can reduce the increase in cholesterol in the blood. Common carp is preffered by many people because the meat taste and protein content. Another advantage of common carp is that it is quite easy to maintain.

Therefore, common carp is in great demand, so the demand for common carp continues to increase. According to fisheries data of West Java Province, common carp production in the last three years (2014-2016) has continued to increase, so that the total production in 2016 reached 213,536 tons (Ministry of Maritime Affairs and Fisheries, Indonesia, 2016).

Picture 1. Common carp (*Cyprinus carpio* L.)

The main problem in common carp cultivation is the occurrence of infectious bacterial diseases that result in a decrease in fish production. To overcome this problem, generally various antimicrobial compounds are used, such as antibiotics and other chemicals. However, the application of antibiotics during fish production can cause other problems, namely bacteria resistant to antibiotics and food safety, because antibiotics accumulate in the fish's body.

Aeromonas hydrophila is a pathogenic bacterium that commonly attacks freshwater fish, including common carp. This bacterium causes haemorrhagic septicemia which is characterized by bulging stomach, ulcus, lesions, abscesses of the skin, exophthalmia, and bleeding, especially in the gills and operculum. This bacterial attack can cause a decrease in fish production up to 80%. The attack of *A. hydrophila* has a large impact on the decline in fish production, and the use of antibiotics causes resistant bacteria and affect the health status of fish, so it is necessary to seek alternative methods that are safer without any side effects to control the attack of *A. hydrophila* in common carp.

Lactic acid bacteria (LAB) is a gram-positive bacteria in the form of coccus or bacil, it does not form spores, optimum temperature ± 40 ºC, generally non motile, anaerobic, catalase negative and oxidase positive, with lactic acid as the main product of carbohydrate fermentation. The special properties of lactic acid bacteria are able to grow at high levels of sugar, alcohol, and salt, capable of fermenting monosaccharides and disaccharides.

Some bacteria, including lactic acid bacteria (LAB), namely *Lactobacillus* spp., *Bacillus* spp. and *Lactococcus* spp. have been commonly used as a probiotic to improve fish growth.

Bacillus sp. isolated from the intestines of fish from coastal areas in Japan produce antimicrobial compounds that can inhibit the growth of pathogenic microorganisms in cultivated fish. The bacteria isolated from the intestines of freshwater fish (*Providencia aeruginosa* VSG-2) have shown a potential as probiotics and antibacterial activity against *A. hydrophila*. Recent research shows that cellular components of Kocuria probiotics SM1 and SM2 have been shown to induce body immunity of rainbow trout against *Vibrio anguillarum* infection. The probiotic cellular component has been used as an immunostimulant to enhance the innate and adaptive immune response of fish, thus providing benefits to prevent disease attacks. Immunostimulants are a group of biological and synthetic compounds that can increase non-specific resistance by interacting directly with cells that activate the immune system in the fish's body. Cells found in leukocytes that can activate the immune system are phagocytic cells.

Based on the results of previous research, isolated isolates of lactic acid bacteria (LAB) from common carp intestine (*Cyprinus carpio* L.) have the potential as immunostimulants to prevent common carp from bacterial infection with *Aeromonas hydrophila*, indicated by antagonistic tests between lactic acid bacteria and fish pathogenic bacteria, showing a high inhibition zone for CcB7, CcB8, and CcB15 isolates. The purpose of this study was to determine the best lactic acid bacteria (LAB) isolates in improving the body's immune system against the attack of the *Aeromonas hydrophila* bacterium.

2. MATERIALS AND METHODS

2. 1. Materials and Tools

The research material used included 144 common carps with an average size of 10 cm, lactic acid bacteria (LAB) isolates, namely CcB7, CcB8, and CcB15, isolated from common carp intestines and *Aeromonas hydrophila* bacteria with a density of 10^8 cfu/mL. The tools used include 12 pieces of aquarium (40×30×30) cm^3, UTE digital scales, autoclave, needle ose, Petri disk, incubator, thermometer, spectrophotometer, and water heater.

2. 2. Method

This study used an experimental method with a completely randomized design consisting of 4 treatments and 3 replications, each treatment using 12 common carps. The treatment given was immersion of test fish in different isolates of LAB, namely A: control (without soaking the LAB isolate), B: CcB7, C: CcB8, and D: CcB15.

2. 3. Procedure

Culture of Lactic Acid Bacteria (LAB)

LAB bacteria isolated from common carp intestine (isolates of CcB7, CcB8, and CcB15) results of previous research were reproduced. Bacterial culture was carried out on de Mann Rogose and Sharpe (MRS) agar medium by inoculation. Media solutions and tools are sterilized in autoclave first. Then the media are poured on a petri disk and left to harden. Furthermore, bacterial isolates were cultured on MRS medium with three quadrant streak method, then the bacteria were incubated under anaerobic conditions for 24 hours in an incubator at 37 ºC. Pure colonies, formed from each BAL isolate, were re-cultured in MRS broth media in a valcon tube, incubated under anaerobic conditions for 24 hours in a 37 ºC incubator. The culture results of

LAB isolates using MRS broth will be turbid when compared to MRS broth control. Before being applied, LAB that has been cultured and calculated using a spectrophotometer to get a density of 10^6 CFU/mL.

Culture of *Aeromonas hydrophila* Bacteria

Nutrient Agar (NA), which has been weighed, dissolved with 100 mL of distilled water in an Erlenmeyer flask, closed using a cotton plug, then heated using a hot plate which was equipped with a magnetic stirrer to homogenize the solution. Then the solution was sterilized using autoclave for 15 minutes at 121 ºC. Sterile SA media were poured aseptically on Petri disks, allowed to cool until frozen and frozen. The bacteria *A. hydrophila* were inoculated on the SA medium aseptically, then incubated at 30 ºC for 48 hours. The bacteria are harvested using an ose needle and put into a test tube, then stored at a temperature of –20 ºC. Bacterial culture is ready for use.

Making *Aeromonas hydrophila* 10^8 CFU/mL bacterial solution

The culture of *A. hydrophila* was taken using an ose needle, then put into a test tube which contained 10 mL of physiological NaCl, the test tube was covered with cotton clogs and then the bacteria were homogenized with vortex. The homogeneous bacteria culture were put into a 2 mL cuvette and calculated using a spectrophotometer at a wavelength of 540 nm and an absorbance value of 0.235 to obtain a density of 10^8 / CFU/mL.

Immersion Fish in a Solution of Lactic Acid Bacteria

The test fish used was the Majalaya strain common carp with an average length of 10 cm, obtained from the Cibiru Fish Seed Center (BBI) Bandung Regency. Test fish are acclimatized for one week to adapt to the new environment and ensure the fish are in a healthy condition. A total of 12 test fishes were put into the aquarium to be immersed in LAB isolate solution 10^6 CFU/mL according to treatment, namely A: Control (without soaking LAB solution); B: CcB7, C: CcB8, and D: CcB15, each isolate repeated three times. Immersion was done for 24 hours, repeated three times with a frequency of 7 days.

Challenging Test with *Aeromonas hydrophila* Bacteria

Tested fish after being treated with LAB isolates were challenged with *Aeromonas hydrophila* 10^8 CFU/mL. Challenging tests were carried out by injecting bacteria ***Aeromonas hydrophila.*** After the fish were tested challenged to observe hematocrit levels, with differential leucocytes and survival rate. Observations were made for seven days.

2. 4. Observation Parameter

Hematocrit of Common carp

Hematocrit measurements on common carp are using method, by means of blood taken from the base of the tail, then applied to a microhematocrit tube (capillary tube) coated with heparin with a capillary system. The capillary tube was introduced to the centrifugator at a speed of 3,000 rpm for 30 minutes. Measurements were made by comparing the volume of blood objects to the volume of plasma blood using hematocrit scale.

Differential Leucocyte of Common carp

The first step is the making of smear preparations. The blood of the fish is dripped onto the glass, prepared and then dried, and soaked in methanol for 30 minutes, and then dripped with giemsa. After the giemsa dries, the preparation is washed and ready to be observed. Measurement of differentiation of leukocytes in carp is done by observing the levels of neutrophils, monocytes, and lymphocytes from fish, and calculated using the calculation below:

$$\% \text{ Limfosit} = \frac{L}{100} \times 100\ \%$$

$$\% \text{ Monosit} = \frac{M}{100} \times 100\ \%$$

$$\% \text{ Neutrofil} = \frac{N}{100} \times 100\ \%$$

Survival Rate

Observation of survival rates was carried out from the first day of common carp infected by *A. hydrophila* until the last day of maintenance. Survival rates are observed by counting the number of fish that die every day. The percentage of survival rate was obtained using the Effendie (1997) method as follows:

$$SR = \frac{Nt}{No} \text{ x } 100$$

where:

SR = Survival Rate (%)
Nt = the number of fish that live at the end of the research (fish)
No = the number of fish that live at the beginning of the research.

2. 5. Data Analysis

Data on hematocrit levels: Differential leukocytes, and survival were analyzed using the ANOVA F test. If there are significant differences between treatments, then proceed with Duncan's multiple distance test at the 5% error level to determine the best treatment.

3. RESULT DISCUSSION

3. 1. Percentage of Common carp Hematocrit levels

Hematocrit observation was carried out before immersion, after immersion, and after an *in vivo* test of the bacterium *Aeromonas hydrophila*. This is useful to determine the condition of fish health by looking at the percentage volume of erythrocyte cells in the blood. Hematocrit levels of test fish are presented in the form of the table below (**Table 1**).

Table 1. Hematocrit levels of Common carp

Treatment	Hematocrit (%) (Immersion)	Hematocrit (%) (*in vivo*)	% Decrease
	Blood	Blood	
Control (A)	44 ± 1.0^{a}	25 ± 2.64^{a}	19
CcB7 (B)	21.6 ± 10.4^{b}	20 ± 2.64^{a}	1.6
CcB8 (C)	37 ± 6.55^{bc}	20 ± 3.0^{ab}	17
CcB15 (D)	35 ± 4.72^{d}	27.5 ± 3.05^{c}	7.5

*Description: The average value followed by letters is significantly different according to Duncan's Multiple Range Test at 95% confidence level.

Based on Table 1, a decrease in hematocrit levels was suspected because the fish experienced anemia and stressed the attack of *Aeromonas hydrophila*. Decreasing hematocrit levels can be used as a clue about the low protein content, vitamin deficiency or fish getting an infection. After being infected with *A.hydrophila*, the number of erythrocytes tended to decrease, compared to after immersion, indicating that there was anemia in fish due to bleeding in the blood-producing organs.

3. 2. Differential Leucocyte of Common carp

Differential leukocytes observations in fish are conducted to determine the form of resistance of the fish body in counteracting the attack of pathogenic bacteria *A.hydrophila*. Leukocytes that are observed include lymphocytes, monocytes, and neutrophils. The percentage of leukocyte differentiation calculations is presented below (**Figures 1** and **2**).

Differential observations of leukocytes were carried out before and after challenging tests, using the bacteria *A. hydrophila*. The percentage of lymphocytes in each treatment after immersion ranged from 74 - 82%. While after the challenge test, the lymphocyte percentage became 65, 67 - 75.33%. Treatment A has a lower lymphocyte percentage when compared to treatment B, C, D due to treatment B, C, D concentration of lymphocytes has increased due to immunostimulant administration in fish through the immersion of LAB isolates in fish.

Based on Table 2, the results lymphocyte calculation (*in vivo*) showed a decrease when compared with lymphocytes before the challenging test. This is because lymphocytes in the fish's body have been used to ward off attacks of pathogenic bacteria. Lymphocytes in the body of fish are not phagocytic but play an important role in the formation of antibodies, reduced number of lymphocytes can reduce the concentration of antibodies causing an increase in disease attacks.

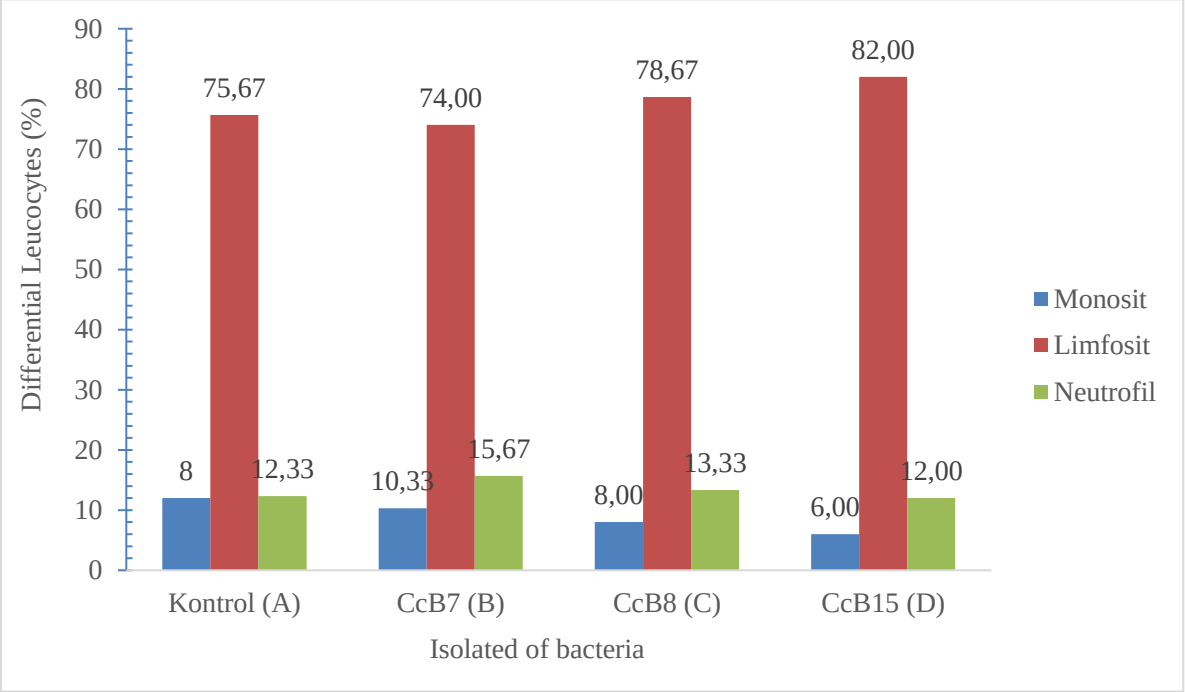

Figure 1. Leukocyte differentiation of Common carp before and after LAB immersion

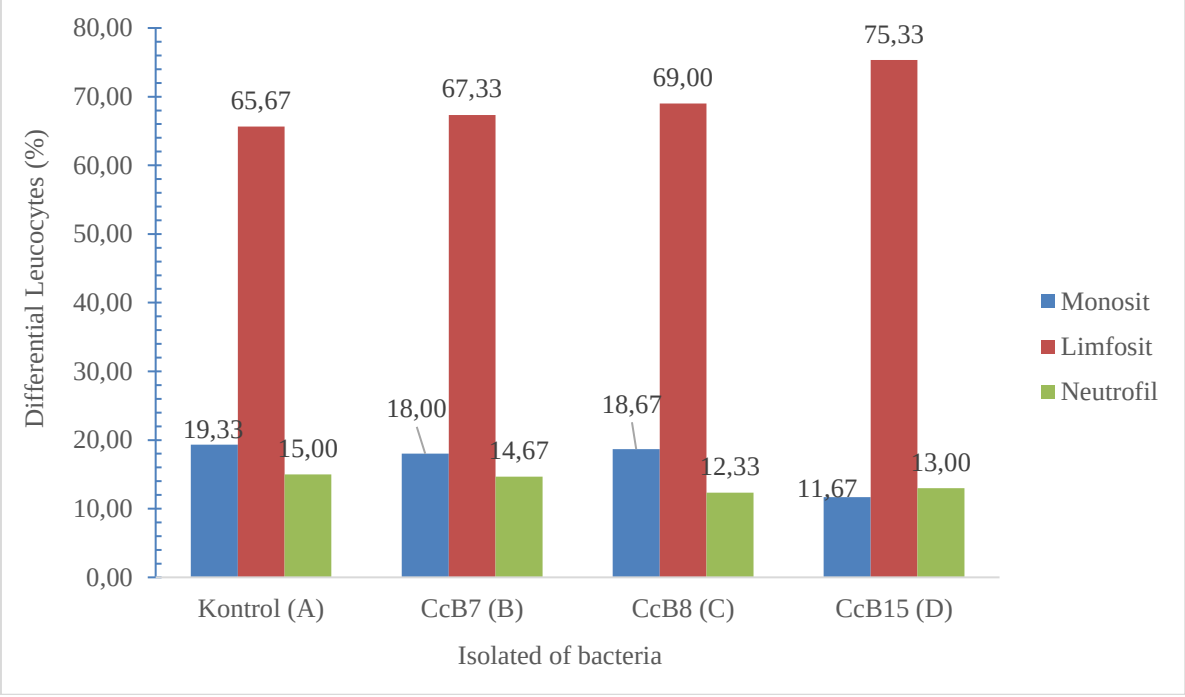

Figure 2. Leukocyte differentiation of Common carp before and after *in vivo*

Table 2. Lymphocytes (%) of Common carp after immersion LAB and *in vivo*

Treatment	Lymphocytes (Immersion)	Lymphocytes (*in vivo*)	% Decrease
	Blood	Blood	
Control (A)	75.67 ± 6.02	65.67 ± 2.08[a]	10
CcB7 (B)	74.00 ± 3.60	67.33 ± 1.52[b]	6.67
CcB8 (C)	78.67 ± 3.21	69.00 ± 4.35[c]	9.67
CcB15 (D)	82.00 ± 2.64	75.33 ± 0.57[d]	6.67

Description: The average value followed by letters is significantly different according to Duncan's Multiple Range Test at 95% confidence level.

Table 3. Monocytes (%) of Common carp after immersion LAB and *in vivo*

Treatment	Monocytes (Immersion)	Monocytes (*in vivo*)	% Enhancement
	Blood	Blood	
Control (A)	8.00 ± 3.46	19.33 ± 1.15[a]	11.33
CcB7 (B)	10.33 ± 2.51	18.00 ± 4.35[b]	7.67
CcB8 (C)	8.00 ± 2.00	18.67 ± 1.52[b]	10.67
CcB15 (D)	6.00 ± 1.73	11.67 ± 1.52[bc]	5.67

Description: The average value followed by letters is significantly different according to Duncan's Multiple Range Test at 95% confidence level.

In **Table 3**, the percentage of monocytes in each treatment after immersion ranged from 6 - 12%, while after the challenge test, the percentage of monocytes in each treatment increased to 12.33 - 19.33%.

Increased levels of monocytes after challenging tests was observed because monocytes phagocytes foreign objects around them were to maintain the resistance of fish to fight the attack of pathogenic bacteria. Macrophages are part of mature monocytes that play a major role in eating organisms and then are destroyed. Monocytes in the blood are short-lived, resulting in fluctuations in the number of monocytes. Monocytes will increase in number when there is inflammation.

The percentage of neutrophils (**Table 4**) in each treatment after immersion ranged from 10 - 13%, while after the challenge test (*in vivo*), the percentage of neutrophils in treatment A

and D has increased, and in treatment B and C has decreased. However, the increase and decrease were not significant. Neutrophils function to defend the body from harmful particles, especially bacteria.

Table 4. Neutrophil (%) of Common carp after immersion LAB and *in vivo*

Treatment	Neutrophil (Immersion)	Neutrophil (*in vivo*)	% Enhancement
	Blood	Blood	
Control (A)	12.33 ± 3.21	15.00 ± 1.00	2.67
CcB7 (B)	15.67 ± 1.15	14.67 ± 4.50	1.00
CcB8 (C)	13.33 ± 3.51	12.33 ± 4.16	1.00
CcB15 (D)	12.00 ± 2.00	13.00 ± 1.00	1.00

Description: The average value followed by letters is not significantly different according to Duncan's Multiple Range Test at 95% confidence level.

3. 3. Survival Rate

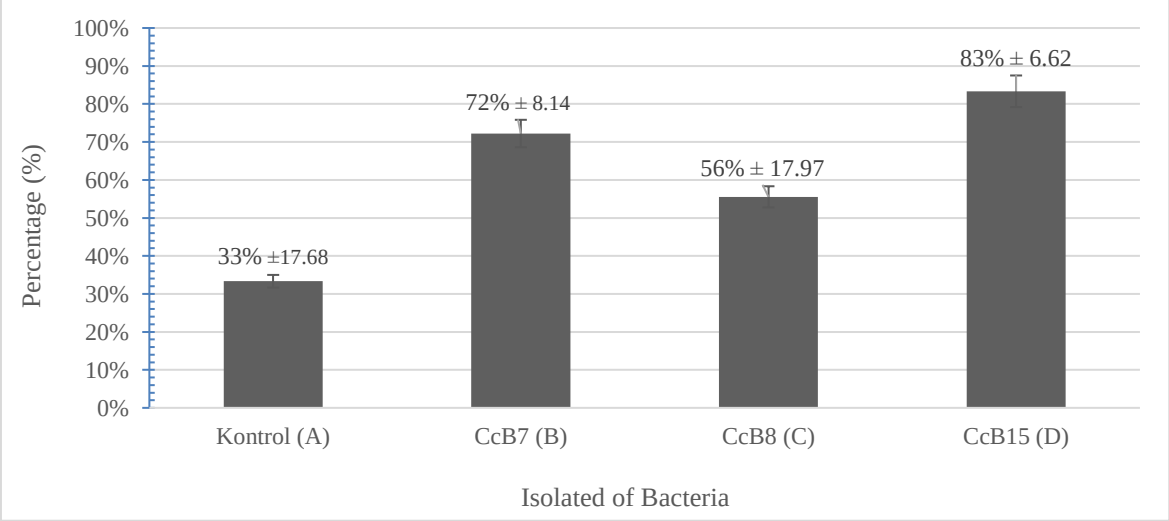

Figure 3. Survival rate of Common Carp after being challenged with *A. hydropila* 10^8 cfu/mL

Based on a variance analysis (ANOVA) test it was shown that between treatments of immersion of LAB isolates on fingerling, Common carp was not significantly different from the survival rate of fish with a significance level of 5%, different according to Duncan's Multiple Range Test at 95% confidence level. However, there was a trend toward the best treatment, namely the immersion treatment of BAL isolates CcB15 code (D), because treatment D as the

highest survival rate was 83%. LAB isolated contain bacteriocin which has a broad spectrum in inhibiting the growth of decomposing bacteria and pathogenic bacteria. Increased immune system in fish, able to counteract the attack of *Aeromonas hydrophila* bacteria, so that the survival rate of fish is high and daily mortality of fish is low.

4. CONCLUSIONS

CcB15 isolate is the best lactic acid bacteria to improve Hematocrit level and Differential Leucocytes as an immune system of fingerling Common Carp infected by *Aeromonas hydrophila* bacteria.

References

[1] Gunapathy Devi, Ramasamy Harikrishnan, Bilal Ahmad Paray, Mohammad K. Al-Sadoon, Seyed Hossein Hoseinifar, and Chellam Balasundaram, Effects of aloe-emodin on innate immunity, antioxidant and immune cytokines mechanisms in the head kidney leucocytes of Labeo rohita against Aphanomyces invadans, *Fish & Shellfish Immunology*, (2019). 10.1016/j.fsi.2019.02.006

[2] Felix K.A. Kuebutornye, Emmanuel Delwin Abarike, and Yishan Lu, A review on the application of Bacillus as probiotics in aquaculture, *Fish & Shellfish Immunology*, (2019). 10.1016/j.fsi.2019.02.010

[3] Dharmaraj Ramesh and Sami Souissi, Effects of potential probiotic Bacillus subtilis KADR1 and its subcellular components on immune responses and disease resistance in Labeo rohita, *Aquaculture Research*, 49, 1, (367-377), (2017).

[4] P. Kumar, K.K. Jain, P. Sardar, M. Jayant, and N.C. Tok, Effect of dietary synbiotic on growth performance, body composition, digestive enzyme activity and gut microbiota in Cirrhinus mrigala (Ham.) fingerlings, *Aquaculture Nutrition*, 24, 3, (921-929), (2017).

[5] Ratchanu Meidong, Kulwadee Khotchanalekha, Sompong Doolgindachbaporn, Takahiro Nagasawa, Miki Nakao, Kenji Sakai, and Saowanit Tongpim, Evaluation of probiotic Bacillus aerius B81e isolated from healthy hybrid catfish on growth, disease resistance and innate immunity of Pla-mong Pangasius bocourti, *Fish & Shellfish Immunology*, 73, (1), (2018).

[6] Yebing Yu, Changhai Wang, Aimin Wang, Wenping Yang, Fu Lv, Fei Liu, Bo Liu, and Cunxin Sun, Effects of various feeding patterns of Bacillus coagulans on growth performance, antioxidant response and Nrf2-Keap1 signaling pathway in juvenile gibel carp (Carassius auratus gibelio), *Fish & Shellfish Immunology*, 73, (75-83), (2018). 10.1016/j.fsi.2017.11.050

[7] Takavar Mohammadian, Mojtaba Alishahi, Mohammad Reza Tabandeh, Masoud Ghorbanpoor, and Dariush Gharibi, Changes in Immunity, Expression of some Immune-Related Genes of Shabot Fish, Tor grypus, Following Experimental Infection with Aeromonas hydrophila: Effects of Autochthonous Probiotics, *Probiotics and Antimicrobial Proteins*, 10, 4, (616-628), (2017). 10.1007/s12602-017-9373-8

[8] Mathialagan Kavitha, Manickam Raja, and Pachiappan Perumal, Evaluation of probiotic potential of Bacillus spp. isolated from the digestive tract of freshwater fish Labeo calbasu (Hamilton, 1822), *Aquaculture Reports*, 11, (59-69), (2018). 10.1016/j.aqrep.2018.07.001

[9] Sekar Jinendiran, Abel Arul Nathan, Dharmaraj Ramesh, Baskaralingam Vaseeharan, and Natesan Sivakumar, Modulation of innate immunity, expression of cytokine genes and disease resistance against Aeromonas hydrophila infection in goldfish (Carassius auratus) by dietary supplementation with Exiguobacterium acetylicum S01, *Fish & Shellfish Immunology*, (2018). 10.1016/j.fsi.2018.10.026

[10] Anran Wang, Chao Ran, Yanbo Wang, Zhen Zhang, Qianwen Ding, Yalin Yang, Rolf Erik Olsen, Einar Ringø, Jérôme Bindelle, and Zhigang Zhou, Use of probiotics in aquaculture of China—a review of the past decade, *Fish & Shellfish Immunology*, (2018). 10.1016/j.fsi.2018.12.026

[11] A. K. Amirkolaie, S. Karimzadeh, and S.P. Miandehy, The effects of dietary Betaplus® and TechnoMos® on growth performance, blood parameters, and intestinal microflora in juvenile kutum, Rutilus kutum (Kamensky, 1901), *Journal of Applied Ichthyology*, 33, 3, (491-497), (2017).

[12] Gülşen Uluköy, Seçil Metin, Ayşegül Kubilay, Şefik Güney, Pınar Yıldırım, Zeynep Güzel-Seydim, Tugba Kok-Tas, and Erkan Gümüş, The Effect of Kefir as a Dietary Supplement on Nonspecific Immune Response and Disease Resistance in Juvenile Rainbow Trout, Oncorhynchus mykiss (Walbaum 1792), *Journal of the World Aquaculture Society*, 48, 2, (248-256), (2016).

[13] Fernando Abasolo-Pacheco, Ángel I Campa-Córdova, José M Mazón-Suástegui, Dariel Tovar-Ramírez, Rubén Araya, and Pedro E Saucedo, Enhancing growth and resistance to Vibrio alginolyticus disease in catarina scallop (Argopecten ventricosus) with Bacillus and Lactobacillus probiotic strains during early development, *Aquaculture Research*, 48, 9, (4597-4607), (2017).

[14] E. Gümüş, A. Kubilay, Ş. Guney, Z. Guzel-Seydim, T. Kok-Tas, S. Metin and G. Ulukoy, Effect of dietary kefir on the growth performance, feed utilization and fatty acid profile of juvenile rainbow trout, Oncorhynchus mykiss, *Aquaculture Nutrition*, 23, 5, (964-972), (2017).

[15] Dharmaraj Ramesh, Sami Souissi, and Tharifkhan Shan Ahamed, Effects of the potential probiotics Bacillus aerophilus KADR3 in inducing immunity and disease resistance in Labeo rohita, *Fish & Shellfish Immunology*, 70, (408), (2017).

[16] Sib Sankar Giri, Jun Woo Jun, Venkatachalam Sukumaran, and Se Chang Park, Evaluation of dietary Hybanthus enneaspermus (Linn F. Muell.) as a growth and haemato-immunological modulator in Labeo rohita, *Fish & Shellfish Immunology*, 68, (310), (2017).

[17] Usha Jyoti Maji, Sriprakash Mohanty, Avinash Pradhan, and Nikhil Kumar Maiti, Immune modulation, disease resistance and growth performance of Indian farmed carp, Labeo rohita (Hamilton), in response to dietary consortium of putative lactic acid bacteria, *Aquaculture International*, 25, 4, (1391), (2017).

[18] H. Doan, S. Doolgindachbaporn, and A. Suksri, Effect of Lactobacillus plantarum and Jerusalem artichoke (Helianthus tuberosus) on growth performance, immunity and disease resistance of Pangasius catfish (Pangasius bocourti, Sauvage 1880), *Aquaculture Nutrition*, 22, 2, (444-456), (2015).

[19] Yebing Yu, Fu Lv, Changhai Wang, Aimin Wang, Weihong Zhao, and Wenping Yang, Effects of Bacillus coagulans on Growth Performance, Disease Resistance, and HSP70 Gene Expression in Juvenile Gibel Carp, Carassius auratus gibelio, *Journal of the World Aquaculture Society*, 47, 5, (729-740), (2016).

[20] Youngjin Park, Mohammad Moniruzzaman, Seunghan Lee, Jeongwhui Hong, Seonghun Won, Jong Min Lee, Hyeonho Yun, Kang-Woong Kim, Daegyun Ko, and Sungchul C. Bai, Comparison of the effects of dietary single and multi-probiotics on growth, non-specific immune responses and disease resistance in starry flounder, Platichthys stellatus, *Fish & Shellfish Immunology*, 59, (351), (2016).

[21] Narayanan Gobi, Balasubramanian Malaikozhundan, Vijayakumar Sekar, Sathappan Shanthi, Baskaralingam Vaseeharan, Rengarajan Jayakumar, and Abdul Khudus Nazar, GFP tagged Vibrio parahaemolyticus Dahv2 infection and the protective effects of the probiotic Bacillus licheniformis Dahb1 on the growth, immune and antioxidant responses in Pangasius hypophthalmus, *Fish & Shellfish Immunology*, 52, (230), (2016).

[22] Takavar Mohammadian, Mojtaba Alishahi, Mohammad Reza Tabandeh, Masoud Ghorbanpoor, Dariush Gharibi, Morteza Tollabi, and Siamak Rohanizade, Probiotic effects of Lactobacillus plantarum and L. delbrueckii ssp. bulguricus on some immune-related parameters in Barbus grypus, *Aquaculture International*, 24, 1, (225), (2016).

[23] Brian Austin and Dawn A. Austin, Vibrios, Bacterial Fish Pathogens, 499-601, (2016). 10.1007/978-3-319-32674-0_10

[24] Mahmoud A.O. Dawood, Shunsuke Koshio, Manabu Ishikawa, Saichiro Yokoyama, Mohammed F. El Basuini, Md. Sakhawat Hossain, Truong H. Nhu, Serge Dossou, and Amina S. Moss, Effects 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), (2016).

[25] Hien Van Doan, Sompong Doolgindachbaporn, and Amnuaysilpa Suksri, Effects of Eryngii mushroom (Pleurotus eryngii) and Lactobacillus plantarum on growth performance, immunity and disease resistance of Pangasius catfish (Pangasius bocourti, Sauvage 1880), *Fish Physiology and Biochemistry*, 42, 5, (1427), (2016).

[26] Sib Sankar Giri, Shib Sankar Sen, Jin Woo Jun, Venkatachalam Sukumaran, and Se Chang Park, Role of Bacillus subtilis VSG4-derived biosurfactant in mediating immune responses in Labeo rohita, *Fish & Shellfish Immunology*, 54, (220), (2016).

[27] Sib Sankar Giri, S. Jai Suda, V. Sukumaran, and Se Chang Park, Dietary emodin affects the growth performance, immune responses, and disease resistance of Labeo rohita against Aeromonas hydrophila, *Aquaculture International*, 10.1007/s10499-015-9910-y, 24, 1, (85-99), (2015).

[28] Bency Thankappan, Dharmaraj Ramesh, Srinivasagan Ramkumar, Kalimuthusamy Natarajaseenivasan, and Kumarasamy Anbarasu, Characterization of Bacillus spp. From

the Gastrointestinal Tract of Labeo rohita—Towards to Identify Novel Probiotics Against Fish Pathogens, *Applied Biochemistry and Biotechnology*, 175, 1, (340), (2015).

[29] Sib Sankar Giri, Shib Sankar Sen, Cheng Chi, Hyoun Joong Kim, Saekil Yun, Se Chang Park, and V. Sukumaran, Effect of cellular products of potential probiotic bacteria on the immune response of Labeo rohita and susceptibility to Aeromonas hydrophila infection, *Fish & Shellfish Immunology*, 46, 2, (716), (2015).

[30] Khaled M. Selim and Rasha M. Reda, Improvement of immunity and disease resistance in the Nile tilapia, Oreochromis niloticus, by dietary supplementation with Bacillus amyloliquefaciens, *Fish & Shellfish Immunology*, 44, 2, (496), (2015).

[31] Dharmaraj Ramesh, Annadurai Vinothkanna, Amit Kumar Rai, and Venkada Subramanian Vignesh, Isolation of potential probiotic Bacillus spp. and assessment of their subcellular components to induce immune responses in Labeo rohita against Aeromonas hydrophila, *Fish & Shellfish Immunology*, 45, 2, (268), (2015).

[32] Sib Sankar Giri, Shib Sankar Sen, Cheng Chi, Hyoun Joong Kim, Saekil Yun, Se Chang Park, and V. Sukumaran, Chlorophytum borivilianumPolysaccharide Fraction Provokes the Immune Function and Disease Resistance ofLabeo rohitaagainstAeromonas hydrophila, *Journal of Immunology Research*, 2015, (1), (2015).

[33] S.S. Giri, V. Sukumaran, S.S. Sen, and P.K. Jena, Effects of dietary supplementation of potential probiotic Bacillus subtilis VSG1 singularly or in combination with Lactobacillus plantarum VSG3 or/and Pseudomonas aeruginosa VSG2 on the growth, immunity and disease resistance of Labeo rohita, *Aquaculture Nutrition*, 20, 2, (163-171), (2013).

[34] Hien Van Doan, Sompong Doolgindachbaporn, and Amnuaysilpa Suksri, Effects of low molecular weight agar and Lactobacillus plantarum on growth performance, immunity, and disease resistance of basa fish (Pangasius bocourti, Sauvage 1880), *Fish & Shellfish Immunology*, 41, 2, (340), (2014).

[35] Qin Zhang, Hairui Yu, Tong Tong, Wanping Tong, Lanfang Dong, Mingzhu Xu, and Zhicheng Wang, Dietary supplementation of Bacillus subtilis and fructooligosaccharide enhance the growth, non-specific immunity of juvenile ovate pompano, Trachinotus ovatus and its disease resistance against Vibrio vulnificus, *Fish & Shellfish Immunology*, 38, 1, (7), (2014).

[36] Oliana Carnevali, Yun-Zhang Sun, Daniel L. Merrifield, Zhigang Zhou, and Simona Picchietti, Probiotic Applications in Temperate and Warm Water Fish Species, Aquaculture Nutrition, (253-289), (2014).

[37] Sib Sankar Giri, V. Sukumaran, and M. Oviya, Potential probiotic Lactobacillus plantarum VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, Labeo rohita, *Fish & Shellfish Immunology*, 34, 2, (660), (2013).

[38] Reza Akrami, Yousef Iri, Hosseinali Khoshbavar Rostami, and Majid Razeghi Mansour, Effect of dietary supplementation of fructooligosaccharide (FOS) on growth performance, survival, lactobacillus bacterial population and hemato-immunological

parameters of stellate sturgeon (Acipenser stellatus) juvenile, *Fish & Shellfish Immunology*, 35, 4, (1235), (2013).

[39] Sib Sankar Giri, Shib Sankar Sen, and V. Sukumaran, Effects of dietary supplementation of potential probiotic Pseudomonas aeruginosa VSG-2 on the innate immunity and disease resistance of tropical freshwater fish, Labeo rohita, *Fish & Shellfish Immunology*, 32, 6, (1135), (2012).

[40] Mahmoud A.O. Dawood, Shunsuke Koshio, Mohamed M. Abdel-Daim, and Hien Van Doan, Probiotic application for sustainable aquaculture, *Reviews in Aquaculture*, (2018).

[41] Takavar Mohammadian, Abdolhossein Jangaran-Nejad, Mehrzad Mesbah, Tahereh Shirali, Pedram Malekpouri, and Mohammad-Reza Tabandeh, Effect of Lactobacillus casei on Innate Immunity Responses and Aeromonas hydrophila Resistance in Shabot, Tor grypus, *Probiotics and Antimicrobial Proteins*, (2019), 10.1007/s12602-018-9510-z

[42] Yanfeng Wang, Liang Chi, Qinghua Liu, Yongshuang Xiao, Daoyuan Ma, Zhizhong Xiao, Shihong Xu, and Jun Li, Effects of stocking density on the growth and immunity of Atlantic salmon salmo salar reared in recirculating aquaculture system (RAS), *Journal of Oceanology and Limnology*, (2018), 10.1007/s00343-019-7350-7