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Application of Fish Probiotics in Catfish Farming Activities using Biofloc System: Low and High Protein Feed Induction

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ARTICLE INFO

Paper Type:
Research Paper

Keywords:
Biofloc, Feed Protein, Sangkuriang Catfish, Bacteria, Indigenous

Article History
Received: 12-03-2025
Revised: 01-06-2025
Accepted: 12-09-2025
Available online: 15-10-2025

ABSTRACT

Catfish farming with the biofloc system is increasingly used because it can increase feed efficiency. However, the main challenge in this system is optimal water quality, especially in relation to the presence of pathogenic bacteria such as *coliform*. This study aims to determine the effect of giving probiotics to feed with high, low, and control protein content on water quality, water and intestinal TPC, and the level of coliform bacteria in the anus of catfish. The method used in this study is experimental in the laboratory of Trunojoyo Madura University. This research was carried out using 3 treatments, namely control, low, and high protein feed. Data related to the results of measuring water quality parameters pH, temperature are presented in the form of a graph. The results data for detecting pathogenic bacteria in sangkuriang catfish *(Clarias gariepinus)* were analyzed qualitatively by calculating the *Total Plate Count* (TPC) value of heterotrophic bacteria growing in *Nutrient Agar* (NA) media and pathogenic bacteria growing in *Eosin Methylene Blue Agar* (EMBA). The results of this study are expected to provide a better understanding of how the use of probiotics affects. Temperature values ranged from 26.71-28.83°C, and pH ranged from 8.34-9.45. Bacterial TPC values ranged from 8.03 to 16.94 CFU/ml. The TC value of bacteria in catfish anus samples ranged from 3.81-7.00 CFU/ml. This study can be a practical solution for farmers to improve sustainable catfish cultivation.

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Cite this article as: Maharani, SD.; Wahyuni, A.; Junaedi, AS.,; Triajie, H; Zainuri, M. (2025). Application of Fish Probiotics in Catfish Farming Activities using Biofloc System: Low and High Protein Feed Induction. *Airlangga Journal of Innovation Management, 6(3), 562-578.* https://doi.org/10.20473/ajim.v6i3.70791

Introduction

Sangkuriang catfish *(Clarias gariepinus)* is a freshwater fishery cultivation commodity that has high economic value, is cheap, and is widely favored by the community, from children to adults. Ciptawati *et al*., (2021) stated that catfish have many advantages compared to other fish, one of which is that they grow quickly and adapt easily to the environment. Based on data from KKP WEB DJPB (2021), it is reported that catfish production has increased by 9.23% per year. The market demand for catfish is increasing day by day, so catfish farmers carry out higher production activities. This can be done through intensive fisheries cultivation activities. The market demand for catfish in the Jakarta area annually is 38,696 tons and 106 tons per day. The market demand for catfish in the South Tangerang area annually is 4,790 tons, and the market demand per day is 13.1 tons, while the Palembang and Karimun areas have a market demand of 13.2 and 0.1 tons per day. The demand for the catfish market in Karimun has increased rapidly. Traders explained that market demand can reach 100 kg per day, but the available stock is only around 50 kg per day. Catfish production in Indramayu in 2010 was a significant increase, reaching 46,060 tons. Indramayu Regency is famous for its center of Catfish. This is due to the high level of catfish production. Based on the results of the analysis of catfish demand data by consumers in Losarang District, Indramayu Regency, it is 15.5 kg per day (Ferdian *et al*., 2012). Intensive fish farming is fish farming with high fish density and requires artificial feed with high protein content (Hariani and Purnomo, 2017). The Table 1 below shows the analysis of catfish market data.

Table 1. The Analysis of Catfish Market Data

Territory	Annual Demand (Ton)	Daily Demand (Ton)	Source
Jakarta	38.696	106	(KKP WEB DJPB, 2021)
Tangerang Selatan	4.790	13,1	(Fauziyah *et al*., 2019)
Palembang	-	13,2	(Sari *et al*., 2022)
Karimun	-	0,1	(Carladiva, 2024)
Indramayu	46,060	0,0155	(Ferdian et al., 2012)

Source: Arranged by author (2024)

Pellet feed in fish farming efforts has a major influence on increasing production. However, the relatively expensive price of pellets is a major obstacle in catfish farming activities because the production costs for feed reach around 60-70% which must be spent by farmers from the total production costs (Mafruchati et al., 2022; Yuriana et al., 2017). This problem can be overcome by carrying out fermentation activities and using a biofloc cultivation system. Fermentation of feed can increase protein value and increase the digestibility of feed so that fermented feed can be utilized by fish efficiently. The biofloc method is an alternative method for solving the problem of wastewater quality from catfish farming activities. Cultivation with a biofloc system is a fish farming technology to improve water quality by utilizing heterotrophic bacteria to convert organic and inorganic waste from feces and fish feed waste into biomass, which can be natural food for fish (Ma'ruf, 2019). Faridah *et al*., (2019) explains the microorganisms involved in the biofloc system, generally Bacillus bacteria. Bacillus bacteria found in probiotics also play a role in the nitrification and denitrification processes in water (Linayati *et al*., 2021). The use of Bacillus sp in probiotics has a positive effect on fish growth, and the enzymes produced by bacteria can help the fish digestion process (Noviana *et al*., 2014).

Changes in water quality that occur in the environment of an aquatic organism can affect fish activity and cause stress in fish, which leads to death (Mafruchati, Othman, et al., 2023). The quality of pond water must be well-controlled so that fish growth is optimal. Decreased water quality can also occur due to organic materials such as feces excreted by fish, which can increase the risk of fish death. The addition of probiotics to fish has been studied by Telaumbanua *et al*., (2023) can increase the process of nutrient absorption in feed because it is assisted by probiotic bacteria in the digestive tract, thereby increasing growth. Catfish cultivation using the biofloc system is increasingly being used to increase feed efficiency. However, the main challenge in this system is optimal water quality, especially in relation to the presence of pathogenic bacteria such as coliform (Mafruchati, Ismail, et al., 2023).

The media used for TPC analysis is *Nutrient Agar* (NA), which supports the growth of various types of heterotrophic bacteria. while detecting coliform bacteria in the anus of fish using *Eosin Methylene Blue Agar* (EMBA) media. TPC analysis, water quality, and the presence of coliform bacteria are closely related because pathogenic bacteria tend to thrive in poorly managed environmental conditions. This study aims to determine the effect of giving probiotics to feed with high, low, and control protein content on water quality, TPC of water and intestines, and the level of coliform bacteria in the anus of catfish. water quality, such as pH, temperature, was also observed to determine the relationship between environmental conditions and bacterial development. The main objective of this study was to determine the differences in water quality values, TPC in water and intestines, and the level of coliform bacteria in each treatment

Literature Review

Definition Probiotics

Djauhari *et al*., (2022) stated that probiotics can increase the process of nutrient absorption in feed because they are assisted by probiotic bacteria in the digestive tract, thereby increasing growth. The bacteria contained in probiotics produce various enzymes that help to hydrolyse feed into simpler molecules, thus facilitating the digestion and absorption process in fish. The main factors that support the life and growth of fish are physical and chemical factors of water, including temperature, pH, dissolved oxygen, and ammonia (Hidayati *et al*., 2024).

Biofloc

Biofloc is a fish farming technique that maximizes the number of catfish stocked in a limited container by utilizing microorganisms that are useful for breaking down catfish waste into food. Catfish waste will turn into small lumps that are a collection of algae, seaweed, and bacteria, which are natural fish food. (Baihaqi *et al*., 2020). Heterotrophic bacteria in biofloc function as bioreactors that control water quality and as a source of protein (Putra *et al*., 2022). Bacteria capable of forming biofloc include Bacillus subtilis, Bacillus Sereus, Zooglea ramigera, Escherichia intermedia, Paracolobacterium aerogenoids, Flavobacterium, Pseudomonas alcaligenes, Sphaerotillus natans, Tetrad, and Tricoda sp.

Water Quality

Temperature

Temperature is a very important parameter to consider in fish farming activities. Temperature is an important parameter in the cultivation process. If the water temperature is unstable, the fish will easily become stressed and die. Besides being unstable, the temperature can also cause fish growth to slow down. Siswanto and Rony, (2018) stated that increasing and decreasing the temperature of the water in the cultivation pond, which is not in accordance with the conditions of the fish, will cause the fish to have difficulty in growing and result in death in a short time. Water temperature is a controller for aquatic life; temperature controls the rate of reproductive activity, metabolism, and life cycle. Temperature can affect fish eating activity; the lower the temperature, the less appetite the fish will have (Nursobah *et al*., 2022). Bacterial growth occurs at a temperature of 30 ° C, and the rate of bacterial growth can be influenced by temperature (Suriani *et al*., 2013). The high and low temperatures greatly affect bacterial growth; if the environmental temperature is not in accordance with the general range, the growth of the enzyme activity will stop (Arivo *et al*., 2017).

pH

The pH value is also one of the factors that affect the productivity of waters. The pH value in water is used as an indicator of the balance of chemical elements and can affect the availability of chemical elements and nutrients that are beneficial to the life of aquatic vegetation. The high and low values of this pH are influenced by fluctuations in O2 and CO2 content. pH can affect the fertility level of waters because water content that is too acidic can cause fish to die, while water content that is too alkaline can cause fish to experience stress and less than optimal growth (Bayu and Sugito, 2017). The pH value also affects bacterial growth. Suboptimal pH conditions will affect the work of enzymes in bacteria. This is because bacteria are unable to adapt to these conditions, so they die because environmental conditions do not support bacteria in carrying out metabolic processes (Suriani *et al*., 2013).

Measurement of bacterial growth

Total Plate Count (TPC)

Total microbes or *total plate count* (TPC) based on SNI 01-2897-2008, is a microbe found in a product that grows on agar media at the specified temperature and incubation time (Lado *et al*., 2017). The total plate count (TPC) test aims to determine the number of microorganisms in a product by counting the bacterial colonies grown on agar plates. The formula for calculating the number of colonies according to (Laili *et al*., 2022) is as follows:

$$\boxed{N = \text{number of colonies per plate x } \frac{1}{Dilution\ factor}}$$

Total Coliform (TC)

Total coliform is a collection of various types of bacteria. Coliform bacteria can detect pathogens in water such as viruses, protozoa, and parasites. These bacteria also have higher resistance than pathogens and can be easily isolated and grown. There are 4 genera of coliform bacteria, namely Escherichia, Enterobacter, Klebsiella, and Citrobacter. Coliform bacteria are generally often referred to as indicators of fecal contamination. Coliform is used to analyze bacteria found in the anus of catfish. Munggaran *et al*., (2024) states that Coliform bacteria can be divided into 2 groups, namely fecal coliform and non-fecal coliform. Fecal coliform is a bacterium that comes from human feces or other warm-blooded animals. While non-fecal coliform is a coliform bacterium that comes from animals or plants that have died.

Methodology

Time and place

This research was conducted in September-December 2024. The research samples used were water samples and catfish from the biofloc system cultivation pond. The method used in this study is an experimental method in the laboratory of Trunojoyo University, Madura. This study was conducted using three treatments, namely low, high protein feed, and control. Data related to the results of measuring water quality parameters pH and temperature is presented in the form of a graph. The results of the data on pathogenic bacteria in sangkuriang catfish were analyzed qualitatively by calculating the *Total Plate Count* (TPC) value of heterotrophic bacteria growing in *Nutrient Agar* (NA) media and pathogenic bacteria growing in *Eosin Methylene Blue Agar* (EMBA). The type of feed in the biofloc system catfish pond is given a low protein content of 14%, while the high protein content is 32% and the control pond is given a medium protein feed content of 20%. The use of control treatments and variations in feed protein levels (low and high) aims to evaluate the effectiveness of probiotics in suppressing the presence of pathogenic bacteria in the body of catfish. Through comparisons between treatments, it can be seen how the biofloc system and fish health respond to differences in feed nutrition and the presence of probiotics, especially in dealing with the pressure of pathogenic microorganisms that may develop in the cultivation environment. Thus, this study not only assesses the growth aspect but also examines the role of treatment on fish resistance to infection.

The method used is Sterilization to keep the equipment in the laboratory sterile and prevent contamination. The tools to be sterilized are first washed using running water and dried with a tissue. Tools made of glass, including Erlenmeyer, beakers, and culture bottles, are sterilized by covering the mouth of the bottle with cotton, then coating it with aluminum foil wrapped in plastic wrap, and then putting it in an oven at a temperature of 121˚C for 15 minutes using an autoclave. The tools and materials are presented in Table below:

Table 2. Tools and materials

No	Tools	Material
1.	*Erlenmeyer*	Alcohol 70%
2.	Analytical balance	Spirit
3.	Beaker glass	Aquades
4.	Petri Dish	Medical cotton
5.	Incubator	Tissue
6.	Autoclave	Plastic wrap
7.	Test tube	*Aluminum foil*
8.	Test tube rack	Paper
9.	Drop pipette	Gloves*latex*
10.	*Pipette Pump*	Face mask
11.	Volume pipette	Label paper
12.	*Magnetic stirrer*	Molasses
13.	Ose needle	*Nutrient Agar* (NA)
14.	Bunsen	*Nutrient Broth* (NB)
15.	Spatula	*Aeromonas Agar* (AA)
16.	*Vortex*	*Eosin Methylene Blue Agar* (EMBA)
17.	Digital scales	*Ampicillin*
18.	UV-Vis Spectrophotometer	Ice
19.	Measuring cup	Plastic 1 kg
20.	*pH meter*	
21.	Refrigerator	
22.	Pan	

23. Mortar and paste
24. Spray bottle
25. Spray bottle
26. Blue tip
27. Jerry can
28. Tray
29. *Dissecting set*
30. Stove
31. Match
32. Culture bottle
33. *Styrofoam box*
34. Ruler

Source: Author (2024)

Work procedures

Probiotic Applications

The application of probiotics is carried out with a ratio of 1:9, namely by using 10 ml of probiotics measured using a measuring cup and then diluted using 90 ml of water, then stir the probiotics that have been mixed homogeneously, Pour the probiotics into a spray bottle and spray them on the feed, then stir until evenly distributed, After that the feed is left for approximately 30 minutes until the feed expands, After 30 minutes of fermentation, the feed can be given directly to the catfish

Total Plate Count (TPC) in water samples and intestinal samples

Sterilize the equipment using an autoclave to be used, Make a dilution medium using 9 ml of distilled water in several test tubes, After that sterilize the stock solution and dilution medium using an autoclave at a temperature of 121 ° C for 15 minutes, Then take a water sample in the high, low, and control protein pools and then dissect and take the intestines of high, low, and control protein pond catfish using a distinguishing set and place them on aluminium foil, weigh the intestines using an analytical scale, The intestines of the catfish are mashed with a mortar and paste, then inoculate 1 ml of bacteria into the stock solution using a micropipette.

Then, take a 10 ml water sample then dissolve it in 90 ml of stock solution. Carry out a series of gradual dilutions every week from 10-4 for pre-treatment, 10-6 for week 0 to 10-20 for week 6. Make (NA) media in Erlenmeyer as much as 2.016 g and add 72 ml of distilled water, then heat using a hot plate and homogenize using a magnetic stirrer until clear and foamy. The (NA) media is covered with cotton and sterilized using an autoclave at a temperature of 121° C for 15 minutes. Next step was to take 1 ml of the last dilution series and do it with 2 repetitions or duplicates, then place it in each cup petri dishes. Then pour NA media into 6 petri dishes, 10 ml each, homogenize by forming the number 8, and wait until it becomes agar, then cover with plastic wrap. After that, incubate in the incubator for 1x24 hours at a temperature of 27°C. Characterize and count the number of bacterial colonies that grow, and calculate using the TPC formula

***Total Bacteria Coliform* (TC) in Catfish Anus Samples**

Taking the anus part of the high, low, and control protein pond catfish using a distinguishing set and placing it on aluminium foil, After the anus is taken, then weigh it using an analytical scale, The anus organ of the catfish is mashed with a mortar and paste, then inoculate 1 ml of bacteria into the mother liquor using a micropipette, Taking 1 ml of a 10-3 dilution series is done by repeating 2 times or duplo and placing a test tube containing distilled water, Making EMBA media in an Erlenmeyer flask of 37.5 g and adding 90 ml of distilled water then heated using a hot plate and homogenized using a magnetic stirrer until clear and foamy, The EMBA media is covered with cotton and sterilized using an autoclave at a temperature of 121 ° C for 15 minutes, Taking 1 ml of a 10-3 dilution series and placing it in each petri dish Waiting until it becomes agar then coated with plastic wrap, Incubating is put in the incubator for 1x24 hours at a temperature of 27°C, Characterize and count the number of bacterial colonies that grow and calculate using the TPC formula.

Data analysis

The results of the next sampling are quantitative descriptive data analysis. Data related to the results of measuring water quality parameters pH, temperature are presented in graphical form. The data results for detecting pathogenic bacteria in sangkuriang catfish *(Clarias gariepinus)* were analyzed qualitatively and descriptively by calculating the *Total Plate Count* (TPC) value of heterotrophic bacteria growing on *Nutrient Agar* (NA) media and pathogenic bacteria growing on *Eosin Methylene Blue Agar* (EMBA)

Results and Discussion

Temperature Values in Aquaculture Ponds

Temperature has a major influence on various biological and chemical processes that occur in the environment, making it one of the main parameters in maintaining ecosystem balance. Temperature measurement results are presented in Figure 1 below:

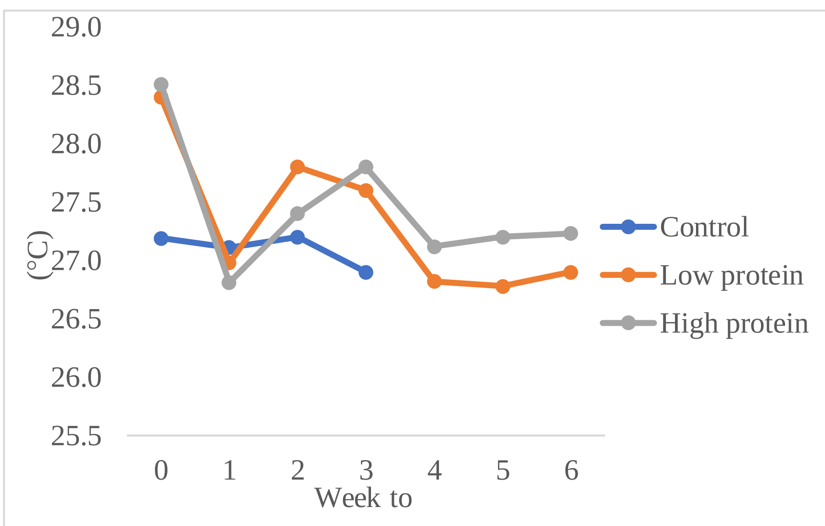

Figure 1. Temperature graph value

Source: Author (2024)

The results of the measurement of the water temperature of the pond during the cultivation of sangkuriang catfish *(Clarias gariepinus)* using the biofloc cultivation system during the study ranged from 26.7 °C to 28.5 °C. The pond treated with low protein produced an average temperature value from week 0 to week 6 of 26.8 °C to 28.4 °C. The pond treated with high protein produced a temperature from week 0 to week 6 of 26.8 °C to 28.5°C while the pond without treatment or control produced a temperature from week 0 to week 3 of 26.9 °C to 27.2°C. The highest temperature value is 28.8°C in the low protein pond, while the lowest value is 26.8°C in the high protein pond. Temperature plays an important role in determining the growth of farmed fish.

The temperature values of the low, high, and control protein ponds are in accordance with SNI No. 3 of 2014 concerning catfish production, where the optimum temperature standard ranges from 25-30°C. The water temperature of the pond in the morning is lower than in the afternoon. This can be influenced by direct sunlight entering the pond and causing an increase in the metabolic process of the catfish's body. Temperature checks are carried out by Annisa & Affandi (2024) in the morning and carried out in the middle of the edge of the pool with a value of 26-28°C. Lumbangaol et al., (2024) in their research, temperature measurements were carried out in the morning and evening. The results of morning temperature measurements ranged between 27.96°C and afternoon temperatures ranged between 29.44°C.

The highest temperature value was shown at 30.41°C, and this value is still optimal for the growth of sangkuriang catfish. The temperature of the cultivated waters is not fixed, but with characteristics showing dynamic changes and many specific factors that affect the temperature of the waters (Himawan et al., 2021), so that its value will change, and the factors that affect temperature changes are sunlight intensity, wastewater, weather, and climate (Muarif, 2016). Temperature values can also be associated with the decomposition process of organic materials involving the activity of microorganisms in decomposing leftover feed, fish waste. Suboptimal pond temperatures can inhibit the less efficient decomposition process (Kurniawan, 2023).

pH Value in Aquaculture Ponds

pH is the negative logarithm of the hydrogen concentration value, which has a value between 0-14. The range of values can indicate whether the waters are classified as acidic, basic, or neutral. The condition of the waters is said to be acidic if the value is below 7 and neutral if the value is 7 (Mafruchati & Ismail, 2023). The degree of acidity (pH) in catfish cultivation waters is important in determining the success of the cultivation business. Unstable pH conditions can cause a decrease in water quality and can result in fish death.

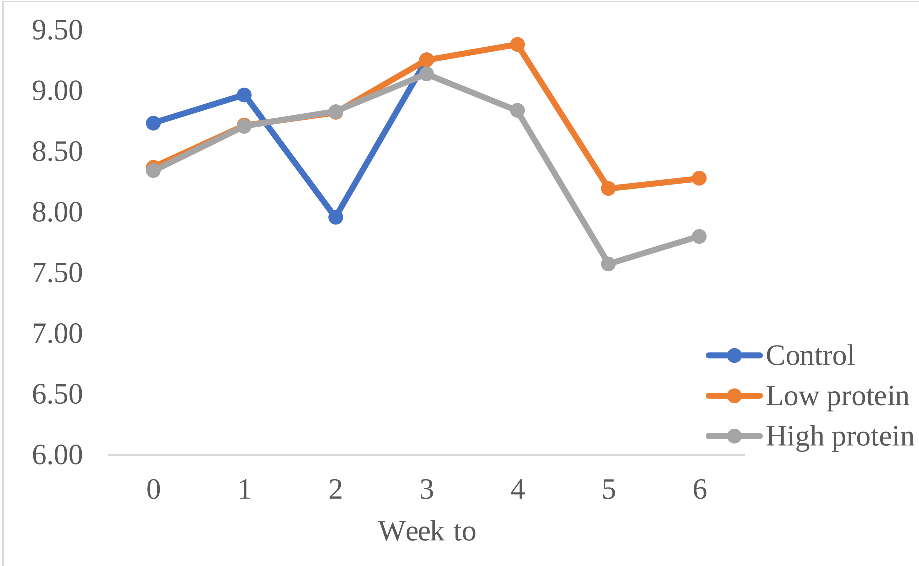

Figure 2. pH graph value

Source: Author (2024)

The results of pH measurement of pond water in the Sangkuriang catfish *(Clarias gariepinus)* biofloc cultivation system during the study ranged from 7.5 to 9.38. Ponds treated with low protein produced an average pH value from week 0 to week 6 of 8.29 to 9.38. Ponds treated with high protein produced an average pH value from week 0 to week 6 of 7.57 to 9.14. While in ponds without treatment or control, the average pH value from week 0 to week 3 was 7.96 to 9.25. The high pH value is 9.38 in the low protein pond, while the lowest value is 7.57 in the high protein pond. Based on the results of the study for 6 weeks, it was found that the pH value has a very fluctuating change. According to the Indonesian National Standard (SNI) Number 3 of 2014, the ideal pH value range for catfish cultivation is 6.5 to 8.5, but according to Sunarma, (2004), the good acidity level for catfish cultivation is 6-9. The highest pH value in the low protein pond is that it does not meet the quality standard. The condition of the catfish pond with low protein that has a high pH value is that the appetite begins to decrease or decrease, its growth is very slow, and cause death in the catfish.

The highest pH value was shown in the low protein pool with an average of 9.45, coinciding with the 4th week, while the lowest value was shown in the high protein pool with a value of 7.50, coinciding with the 6th week. These results can be compared with the research results Lumbangaol *et al*., (2024) which concerns the provision of different probiotics in Sangkuriang catfish nursery ponds and measuring the morning pH in the morning and evening. The morning pH measurements ranged from 8.03 to the highest of 8.11, while in the afternoon the pH value ranged from 9.26 to the highest of 9.43. The pH value has passed the threshold based on SNI 2014, which ranges from 6.5 to 8.5.

The pH value in the afternoon is higher than in the morning because it is influenced by the photosynthesis activity of phytoplankton, where high sunlight intensity can increase the rate of photosynthesis and phytoplankton absorb carbon dioxide (CO_2) from water, which produces oxygen. A decrease in CO_2 can cause an increase in pH in the afternoon. Giving probiotics to fish ponds can also affect the pH value. The activity of decomposing organic matter is related to microorganisms. Microorganisms in probiotics are more efficient in decomposing organic matter without causing the pH to decrease. Probiotics can also increase the diversity and abundance of beneficial decomposing bacteria that support a more efficient decomposition process.(Kurniawan *et al*. 2021). Factors in administering probiotics include the type of probiotic, the dose given, and the condition of the pond.

Total Plate Count **(TPC) Value of Heterotrophic Bacteria in Water and Intestinal Samples**

Total Plate Count (TPC) is a method that has been developed by the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Total microbes or total plate count (TPC) based on SNI 01-2897-2008, is a microbe found in a product that grows on agar media at a specified temperature and incubation time (Lado et al., 2017).

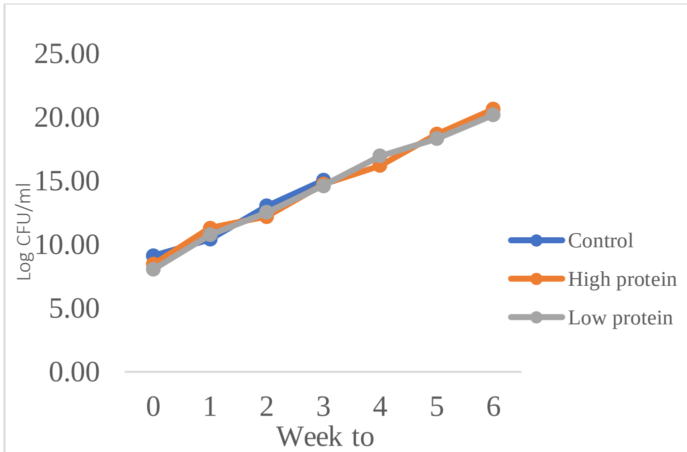

Figure 3. TPC diagram of heterotrophic bacteria in water samples

Source: Author (2024)

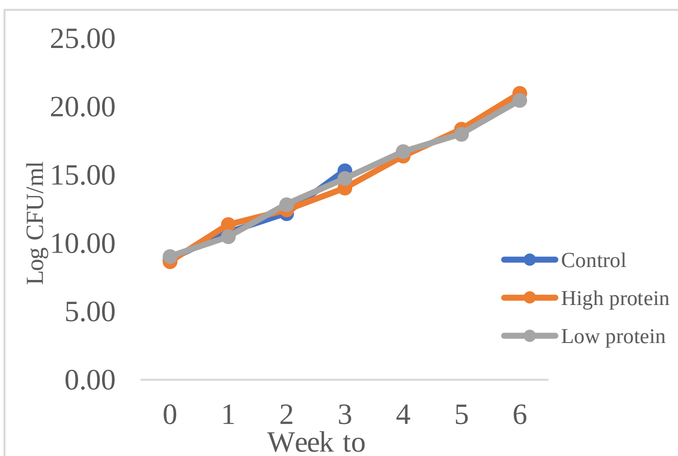

Figure 4. TPC diagram of heterotrophic bacteria in intestinal samples

Source: Author (2024)

The results of the *total plate count* (TPC) measurement of bacteria in water samples of sangkuriang catfish ponds (*Clarias gariepinus*) using a biofloc cultivation system with high, low and medium protein treatments. The growth medium used to grow the bacteria is *Nutrient Agar* (NA). The total plate count (TPC) value is calculated by adding up the bacterial colonies that grow in the petri dish at each dilution level, and then the data is converted using the formula (Log) to facilitate the analysis of bacteria in water samples. The dilution used in this study started from 10-6 to 10-20, but in the control pond, dilutions were carried out from 10^{-6} to 10^{-12}. The results of the study obtained heterotrophic bacteria in catfish cultivation water samples with low protein treatment in week 0 and week 6 were 8.04 log CFU / ml and 20.17 log CFU / ml, High protein treatment in week 0 and week -6 was 8.43 log CFU / ml and 20.62 log CFU / ml, while in the control pond in week 0 and week 3 was 9.12 log CFU / ml and 15.03 log CFU / ml. The highest *total plate count* (TPC) value from the pond water sample was in the high protein treatment in week 6, with a value of 20.62 log CFU / ml, while the lowest value was in the low protein treatment in week 0, with a value of 8.04 log CFU / ml.

The results obtained by heterotrophic bacteria with *Nutrient Agar* (NA) media of catfish intestine samples in low protein treatment in week 0 and week 6 were 9.03 log CFU/ml and 20.46 log CFU/ml, treatment with high protein in week 0 and week 6 was 8.65 log CFU/ml and 20.97 log CFU/ml, while the *Total Plate Count* (TPC) value of the intestine in the control pond in week 0 and week 3 was 8.82 log CFU/ml and 15.32 log CFU/ml. The bacterial value increased every week because the bacteria were able to live in the cultivation pond and the catfish intestines. Pond water contains various types of bacteria originating from leftover feed, as well as natural microorganisms present in the aquatic environment. Wu *et al*., (2018) stated that microorganisms in pond water can enter the fish's digestive system through the gill filtration process. This causes the microbes in pond water to reflect the microbial activities in the fish's intestines. Nayak, (2010) explained that if pond water is contaminated with pathogenic bacteria such as *Aeromonas sp*, then infection in the fish's intestines will also increase and can cause digestive disorders.

Duncan and Hannah, (2012) emphasized that bacteria in water and fish intestines can also be influenced by several environmental factors such as temperature, pH, DO, and fish density. When environmental conditions support the growth of beneficial microbes, the number of probiotic bacteria in the fish's intestines tends to increase, which can help increase fish resistance to infection. Conversely, when the water quality value is poor due to the presence of toxic compounds such as ammonia and nitrite, the total plate count (TPC) value in the water increases, disrupting microbial balance in the fish's intestines. De Schryver *et al*., (2008) confirmed that the *total plate count* (TPC) value in water and intestines that increased gradually and stably indicated that optimal microbial balance had been achieved. Therefore, the analysis of *total plate count* (TPC) in water and fish intestines can be an indicator to assess the effectiveness of probiotics in improving water quality and fish health in biofloc fish farming systems.

***Total Coliform* (TC) Bacteria from Anus Samples of Sangkuriang Catfish (*Clarias gariepinus*)**

Measurement of total coliform from the anus samples of Sangkuriang catfish used the pour plate method with media*Eosin Methylene Blue Agar* (EMBA). The results of *total coliform* (TC) measurements are presented in Figure 5 below:

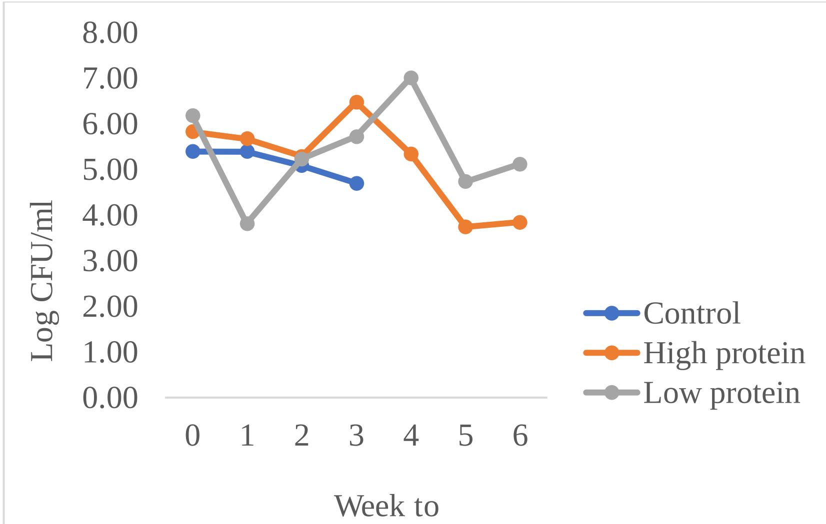

Figure 5. Total Coliform (TC) anus sample

Source: Author (2024)

The results of the study of Total Coliform values in the anus of catfish from the low protein pond treatment were highest in the 4th week, ranging from 7.01 log CFU/ml, and the lowest value was 3.81 log CFU/ml in the 1st week. The results of the study on the high protein treatment were highest in the 3rd week ranging from 6.48 log CFU/ml and the lowest value was in the 5th week ranging from 3.74 log CFU/ml, while in the control pond the highest value was shown in weeks 0 and 1 with a value of 5.39 log CFU/ml, while the lowest value was in week 3 with a value of 4.70 log CFU/ml. The results of the three treatments can be concluded that the highest value was in the low protein pond with a value of 7.01 log CFU/ml, and the lowest value was in the high protein pond with a value of 3.74 log CFU/ml.

Catfish tend to be more interested in feed containing high protein because high protein has good quality and is nutritious, so that the remaining feed in the high protein pond is small, which can reduce the pathogenic bacteria. This is emphasized by Ahmad *et al*., (2017) that good feed for fish is feed that has a higher protein content than carbohydrates because protein is the main source of energy for fish.

Figure 6. *Coliform* bacteria

Source: Author (2024)

The coliform value in low protein is higher than in high protein. This is because fish must consume more to meet these nutrients. However, in low-protein feed, it is less digested by fish, and nutrients are not absorbed properly and excreted as organic waste, namely feces. High-protein feed plays an important role in optimal catfish growth. The digestive system in fish starts from the mouth, oral cavity, pharynx, esophagus, stomach, intestines, to the anus. After being eaten by fish, the feed will go through a digestion process involving protease enzymes in the stomach and small intestine, which function to break down proteins into peptides and amino acids, and then nutrients are absorbed by the intestines and distributed throughout the body. If high protein feed is arranged in a tub, the efficiency of protein utilization increases, so that it can reduce organic waste excreted by fish in the form of feces.

Conversely, if the protein in the feed cannot be digested, it is wasted into the excretion system and increases the population of bacteria in the aquatic environment, including coliform bacteria (Luqman et al., 2019; Mafruchati et al., 2022). The use of feed is also combined with probiotics that can control the population of potentially harmful coliform bacteria. Therefore (Merrifield *et al*., 2010) said that the combination of high protein feed and probiotics can increase feed efficiency, as well as suppress the growth of coliform bacteria in the anus, which has the potential to cause infections in catfish.

Conclusion

The results of temperature measurements of low protein 26.7-28.4, high protein 26.8-28.5, and control 26.9-27.2, while the pH value of low protein 8.19-9.38, high protein 7.57-9.14, and control 7.96-9.25. The TPC value in the intestine was obtained 8.04-20.17 log CFU/ml. The TC value of catfish anus samples with low protein 3.81-7.01 log CFU/ml, high protein, 3.74-6.48 log CFU/ml, and control, 5.08-5.39 log CFU/ml. The best treatment is in a probiotic pool with high protein treatment where the value in the high protein treatment is low. The results of this study provide an opportunity to further explore the mechanism of action of probiotic microbes in the biofloc system, especially related to the relationship between microorganism communities, nutrient utilization and the role of probiotics in increasing the resistance of fish to environmental stress, in addition to the need for further research related to the optimization of the most effective dose and frequency of probiotics in various more complex cultivation conditions

Author's Contribution

All authors have contributed to the design of the study, data collection, and preparation of the results and discussion sections. All authors revised the manuscript prior to publication and contributed to the final draft.

Acknowledgements

The author would like to thank the Institute for Research and Community Service for the 2024 internal independent grant given to researchers to carry out community service activities on catfish cultivation using the biofloc system.

Declaration of Competing Interest

The study is independent internal grant research that does not have a tendency or interest in the various parties involved. The data obtained is pure data used for science and knowledge.

Funding

This research has been funded by the Institute for Research and Community Service for an internal independent grant in 2024, which is given to researchers to conduct research activities on the topic of the application of probiotic fish probiotics in biofloc system catfish farming activities: induction of low and high protein feeds.

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