Biosaintifika 15 (1) (2023): 75-86 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

The Effect of Probiotic *Lactobacillus paracasei* on The Performance of Guppy Fish (*Poecilia reticulata* var. Mosaic)

Siti Nur Jannah[1]*, Resanti Adityani[1,2], Susiana Purwantisari[1]

[1]Department of Biology, Diponegoro University. Jl. Prof Soedarto, Tembalang, Semarang, 50275 Central Java, Indonesia.
[2]Stem Cell and Cancer Research (SCCR) Laboratory, Semarang

*Corresponding E-mail: nurjannah.suroso@gmail.com

Submitted: 2022-09-07. Revised: 2023-01-08. Accepted: 2023-03-15

Abstract. Guppy fish (*Poecilia reticulata* var. Mosaic) is a type of ornamental fish that has many enthusiasts. Guppy fish culture still has low growth and viability in guppy fish culture. The previous solution was to apply antibiotics, but as time goes by antibiotics cause bacterial resistance. Another solution is the application of probiotics to feed. *L.paracasei* is one of the lactic acid probiotic bacteria and has never been applied to guppies. This study aimed to analyze the effect of the probiotic on performance (growth and viability, gill and gut histology, and LAB (Lactic Acid Bacteria *L.paracasei*) total) in guppies. 180 fish were randomly assigned into four triplicates groups and there were four treatment groups: 0 (Control), 5, 10, and 15 mL. kg-1 feed. As the result of this research, the highest body weight and length growth performance are found in the P3 group. Meanwhile, there were no significant differences between the groups of guppies' viability. Gill and intestinal histology showed that the control group was the best. This study concludes that 15 mL. kg-1 is considered the most effective for improving the performance of guppy fish. The addition of *L.paracasei* can be used as a feed supplement to improve the performance of guppy fish.

Keywords: Guppy, histology, L.paracasei, performance, viability.

How to Cite: Jannah, S. N., Adityani, R., & Purwantisari, S. (2023). The Effect of Probiotic *Lactobacillus paracasei* on The Performance of Guppy Fish (*Poecilia reticulata* var. Mosaic). *Biosaintifika: Journal of Biology & Biology Education*, *15*(1), 75-86.

DOI: http://dx.doi.org/10.15294/biosaintifika.v15i1.39899

INTRODUCTION

Guppy fish (*Poecilia reticulata*) is one type of ornamental fish that has many enthusiasts because guppy fish have various colors and patterns. Guppies are also known as million fish or rainbow fish. According to Zezen et al., (2022), Indonesia's maritime ornamental fish market share is 20 percent of the global world market Share. The number of Indonesian freshwater ornamental fish species are estimated at around 36 percent of ornamental fish species worldwide, as many as 400 species, one of the favorite ornamental fish commodities from Indonesia is guppy fish which dominate as much as 25% in the world market. KKP (2019) reports that Indonesia is likely to be an exporter of freshwater ornamental fish, especially guppies. It was noted that Indonesia, especially the Special Region of Yogyakarta (DIY) exported 1.2 million guppy fish seeds to the Philippines. Besides DIY, guppy fish export activities were preceded by Bali, Jakarta, and Bandung. This is evidenced by the value of domestic ornamental fish production which increased by an average of 13.17% from 2015 to 2018, indeed guppies with the highest value of 82.5%. This causes guppy fish to be widely used as an ornamental fishery business field which is seen as having bright prospects.

Guppy fish farming is said to be successful if it can produce fish with good performance, good growth and high viability. An important factor influencing the growth and viability of guppies is environmental management and feed management, which are determinants of the level of feed consumption and water quality of the guppy fish habitat. The management of the provision and the management of the environment in fish farming have a close relationship and influence each other. Poor feed management will affect air quality, as well as poor environmental management will reduce feed consumption by fish (Waite et al., 2014; Ahmed, 2019). However, in real practice in the field, there is still a low level of viability and growth in guppy fish culture. This is due to poor feed management, as well as a decrease in air quality followed by the emergence of disease, which ultimately results in the death of fish.

Based on these problems, it is necessary to have additional ingredients in the feed to increase fish growth by maximizing the absorption rate of feed in the intestines and increasing fish viability by maintaining water quality so that fish avoid disease. Antibiotics have been commonly used as additives in feed to promote growth and prevent pathogens. Antibiotics have been used in aquaculture since 1950. Antibiotics are used to increase fish growth by utilizing their ability to increase food absorption or act as AGP (Antibiotic Growth Promoters) and can treat infections from pathogenic bacteria. The antibiotics commonly used in aquaculture are oxytetracycline, tetracycline, amoxicillin, ampicillin, erythromycin, sulfonamides, and quinolones (Boeckel et al., 2015; Schar et al., 2020). Antibiotics in guppy farms had resistant issues in ampicillin and physical defects in chloramphenicol application in early fry (Anjur, 2021). Antibiotics also inhibit and kill the normal beneficial flora in the intestine and damage the natural environment which can have an impact on the absorption of fish nutrients (Maynard et al., 2012).

The right solution based on previous research is to use probiotics added to guppy fish feed. Probiotics are live microorganisms (bacteria, yeast, or fungi), which can have a good effect on body health and are beneficial to the host. The workings of this probiotic are to improve the balance of microbes in the digestive tract of living things (Jannah et al., 2016). The role of probiotics in aquaculture is as a stimulant, increasing feed conversion ratio, inhibiting the growth of pathogenic bacteria, producing natural antibiotic abilities, and improving air quality (Steenbergen et al., 2015).

Previous research related to probiotic testing on guppy fish has been carried out by (Khine, 2018), four species of bacteria of the genus *Bacillus* namely *B. megaterium, B. larvae, B. fimus,* and *B. megatrium* can improve air quality including dissolved oxygen (DO). and the amount of ammonia and guppies treated with these 4 probiotics were all alive until the end of the observation, namely on the 21st day. (Schmidt et al., 2017) also stated that two species of probiotic bacteria *Phaeobacter inhibins* and *Bacillus pumilus* can reduce mortality and increase the microbiome in the digestive tract of guppies. Tests of probiotics from the genus *Lactobacillus* have never been carried out on guppies.

Previous research related to the probiotic bacterial species *L.paracasei* on fish has been carried out by (Ljubobratovic et al., 2017), who stated that the administration of the probiotic *L.paracasei* had a positive effect on larval growth, protein digestibility, and bone development, as well as reducing the potential for pathogens in *Sander lucioperca* fish. Research related to the application of the probiotic *L.paracasei* to guppies has not been carried out, so it needs to be studied further to add references to probiotics in the world of fisheries. This study aimed to analyze the effect of the probiotic on performance (growth and viability, gill and gut histology, and LAB (Lactic Acid Bacteria *L.paracasei*) total) in guppies. Based on this background, a study was conducted on the effect of the probiotic *L.paracasei* on performance (growth and viability), histology of gills and intestines, and total LAB (Lactic Acid Bacteria) in guppies (*Poecilia reticulata* var. Mozaic).

METHODS

Probiotic Preparation

L.paracasei which has been isolated from chicken intestines, was rejuvenated on MRSB media. *L.paracasei* that has been purified is inoculated on MRSA media with added $CaCO_3$ and incubated for 48 hours at 37^o C. Probiotics are harvested by taking the isolates put in sterile physiological saline solution of 0.85% NaCl and equating the turbidity with the standard Mc. Farland. According to (Kamaliah, 2017), the manufacture of the Mc Farland 0.5 standard by mixing 0.05 ml of a 1.175% solution of barium chloride ($BaCl_2$) and 9.95 ml of a 1% solution of sulfuric acid (H_2SO_4) in a test tube. Sterile physiological NaCl/sterile distilled water was put into a sterile test tube according to the desired volume, then 1 ose of bacterial culture was mixed and the color of turbidity was adjusted to the standard Mc Farland 0.5. Mc Farland 0.5 indicates the number of colonies 1.5 x 10^8 CFU/mL.

(Dalynn, 2014) added that the density accuracy of Mc. Farland can be tested using a spectrophotometer with absorbance values between 0.08 to 0.1 at a wavelength of 625 nm. Bacterial population density can also be determined by calculating the population using Total Plate Count with serial dilutions up to 10 times.

Feed Preparation

Feed preparation is done by mixing probiotics with commercial feed made for guppies. The concentration of probiotics was in accordance with the treatment is 5, 10, and 15 mL. kg-1 of feed. The control treatment (P0) was prepared using the same feed but without the addition of probiotics. Preparation of feed in other treatments, namely by mixing all the feed sprayed with probiotics as much as the required dose when it will be given to the fish.

In Vivo Study Design

This study used a completely randomized design, non-factorial with 4 levels of treatment factors and 3 replications. The population was 180 guppies with uniform weight which were divided into 4 treatment groups. Each treatment group was repeated 3 times, each experimental unit consisted of 45 guppies. The repetition factor consists of P0 (Control: Commercial feed without probiotics), P1 (Commercial feed with probiotics 5 ml/1 kg feed), P2 (Commercial feed with probiotics 15 ml/1 kg feed), and P3 (Commercial feed with probiotics 25 ml/1 kg feed). The research variables consisted of probiotic *L.paracasei'* doses as independent variables, fish performances (that include growth and viabilities), histology of gill and gut, and total LAB of Guppy Fish (*Poecilia reticulata* var. Mosaic). Before giving treatment, guppies were acclimatized for 1 day. Then feeding is done twice a day, in the morning and evening. Feed containing probiotics was given only in the first week of treatment.

Performance Parameters

Guppie's performance is seen from their growth and viability. The growth measured included growth in body weight and growth in body length. Body weight growth is measured by the formula :

$$\mathbf{W = Wt - Wo} \quad \text{(Lugert et al., 2014)}$$

Formula description:
W : Weight growth (g)
Wt : Final weight of fish fry (g)
Wo : Initial weight of fish fry (g)

Body length growth is measured by the formula

$$\mathbf{L = Lt - Lo} \quad \text{(Lugert et al., 2014)}$$

Information :
L : Growth Length (cm)
Lt : Final length of fish fry (cm)
Lo : Initial length of fish seed (cm)

Performance is also measured by the survival rate or viabilities, based the formula :

$$SR = \frac{Nt}{No} \times 100\% \quad \text{(Muchlisin et al., 2016)}$$

SR = Survival rate / survival rate (%)
Nt = Number of fish at the end of the study (tails)
No = Number of fish at the beginning of the study (tails)

Evaluation of Water Quality

Water quality, including dissolved oxygen (DO), ammonia content, pH, and water temperature of the guppies habitat was observed 3 times during the study, at the beginning of the treatment, the end of the 2nd week, and the end of the treatment (Rachmawati et al., 2016). The pH of the water was measured using a pH meter. Water temperature was measured using a digital aquaculture thermometer. The dissolved oxygen content was measured using a DO meter, while the ammonia content was measured using an ammonia meter.

Histological Analyse

Surgery was carried out on fish that took 1 sample from the control treatment (P0), 5 mg/L (P1), 10 mg/L (P2), and 15 mg/L (P3) treatment at the end of the treatment. Fish were terminated by lethal surgery. After the fish died, a necropsy was carried out to isolate the gill organs by opening the head area under the operculum, while the isolation of the intestinal organs was done by opening the abdomen by drawing a straight line starting from the pelvis to the fish's anal. Organs that have been isolated and cleaned of blood and other impurities using 0.9% physiological saline. Physiological salt is an isotonic solution made from 0.9 g of NaCl crystals and dissolved in 100 ml of distilled water. Preparation of gill and intestines of guppy sections using the paraffin method. Sections staining using hematoxylin-eosin dye. The preparations that have been made were observed using a microscope and photomicrograph to analyze several parameters, histomorphology (length, width, and surface area of secondary lamella and the distance between secondary lamellae) and histopathology for gill organs and histomorphology (length, width, and surface area of villi) for intestinal organs.

Data analysis

The data to be obtained from the research are quantitative and qualitative data. Quantitative data in the form of body weight, body length, viability/survival rate of guppies, total LAB in guppies, and gill and gut histomorphometry data. Quantitative data were tested statistically. The statistical test used was the normality test and the homogeneity test. Normality tests and homogeneity tests were carried out to ensure that the data were distributed normally and homogeneously as a prerequisite for conducting a Completely Randomized Design (CRD). Subsequent analysis using ANOVA. If there is a significant difference, the Tukey test is carried out at a 95% confidence level to determine the difference in the mean value of the treatment, so that the best treatment results may be obtained using the SPSS application (Hamdani et al., 2018).

Qualitative data was obtained in the form of observations of cut preparations of intestinal organs and gills using a microscope and photomicrograph. Qualitative data on gill histology condition was used as a parameter of gill damage. stated that gill damage from mild to severe sequentially, namely edema, hyperplasia at the base of the lamellae, a fusion of two lamellae, hyperplasia at the base of the lamellae, fusion of two lamellae, hyperplasia of almost all secondary lamellae, and damage or loss of gill filament structure (pollution). heavy). Edema is characterized by the accumulation of fluid at the base of the lamellae. Hyperplasia is the union of the base of the lamellae with one another.

RESULTS AND DISCUSSION

Analysis of variance at 95% confidence level on fish performance data, which weight growth, length growth, and viabilities of guppy fish showed that probiotic *L.paracasei* additive in the feed had a significant effect on the performance of guppy fish. The mean values of performance variables are presented in Table 1.

Table 1. The mean value of performance variable of guppy fish after the administration of probiotic *L.paracasei* for 1 week

Components	Concentration of probiotic *L.paracasei* (mL/kg feed)			
	P0	P1	P2	P3
Weight Growth	$0.043^{a} \pm 0.002$	$0.054^{b} \pm 0.002$	$0.064^{c} \pm 0.002$	$0.087^{d} \pm 0.002$
Length Growth	$0.655^{a} \pm 0.095$	$0.705^{b} \pm 0.040$	$1.057^{c} \pm 0.061$	$1.338^{d} \pm 0.030$
Viabilities	$95.56^{a} \pm 0.04$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$	$100.00^{a} \pm 0.00$

Note: The data displayed are the average ± standard deviation. Different superscripts in the same column showed significant differences between treatments (P < 0.05)

Based on the result, weight growth and length growth in guppy fish treated with probiotic *L.paracasei* was higher than the control (P<0.05). This means that probiotic *L.paracasei* can increase weight growth and length growth in guppy fish. This may be due to the success of the probiotic *L.paracasei* in giving a good impact on the components that support the growth of guppies. These components include increased absorption of food in fish. The absorption of food in fish increases if there is an elongation in the surface area of the digestive tract in fish. This is to research conducted by (Guardiola et al., 2017), that microbiota and digestive enzyme activity in the intestine stimulated by probiotics originating from outside the host may increase nutrient digestibility and feed utilization and ultimately improve growth performance. An increase in the surface area of villi has been investigated by Hossain et al., (2022), that the increase in villi surface area could be caused by the increase in short-chain fatty acids induced by probiotics. Short-chain fatty acids are produced from the fermentation process carried out by probiotic bacteria. These fatty acids play a role in stimulating the multiplication of intestinal epithelial cells.

Increased food absorption activity may also be caused by optimal enzyme activity. In previous research about the application of the probiotic genus Lactobacillus in feed conducted by (Dawood et al., 2020), there was an increase in the activity of lipase and protease digestive enzymes in nile tilapia fish treated with feed. Probiotics may increase the activity of digestive enzymes due to the ability of probiotics to produce exogenous enzymes including lipase and protease. This is following with (Sewaka et al., 2019), the main digestive enzymes produced by fish are proteases, lipases, and amylase, which play a role in digestion and feed assimilation. If the activity of this enzyme increases, the overall metabolism of the body may increase. Zheng et al., (2019) also added that microorganisms and their exoenzymes have an important role in the digestive process by increasing the total intestinal enzyme activity and stimulating the production of endoenzymes that may improve food digestibility and nutrient utilization. Exogenous enzymes have a wider pH range than endogenous enzymes. Exogenous enzymes may be active during the digestion period and allow for better hydrolysis of the substrate.

The viability of guppies obtained in each treatment is relatively the same. In the P0 treatment (control), the survival rate of guppy fish was the lowest, 95.56%, while groups P1, P2, and P3 had the same survival rate, 100%. The results of the ANOVA test showed that there was no significant difference in the survival rate of guppy fish between treatments P0, P1, and P2 against P3. Based on these results indicate that the probiotic *L.paracasei* does not affect the survival rate of guppies. The mean value of the lowest guppy survival rate was in the P0 (Control) group. This is probably due to the absence of probiotics added to the feed. The addition of probiotics in the feed showed that a higher concentration of probiotics could increase the survival rate of guppy fish. Factors that affect the viability of guppies are feeding management and environmental management. It is suspected that the provision of probiotics has little or no effect on the environment of the guppy fish, so it does not affect the survival of the fish. The survival rate is also influenced by the immunity possessed by the fish. Probiotic *L.paracasei* in the feed may increase fish immunity. This is following the research conducted by (Van Doan et al., 2021), that *L. paracasei* l61-27b added to tilapia feed significantly increased growth performance and also stimulated mucus layer immunity. (Caipang & Lazado, 2015) added that probiotics have been shown to have a beneficial impact on the immune system of the intestinal mucosa, and skin and association with fish gills. Probiotics and their derivatives may act locally on the mucus layer or systemically. (Kelly & Salinas, 2017) added that these microorganisms enter the host's bloodstream or activate immune cells that migrate from mucosal sites to systemic lymphoid tissue. In addition, microorganisms may exert immunostimulator or immunosuppressive effects not only on non-specific but also on specific immune cells. Improving fish immunity has an impact on better survival rates.The survival rate of guppies which did not significantly affect between groups was probably also due to all groups of fish in an environment that still supported the existence of life. A supportive environment is indicated by water quality that meets the requirements for guppies to live. The environmental water quality of the guppy fish habitat measured in this study were temperature, pH, ammonia levels, and DO levels, which are shown in Table 2.

Table 2. The mean value of environmental water quality in all group on day 0, 15 and 30.

No	Group	Day	Temperature (°C)	pH	Ammonia (mg/L)	DO (mg/L)
1	P0	0	26.53	7.93	0	5.97
		15	26.47	7.93	0.25	5.80
		30	26.43	8.17	0.25	5.93
2	P1	0	26.57	8.37	0	6.13
		15	26.67	8.00	0.25	6.07
		30	26.53	8.00	0.25	6.37
3	P2	0	26.70	8.07	0	5.93
		15	26.60	7.97	0.25	5.67
		30	26.53	8.03	0.25	6.10
4	P3	0	26.57	7.97	0	6.10
		15	26.57	7.93	0.25	6.07
		30	26.67	8.10	0.25	5.97

The mean value of temperature on the 0, 15th, and 30th days was included in the normal range for guppies' life requirements. This is following with Cline (2019), that the water temperature parameter where the guppies live is suitable, which is between 18 - 28°C. This also shows that the administration of *L.paracasei* does not make the water temperature fluctuate significantly and has an impact on fish life. The mean value of pH on the 0,15th, and 3rd day was included in the normal range for guppy fish's life requirements, but there were also those whose values exceeded the normal range. According to Cline (2019), the parameters of the pH value of the water where the guppies live that are suitable are between 7 - 8. The pH value that exceeds the normal range is probably caused by high ammonia levels in the waters.

The mean value of ammonia level at the beginning of the treatment was still within the normal range for guppy fish life requirements, while the average ammonia level on the 15th and 30th days showed values exceeding the normal range. This indicates that the probiotic *L.paracasei* has not been able to reduce ammonia levels in the treatment. The high ammonia level was probably caused by the feed used which was high in protein and was given regularly and uncontrolled as ammonia levels increased. Sometimes the feed given is left over, causing spoilage in the guppies' environment. In addition, ammonia may be sourced from fish feces during the study. This is in accordance with (Handajani et al., 2018) that ammonia produced from fish farming comes from feces, fish excretion results, and feed residues. Ammonia as a result of excretion is excreted from the body through the branchial epithelium of the gills and ureters. Most of the ammonia comes from the feed left in the environment where the fish live. (Zhou & Boyd, 2015) also added that 20–40% nitrogen sourced from the feed is used to build body biomass, while the remaining 60–80% is disposed of in the aquatic environment as feces and uneaten feed residue. Other causes besides the presence of feed residues are also caused by the effectiveness of the method of giving probiotics that are suitable for reducing ammonia levels. Probiotics given by the direct method to aquatic media are more effective in reducing excess ammonia levels in the fish's living environment. This is because probiotics may directly contact the environment and reduce ammonia levels through the ammonia reshuffle process. This is in accordance with (Cha et al., 2013), that the application of probiotics directly in the waters may reduce the ammonia in the fish water environment effectively. (Andriani et al., 2018) also added that ammonia in the water decreased due to the ammonia reshuffle mechanism. The breakdown of ammonia into simple compounds occurs in two stages. The first step is nitrification, where ammonia is oxidized to nitrite (nitrite) and nitrite is oxidized to nitrate (nitration), while the second step is denitrification. At this stage, the nitrate is reformed into hydrogen gas (H2). (Trisna et al., 2013) added that probiotic bacteria may carry out the ammonia reform process. Bacteria perform this including nitrifying bacteria. (Liu et al., 2014) further revealed that the probiotic bacteria of the genus *Lactobacillus* may carry out the ammonia breakdown process, but the pathway for the conversion is not known for certain. The mean value of DO levels on the 0, -15, and 30 days was still in the normal range for the life requirements of guppies. Probiotic *L.paracasei* in this study was able to maintain the stability of DO levels until the 30th day. This is following research conducted by Mishra & Sharma (2021), that the administration of probiotics in tilapia rearing for 17 weeks showed DO levels remained in the normal range.

Another variable in this study is the histomorphology of gills. Analysis of variance at a 95% confidence level on this variable, consists of secondary lamellae length, secondary lamellae width, secondary lamella surface area, and distance between secondary lamellae The mean values of gill histomorphology are presented in Table 3.

Table 3. The mean value of gill hystomorphology variable of guppy fish after the administration of probiotic *L.paracasei* for 1 week

Secondary lamellae properties	Concentration of probiotic *L.paracasei* (mL/kg feed)			
	P0	P1	P2	P3
Secondary lamellae length	20.88[a] ± 2.05	23.49[a] ± 0.94	23.35[a] ± 2.33	25.43[a] ± 4.62
Secondary lamellae width	3.86[a] ± 0.56	5.22[a] ± 2.12	4.49[a] ± 1.27	5.83[a] ± 0.87
Secondary lamellae surface area	81.45[a] ± 20.61	121.76[a] ± 45.95	103.36[a] ± 26.15	145.63[a] ± 7.05
Distance between secondary lamellae	14.61[a] ± 1.24	11.50[b] ± 2.81	11.83[b] ± 0.34	7.16[b] ± 0.84

The mean value of secondary lamellae length, secondary lamellae width, and secondary lamellae surface area showed that probiotic *L.paracasei* additive in the feed didn't show a significant difference in the gill histomorphology of guppy fish (P>0.05). On the other hand, the distance between the secondary lamellae, the mean value showed that there was a significant difference between the probiotic-treated fish groups (P1, P2, and P3) and the P0 (Control) group, while the P1, P2, and P3 groups did not differ significantly from each other. This indicates that the application of probiotic *L.paracasei* in the feed did not significantly affect the distance between the secondary lamellae of guppies. The results of gill histomorphology data indicate that the probiotic *L.paracasei* does not affect the gills to develop changes in response to oxygen deprivation. The response was characterized by the secondary lamellae getting longer and wider, the surface area is increased and the distance between the secondary lamellae getting narrower. This is following Esam et al., (2022), who that fish that live in periodic or chronic hypoxia and cannot breathe air generally show changes in gill morphology that increase diffusion capacity. The gill lamellae will turn bigger and the gill filaments will turn longer. The surface area of the gills is higher than that of fish that live in normal dissolved oxygen levels.

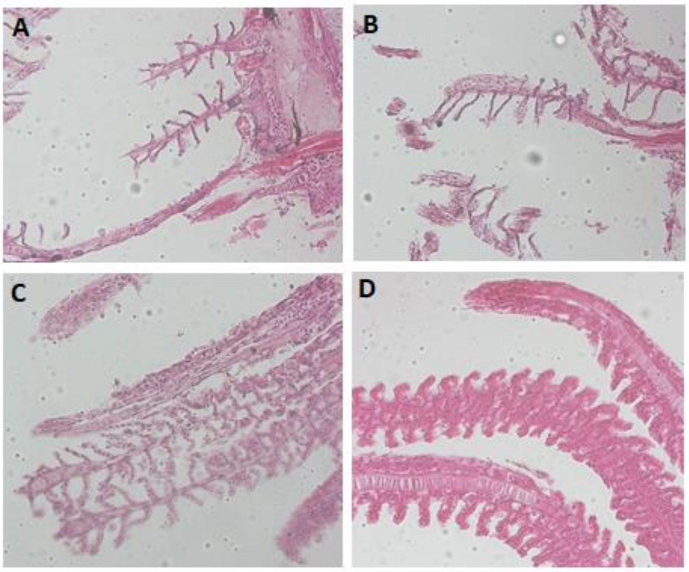

Figure 1. Histomorphology of gill in Guppy fish after the administration of probiotic L.paracasei for 1 week

The gill histomorphology are also seen by histopathology aspect. Gill histopathological observation is shown in Table 4.

Table 4. Types of gill pathology of guppy fish after the administration of probiotic *L.paracasei* for 1 week

Types of gill pathology	Pathology Score	PO (%)	P1 (%)	P2 (%)	P3 (%)
Secondary lamellae edema	0	100	100	90	100
	1	0	0	10	0
	2	0	0	0	0
	3	0	0	0	0
Secondary lamellae hyperplasia	0	100	80	80	80
	1	0	20	20	20
	2	0	0	0	0
	3	0	0	0	0
Secondary lamellae fusion	0	100	20	50	80
	1	0	30	25	20
	2	0	50	25	0
	3	0	0	0	0

There was no secondary lamellae edema in groups P0 (Control), P1, and P3, while in group P2, 10% secondary lamellae edema was found. The condition of secondary lamellae hyperplasia was not found in the P0 (control) group, while in the P1, P2, and P3 groups, secondary lamellae hyperplasia was found at a score level of 1 (mild) which was 20% each. Secondary lamellae fusion conditions were not found in group P0 (Control, while in group P1 secondary lamellae fusion was found at the level of score 1 (mild) by 30% and score 2 (moderate) by 50%. Group P2 found secondary lamellae fusion at the level of score 1. (mild) by 25% and score 2 (moderate) by 25%, while in the P3 group, secondary lamellae fusion was found at the level of score 1 (mild) of 20%.

The level of pathology in the gills associated with toxicity is grade I, edema occurs in the lamellae and the release of epithelial cells from the underlying tissue; in grade II, there is hyperplasia of the proximal basal secondary lamella; in grade III, hyperplasia leads to fusion of the two secondary lamellae; grade IV, almost all secondary lamellae are hyperplastic; grade V, loss of secondary lamellae structure and filamentous damage. Based on the results of the observations, it may be seen that the level of gill pathology in the P0 (control) group has not yet entered the pathological stage, while the P1, P2, and P3 groups enter into stage III, where hyperplasia causes secondary lamellae fusion (Bechmann et al., 2019).

The level of gill pathology obtained in this study stated that the administration of probiotics affected gill pathology. The provision of probiotics has an impact on the environment in which fish live. The level of pathology that exists is due to toxicity in the environment where the fish live. Ammonia levels may affect water toxicity. This is following (Mangang & Pandey, 2021), that the histopathology of fish gills is influenced by ammonia levels in the aquatic environment. Ammonia in high levels causes the level of gill histopathology to increase. Parvathy et al., (2022) added that the removal of squamous epithelium, necrosis, hyperplasia, and secondary squamous fusion in the gills is affected by toxins in the environment where the fish live. Toxic conditions that cause glycoproteins in mucus-coated cells may be associated with fusion and hyperplasia of the gill lamellae, thereby altering the negative charge of the epithelium and facilitating adhesion to adjacent lamellae. Esam et al., (2022) also add that the occurrence of changes such as hyperplasia, epithelial removal, and partial fusion of certain secondary lamellae, are examples of a defensive response to stress that contribute to an increase in the distance between the external environment and the blood, thereby preventing the entry of pollutants and resulting in inhibition of absorption. oxygen.

The gill pathology level data explained that probiotics were unable to improve gill histopathology, possibly due to the administration of probiotics only in the first week of the study. The role of the probiotic *L.paracasei* may be reduced due to the discontinuation of its administration after 1 week. One previous research about the application of Lactobacillus in fish feed was conducted by Thuraisami (2019) that administration of *L. rhamnosus* in feed of *M. montanus* can improve the histomorphology and histopathology of gills after 60 days of exposure.

Another variable in this study is the histomorphology of the intestinal organ. Analysis of variance at a 95% confidence level on this variable, consists of villi length, villi width, and villi surface area. The mean values of intestinal histomorphology are presented in Table 5.

Table 5. The mean value of intestinal hystomorphology variable of guppy fish after the administration of probiotic *L.paracasei* for 1 week

Villi properties	Concentration of probiotic *L.paracasei* (mL/kg feed)			
	P0	P1	P2	P3
Villi length	156.780^{b} ± 21.418	121.079^{a} ± 7.173	167.398^{b} ± 9.303	146.867^{ab} ± 8.582
Villi width	58.572^{a} ± 3.273	52.686^{a} ± 3.614	44.900^{a} ± 2.529	53.940^{a} ± 9.109
Villi surface area	9163.538^{b} ± 776.467	6389.083^{a} ± 892.60	7473.458^{ab} ± 62.651	6890.339^{a} ± 790.693

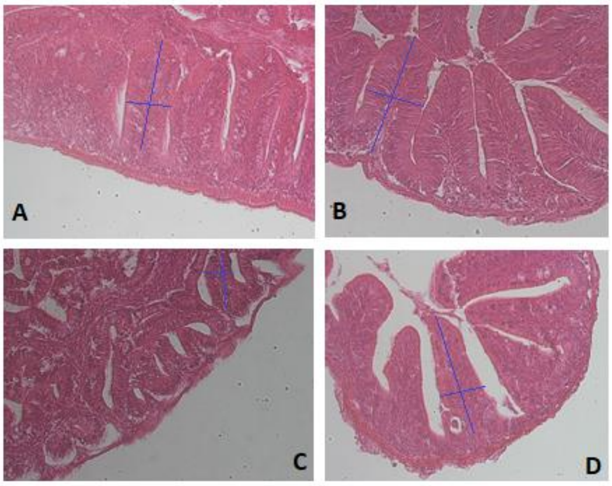

Figure 2. Histomorphology of intestinum in Guppy fish after the administration of probiotic *L.paracasei* for 1 week

The mean value of villi length, villi width, and villi surface area showed that probiotic *L.paracasei* additive in the feed had a significant difference in intestinal histomorphology of guppy fish (P>0.05), but group P0 (Control), had the highest villi surface area of 9163.538 m2. This shows that the administration of probiotic *L.paracasei* has an effect on the surface area of the intestinal villi of guppies but does not fulfill the hypothesis that feeding containing probiotics has a better impact than feeding that does not contain probiotics on the surface area of the intestinal villi of guppy fish. This is probably caused by the administration of probiotics only in the first week of the study. The role of the probiotic *L.paracasei* may be reduced due to the discontinuation of its administration after 1 week. Based on research conducted by (Adeshina et al., 2020), the administration of the probiotic *Lactobacillus acidophilus* in feed given to *Cyprinus caprio* may significantly increase the intestinal surface area by giving 56 days of treatment.

Regarding the increase in fish growth, it is suspected that there is a relationship with the effect of the probiotic *L.paracasei* on the intestinal villi of guppies. The digestibility of fish to feed is not only influenced by intestinal histomorphology. According to Mzengereza et al., (2021), the level of fish digestibility of a type of feed depends on the quality of the feed, the composition of the feed ingredients, the nutritional content of the feed, the type and activity of digestive enzymes in the fish digestive system, the size and age of the fish and the physical and chemical properties of the waters. Probiotics have been linked to the modulation of the gut microbiota and the activity of digestive enzymes in the gut that may improve nutrient digestibility. The main digestive enzymes produced by fish are proteases, lipases, and amylase which play a role in digestion and feed assimilation. If the activity of this enzyme increases, the overall metabolism of the body may increase. According to research conducted by Dawood et al., (2020), Tilapia which fed a feed with the addition of the probiotic *Lactobacillus plantarum* showed an increase in the activity of the digestive enzymes lipase and protease. Digestive enzymes showed increased activity and resulted in high feed efficiency.

This study also analyzed the total LAB in the gut of guppy fish, which was intended to determine whether the addition of probiotic *L.paracasei* to the diet of guppy fish could affect the total LAB in the intestines of guppy fish. Analysis of variance at a 95% confidence level on total LAB based on the mean values of total LAB in each group is presented in Table 6.

Table 6. The mean value of total LAB variable of guppy fish after the administration of probiotic *L.paracasei* for 1 week

LAB	Concentration of probiotic *L.paracasei* (mL/kg feed)			
	P0	P1	P2	P3
Total LAB	$7.8 \times 10^{6\ a}$	$9.4 \times 10^{6\ b}$	$1.0 \times 10^{7\ c}$	$1.4 \times 10^{7\ d}$

The results showed an increase in the number of lactic acid bacteria along with an increase in the number of probiotics added, P3 group had the highest total number of lactic acid bacteria in the intestine compared to other treatments, 1.4 x 107 CFU. The results of the ANOVA test on the total number of lactic acid bacteria showed a significant difference between the treatment group and the control group. This shows that the probiotic *L.paracasei* added to the feed affects the total lactic acid bacteria in the gut of Guppy fish. This is following research conducted by (Alishahi et al., 2018) related to the probiotic bacteria *Lactobacillus plantarum* and *Lactobacillus bulgaricus* may increase the total amount of LAB in the intestines of tilapia. The difference in the number of LAB in each treatment was suspected to be the success of the attachment of the probiotic *L.paracasei* in the digestive tract of guppies. This is supported by research conducted by Wuertz et al., (2021) that allochthonous LAB or lactic acid bacteria originating from outside the host's body given to rainbow trout showed the presence of allochthonous LAB in the analysis of intestinal mucosa samples of Rainbow trout. LAB generally has good qualities in terms of adaptation. According to Iorizzo et al., (2022), LAB has potential abilities, such as adaptation to different cell types; can produce hydrogen peroxide acid and bacteriocin to inhibit the colonization of pathogens; is non-invasive, non-carcinogenic, and non-pathogenic; and may aggregate to form a normal balanced microbiota.

This research can contribute to ornamental fish farmers providing *L.paracasei* as a substitute in commercial feed to improve the performance of *Poecilia reticulata*.

CONCLUSION

The addition of probiotic *L.paracasei* in the feed may increase the performance growth and viability of guppy fish, also the total LAB. The addition of probiotics has not been able to improve the histology of the gill and intestinal of guppy fish but may increase the total LAB in the intestinal organ of guppy fish. Further study may consider elongating the treatment time to determine the effect of probiotics on the gill and intestinal histomorphology of guppy fish.

ACKNOWLEDGEMENT

This research is supported by the RPP research program from Diponegoro University

REFERENCES

Adeshina, I., Abubakar, M. I. O., & Ajala, B. E. (2020). Dietary supplementation with Lactobacillus acidophilus enhanced the growth, gut morphometry, antioxidant capacity, and the immune response in juveniles of the common carp, Cyprinus carpio. *Fish Physiology and Biochemistry*, *46*(4), 1375–1385. https://doi.org/10.1007/s10695-020-00796-7

Ahmed, N. (2019). Global Aquaculture Productivity , Environmental Sustainability , and Climate Change Adaptability. *Environmental Management*, 159–172. https://doi.org/10.1007/s00267-018-1117-3

Alishahi, M., Tulaby Dezfuly, zahra, Mohammadian, T., & Mesbah, M. (2018). Effects of Two Probiotics, Lactobacillus Plantarum and Lactobacillus Bulgaricus on Growth Performance and Intestinal Lactic Acid Bacteria of Cyprinus Carpio. *Iranian Journal of Veterinary Medicine*, *12*(3). https://doi.org/10.22059/ijvm.2018.235444.1004816

Andriani, Y., Kamil, T. I., & Iskandar, I. (2018). Efektivitas probiotik BIOM-S terhadap kualitas air media pemeliharaan ikan nila nirwana Oreochromis niloticus. *Depik*, *7*(3), 209–2017. https://doi.org/10.13170/depik.7.3.9043

Anjur, N. (2021). *An update on the ornamental fish industry in Malaysia : Aeromonas hydrophila - associated disease and its treatment control*. *14*, 1143–1152.

Bechmann, K. R., Arnberg, M., Gomiero, A., Westerlund, S., Lyng, E., Berry, M., Agustsson, T., Jager, T., & Burridge, L. E. (2019). Ecotoxicology and Environmental Safety Gill damage and delayed mortality of Northern shrimp (Pandalus borealis) after short time exposure to anti-parasitic veterinary medicine containing hydrogen peroxide. *Ecotoxicology and Environmental Safety*, *180*(February), 473–482. https://doi.org/10.1016/j.ecoenv.2019.05.045

Boeckel, T. P. Van, Brower, C., Gilbert, M., Grenfell, B. T., & Levin, S. A. (2015). *Global trends in antimicrobial use in food animals*. *16*, 1–6. https://doi.org/10.1073/pnas.1503141112

Caipang, C. M. ., & Lazado, C. . (2015). Nutritional impacts on fish mucosa immunostimulants pre- and probiotics. Mucosal Health in Aquaculture.

In *Mucosal Health in Aquaculture* (pp. 211–272).

Cha, J. H., Rahimnejad, S., Yang, S. Y., Kim, K. W., & Lee, K. J. (2013). Evaluations of Bacillus spp. As dietary additives on growth performance, innate immunity and disease resistance of olive flounder (Paralichthys olivaceus) against streptococcus iniae and as water additives. *Aquaculture*, *402–403*, 50–57. https://doi.org/10.1016/j.aquaculture.2013.03.030

Cline, D. (2019). *Water Quality in Aquaculture*. Freshwater-Aquaculture.freshwater-aquaculture.extension.org/water-quality-in-aquaculture/

Dalynn, B. (2014). *McFarland Standard – for in vitro use only*, *Catalogue No TM50-TM60*.

Dawood, M. A. O., Magouz, F. I., Salem, M. F. I., Elbialy, Z. I., & Abdel-Daim, H. A. (2020). Synergetic Effects of Lactobacillus plantarum and β-Glucan on Digestive Enzyme Activity, Intestinal Morphology, Growth, Fatty Acid, and Glucose-Related Gene Expression of Genetically Improved Farmed Tilapia. *Probiotics and Antimicrobial Proteins*, *12*(2), 389–399. https://doi.org/10.1007/s12602-019-09552-7

Esam, F., Khalafalla, M. M., Gewaily, M. S., Abdo, S., Hassan, A. M., & Dawood, M. A. O. (2022). Ecotoxicology and Environmental Safety Acute ammonia exposure combined with heat stress impaired the histological features of gills and liver tissues and the expression responses of immune and antioxidative related genes in Nile tilapia. *Ecotoxicology and Environmental Safety*, *231*, 113187. https://doi.org/10.1016/j.ecoenv.2022.113187

Guardiola, F. A., Bahi, A., Bakhrouf, A., & Esteban, M. A. (2017). Effects of dietary supplementation with fenugreek seeds, alone or in combination with probiotics, on gilthead seabream (Sparus aurata L.) skin mucosal immunity. *Fish and Shellfish Immunology*, *65*, 169–178. https://doi.org/10.1016/j.fsi.2017.04.014

Hamdani, Waspodo, S., & Damayanti, A. A. (2018). Penggunaan Probiotik pada Pakan Ikan Bawal Bintang (Trachinotus blochii). *Jurnal Perikanan*, *8*(2), 16–19.

Handajani, H., Widanarni, Setiawati, M., Budiardi, T., & Sujono. (2018). Evaluation of digestibility and ammonia excretion of fish meal and fish silage fed to juvenile Indonesian shortfin eel (Anguilla bicolor). *AACL Bioflux*, *11*(2), 495–504.

Hossain, M. K., Islam, S. M. M., Rafiquzzaman, S. M. Nuruzzaman, M., Hossain, M. T., & Shahjahan, M. (2022). Multi-species probiotics enhance growth of Nile tilapia (Oreochromis niloticus) through upgrading gut, liver and muscle health. *Aquaculture Research*, *53*, 5710– 5719. https://doi.org/10.1111/are.16052

Iorizzo, M., Albanese, G., Letizia, F., Testa, B., Tremonte, P., Vergalito, F., Lombardi, S. J., Succi, M., Coppola, R., & Sorrentino, E. (2022). *Probiotic Potentiality from Versatile Lactiplantibacillus plantarum Strains as Resource to Enhance Freshwater Fish Health*.

Jannah, S.N., Dinoto, A., Wiryawan, K.G., & Rusmana, I. (2016). Molecular diversity pattern of intestinal lactic acid bacteria in Cemani chicken, Indonesian native chicken, as revealed by terminal restriction fragment length polymorphisms. Malaysian Journal of Microbiology, 12(1), 102-111. https://www.researchgate.net/publication/298792445.

Kamaliah, K. (2017). Kualitas Sumber Air Tangkiling yang Digunakan sebagai Air Baku Air Minum Isi Ulang dari Aspek Uji MPN Total Coliform. *Media Ilmiah Teknik Lingkungan*, *2*(2), 5–12. https://doi.org/10.33084/mitl.v2i2.122

Kelly, C., & Salinas, I. (2017). Under pressure: Interactions between commensal microbiota and the teleost immune system. *Frontiers in Immunology*, *8*(MAY), 1–9. https://doi.org/10.3389/fimmu.2017.00559

Khine, A. A. (2018). Probiotic effect of Bacillus species isolates on Poecilia reticulate Peters, 1859 (Guppy) and water quality. *1st Myanmar – Korea Conference*, *1*(1), 1–12.

KKP. (2019). *UPI Menengah Besar*. Kementerian Kelautan Dan Perikanan Republik Indonesia.

Liu, D. M., Wang, P., Zhang, X. Y., Xu, X. L., Wu, H., & Li, L. (2014). Characterization of nitrite degradation by Lactobacillus casei subsp. rhamnosus LCR 6013. *PLoS ONE*, *9*(4). https://doi.org/10.1371/journal.pone.0093308

Ljubobratovic, U., Kosanovic, D., Vukotic, G., Molnar, Z., Stanisavljevic, N., Ristovic, T., Peter, G., Lukic, J., & Jeney, G. (2017). Supplementation of lactobacilli improves growth, regulates microbiota composition and suppresses skeletal anomalies in juvenile pike-perch (Sander lucioperca) reared in recirculating aquaculture system (RAS): A pilot study. *Research in Veterinary Science*, *115*(July), 451–462. https://doi.org/10.1016/j.rvsc.2017.07.018

Lugert, V., Thaller, G., Tetens, J., Schulz, C., & Krieter, J. (2014). *A review on fish growth* *calculation : multiple functions in fish production and their specific application*. 1–13. https://doi.org/10.1111/raq.12071

Mangang, Y. A., & Pandey, P. K. (2021). ll and kidney tissues of Osteobrama belangeri (Valenciennes, 1844) exposed to different sub-lethal unionized ammonia. *Aquaculture*, *542*.

Maynard, C. L., Elson, C. O., Hatton, R. D., & Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature*, *489*(7415), 231–241. https://doi.org/10.1038/nature11551

Mishra, V., & Sharma, R. (2021). *Impact of probiotic supplementation on water quality and behaviour parameters in Cyprinus carpio*. *March*. https://doi.org/10.22271/fish.2021.v9.i1e.2430

Muchlisin, Z. A., Afrido, F., Murda, T., Fadli, N., Muhammadar, A. A., Jalil, Z., & Yulvizar, C. (2016). The Effectiveness of Experimental Diet with Varying Levels of Papain on The Growth Performance, Survival Rate and Feed Utilization of Keureling Fish (Tor tambra). *Biosaintifika: Journal of Biology & Biology Education*, *8*(2), 172. https://doi.org/10.15294/biosaintifika.v8i2.5777

Mzengereza, K., Ishikawa, M., Koshio, S., Yokoyama, S., Yukun, Z., Shadrack, R. S., Seo, S., Kotani, T., Dossou, S., Basuini, M. F. El, & Dawood, M. A. O. (2021). *novo Camelina Meal in Diets of Red Seabream (Pagrus major)*.

Parvathy, A., Das, B., Jifiriya, M., Varghese, T., Pillai, D., & Rejish Kumar, V. (2022). Ammonia induced toxico-physiological responses in fish and management interventions. *Rev Aquac*, 1–28. 10.1111/raq.12730

Rachmawati, D., Samidjan, I., & Prayitno, S. B. (2016). Aplikasi Teknik Probiotik Terhadap Kualitas Air Media Budidaya Ikan Lele Sangkuriang (Clarias gariepinus) di Desa Tambaksari, Kecamatan Rowosari, Kabupaten Kendal. *PENA Akuatika*, *14*(1).

Schar, D., Klein, E. Y., Laxminarayan, R., Gilbert, M., & Boeckel, T. P. Van. (2020). Global trends in antimicrobial use in aquaculture. *Scientific Reports*, 1–9. https://doi.org/10.1038/s41598-020-78849-3

Schmidt, V., Gomez-Chiarri, M., Roy, C., Smith, K., & Amaral-Zettler, L. (2017). crossm Probiotics Reduces Antibiotic-Associated Mortality in Fish. *American Society for Microbiology*, *2*(6), 1–13.

Sewaka, M., Trullas, C., Chotiko, A., Rodkhum, C., Chansue, N., Boonanuntanasarn, S., & Pirarat, N. (2019). Efficacy of synbiotic Jerusalem artichoke and Lactobacillus rhamnosus GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against Aeromonas veronii in juvenile red tilapia (Ore. *Fish and Shellfish Immunology*, *86*, 260–268. https://doi.org/10.1016/j.fsi.2018.11.026

Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J. A., & Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, Behavior, and Immunity*, *48*, 258–264. https://doi.org/10.1016/j.bbi.2015.04.003

Trisna, D. E., Sasanti, A. D., & Muslim. (2013). Populasi Bakteri, Kualitas Air Media Pemeliharaan dan Histologi Benih Ikan Gabus (Channa striata) yang Diberi Pakan Berprobiotik. *Jurnal Akuakultur Rawa Indonesia*, *1*(1), 90–102.

Van Doan, H., Lumsangkul, C., Jaturasitha, S., Meidong, R., Hoseinifar, S. H., & Dawood, M. A. O. (2021). Modulation of growth, skin mucus and serum immunities, and disease resistance of Nile tilapia fed host-associated probiotic (L.paracasei l61-27b). *Aquaculture Nutrition*, *July*, 1–10. https://doi.org/10.1111/anu.13314

Waite, R., Beveridge, M., & Brummett, R. (2014). *Improving Productivity and Environmental Performance of Aquaculture*. World Resources Institute.

Wuertz, S., Schroeder, A., & Wanka, K. M. (2021). *Probiotics in Fish Nutrition — Long-Standing Household Remedy or Native Nutraceuticals ?*

Zezen, A., Abidin, Z., Murdianingsih, Y., & Ruhiyat, I. (2022). *IoT-Based Guppy Aquaculture Monitoring System Using C 4 . 5 Method on Thingspeak Platform*. *6*(2). https://doi.org/10.29099/ijair.v6i2.387

Zheng, C., Wu, J., Zi, J., Yang, S., & Hui, S. (2019). *Exogenous enzymes as functional additives in finfish aquaculture*. *September*, 1–12. https://doi.org/10.1111/anu.12995

Zhou, B. L., & Boyd, C. E. (2015). *Ammonia nitrogen management in aquaculture ponds*. *October*, 1–6.