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EFFECTS OF *Bacillus* sp PROBIOTICS ON GROWTH AND SURVIVAL OF *Carassius auratus* IN URBAN FISH FARMING

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ABSTRACT

This study aims to determine the optimum density of *Bacillus* sp. bacteria applied to feed to increase the growth of *C. auratus* maintained in a fish farming system in an 80 L bucket. As a form of urban farming, this research was conducted in the yard of Building 1 of the Faculty of Fisheries and Marine Sciences, UNPAD. This study used an experimental method with a Completely Randomized Design model consisting of 4 treatments and three replications, namely treatment A (control), treatment B (*Bacillus* sp 10^6 CFU/mL), treatment C (*Bacillus* sp. 10^8 CFU/mL), and treatment D (*Bacillus* sp 10^{10} CFU/mL). Observations were made for 60 days. Observation parameters were weight growth, length growth, specific growth rate, feed efficiency, survival, and water quality. The results of this study indicate that treatment C (*Bacillus* sp 10^8 CFU/mL) was the most effective in increasing the growth of *C. auratus*, as seen from the absolute weight growth of 61.20 g, absolute length growth of 1.30 cm, specific growth rate of 0.77%/day, feed efficiency of 38%, and fish survival rate of 96% which were significantly different compared to the others.

Keywords: *Bacillus* sp, Specific Growth Rate, Feed Efficiency, Urban Farming

1. INTRODUCTION

As a country with a tropical climate, Indonesia has an enormous potential in ornamental fish production, with an estimated output of hundreds of millions per year. Ornamental fish such as *Carassius auratus* have become one of the primary commodities thanks to their attractive colors and shape. However, the increased population density and urbanization have shrunk the lands and water available to farm fish conventionally in urban areas. This calls for innovation in an efficient and sustainable aquaculture system.

One massively developed solution is the "budikdamber" (an acronym of cultivation fish in a bucket, fish farming in a bucket) system. It integrates aquaculture and hydroponics in a limited space. Such a system is known for its efficiency in using water and is feasible to be applied in a narrow urban environment[1]. Despite its more general usage for consumption fish, adapting this system to ornamental farming, such as comet fish, has great potential in urban aquaculture diversification.

In the "budikdamber" system, water quality is the crucial factor that affects the fish's growth and survival. The use of probiotics, especially from the *Bacillus* genus, has been widely studied thanks to their ability to increase fish health, feed efficiency, and reduce the ammonia level and other toxic compounds[2-3]. *Bacillus subtilis* and *B.cereus* are known to have the ability to enhance the comet fish's growth

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and resistance to environmental stress and bacterial infection[4].

The current research aims to evaluate the ability of *Bacillus* sp to increase *C. auratus* growth and survival in the "budikdamber" system and to determine the optimal dose that can be most effectively applied in a sustainable urban farming context.

2. RESEARCH METHOD

Time and Place

This research was conducted in three locations within the Padjadjaran University: (1) the yard of Building I at Faculty of Fisheries and Marine as the main farming site, (2) Microbiology and Biotechnology Laboratory of Building three at Faculty of Fisheries and Marine for preparing and culturing of *Bacillus* sp bacteria, and (3) Aquatic Animal Physiology Laboratory of Building 2 at Faculty of Fisheries and Marine for analyzing the physiological parameters and water quality. The research lasted for three months, starting from December 2023 through February 2024.

Method

This research used an experiment as its method with a Completely Randomized Design (CRD), consisting of four treatments, each repeated three times. The applied treatments were as follows: treatment A (control), treatment B (*Bacillus* sp 10^6 CFU/mL), treatment C (*Bacillus* sp 10^8 CFU/mL), and treatment D (*Bacillus* sp 10^{10} CFU/mL). For 60 days, the maintenance was observed. The parameters used during this observation were fish growth, which covered absolute weight growth (g), absolute length growth (cm), specific growth rate (SGR; %/day), feed efficiency (FCR), survival rate (% survival), and water quality, including: temperature (°C), pH, DO (dissolved oxygen, mg/L) and ammonia (mg/L).

Procedures

This research was carried out in two main stages: preparation and implementation. The former included *Bacillus* sp. culture, feed fortification with probiotics, research container preparation, and water spinach planting. The latter involved maintaining the test fish, observing the water quality, and collecting the data.

Bacillus sp Bacterial Culture

The *Bacillus* sp isolate was cultured using scratching in a sterile Nutrient Agar (NA) medium. The NA medium was poured into a petri dish aseptically and left idle until it reached the ambient temperature. The inoculation was carried out using the scratching method, before being incubated at 37 °C for 24 hours[5].

The bacteria that started to grow were taken using ose needle and suspended in sterile distilled water, before being homogenized using a vortex mixer. The bacterial cell density was determined using a spectrophotometer UV-Vis at 600 nm wavelength to confirm the *Bacillus sp.* density at 10^{10} CFU/mL[6].

Dilution and Dose Standardization

The bacteria suspension at an initial density of 10^{10} CFU/mL was diluted in series until 10^8 and 10^6 CFU/mL concentrations were obtained. The dilution was performed using a 10-fold method, i.e., mixing 1 mL of bacteria suspension into 9 mL of sterile distilled water and being homogenized.

Feed Fortification with Probiotic

The commercial feed PF 1000 was weighted and mixed with *Bacillus* sp. suspension at 10 mL bacteria to 1 kg feed ratio. A natural binder in egg white was added at 2% of the total feed weight to increase the adhesiveness. The mixture was dried at ambient temperature before it was fed to the fish[7].

Maintenance Container Preparation

The bucket with 80L capacity was cleaned using flowing water and rubbed, then sun dried for ±2 hours under the sunlight. Once it was dry, the bucket was filled with 30 L of clean water, and an aeration system was installed.

Water Spinach Planting

The plastic glasses were perforated at their bottom and filled with charcoals 3 cm thick. Each bucket had six planting glasses tied to it using a rope. The water spinach seeds were submerged for 24 hours; the sinking seeds were selected and planted using rockwool as the media, then they were placed inside the plastic glasses after their 7-day adaptation time.

Absolute Weight Growth

The absolute weight growth was calculated by weighing the fish's total weight at the study's beginning and end. This parameter was used to find out the increase in the total biomass weight during the maintenance period[5].

$$W = Wt - W0$$

Note:

W	=	Absolute growth weight (g)
Wt	=	biomass weight of test fish at the end of the study (g)
W0	=	Biomass weight of test fish at the beginning of the study (g)

Absolute Length Growth

The fish's total length was measured using a ruler (0.1 cm accuracy) at the beginning and the end of the study to discover how much the fish's body length had increased as a result of the treatments[8].

$$L = Lt - L0$$

Note:

L	=	Absolute length growth (cm)
Lt	=	Test fish length (TL) at the end of the study (cm)
L0	=	Test fish length (TL) at the beginning of the study (cm)

Specific Growth Rate

SGR constitutes a growth parameter that depicts the rate at which the fish body weight grows per day exponentially, and is expressed as a percentage

$$SGR = \frac{[\ln(Wt) - \ln(W0)]}{T} \times 100$$

Note:

SGR	=	Specific growth rate (%/day)
W0	=	Seed average weight at the beginning of the study (g)
Wt	=	Average weight at t-th day (g)
T	=	Maintenance duration (day)

Feed Efficiency

Feed efficiency depicts how effectively the feed is consumed by the fish and turned into body weight gain. The calculation considers the total weight of dead fish[9].

$$FE = \frac{(Wt + D) - W0}{F}$$

Note:

FE	=	Feed efficiency (%)
Wt	=	Test fish weight at the end of the study (g)
Wo	=	Test fish weight at the beginning of the study (g)
D	=	Total weight of dead fish during maintenance (g)
F	=	Total amount of feed given (g)

Survival Rate

The fish's survival was calculated based on the number of fish surviving until the end of the maintenance period, expressed as a percentage.

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

Note:

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

Water Quality

The water quality parameters were observed as supporting data to evaluate the environmental condition during the test fish maintenance period. The observation was done once every 10 days regularly to ensure that the farming environment remains within the optimal range to support the comet fish's growth and survival.

The temperature was measured in the morning (±08.00 WIB) to get the actual temperature of the farming media. The dissolved oxygen and pH were measured in situ using portable tools, and the ammonia concentration was observed using a color-based kit test.

Data Analysis

The obtained data on the absolute weight growth, absolute length growth, specific growth rate, feed efficiency, and survival rate were analyzed using analysis of variance (ANOVA) at a 95% confidence level. When significant differences were found between treatments, further tests would be taken using the Duncan test. The water quality was analyzed descriptively.

3. RESULT AND DISCUSSION

Absolute Weight Growth

For this absolute weight growth (W) parameter in the research, after being treated for 60 days, the *C. auratus* showed varied absolute weight growth values in all treatments. Treatment A (control) yielded the lowest average absolute weight growth value, at 42.23 ± 1.0 g, and treatment C (10^8 CFU/mL) indicated the highest value at 61.20 ± 0.1 g on average, as seen in Figure 1.

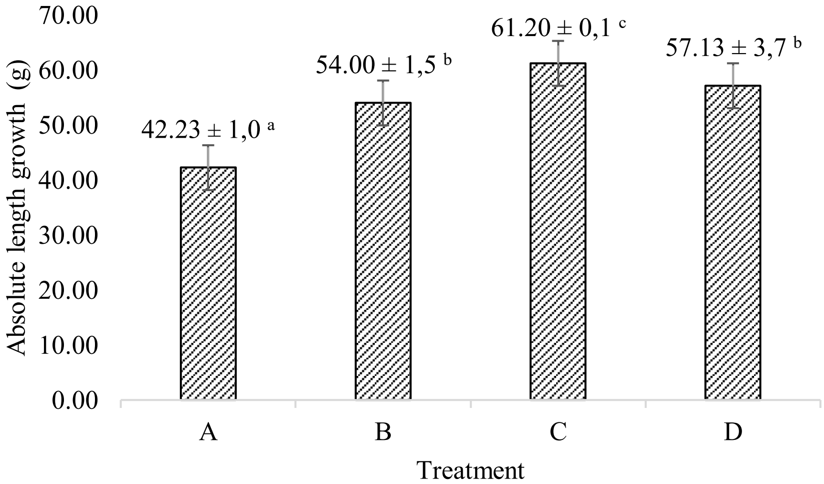

Figure 1. Absolute length growth of *C. auratus* for 60 days in maintenance

Treatment A (control) produced the lowest weight growth, and treatment C (10^8 CFU/mL) generated the highest. Adding *Bacillus* sp to the *C.auratus* feed in treatments B, C, and D significantly increased the absolute weight. *Bacillus* sp played the role of a probiotic, which helped increase the fish digestion through the so-called colonization process in the digestive tract, suppress the pathogenic bacteria, and improve the activities of such enzymes as protease and amylase, which made the nutrient absorption easier[5,10].

Treatment C (10^8 CFU/mL) showed the highest result because the intestinal microflora was balanced and the fish's internal environment was stable. However, the weight loss in treatment D (10^{10} CFU/mL) might be caused by the microbe overpopulation that triggered competition between bacteria in the digestive tract, leading to a disruption to the digestion process[11].

Absolute Length Growth

For the absolute length growth (L) parameter in this research, upon being treated for 60 days, the *C. auratus* showed varied absolute length growth values in all treatments. Treatment A (control) yielded the lowest absolute length growth average value at 0.83 ± 0.1 cm range, and treatment C, which added *Bacillus* sp. at 10^8 CFU/mL to the feed, indicated the highest value at 1.30 ± 0.1 cm on average, as can be seen in Figure 2.

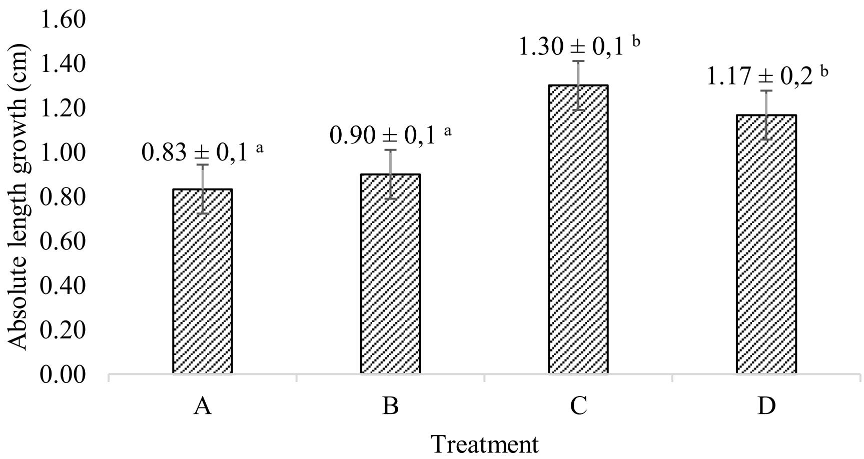

Figure 2. Absolute length growth of *C. auratus* for 60 days in maintenance

From the statistical analysis, ANOVA was performed at a 95% confidence level, and it was found that the F statistic > F table, meaning that the treatment with *Bacillus sp*. bacteria addition to the feed significantly affected the fish's final length. Based on this, it was found that adding *Bacillus* sp to the feed at 10^8 CFU/mL density was the optimal treatment for the *C. auratus* growth. The *C. auratus* length increased proportionally with its weight gain, i.e., as its weight increased, its length also increased.

This simultaneous increase in the *C. auratus* length and weight gain indicated proportional growth. Treatment C remained the best one, which was supported by Adlikahfi et al.[12] who found that adding *Bacillus* sp as a probiotic at 10^8 CFU/mL concentration significantly enhanced tilapia fish's length growth. The length growth was also affected by the increased metabolism efficiency and nutrient absorption, which were driven by the probiotic work in optimizing the intestinal villi structure and improving the digestive enzyme activities[9].

Specific Growth Rate

Specific growth rate is a parameter that explains the percentage of fish growth per day. The specific growth rate value is tightly related to the absolute weight growth. The fish's optimal weight gain can increase the specific growth rate value in a time unit.

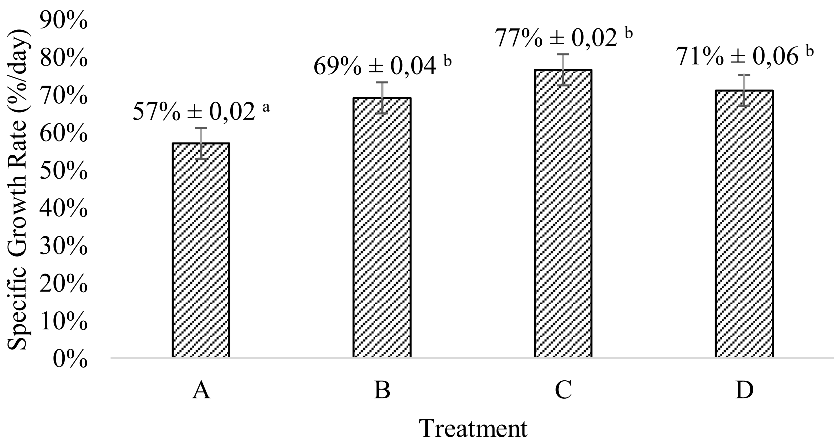

Figure 3. Specific growth rate of *C. auratus* for 60 days in maintenance

Figure 3 shows the result of the increase of *C. auratus*, observed once every 10 days. Treatment A had the lowest specific growth rate value at 57 ± 0.02 % per day, and treatment C had the highest value at 77 ± 0.02 % per day. Based on the statistical analysis, ANOVA at a 95% confidence level, it was found that the F statistic > F table. This meant that the treatment with *Bacillus* sp bacteria addition to the feed had different significant effects on the specific growth rate. The Duncan test result showed that there was a significant difference between treatments.

The highest SGR was found in treatment C, proving that the probiotic helped accelerate daily growth. This was consistent with Harianti et al.[8], who found that *Bacillus* sp increased the fish's appetite and growth since it could produce essential amino acids such as tryptophan, which triggered the production of serotonin. Serotonin played an important role in regulating appetite and homeostasis; hence, the fish would consume its feed more actively and could convert it into biomass faster[13]. This physiological effect made a great contribution to the SGR value.

Feed Efficiency

Feed efficiency is an alternative parameter used in addition to FCR and SGR. Feed efficiency is used to figure out the effectiveness of feeding in improving farming productivity. This feed efficiency parameter explains how efficient the feeding is in enhancing the fish's growth percentage[14].

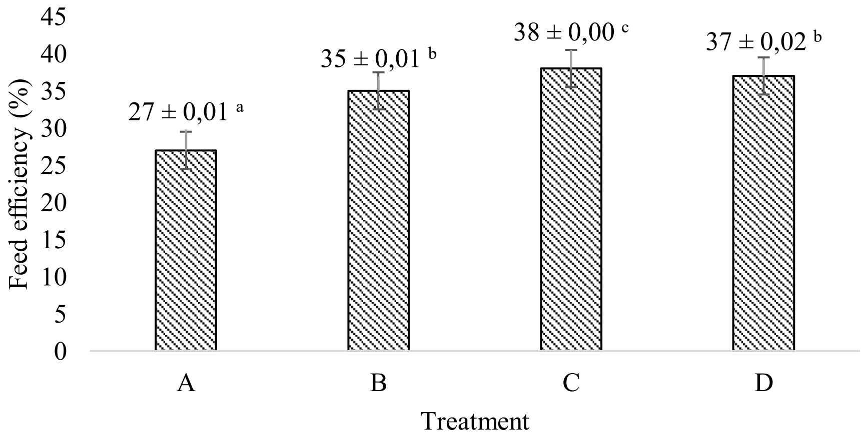

Figure 4. Feed efficiency of *C. auratus* for 60 days in maintenance

Figure 4 shows the feed efficiency of *C. auratus* during the research. Treatment A had the lowest feed efficiency at 27 ± 0.01%, and treatment C had the highest feed efficiency at 38±0.00%. Based on the statistical analysis, ANOVA at a 95% confidence level, it was found that the F statistic > F table. This meant that the treatment of adding Bacillus sp. bacteria to the feed had a significant effect on feed efficiency. The Duncan result showed that there was a significant difference between treatments. Treatment C (10^8 CFU/mL) produced the highest feed efficiency value, and treatment A (control) yielded the lowest feed efficiency value.

The increased feed efficiency in treatment C showed that the feed containing *Bacillus sp* was more effectively converted into growth. This bacterium secreted various enzymes such as amylase, lipase, and protease that broke complex nutrients into readily absorbable forms[11]. Fauzana et al.[10] reported that probiotics could improve feed efficiency up to 25% in biofloc and aquaponic systems, can to improved quality of intestinal microflora and reduced wasted feed residue.

Survival Rate

After treatment, the comet fish's survival rate in all treatments was found to have varied values. Treatment A had the lowest survival rate percentage at 92 ± 3.33 %. And treatments B and C had the highest survival rate at 96 ± 3.85 % and 96 ± 1.92 % respectively, as seen in Table 2.

Table 2. Survival rate of *C. auratus* for 60 days in maintenance

Treatment	SR (%)
A (Control)	92 ± 3,33
B (*Bacillus* sp 10^6 CFU/ mL)	96 ± 3,85
C (*Bacillus* sp 10^8 CFU/ mL)	96 ± 1,92
D (*Bacillus* sp 10^{10} CFU/ mL)	94 ± 3,85

From the statistical analysis, ANOVA performed at a 95% confidence level, it was found that the F statistic < F table, meaning that the treatment by adding *Bacillus* sp bacteria to the feed had no significant effect on the *C. auratus* survival rate.

Despite the insignificant differences in survival rate between treatments, there was a tendency for treatments B and C to have the highest SR value (96%). This was because of the positive influence of probiotics on the fish's immune system. Zahara et al.[15] showed that adding *Bacillus* sp increased the number of leukocytes and phagocytes, which served as an indicator of the fish's nonspecific immune system to infection. This treatment also lowered the fish's physiological stress and improved its resistance to environmental changes.

Water Quality

During the 60-day maintenance period, the water quality was observed by measuring the temperature, pH, DO, and ammonia in the maintenance media in all treatments. The water quality was measured once every 10 days during the maintenance to determine the water quality in the fish maintenance media. The data on this water quality during the maintenance period are presented in Table 3.

Table 3. Observation of the water quality of *C. auratus* for 60 days in maintenance

Treatment	Temperature (°C)	pH	DO (mg/L)	Ammonia (mg/L)
A	24,0 - 27,5	7,5 - 7,8	6,5 - 7,4	< 0,1
B	24,3 - 28,0	7,5 - 7,7	6,5 - 7,8	< 0,1
C	24,1 - 28,8	7,5 - 7,7	6,4 - 7,6	< 0,1
D	24,2 - 27,5	7,6 - 7,8	6,7 - 7,6	< 0,1
Standards	*26 – 30	*6,5 - 8,5	*> 5	**< 0,2

Note: *SNI[16]; **PP Number 22 of 2021[17]

The water quality parameter during the study was still limited to the optimal limits based on PP No. 22 of 2021[17]; Maulana et al.[18] the temperature ranged between 24.0 and 28.8°C, even if it was a little lower than the 26–30°C[19]. The temperature fluctuated since the farming system was in an open bucket without sunlight exposure. The pH ranged between 7.5 and 7.8, the dissolved oxygen (DO) ranged between 6.4 and 7.8 mg/L, and the ammonia was <0.1 mg/L, all in compliance with the ornamental fish farming quality standards. The application of *Bacillus* sp was also proven capable of helping maintain the water quality through ammonia and organic material degradation[11].

4. CONCLUSION

Based on the research that had been conducted, it could be concluded that adding *Bacillus* sp bacteria to the feed could improve the growth performance of *C. auratus* maintained in a fish farming system in an urban area (urban farming). Adding *Bacillus* sp at 10^8 CFU/mL density was the most effective treatment in improving the *C. auratus* growth performance. This was proven by several growth parameters, namely absolute weight growth (61.20 g), absolute length growth (1.30 cm), specific growth rate (0.77 %/day), feed efficiency (38 %), and fish survival rate at 96%.

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