

# THE EFFECT OF ADDING DIFFERENT TYPES OF PROBIOTICS IN FISH FEED ON THE GROWTH AND SURVIVAL RATE OF CARP (*Cyprinus carpio*)

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## ABSTRACT

This study aimed to determine the effect of giving different types of probiotics in fish feed on carp's growth and survival rate (*Cyprinus carpio*). It was an experimental study with a completely randomized design that included four treatments and three replications. The treatment was divided into (A) 0 mL/kg addition of probiotics, (B) 10 mL/kg addition of probiotics, (C) 15 mL/kg addition of probiotics, and (D) 3 mL/kg addition of probiotics. The stocking density was 20 fish/aquarium, and the feeding frequency was three times per day. This study showed that adding different types of probiotics had a significant effect ( $p < 0.05$ ) on length growth, weight growth, and survival rate of carp. The best results for getting the highest length growth were obtained in treatment B with a value of 1,96 cm, and the highest increase in weight growth was also obtained in treatment B with a value of 1,49 g. Moreover, the highest survival rate observed was also obtained in treatment B, with a percentage of 85%.

**Keywords:** *Cyprinus carpio*, Survival, Feed, Growth, Probiotic.

## 1. INTRODUCTION

Carp (*Cyprinus carpio*) is a freshwater species with good cultivation prospects. Carp is a type of freshwater fish with high economic value and is popular with the public. Carp is a freshwater fish species that has long been cultivated and is well domesticated globally. Indonesia has a strain of carp that is known to the public. Some strains of carp that are cultivated are majalaya, punten, sinyonya, domas, red /angkringan, kumpai, etc. One of the efforts made to increase carp growth is by giving probiotics.

Rinaldi<sup>1</sup> states that probiotics in aquaculture can be provided through feed because they can affect the digestive tract, so they will significantly help the process of food absorption in fish digestion. Probiotics are live microorganisms that benefit living things, including fish.

Microorganisms contained in probiotics can help digest food in the bodies

of animals and humans so that food containing probiotics can be digested and absorbed by the body properly. Microorganisms in the digestive tract are essential in increasing digestibility to accelerate the digestive process and fish growth.

The problem that often occurs in carp farming is the slow growth rate, an obstacle farmers often face in the fish farming business. Alternative efforts can be made to increase the growth and survival of carp, one of which is by giving probiotics because these probiotics can overcome the slow growth of carp. Probiotic Probio-7, probiotic Effective Microorganisms 4 (EM4) and probiotic Biopoccal Multiguna (organic feed supplement) are the types of probiotics used. The bacteria in probiotic probio-7 consists of 7 bacteria, namely *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Aspergillus*

*oryzae*, *Rhodopseudomonas*, *Actinomecetes*, and *Nitrobacter*.

Bacteria in probiotic Effective Microorganisms 4 (EM4) consists of 5 bacteria: *Lactobacillus* sp., *Rhodopseudomonas* sp., *Actinomycetes* sp., *Streptomyces* sp., and yeast<sup>2</sup>. The Biopoccal Multiguna probiotic (organic feed supplement) consists of 5 bacteria: *Bacillus* sp., *B.megaterium*, *B.mycoides*, *B.amyloliquefaciens*, and *B.altitudinis*.

This study aims to determine the effect of giving different types of probiotics in feed on carp growth rate and survival, as well as to determine the type of probiotic that produces the best growth and survival of carp fry.

## 2. RESEARCH METHOD

### Time and Place

This research was conducted from July to August 2022 at the Tatelu Freshwater Aquaculture Centre (BPBAT), Tatelu Village, Dimembe District, North Minahasa Regency, Sulawesi Utara Province.

### Method

The research was experimental, using a completely randomized design (CRD) consisting of 4 treatments and three replications with 12 experimental units<sup>3</sup>. The placement of each experimental unit was randomized, so the treatments applied in the study were as follows:

- A = Probiotic 0 mL/kg feed (control)
- B = Probiotic probio-7 10 mL/kg feed
- C = Probiotic EM4 15 mL/kg feed
- D = Biopoccal probiotic 3 mL/kg feed

### Procedure

#### Container Preparation

The container used in this study is an aquarium measuring 60x40x40 cm. Firstly, the aquarium was cleaned and dried. After the aquarium is dry, it is labeled and placed according to the specified layout. Then, the aquarium was filled with 40 L of water. The number of fish used in this study was 20 in

one aquarium with a density of 2 fish/L and a seed size of 4-6 cm<sup>4</sup>.

### Feed Preparation with Probiotics Added

The initial preparation stage for making feed is probio-7 as much as one bottle cap, one teaspoon of sugar is put into a measuring cup, and 500 mL of water is added and then allowed to stand for  $\pm$  5-10 minutes. A biopocall multipurpose probiotic of 3 mL was dissolved in 20 mL of water, and an EM4 probiotic of 15 mL/kg was fed. Next, the probiotics were taken using a dispo, put into a spray bottle, and sprayed on 1 kg of feed. Then, the feed was dried for  $\pm$  30 minutes. After drying, the feed is ready for use<sup>5</sup>.

### Maintenance of Test Fish

The test fish came from the Tatelu Freshwater Aquaculture Centre. Maintenance of test fish was carried out for 35 days. During maintenance, the fish were given commercial feed containing probiotics with a frequency of feeding three times a day at 08.00, 13.00, and 18.00 WIB. Feeding was 10% of fish biomass<sup>6</sup>.

### Water Quality Measurement

The water quality parameters measured are temperature, pH, and dissolved oxygen (DO), which are done every two days, while ammonia is measured once a week. Water quality measurements are taken in the morning. Sprinkling is done every other day after water quality measurement.

### Absolute Length Measurement

Body length measurements of carp were measured every seven days during the study. Body measurements were made by placing fish fry in a tray and measuring the total length, starting from the mouth's tip to the tail's tip. Measurements using a ruler, the measurement results were recorded in a table and then calculated the average total body length of fish per individual<sup>7</sup>.

### Absolute Weight Measurement

The body weight of carp was weighed every seven days during the study using a digital scale, and the average weight of carp fry per individual was calculated. Weighing of carp fry was carried out using the wet method, and the container was given a little water, placed on the scale, and then neutralized. Then, the carp fry was put into the container and recorded and calculated in the table<sup>8</sup>.

### Variables Observed

The variables observed in this study were the growth of carp fry, including absolute length growth, weight growth, survival, and water quality. Calculation of Absolute Length of carp using the formula Denoh & Sumantriyadi<sup>7</sup> as follows:

$$L = Lt - Lo$$

Description:

- L = Length growth of fish (cm)
- Lt = Final length of the study (cm)
- Lo = Initial length of the study (cm)

The absolute weight calculation can be calculated using the formula according to Idawati et al.<sup>8</sup>.

$$W = Wt - Wo$$

Description:

- W = Fish weight gain (g)
- Wt = Final weight of the study (g)
- Wo = Initial weight of the study (g)

The formula used to determine the percentage of survival of test fish according to Effendi<sup>9</sup>:

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

Description:

- SR : Survival
- Nt : Final fish count at the end of the study
- No : Number of fish at the beginning of the study

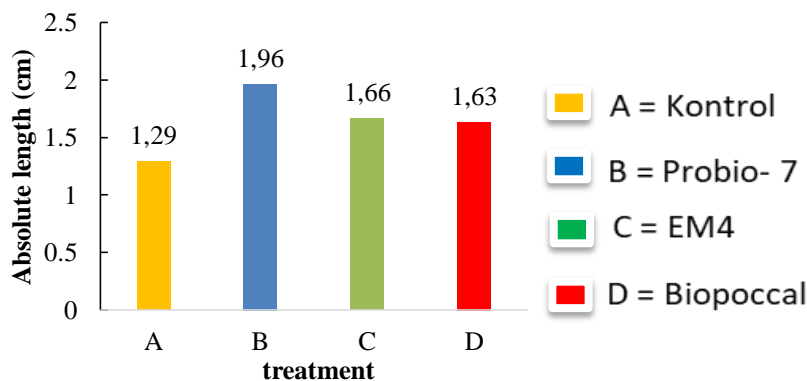
### Data Analysis

The data obtained were fish growth rate and water quality measurement data. The data obtained were then analyzed using Analysis of Variance (ANOVA) to determine the effect of the treatment given on growth. If the analysis of variance showed that the treatment showed a significantly different effect or significantly different, then the Duncan test was conducted to determine the difference between treatments.

## 3. RESULT AND DISCUSSION

### Absolute Length Growth of Carp (*C. carpio*)

The results of measuring the length of carp during the study showed that the highest length gain was found in treatment B (probio-7) with a dose of 10 ml/kg feed which resulted in a length of 1.96 cm. More clear data on carp's length gain can be seen in Figure 1.



**Figure 1.** Absolute length growth of carp (*C. carpio*)

Figure 1 shows that probiotics provide the results of carp growth,

experiencing length increases with different averages in each treatment. The average

length of carp is different, namely in treatment A (control) produces a length of 1.29 cm, in treatment B (probio-7) produces a length of 1.96 cm, treatment C (EM4) produces a length of 1.66 cm and in treatment D (Biopocaal) produces a length of 1.63 cm. The highest growth result in length during the study was found in treatment B (probio-7), which was 1.96.

The results above show that the provision of probiotics in feed can affect the growth of length in fish because it has *Bacillus* sp. bacteria, which can affect feed consumption in fish. This agrees with Sainah et al.<sup>10</sup>, which states that *Bacillus* sp.

is one type of bacteria believed to increase fish digestibility to increase fish growth.

The addition of probiotic bacteria can be used as a source of enzymes in feed; probiotics are photosynthetic bacteria that can increase the efficiency of feed utilization and fish growth efficiency. Probiotic species such as *Lactobacillus* sp., *Actinomyces* sp., and yeast are often required in probiotics to be added to feed, which aims to increase feed digestibility by increasing digestive enzymes that can hydrolyze proteins into simpler compounds so that they are easily absorbed and used as deposits for growth<sup>11</sup>.

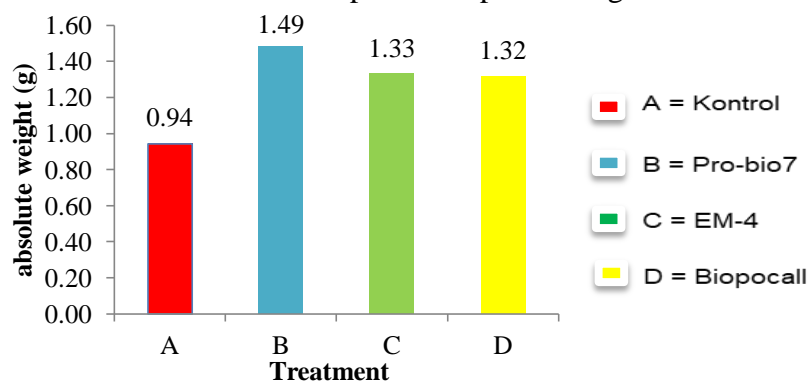


Figure 2. Absolute weight growth of carp (*C. carpio*)

### Absolute Weight Growth of Carp (*C. carpio*)

Based on the research, the highest absolute weight growth was obtained in treatment B with a dose of probiotics 10 mL/kg of feed, producing a weight of 1.49 g (Figure 2).

Based on the observations, the treatment with the highest value is B with a probiotic dose of 10 mL/kg feed. In contrast, treatments C and D have an average weight that is not significantly different, and treatment A (control) without probiotic administration has the lowest average weight value of 0.94 g. The results in the figure above are due to the activity of probiotic bacteria *Lactobacillus* sp., where these bacteria can produce lactic acid from sugar and other carbohydrates produced by photosynthetic bacteria and *S. cerevisiae*.

Arief<sup>12</sup> stated that *Lactobacillus* sp. bacteria balance fish digestibility by converting carbohydrates into lactic acid,

which can reduce pH, thus stimulating endogenous enzyme production to increase nutrient absorption, feed consumption, and growth and block pathogenic organisms. The amount of decomposing bacteria consumed will help the digestive process of fish. This is because these bacteria can produce protease, amylase, and lipase enzymes and improve the balance of bacteria in the digestive tract<sup>13</sup>. *S. cerevisiae* bacteria can improve diet, thus showing better growth and feed efficiency<sup>14</sup>.

Duncan's further test results showed that treatment A significantly differed from treatment D, C, and B while treatment D, C, and B showed no significant difference. The highest absolute weight growth was found in treatment B with a dose of 10 mL/kg feed. Internal and external factors influence growth. Internal factors largely depend on the condition of the fish body, for example, the ability of fish to utilize the remaining energy and protein after

metabolism for growth. Meanwhile, external factors such as environmental factors and feed influence fish growth. Both factors will balance the state of the fish body while in the maintenance medium and support fish growth. According to Selfiana<sup>15</sup>, growth is a complex biological process where many factors affect it. Growth in individuals is tissue growth due to mitotic cell division, which occurs when

an excess input of energy and amino acids (protein) is derived from food.

### Survival Rate of Carp (*Cyprinus carpio*)

Based on the research results, the highest survival rate was obtained in treatment B, with a survival percentage of 85%. The results of carp survival can be seen in Figure 3.

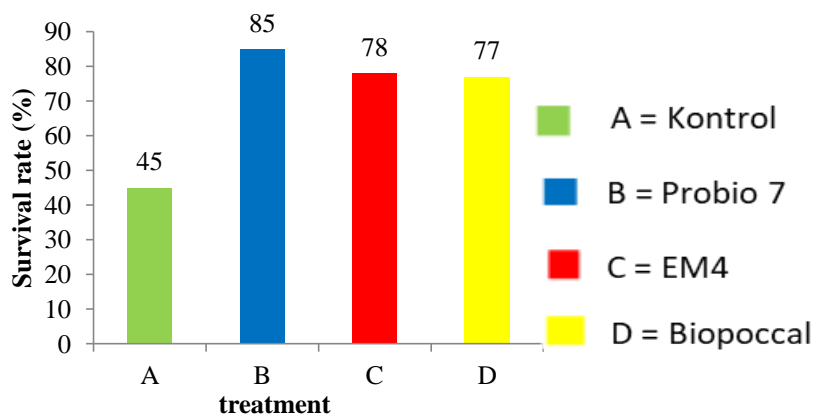


Figure 3. Survival of carp (*C. carpio*)

Based on the observation of the survival of carp seeds, the highest percentage of survival was obtained at a dose of 10 mL/kg of feed, namely 85%, followed by treatment C with a dose of 15 mL/kg of feed at 78% and treatment D with a dose of 3 mL/kg of feed at 77%, while the lowest survival was in treatment A (control) without the use of probiotics with a survival percentage of 45%.

The quality of the environment strongly influences the survival rate of carp fry; this is because if there is a change in the environment, it can cause stress, and the fish's appetite will decrease, which can cause death. The use of probiotics in aquaculture is to increase the growth and survival of fish; this agrees with Taufik et al.<sup>16</sup> which states that an increase in organic matter in the media will become toxic in maintenance water, the impact will trigger the onset of disease and lack of appetite resulting in low growth rates and survival of cultured fish. *Bacillus* sp. bacteria increase survival and growth rates, improve fish health status, and reduce

pathogenic vibrio<sup>17</sup>. Types of bacteria such as *Lactobacillus* sp, *Actinomycetes* sp, *Streptomyces* sp, and yeast are often required in probiotics to be added to feed, which aims to increase digestive enzymes that can hydrolyze proteins into simpler compounds so that they are easily absorbed and used as deposits for growth.

Duncan's further test results showed that treatment A significantly differed from treatments D, C, and B, while treatments D, C, and B showed no significant difference. The highest survival rate is in treatment B, with a percentage of 85%.

The above results show a significant effect on fish survival, so Duncan's test is carried out with the analysis results, which can be seen in the figure above. The above results are because microbes in probiotics can increase immunity in seeds, post-larva digestive tract, baker's yeast extract, and *S.cerevisiae* containing  $\beta$ -glucan produced by digestive glands. The degradation product is a short-chain polysaccharide, which is then converted into glycogen via the UDP glucose pathway. Excess  $\beta$ -glucan

that is not degraded will be absorbed by the intestine, joining the hemolymph as an immunostimulant that can boost the fish's immune system<sup>18</sup>.

### Water Quality

Based on the results of measurements during the maintenance of carp, the following results were obtained:

**Table 1.** Water quality measurements

| Treatment | Temperature (°C) |           | DO (mg/L) |           | pH      |           | Ammonia (mg/L) |
|-----------|------------------|-----------|-----------|-----------|---------|-----------|----------------|
|           | Morning          | Afternoon | Morning   | Afternoon | Morning | Afternoon |                |
| A         | 25,26            | 26,2      | 7,56      | 7,43      | 7,68    | 7,68      | 0,26           |
| B         | 25,16            | 26,3      | 7,6       | 7,43      | 7,66    | 7,72      | 0,64           |
| C         | 25,38            | 25,88     | 7,6       | 7,36      | 7,67    | 7,69      | 0,14           |
| D         | 25,3             | 25,83     | 7,6       | 7,4       | 7,65    | 7,59      | 0,11           |

Results above this temperature range are the optimal range for the life of carp recommended temperature range is 25-30°C<sup>19</sup>. The temperature obtained in the morning ranged from 25.38-25.30°C, and in the afternoon ranged from 25.88-26.5°C. Dissolved oxygen plays an essential role in cultivation. The amount of dissolved oxygen is sufficient and constantly needed by microbes to decompose organic matter. Oxygen is also a limiting factor in hatchery activities because fish in this phase have a high metabolic rate and oxygen levels.

Fish need oxygen to burn their fuel (food) to move, swim and reproduce. The results of dissolved oxygen data obtained during the study in the morning ranged from 7.5-7.6 mg/L, and in the afternoon ranged from 7.36-7.49. The recommended range of dissolved oxygen is more than 5<sup>19</sup>. The pH value in the study in the morning ranged from 7.68-7.77, and in the afternoon ranged from 7.72-7.77. The pH range for carp survival is 6.5-8.5 (SNI: 01-6131-1999). pH is also often used to indicate the acidity of a water body influenced by the concentration of CO<sub>2</sub> and acidic compounds, and pH is often used to determine the good and bad state of the cultivation media. Carp have a long tolerance to pH acidity, 6.5-8.5, and optimum at pH 7.

Ammonia was measured every 7 days, and the graph shows that the ammonia value from the highest in treatment B to the lowest value in treatment D. The ammonia range for carp rearing is less than 0.02<sup>19</sup>. The primary source of

ammonia in water results from the breakdown of organic matter, while feed is the largest source of organic matter in intensive aquaculture. Most of the feed used and given will be utilized in the form of feces and ammonia in water. Free non-ionized ammonia is a nitrogen source that aquatic plants can use directly but is toxic to aquatic organisms<sup>20</sup>.

The difference in ammonia values in the first week and last week's measurements is due to differences in the rainy season and summer, where the rainy season pH tends to rise, and ammonia values rise; this is in line with the opinion of Effendi<sup>9</sup>, at pH more than 7 ammonia will be toxic to aquatic biota and is present in large quantities.

The high value of ammonia in treatment B due to an increase in the weight of each seed measurement, which can affect the amount of feed given at the time of maintenance; this agrees with Sari et al.<sup>21</sup>, stating that the increase in ammonia levels is produced from the waste of fish metabolism due to protein breakdown, both the fish itself in the form of feces and urine and the rest of the feed.

The application of probiotics is significant in improving water quality, especially dissolved oxygen ammonia, for optimal fish-rearing media, in addition to enriching natural food and having a beneficial effect on the health and life of fish. Probiotic decomposing bacteria is appropriate to improve water quality so that the growing medium for fish becomes optimal. A potential alternative for disease

prevention measures is probiotic microorganisms<sup>22</sup> because probiotics can accelerate the decomposition of organic matter in water and produce nutrients that can be utilized for the natural development

of plankton. Kesarcodi-Watson et al.<sup>23</sup> mentioned that the role of probiotic bacteria is to degrade waste organic matter and ammonia, which will then be recycled into natural food.

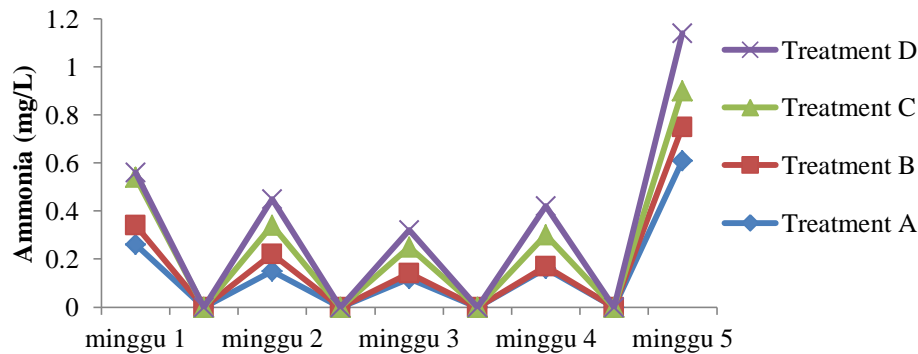


Figure 4. Ammonia graph of carp (*C. carpio*)

Improved water quality has mainly been associated with *Bacillus* sp. because gram-positive bacteria are better at converting organic matter back to CO<sub>2</sub> than gram-negative bacteria and can minimize the build-up of dissolved organic carbon and particulates. Ammonia levels in treatment D with a dose of 3 ml/kg feed are lower due to the presence of probiotic bacteria that can reduce ammonia levels by utilizing the remaining feed and feces that settle at the bottom of the maintenance media to be used as a source of protein for these bacteria through the assimilation process. Andriani et al.<sup>24</sup> stated that *Saccharomyces* in the form of yeast can assimilate ammonia to reduce ammonia levels in the cultivation media. According

to Widanarni et al.<sup>2</sup>, heterotrophic bacteria contained in probiotics can use leftover feed and feces in the maintenance media to assimilate nitrogen and organic carbon into microbial protein.

#### 4. CONCLUSION

Using different types of probiotics has an effect ( $p < 0.05$ ) on carp growth rate and survival in treatment B, with probiotic probio-7 producing the best growth rate and survival. Based on the above results, the authors provide suggestions for further research on different types of probiotics in feed on the growth and survival of carp by increasing the stocking density of seeds in the study.

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