

Effect of *Lactobacillus casei* FNCC 0090 to improve gastrointestinal bacterial abundance, immune system and water quality in catfish farming

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Abstract. 'Aini N, Bachruddin M, Fatimah, Andriyono S, Wahyuningsih SPA. 2024. Effect of *Lactobacillus casei* FNCC 0090 to improve gastrointestinal bacterial abundance, immune system and water quality in catfish farming. *Biodiversitas* 25: 2130-2138. Catfish is the most widely cultivated type of fish. However, along with the increase in catfish production as an effort to fulfill the fishery stock, there is an obstacle to the emergence of disease infection caused by *Aeromonas hydrophila*. For this reason, good fisheries management is required. One of the efforts to deal with this is by giving probiotics *Lactobacillus casei* FNCC 00990. *Lactobacillus casei* FNCC 0090 is expected to improve the immune system of catfish through improving the blood profile of fish and increasing the number of lactic acid bacteria in the gastrointestinal tract of catfish. This research aims to determine the effect of administering the probiotic *L. casei* FNCC 0090 on the abundance of microbes in the fish digestive tract, immune system, and water quality in cultivating catfish infected with *A. hydrophila*. The research design was a completely randomized design with five treatments and five replications. The treatments were A (administering 5% probiotics and infected with 0.1 mL x 10⁸ CFU/mL), B (10% probiotics and infected), C (15% probiotics and infected), KP (positive control with 0% probiotics and infected with pathogens), KN (negative control, without probiotics and pathogen infection). Fish are kept for 42 days; on the 35th day, the fish were infected with *A. hydrophila* by intramuscular injection. Probiotics were mixed with feed with a total of 10⁸ CFU/mL. Data in the form of BAL counts and total bacterial counts of fish digestive tracts, blood profiles, and water quality were analyzed using Statistical Package for the Social Sciences (SPSS) software version 23.0 (IBM SPSS Statistics). The results showed that the addition of probiotic *Lactobacillus casei* FNCC 0090 with a density of 10⁸ CFU/mL can increase the abundance of bacteria both the total number of bacteria and the number of lactic acid bacteria contained in the gastrointestinal tract of catfish. Giving probiotics can also improve the immune system and water quality in catfish farming ponds infected by pathogenic bacteria *Aeromonas hydrophila* as much as 0.1 mL x 10⁸ CFU/mL. The concentration of probiotics that produces the best impact compared to the control treatment is 15%.

Keywords: *Clarias gariepinus*, fish stocks, fisheries management, LAB, *Lactobacillus casei*, probiotic

INTRODUCTION

The fish digestive tract is a very complex ecosystem for various kinds of microflora (Yukgehnaish et al. 2020). The fish's digestive tract includes the mouth to the anus, including the fish's stomach, and intestines (Cho et al. 2023). The main function of the fish's digestive tract is to digest food so that it can be absorbed by the fish's body (Nugraha et al. 2020). In carrying out this process, there is a symbiosis that depends on each other (Apriliyanto et al. 2021). There are even several types of good bacteria that are deliberately added to increase fish growth, feed efficiency, and the fish's immune system in the fish's digestive tract (Abdul-Malik et al. 2023). Intestinal microflora plays an important role in digesting food, fermenting unused energy substrates, the immune system, preventing the growth of disease-causing pathogens, regulating intestinal development, and producing vitamins for the host (Burgos et al. 2018).

The number of microbes contained in the fish digestive tract varies between 10⁷ and 10¹¹ per gram (Ray and Ringø 2014). The diversity of microflora in the fish digestive tract is influenced by various factors, such as age, genetics, diet, and environment (Karimi et al. 2022; Diwan et al. 2023). The eating habits of different fish (herbivores, carnivores, omnivores, detritivores) affect the number and type of microbes (Egerton et al. 2018).

One of the largest fishery commodities that has been widely cultivated is African catfish (*Clarias gariepinus*) (Kusuma et al. 2022). Catfish are widely cultivated because they have an easy cultivation process, high economic value, adaptability to various types of feed, and can be cultivated in small areas of land (Aini et al. 2020; Putra et al. 2020). However, in carrying out the aquaculture business process many obstacles must be faced. Poor air quality, expensive fish food prices, and many pathogens that attack fish during the cultivation process (Hai 2015; Dawood 2021; Kari et al. 2022a). One of the pathogenic bacteria that most often

attacks catfish is *Aeromonas hydrophila* (Zhang et al. 2020; Pangastuti et al. 2021). To overcome this problem, catfish cultivation uses antibiotics and other chemicals (Tabassum et al. 2021). However, continuous use of antibiotics and chemicals can hurt human health, fish, and ecosystems (Sumon et al. 2022).

In an effort to maintain fish stocks, good fisheries management is required. In recent years, to overcome the negative impacts of using chemicals in aquaculture, probiotics have been used as an alternative problem solution. Probiotics are a group of good microbes that are safe for the host (Aini and Hariani 2019). There are many benefits of adding probiotics, namely increasing fish growth and productivity (Hassan et al. 2022; Mondal et al. 2022), improving the immune system and nutrient metabolism (El-Saadony et al. 2021; El-Kady et al. 2022), improving water quality (Thurlow et al. 2019), increasing feed efficiency (Serradell et al. 2023), increasing resistance to disease infections (Cavalcante et al. 2020; Hardi et al. 2022), improving water quality in cultivation ponds (Tabassum et al. 2021), improving the balance of microflora in the fish digestive tract (Tan et al. 2019; Wang et al. 2019) and modulating the hematological index to determine the health status of fish (Michael et al. 2019). One type of bacteria that has been shown to have a role as a probiotic is *Lactobacillus casei* (Gündüz 2005; Heimbach 2012; Aini and Hariani 2019; Safari et al. 2022).

Lactobacillus casei FNCC 0090 bacteria are resistant to bile salts and low pH so they can live in the intestine and stomach organs (Aini and Hariani 2019). In addition, *L. casei* bacteria have antimicrobial activity and can enhance the fish's immune system (Wang et al. 2021). Research conducted by Luo et al. (2022) showed that giving probiotics was able to increase the abundance of microflora found in the digestive tract of host animals. Microflora diversity is an indicator of good digestion. If digestion is good, the host animal also tends to live a healthy life. The use of probiotics in aquaculture is expected to improve water quality, considering that water quality is an important factor in the catfish cultivation process (Qiu et al. 2023).

The presence of pathogenic bacteria *A. hydrophila* can harm fish health. Fish health is generally monitored by clinical symptoms that appear from the outside of the fish body, morphological appearance and fish behavior (Fazio 2019). For this reason, a hematological examination of fish needs to be done to determine the health status of fish. For this reason, this study aimed to determine the effect of *L. casei* FNCC 0090 probiotic supplementation on the abundance of gastrointestinal bacteria, the immune system, and water quality in cultivated catfish exposed to *A. hydrophila* infection.

MATERIALS AND METHODS

Ethical statement

The study was conducted according to the ethical commission, Animal Care and Use Committee (ACUC) Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia No.: 2.KE.163.09.2018.

Study design

The research design used was a Completely Randomized Design (CRD) with 5 treatments (giving probiotics at a concentration of 0% (positive and negative control), 5%, 10%, and 15% with 5 repetitions. The factor manipulated was the probiotic concentration. The probiotic concentrations used in this study are a continuation of previous research (Aini and Hariani 2019; Aini et al. 2024). Below are the types of treatments in research: (i) A: Feed + 0% probiotics + pathogen infection, (ii) B: Feed + 5% probiotics + pathogen infection, (iii) C: Feed + 10% probiotics + pathogen infection, (iv) KN: Negative Control (feed + 0% probiotics + pathogen infection), (v) KP: Positive Control (feed + 0% probiotics + no pathogen infection).

Re-culture of *L. casei* and *A. hydrophila*

Re-culture of *L. casei* bacteria was carried out on MRSB media (de Man Rogosa Broth, Himedia) and *A. hydrophila* bacteria on NB media (Nutrient Broth, Himedia). The tube needle is heated over a Bunsen lamp until it is hot and allowed to cool slightly and then used to collect bacterial colonies in a petri dish. Bacterial colonies taken at the tip of the tube needle were dipped into a test tube containing 10 mL of sterile MRS Broth medium and incubated for ± 48 h at 35°C until a cell density of at least 10^8 Colony Forming Units/mL was obtained. For *A. hydrophila* bacteria, they were incubated at 30°C for 48 hours. To determine the number of bacterial cells after the re-culture process, the steps taken are to adjust to adjust the incubation time to the OD value on the standard curve that has been created (Aini et al. 2024).

Experimental fish

The catfish used are fish that are 25-30 cm long with an average weight of 50-60 g. Before the acclimation process, the fish are soaked in methylene blue solution at a dose of 1 drop of methylene blue for 25 L of water. The fish are placed in the aquarium for 5 × 24 hours for acclimation. During acclimation, the fish were fed commercial Hi-Pro-Vite 1000 food produced by PT. Central Proteina Prima, Tbk. (33% protein content). After acclimation, test fish were taken randomly and put into aquariums with a density of 8 fish per pond.

During treatment, fish were fed with additional *L. casei* probiotics. The feed is placed in a basin and sprayed using probiotics according to the treatment. Add probiotics by spraying using a spray bottle. The feed that has been sprayed is then homogenized and left for 30 minutes in a closed container. Fish were kept in aquariums with a water volume of 100 L with each aquarium provided with aeration. Each aquarium contains 8 fish. They were feeding twice a day at 06.00 and 18.00 with a feeding rate of 3% of the catfish's body weight. Water changes are carried out every 3 days by taking $\pm 70\%$ of the total water volume using a hose (Aini et al. 2024).

Challenge test

Fishes were kept for 42 days. On the 8th day of rearing, catfish were infected with *A. hydrophila* at 1×10^8 CFU/mL by intraperitoneal injection. Before being injected into catfish,

a pathogenicity test for *A. hydrophila* was carried out to determine the LD₅₀ value. Based on the results of the pathogenicity test, it was found that the lethal dose for 50% of the test animals was 0.1 mL × 10⁸ CFU/mL (Aini et al. 2024).

Blood analysis

Fish blood was taken from the caudal vein using a sterile syringe through the following tail ablation technique. In each pond, 1 mL of fish blood was taken and placed in an Eppendorf tube containing EDTA so that the blood did not clot during the testing process. Testing the blood profile of catfish using a hematology analyzer. Hematological parameters like Leukocyte (WBC), Monocyte (MON), Lymphocyte (LYM), Granulocyte (GRAN) Erythrocyte (RBC), Hemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets (PLT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Procalcitonin (PCT), Platelet-Large Cell Ratio (PLCR).

Isolation and enumeration of culturable intestinal microbiota

At the end of the study, the fish were fasted for 24 hours. The fish were dissected and their intestinal organs were removed, then the fish intestines were stored at -80°C until it was time to isolate the bacteria. In each treatment, 3 fish were taken to have their intestines removed. Isolation and enumeration of bacteria in the catfish digestive tract using the TPC method using Nutrient Agar (Himedia) to calculate the abundance of total bacteria, while MRSA+CaCO₃ media to calculate the abundance of lactic acid bacteria. A total of 10 g of fish intestines were crushed by adding 90 mL of 0.85% physiological water. This mixture was planted in duplicate in sterile petri dishes and incubated for ±48 hours at 37°C. After incubation, colonies were observed, recorded, and expressed as the number of colony-forming units per mL (CFU/mL). A total of 5 most dominant colonies from each NA and MRSA media were purified and continued with further observations both macroscopically and microscopically.

The identification process is carried out by knowing the characteristics of the bacteria that grow. The isolate used in this identification is a pure culture. Colony morphology observations were carried out macroscopically and microscopically. Macroscopically, it includes the shape of the bacterial colony, the color of the colony, the edge of the colony, and the elevation of the colony. Microscopically, the cell shape and arrangement of bacterial cells were observed.

Water quality measurement

Water quality measurements are carried out every 3 days. The test parameters and equipment used in this research were temperature measured using a Celsius scale thermometer, DO (Dissolved Oxygen) calculated using a DO meter, pH measured using a pH meter, and ammonia levels calculated using an ammonia test strip.

Data analysis

The treatment of *L. casei* on the parameters of bacterial abundance in the catfish digestive tract, immune system, and water quality in the catfish rearing ponds obtained were compared and analyzed statistically (SPSS v.26 program). The data were tested for normality and homogeneity respectively using the Kolmogorov Smirnov test and the Levene test. All data obtained were normally distributed and homogeneous, so the data were analyzed using Analysis of Variance (ANOVA) with a significance degree of 5% then continued with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Abundance of bacteria in the fish digestive tract

The total number of catfish gut bacteria is a group of bacteria that grow in the catfish gut as a whole. The total bacterial group consists of normal catfish intestinal flora as well as a combination of normal intestinal flora and probiotic bacteria in feed that successfully enters the fish's digestive tract. The total number of bacteria was calculated by isolating the bacteria found in the fish intestines using NA (Nutrient Agar) media. Based on Table 1, treatment KP had the lowest total bacterial count, namely log 5.095±0.76 CFU/g. Treatment C had the highest total bacterial count, namely log 5.541±0.43 CFU/g. However, statistically, there was no significant difference between the control treatment and the probiotic treatment. Meanwhile, for the LAB count parameter, it is known that treatment A had the lowest LAB count, namely 3.87±0.05 CFU/g. Treatment C had the highest LAB count compared to all treatments, namely 4.94±0.45 CFU/g. When compared with the other four treatments, treatment C was significantly different. Treatment C was the best treatment to increase the number of catfish intestinal BAL.

Based on Table 2, there are 5 colonies that dominate most in each treatment. All of them have the form of bacillilli with the Gram genus being Gram-positive. Most of the colonies were found in treatments with the addition of probiotics, especially at a concentration of 15% (in treatment C).

Table 1. Abundance of bacteria in the digestive tract of catfish with various treatments

Treatments	LAB	Total bacteria
KP	4,17±0,36 ^a	5,095±0,76 ^a
KN	3,97±0,20 ^a	5,062±0,54 ^a
A	3,87±0,05 ^a	5,151±0,21 ^a
B	4,23±0,18 ^a	5,349±0,46 ^a
C	4,94±0,45 ^b	5,541±0,43 ^b

Note: Different letters indicate significant differences between treatment groups: A. Feed + 0% probiotics + pathogen infection; B. Feed + 5% probiotics + pathogen infection; C. Feed + 10% probiotics + pathogen infection; KN. Negative Control (feed + 0% probiotics + pathogen infection); KP. Positive Control (feed + 0% probiotics + no pathogen infection)

Table 2. Most commonly found LAB colonies

Character	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
Colony shape	Round	Round	Round	Round	Round
Color	White	White	Cream	Cream	White
Edge	Smooth	Smooth	Smooth	Smooth	Smooth
Elevation	Convex	Plateau	Flat	Plateau	Convex
Cell shape	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Cell arrangement	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus
Gram	+	+	+	+	+
Catalase	-	-	-	-	-
Motility	-	-	-	-	-
Most often found in treatment	C	B	C	A	C

Table 3. Catfish blood profile

Parameters	KP	KN	A	B	C
WBC (10 ³ /μL)	37.73±1.53 ^a	41.03±1.40 ^b	37.03±0.71 ^a	37.00±0.50 ^a	43.37±1.19 ^c
LYM (%)	94.87±0.76 ^a	95.33±0.12 ^a	94.67±1.17 ^a	96.20±1.08 ^a	96.27±0.68 ^a
MON (%)	3.57±0.55 ^a	3.23±0.21 ^a	3.17±0.67 ^a	2.70±0.82 ^a	3.13±0.51 ^a
GRAN (%)	1.57±0.23 ^{ab}	1.53±0.25 ^{ab}	1.77±0.55 ^b	1.10±0.26 ^a	1.85±0.05 ^b
RBC (10 ⁶ /μL)	2.58±0.36 ^a	3.04±0.06 ^b	2.66±0.17 ^{ab}	2.84±0.03 ^{ab}	3.15±0.23 ^c
HGB (g/dL)	9.77±0.86 ^a	11.63±0.21 ^c	10.13±0.49 ^{ab}	10.30±0.52 ^{ab}	11.10±0.72 ^c
HCT (%)	34.23±2.84 ^a	36.40±0.92 ^a	35.00±3.66 ^a	31.97±2.41 ^a	29.63 ±7.27 ^a
MCV (fL)	117.63±2.25 ^a	121.40±1.59 ^a	117.27±4.79 ^a	116.63±3.96 ^a	114.40±4.60 ^a
MCHC (g/dL)	31.33±1.00 ^a	31.77±0.46 ^a	32.63±0.76 ^a	33.23±1.29 ^a	35.87±5.59 ^a
PLT (10 ³ /μL)	52.33±8.33 ^c	45.00±2.65 ^{ab}	39.67±3.79 ^b	25.67±3.51 ^a	42.33±0.58 ^b
MPV (fL)	8.47±0.60 ^a	7.97±0.15 ^a	8.50±0.10 ^a	7.90±0.20 ^a	8.43±0.55 ^a
PDW (%)	6.80±0.36 ^b	6.73±0.85 ^b	5.87±0.25 ^{ab}	5.40±0.17 ^a	6.10±0.50 ^{ab}
PCT (%)	0.05 ±0.01 ^b	0.03±0.01 ^{ab}	0.04±0.01 ^{ab}	0.02±0.01 ^a	0.03±0.00 ^{ab}
PLCR (%)	9.30±0.26 ^{ab}	7.80 ±1.30 ^{ab}	12.30±1.42 ^b	6.23±3.62 ^a	12.37±3.78 ^b

Note: Different letters indicate significant differences between treatment groups. A. Feed + 0% probiotics + pathogen infection; B. Feed + 5% probiotics + pathogen infection; C. Feed + 10% probiotics + pathogen infection; KN. Negative Control (feed + 0% probiotics + pathogen infection); KP. Positive Control (feed + 0% probiotics + no pathogen infection)

Table 4. Water quality in catfish cultivation ponds with the addition of probiotics

Water quality	KP	KN	A	B	C
Temperature (°C)	25.3 ^a	25.3 ^a	25.1 ^a	25.0 ^a	25.4 ^a
pH	6.9 ^a	6.8 ^a	6.8 ^a	7.0 ^a	6.9 ^a
DO (ppm)	5.7 ^a	5.9 ^a	6.3 ^{ab}	6.5 ^b	6.9 ^c
Ammonia levels (mg/L)	1.0 ^a	1.0 ^a	0.8 ^{ab}	0.8 ^{ab}	0.5 ^c

Note: Different letters indicate significant differences between treatment groups: A. Feed + 0% probiotics + pathogen infection; B. Feed + 5% probiotics + pathogen infection; C. Feed + 10% probiotics + pathogen infection; KN. Negative Control (feed + 0% probiotics + pathogen infection); KP. Positive Control (feed + 0% probiotics + no pathogen infection)

Based on Table 3, it is known that in general, there was an increase in almost all blood profile parameters along with increasing concentrations of the probiotics given. The highest number of white blood cells was in treatment C, namely 43.37±1.19 and this number was significantly different compared to the control treatment and other treatments. This also applies to other parameters such as granulocytes and RBC (Red Blood Cells).

The addition of probiotics can improve water quality as evidenced by the increase in DO values along with the increase in probiotic concentration, as well as a significant decrease in ammonia levels in treatment C compared to the control ss shown in Table 4.

Discussion

In the process of cultivating catfish, fish health is the main factor that must be considered (Sudrajad et al. 2023). Monitoring fish health can be done by looking at the blood profile (Yukgehnash et al. 2020; Hendam et al. 2023). In this study, probiotics in the form of *L. casei* were used in the hope of improving the health quality of catfish by improving the quality of the blood profile, the abundance of bacteria in the digestive tract, and water quality in the process of cultivating catfish infected with *A. hydrophila*.

Lactobacillus casei plays a role in balancing the microflora found in the catfish digestive tract (Song et al. 2023). This is indicated by the increasing number of LAB and the total number of bacteria found in the fish's digestive tract along with increasing concentrations of the probiotics provided. When given 15% probiotics, the amount of LAB in the fish intestines was at the highest level compared to other treatments.

There were five colonies that seemed to dominate and were found in almost all samples. All of them are thought

to be from the lactic acid bacteria group. This is because the five colonies grew on MRSA+CaCO₃ media. According to Aritonang et al. (2017) the addition of CaCO₃ compounds to MRSA media used to select bacteria that produce lactic acid, so that the clear zone formed on the MRSA media is due to the reaction of the organic acid produced by LAB with the compounds contained in the media. The CaCO₃ compound reacts with lactic acid and forms a new compound, namely calcium lactate (C₆H₁₀CaO₆), giving rise to a clear color on the media (Lawalata and Satiman 2015).

Lactobacillus casei significantly improved the composition of intestinal microorganisms, inhibited pathogenic bacteria, and improved the abundance of probiotics. Blood profile parameters in fish are said to be a reflection of physiological processes, fish health status, and the state of fish immunity (Liu et al. 2023). Giving probiotics to fish can stimulate the hematopoietic organs so that it has a role in increasing the amount of oxygen carried by red blood cells and immune response components (Haque et al. 2021). The results of research conducted by Kong et al. (2020) show that the addition of *L. casei* to crucian carp, *Carassius auratus*, can significantly increase the immune response. As a probiotic agent, the way *L. casei* enhances the immune system of fish is through several actions in the fish gastrointestinal tract. Probiotic organisms are competitive and grow in the gut, inhibiting the colonization of pathogenic bacteria. Probiotics are also capable of producing certain inhibitory substances that inhibit pathogenic organisms, such as bacteriocins. Due to nutrient competition between probiotics and pathogenic microbes, probiotics will utilize nutrients that make them unavailable to pathogens. At the same time, there is an increase in macrophage activity and antibody levels in fish so that the fish immune system becomes better (Zorriehzahra et al. 2016).

Fish that have been fed a diet containing a probiotic mixture will have higher RBC levels compared to fish without the probiotic control. The part of blood cells that has an important role in the body's defense system against pathogens is leukocytes (Yu et al. 2015). One of the leukocytes that have this important role is the phagocytic cells consisting of monocytes and neutrophils. The parameter used to measure phagocytic cell activity is phagocytosis activity (DeLeo and Quinn 2019). The increased immune response after probiotic administration is due to the immunomodulatory effect of probiotics. Probiotics that enter the fish gastrointestinal tract will interact with Pattern Recognition Receptors (PRRs) through Microbial Associated Molecular Patterns (MAMPs), which can trigger an immune response (Song et al. 2014). However, the functional effects of WBC derived from tissues outside the blood (thymus, spleen, and kidney) are also related to the place of production (Yanuhar et al. 2021). Fish-fed diets containing probiotics can have higher levels of WBCs and can react better to stressors, thus proving to have superior immune responses compared to fish-fed basal diets (Choi et al. 2022). These observations also suggest that fish-fed probiotic-supplemented diets are healthier than the control (Michael et al. 2019).

Research conducted by Aini et al. (2024) showed that the administration of *L. casei* can increase lysozyme

enzyme levels and phagocytosis activity in catfish infected with *A. hydrophila*. Phagocytosis is carried out by macrophage cells which in this case may come from monocytes. Based on the results of the study, it is known that monocyte levels in the fish group given probiotic *L. casei* are better than the control group so the catfish immune system becomes better with monocytes that are ready to be active when there are pathogens that attack. In line with research conducted by Kong et al. (2019) that the administration of *L. casei* to Crucian carp triggers the generation of appropriate immune responses in protecting fish from disease infections caused by the pathogen *A. veronii*. Another study using *L. casei* in rainbow trouts was found to improve the immune system of fish through increased IgM levels (Zhao et al. 2012).

In this study, in general, the hematology profile values of the fish tended to be good along with increasing probiotic doses. However, several hematology parameters decreased. This is likely because probiotic administration takes a longer time to affect the hematological status of the fish. Further research is needed to change the method of administering probiotics by mixing them in water. In addition, administering *A. hydrophila* infection acts as a vaccine agent for catfish (Mulia et al. 2022). Research conducted by Tabassum et al. (2021) shows that giving probiotics. Research conducted by (Chao et al. 2023) showed that adding the probiotic *L. casei* to crucian carp can improve the fish's immune system.

The probiotics can offer a promising approach for controlling bacterial, fungal, and parasitic infections in fish. The *Bacillus* sp. has also been recognized as a good fish probiotic involved in the overall development of *B. subtilis* and *B. circulans* enhanced fish growth and eliminated the anti-nutritional factor mimo sine. *Lactobacillus* sp. also shows good probiotic potential, inhibiting activities against harmful fish pathogens. Gut microbiota is a group of microorganisms that live in the digestive tract of fish. They participate in all biochemical processes that occur in the body and can modulate the host's immune system (Yukgehnaish et al. 2020). Giving probiotics to fish will have a good impact because probiotics are known to have a role as immunostimulants. The role of probiotics starts from the fish's gastrointestinal tract by activating mucosal immunity in the gut, serum immunity, antioxidant response until the fish's whole body immunity increases (Romano 2021).

The number of RBCs in normal fish ranges from 1-3×10⁶ cells/mm³ (Hastuti and Subandiyono 2012). After the infection process of catfish, the number of RBCs decreased in all treatments, except in treatment C where fish erythrocytes were still at a higher level compared to treatment KN without the pathogen infection process. The decrease in total erythrocytes in treatment with pathogenic infections is thought to be caused by the infecting *A. hydrophila* bacteria causing damage to the kidney organs. The presence of *A. hydrophila* injected intraperitoneally into the fish body causes damage to fish internal organs, especially hemopoietic organs such as the liver, spleen and kidneys. The kidney head in fish will be the most severely damaged compared to others because the kidney is the main hematopoietic organ in fish. In fish, the kidney is an

organ that produces erythrocytes. Damaged fish kidneys cause erythrocyte production to decrease. Apart from that, the number of erythrocytes is closely related to hemoglobin and hematocrit levels (Indriani et al. 2020). Research conducted by Sudrajat et al. (2023) showed that the provision of probiotics *Bacillus* sp. in catfish infected with *A. hydrophila* can reduce the number of pathogenic bacteria found in the liver and kidney organs compared to the control treatment without probiotic treatment.

Aeromonas hydrophila produces exotoxin in the form of hemolysin. Hemolysin is an enzyme that can lyse red blood cells and liberate hemoglobin (Zhang et al. 2020). This situation causes the average erythrocytes of the test fish to generally decrease or be lower than normal until the 42nd day after the challenge test. According to Wedemeyer and Yasutake (1977), a low number of erythrocytes indicates that the fish is suffering from anemia and kidney damage and that the fish is in a state of stress.

Administration of *A. hydrophila* in fish pathogenicity intraperitoneally will release exotoxins in the form of aerolysins. These toxins work systemically and enter the bloodstream resulting in hemolysis, namely the rupture of erythrocytes and the decreasing hemoglobin levels in the blood and causing anemia affecting fish appetite, resulting in decreased fish survival (Kari et al. 2022b). A decrease in hemoglobin and erythrocyte levels, it will trigger the kidney in the head kidney to produce erythropoietin hormone. This hormone is used for the process of erythropoiesis in the kidney marrow to form myeloid progenitors from myeloid progenitors. Furthermore, they differentiate into erythroids (Hasan and Banerjee 2020). This phase in the presence of glycine produced by *L. casei* will bind with succinyl CoA to amino levulinic acid synthesized by ALAs enzyme. The erythroid then differentiates into erythroblasts. In this phase, levulinic acid binds to porphyrine to form heme molecules synthesized by Fe chelatase. Erythroblasts will differentiate into erythrocytes. In this phase, the heme molecule will bind to globin to form hemoglobin. With the formation of erythrocytes and hemoglobin levels increasing, oxygen transport in cellular respiration will increase cell respiration will increase (Salam et al. 2021).

Water quality has an important role in the catfish cultivation process, especially for fish survival parameters. Water can be an intermediary for the transmission of disease seeds. If the water used in aquaculture is polluted or has a quality that does not meet the requirements for catfish cultivation, the cultured fish will be attacked by disease seeds or parasites that live in the water (Kartikaningsih et al. 2022). The ideal conditions for the growth and development of catfish seeds in waters are a pH of 6.5-9, a temperature of 24-26°C and a DO level of more than 3 ppm (Ratnasari 2011). Based on the results of water quality measurements, show that all waters where fish are kept have ideal water quality for the growth of catfish. The pH level in this study is still stable and in accordance with the catfish farming guidelines. Buffering agents that play a role in resisting fluctuating pH changes are alkanes through interactions between carbonate-bicarbonate with neutralizing acids or acids and bases (Makori et al. 2017). The action taken by probiotics is to convert CO₂ into carbonate. This

may be the reason why acidic and alkaline water can be neutralized by probiotics (Tabassum et al. 2021). Although there was no significantly different effect on water temperature and pH parameters, the provision of probiotic *L. casei* with a higher concentration than the control was found to have a role in increasing DO values and significantly reducing ammonia levels in the water. In this study, it was found that the addition of probiotic *L. casei* had no significant effect on changes in water temperature. There is no clear evidence that either the addition of *L. casei* or other types of probiotics can regulate water temperature (El-Kady et al. 2022). However, in other studies, it was mentioned that the addition of *L. casei* can generally improve water quality, the immune system and the growth rate of catfish (Aini and Hariani 2019).

Probiotic bacteria are microorganisms whose role is to decompose organic matter. Gram-positive bacteria can convert it into carbon dioxide. Gram-negative bacteria can convert organic matter into bacterial biomass or slime. Nitrogen compounds contained in water are used for phytoplankton growth. The presence of phytoplankton can trigger an increase in DO caused by an increase in the photosynthesis process so that oxygen is produced (Aini et al. 2024).

Probiotics also stabilize the growth of phytoplankton communities in water by decomposing organic matter in water. Thus, the addition of probiotics can significantly improve water quality by reducing ammonia concentrations (George et al. 2016). Certain probiotic bacteria are even capable of acting as algicidal agents that can kill microalgae that have a negative impact on water (Hassan et al. 2022). Toxins resulting from metabolic waste in the form of ammonia and nitrate can be eliminated by probiotic bacteria through the addition of cultures that can process nitrification. The presence of probiotic bacteria is also useful for increasing the diversity of normal flora in waters and improving the balance of the microbial community in them (Gobi et al. 2018).

In conclusion, the addition of probiotic *Lactobacillus casei* FNCC 0090 with a density of 10⁸ CFU/mL can increase the abundance of bacteria both the total number of bacteria and the number of lactic acid bacteria contained in the gastrointestinal tract of fish. Giving probiotics can also improve the immune system and water quality in catfish farming ponds infected by pathogenic bacteria *Aeromonas hydrophila* as much as 0.1 mL × 10⁸ CFU/mL. The concentration of probiotics that produces the best impact compared to the control treatment is 15%.

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