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Effects of Dietary Levels of Probiotics on the Growth and Survival of the Snakehead Fish (*Channa striata*) Cultivated in the Bucket System

Azka Fathir Rizki[*1], Roffi Grandiosa[2], Rusky Intan Pratama[2], Iskandar[2]

[1]Study Program of Fisheries, Faculty of Fisheries and Marine Sciences, Padjadjaran University, West Java, Indonesia

[2]Department of Fisheries, Faculty of Fisheries and Marine Sciences, Padjadjaran University, West Java, Indonesia

[*]**Corresponding Author: azka19005@mail.unpad.ac.id**

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ABSTRACT

This research aimed to analyze the effects of different dietary levels of probiotics on the growth and survival rate of the snakehead fish (*C. striata*) cultivated in a bucket system. The study employed a completely randomized design (CRD) experimental method with five treatments and three replications. The fish were fed commercial feed supplemented with probiotics at concentrations of 5, 10, 15, and 20ml.kg^{-1} of feed, while the control group received commercial feed without probiotics. Each treatment group contained 10 fish, ranging from 7 to 9cm in length, and was stocked in an 80-liter bucket for a 42-day rearing period. The feed provided was 5% of the fish biomass. The addition of probiotics to the feed had a significantly different effect on the fish compared to the control group, impacting absolute length growth, absolute weight growth, specific growth rate, feed conversion, and survival rate. The 10 ml.kg^{-1} concentration resulted in the highest absolute length growth (2.33cm) and the highest specific growth rate (2.08%). The 20ml.kg^{-1} concentration led to the highest absolute weight growth (5.43g) and the best feed conversion ratio (2.35). The 5ml.kg^{-1} concentration achieved the highest feed efficiency (45.02%). These results indicate that the addition of commercial probiotics to the diet significantly influences the growth performance of snakehead fish reared in a bucket system.

INTRODUCTION

The snakehead fish (*Channa striata*) is one type of freshwater fish that has high economic value and can be utilized in various sizes ranging from small (juvenile) to large (adult). The snakehead fish fry are usually used as natural food for ornamental fish or predatory fish such as the flowerhorn, arowana, and giant featherback. The adult snakehead fish are used for consumption. The utilization of various snakehead fish ranging from small to adult sizes has an impact on increasing the need for the snakehead fish **(Muslim, 2017)**.

The snakehead fish have a cannibalistic nature that has a negative impact. The cannibalism of the snakehead fish can be reduced through the use of optimum density

(Saputra *et al*., 2018). However, the snakehead fish have highly beneficial properties for cultivation or aquaculture business. They have quite high resistance to poor aquatic environmental conditions. The snakehead fish are still able to survive in conditions of water shortages because they have well developed air breathing organs so that they can utilize free oxygen in the air for their breathing process **(Muslim, 2017)**. Moreover, they are able to tolerate unfavorable conditions such as low oxygen levels and low pH, pH range of 5.5–8.0 was suitable for the early stage of the snakehead fish. While, a pH range of 5.5–6.5 was better for the survival and growth of the snakehead fish in the fry to juvenile stage **(Ly *et al*., 2024)**.

The snakehead fish cultivation can be done in various media including buckets. The lack of empty land that can be used for freshwater cultivation makes cultivation in buckets or commonly known as ‘bucket’ a solution. ‘Bucket’ is also one of the solutions to the problems faced, such as the rapid increase in the world's population, requiring food products to also increase to meet food sources **(Purnaningsih *et al*., 2020)**. This method of fish cultivation does not require a large land area. It is easy to implement, uses small-scale media, is flexible, and does not require electricity for operation **(Zen *et al*., 2020)**. Based on research by **Damayanti *et al.* (2023)**, the stocking density in the ‘*bucket*’ system has an impact on decreasing water quality, due to the large amount of leftover feed and feces produced. One solution to this problem is to utilize the use of probiotics in the ‘bucket’ system.

Probiotics can function as immunostimulants, increase the feed conversion ratio (FCR), inhibit pathogenic bacteria's growth, produce antibiotics, and maintain and improve water quality **(Watson *et al.*, 2008)**. Several studies have reported that the application of probiotics mixed into feed has a positive effect on the average daily growth, feed efficiency, and survival of mantap carp **(Rostika *et al.*, 2020)**. The addition of commercial probiotics containing yeast microbes, Lactobacillus bacteria, Acetobacter, prebiotics, pro-amino, pro-active compounds, multivitamins, and minerals to artificial feed significantly improves average daily growth and enhances feed utilization efficiency. However, it does not have a significant effect on the survival of gourami (*Osphronemus gouramy*) fry **(Suminto & Chilmawati, 2015)**.

. The study by **Khayrurraja *et al*. (2023)** found that the addition of chitosan and liquid probiotics to feed significantly affected the absolute length and weight growth, specific growth rate, and feed efficiency of gourami (*Osphronemus gouramy*). Similar research on the effect of probiotics for fish raised in buckets or aquaponic systems has been conducted on catfish by several researchers. The use of different probiotics in aquaponic systems significantly influenced the feed conversion ratio and biomass of catfish (Clarias sp.) **(Sukoco *et al.*, 2016)**. Additionally, the addition of probiotics was shown to improve the growth rate and survival rate of catfish **(Primashita *et al.*, 2017)**.

The results of the study by **Hartini *et al.* (2013)** showed that snakehead fish (*C. striata*) fry raised in ponds with the addition of probiotics had a positive effect on the quality of the media water, survival (SR), and growth of the snakehead fish fry when compared to those raised without probiotics. The study by **Saputra *et al.* (2022)** showed that the local domesticated snakehead fish raised with probiotics had a relatively higher survival rate and relatively faster growth compared to those not given probiotics. Research on the effect of probiotics on fish raised in the 'bucket' system is important as information for the success of cultivation with the 'bucket' system and can produce data for further research related to *bucket*. Therefore, this study aimed to analyze the effect of giving probiotics in feed on the growth and survival rate of snakehead fish (*C. striata*) kept in the *bucket* system.

MATERIALS AND METHODS

The research was conducted for approximately 42 days. The research location was at an altitude of ± 828 meters above sea level. The research method used was an experimental design with a completely randomized design (CRD) consisting of five treatments and three replications, so the experiment was carried out on 15 experimental units.

1. Bucket preparation

The cultivation container used is an 80-liter bucket (Fig. 1). The bucket has a hole in the lid and is equipped with a drain thread. After cleaning and sterilizing the bucket, it is positioned and arranged properly. The bucket is then filled with water up to two-thirds of its capacity. Water spinach plants are placed in net pots, which are positioned in each hole in the bucket lid.

Fig. 1. Bucket for fish rearing

2. Fish preparation and rearing

The test fish were maintained in a bucket containing 10 snakehead fish fry measuring 7-9cm and weighing 4-5g in each treatment. Feeding was carried out twice a day. The amount of feed given was 5% of the fish biomass in each bucket and adjusted according to the fish's growth every 7 days.

3. Probiotic and feed preparation

A total of 1kg of commercial feed was weighed for each treatment. Probiotics were measured using a measuring cup and added to a spray bottle. The treatments were as follows: (A) no probiotics added to the feed (control), (B) probiotics added at 5ml.kg^{-1} of feed, (C) 10ml.kg^{-1} of feed, (D) 15ml.kg^{-1} of feed, and (E) 20ml.kg^{-1} of feed. The probiotics used were commercial products containing several types of microorganisms, including *Bacillus* sp., *Saccharomyces* sp., and *Lactobacillus* sp. **(Andriani *et al*., 2018)**. The probiotics were then sprayed onto the feed according to the treatment. After mixing, the feed was air-dried before use.

4. Water quality

Water sample quality parameter tests consisting of temperature, pH, dissolved oxygen and ammonia levels were carried out *in situ*. Siphoning or water changes of 30% was carried out every 7 days during the research.

5. Data analysis

Observation parameters such as absolute length growth, weight growth, daily growth rate, feed conversion ratio, feed efficiency, survival and water quality were calculated every 7 days. Data from observations of length, weight, growth rate, FCR, feed efficiency, and survival rate were tabulated in tables and graphs. The data were analyzed using the analysis of variance (ANOVA) with a 95% confidence level. If the sample averages were significantly different, Duncan's multiple range test was conducted. Water quality data, including temperature, pH, DO, and ammonia levels, were analyzed descriptively.

RESULTS AND DISCUSSION

1. Absolute length growth

The results of the absolute length growth of snakehead fish during the research are presented in Fig. (2).

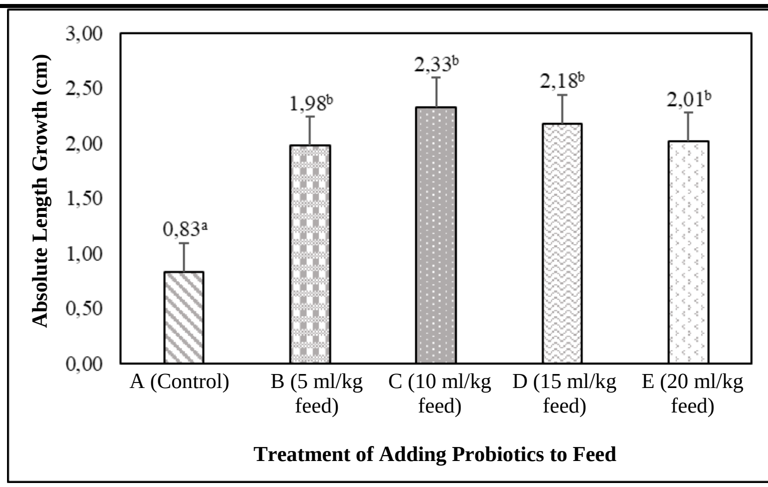

Fig. 2. Absolute length growth of snakehead fish

The highest absolute length growth of 2.33cm was obtained in treatment C. ANOVA analysis at a 95% confidence level showed a significant difference between the control treatment and the treatments with probiotics added to the snakehead fish feed, particularly in the absolute length growth of the fry. Based on Duncan's test, treatment B was found to be the most efficient for absolute length growth of snakehead fish raised in the bucket system, as its growth was not significantly different from that of treatments C, D, and E, which received higher probiotic concentrations.

The addition of probiotics to the feed of snakehead fish raised in the bucket system resulted in absolute length growth ranging from 1.98 to 2.33cm. The variation in length growth can be influenced by both internal and external factors. Internal factors include genetic traits, disease resistance, and the ability to utilize food, while external factors involve the physical, chemical, and biological properties of the water. Among these, food quality and water temperature are key factors that can impact fish growth. Fish growth occurs when the amount of food provided exceeds the body's maintenance needs **(Prihadi, 2007)**.

The increased absolute length growth in treatment C may be attributed to better digestibility of the food, facilitated by the probiotics in the feed. This is in line with **Ahmadi *et al.* (2012)**, who suggested that growth rates can be influenced by bacterial activity in the digestive tract and differences in probiotic bacterial numbers in commercial feed. Probiotic bacteria work optimally when their colonies are adequately represented in the feed, improving the fish's digestibility and, ultimately, promoting growth **(Arief *et al*., 2014)**.

2. Absolute weight growth

The results of the absolute weight growth of the snakehead fish during the research are presented in Fig. (3).

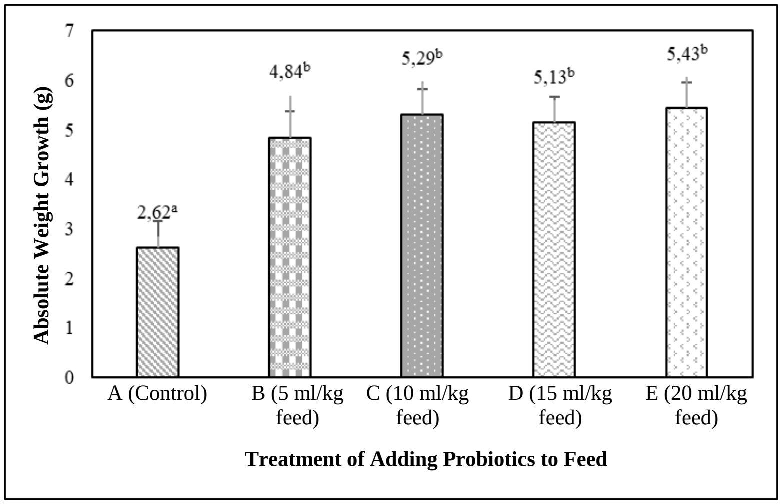

Fig. 3. Absolute weight growth of snakehead fish

The increase in absolute weight growth observed in several treatments is believed to be a result of enhanced digestibility in fish, enabling them to absorb more nutrients. This improvement is attributed to the influence of probiotics in the feed provided to the fish. Bacteria from genera like *Bacillus* and *Lactobacillus* can support the host's nutrition by supplying essential nutrients such as fatty acids and vitamins. According to **Aini *et al.* (2024)**, the use of *Bacillus* and *Lactobacillus* bacteria increased the crude protein content of the feed. The higher the probiotic concentration, the greater the increase in crude protein content. Additionally, probiotics help reduce fiber and BETN (extract material without nitrogen) levels in the feed by breaking down complex crude fibers into simpler forms of carbohydrates.

Lactobacillus species, in particular, produce a variety of enzymes, including lactases, proteases, peptidases, bile salt hydrolases, phytases, fructanases, amylases, and esterases **(Maske *et al.*, 2021)**. These enzymes assist in hydrolyzing feed nutrients that are still in complex forms into simpler molecules, facilitating the digestion and absorption processes in the fish's digestive tract **(Putra *et al.*, 2021)**.

As a result, snakehead fish that were fed probiotics showed significantly higher absolute weight growth compared to those that were not given probiotics, even when raised in a constrained environment such as the bucket system.

3. Specific growth rate

The specific growth rate of the snakehead fish during the research is presented in Fig. (4).

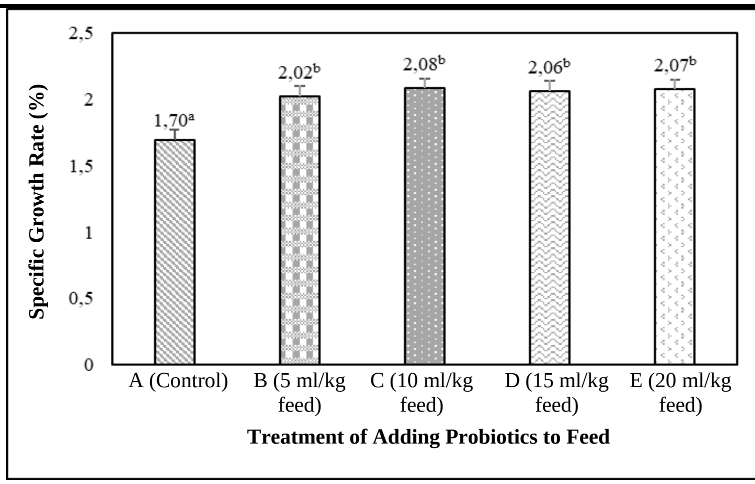

Fig. 4. Specific growth rate of snakehead fish

The highest specific growth rate of 2.08% was achieved in treatment C. This increase in the growth rate of the snakehead fish fry with the addition of probiotics to the feed suggests that the fry were able to better utilize the feed's nutrients. These nutrients were stored in the body and converted into energy. This energy supports various physiological processes, including movement, basic metabolism, reproduction (production of sexual organs), maintenance of body parts, and the replacement of damaged cells. Any excess energy is then used for growth.

The observed growth indicates that the fish were efficiently utilizing the feed nutrients for body functions and energy production, which is critical for their overall development **(Syahruddin, 2021)**. Fish growth is closely linked to the availability of protein in their diet, as protein serves as a key source of energy and an essential nutrient for growth. According to **Yulisman *et al.* (2012)**, the snakehead fish requires feed with a protein content of 40% to achieve optimal growth. In this study, the commercial feed provided had a protein content ranging from 39 to 41%, which is likely contributed to the optimal growth of the snakehead fish fry.

4. Feed conversion ratio

Feeding with the addition of different amounts of probiotics can improve the feed conversion ratio of the snakehead fish fry, as shown in Fig. (5).

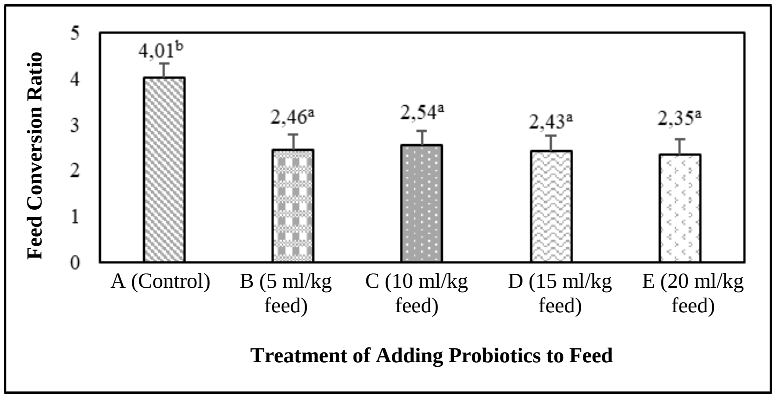

Fig. 5. Feed conversion ratio of snakehead fish

The addition of probiotics to the feed of the snakehead fish fry reared in the '*bucket*' system makes the feed more efficient to be converted into meat. The feed conversion ratio of snakehead fish given control treatment was significantly different from the treatment of the snakehead fish given probiotics in their feed. The highest feed conversion ratio was obtained in the snakehead fish fry given treatment A with a feed conversion ratio of 4.01 during the study. The lowest feed conversion ratio of 2.35 was obtained in treatment E. This means that the best feed conversion ratio in this study required 2.35kg of feed to form 1kg of fish meat. The lower the FCR value, the better the feed utilized and absorbed by the fish. The lower the feed conversion value, the better because the amount of feed used to produce a certain weight is less **(Pascual, 2009)**.

The results of the study with the addition of probiotics to the feed of the snakehead fish raised in the '*bucket*' system produced a feed conversion ratio ranging from 2.35 - 2.54, which is better than the treatment without the addition of probiotics. The activity of digestive enzymes is thought to have an effect on increasing fish appetite, and the amount of fish feed consumption becomes optimal so that it can be used as energy for fish. The feed ratio conversion value can be influenced by the activity of digestive enzymes in snakehead fish because enzymes help in the process of absorbing nutrients contained in the feed, so that it can increase the effectiveness value of the feed **(Lestari *et al*., 2022)**. Enzymes are biological catalysts that accelerate biochemical reactions within living organisms. In the context of fish nutrition, enzymes play a crucial role in breaking down complex nutrients in feed into simpler, more readily absorbable forms. This improved digestibility can enhance feed utilization and ultimately promote growth. The latter one produces numerous digestive enzymes such as cellulases, collagenases, proteases, amylases, allowing the digestion of complex organic macromolecules **(Liang *et al.*, 2022)**. The feed conversion ratio value can be an illustration of the level of efficiency of the feed given, the smaller the feed conversion value, the higher the feed efficiency value **(Saputra *et al*., 2018)**.

5. Feed efficiency

The feed efficiency (FE) value reflects the relationship between the amount of fish weight gained and the amount of feed consumed. According to **Kordi (2011)**, a higher feed efficiency value indicates that the fish are utilizing feed more efficiently. Probiotics can enhance fish growth by improving digestion, nutrient absorption, and the overall utilization of nutrients from feed. This is supported by the findings of **Chiu *et al*. (2021)**, who observed an increased feed efficiency in fish supplemented with probiotics, indicating better feed utilization. In this study, treatment B, where probiotics were added at 5ml.kg-1 of feed, was found to be the most efficient. The provision of probiotics in this treatment resulted in higher feed efficiency compared to the other treatments, as shown in Fig. (6). This suggests that even at a lower concentration, probiotics significantly improved the fish's ability to convert feed into body mass more effectively.

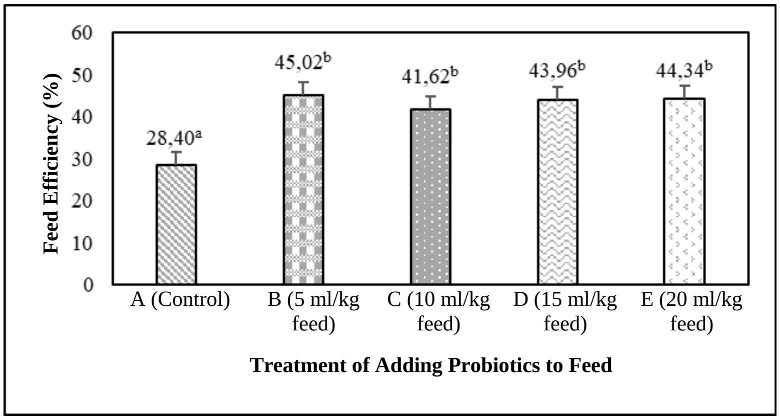

Fig. 6. Feed efficiency of snakehead fish

The highest feed efficiency value of 45.02% was obtained in treatment B. The addition of probiotics to the feed of the snakehead fish fry reared in the '*bucket*' system had a significant effect on the feed efficiency of the snakehead fish fry. The addition of probiotics to the feed of the snakehead fish reared in the '*bucket*' system resulted in a feed efficiency ranging from 41.62 - 45.02% during the research period. According to **Kandida *et al.* (2013)**, the amount of feed consumed by fish is greatly influenced by the quality of the feed, environmental conditions, and fish conditions. The increase in feed efficiency in several treatments is thought to occur due to an increase in the quality of the feed given probiotics. Higher feed efficiency indicates better quality of the feed given **(Putra *et al*., 2020)**.

Different probiotic dosage treatments may not show significant differences in some growth parameters and feed efficiency may be caused by biological variability. Fish within the same group can exhibit significant individual variation in growth rates and feed utilization. This variation can make subtle differences caused by different treatment doses.

6. Survival rate

Fish survival was calculated based on the comparison of the number of fish alive at the end of research with the beginning of research. Fish survival can be used as one indicator of success in cultivation activities. The survival of the snakehead fish during the study is presented in Fig. (7).

The highest survival rate of 97% was obtained in treatments C, D, and E. The addition of probiotics to the feed of the snakehead fish fry raised in the *bucket* system did not have a significant effect on the survival of the snakehead fish fry. The high value of fish survival cannot be separated from various factors that influence it. It is necessary to prepare a clean container that is good for fish life, provide sufficient and regular feed, and good and optimal water quality for the life of the fish being raised. Fish survival was determined by the availability of feed, the amount of food, health, and the cultivation environment **(Armando *et al*., 2021)**. According to **Effendie (2002)**, fish survival is also influenced by human handling. The quantity and quality of feed that is sufficient and good and supported by good environmental conditions greatly supports the survival of the snakehead fish **(Agustin *et al*., 2022)**.

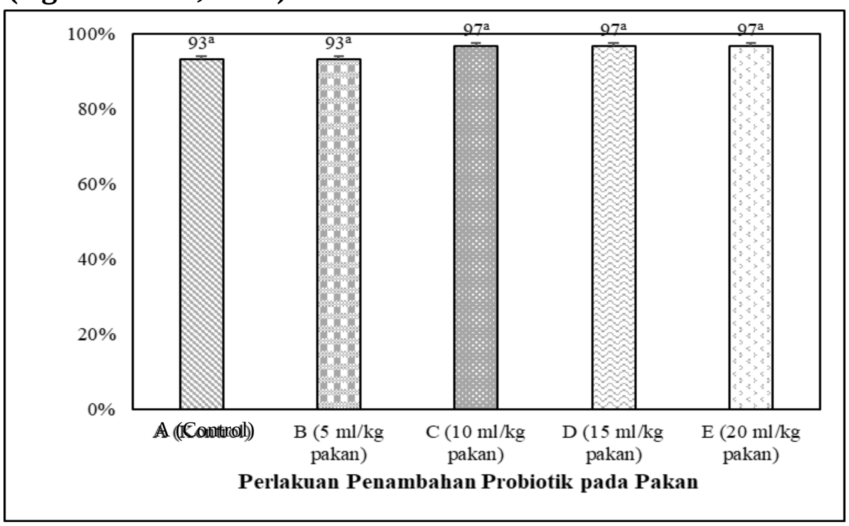

Fig. 7. Survival rate of snakehead fish

The survival rate of the snakehead fish given both control and probiotic treatments was notably high, indicating that the fish are capable of adapting and surviving in the *bucket* system under controlled conditions. This suggests that the low stocking density in the *bucket* system plays a role in reducing the risk of cannibalism, which is often a concern in intensive fish cultivation systems.

However, fish deaths were most commonly observed during the 5th and 6th weeks of the study, with three fish dying per week during this period. This mortality is believed to be linked to environmental stressors, particularly temperature fluctuations. As mentioned, decreasing water temperatures can cause stress for fish, which may lead to compromised immune systems, making them more susceptible to diseases or even causing direct physiological harm, ultimately resulting in death **(Nugraha, 2019)**.

Given these observations, it's important to monitor and control environmental conditions such as water temperature more closely in the bucket system to prevent further stress and ensure higher survival rates during cultivation.

7. Water quality

Water quality can determine the success of fish farming. Optimum cultivation water quality is needed to obtain optimal fish growth and high survival. The quality of cultivation water must at least meet the quality standards so that fish can live properly. The results of water quality measurements in the study during the 42-day research period of the snakehead fish raised in the '*bucket*' system are presented in Table (1).

The water quality parameters in the study showed that the addition of probiotics to the feed did not significantly affect the water temperature, but probiotics had a positive impact on water quality in terms of ammonia levels.

While the temperature range in the bucket system (22 - 25°C) was slightly outside the optimum range for the snakehead fish, it was still within the tolerable limits for their growth and reproduction, as the snakehead fish can generally tolerate temperatures between 23°C and 27°C **(Muflikha *et al.*, 2008)**.

Regarding ammonia levels, the recorded range of 0 - 0.25 mg/L was slightly above the optimal ammonia level for the snakehead fish, which is typically below 0.02 mg/L **(BPBAT Mandiangin, 2014)**. However, the fish were able to tolerate this level, as ammonia concentrations between 0.30 and 0.70 mg/L can still support growth **(Bijaksana, 2011)**.

A significant benefit of adding probiotics to the feed is the improvement in water quality, specifically in terms of ammonia reduction. The probiotics, particularly Bacillus bacteria, can enhance water bioremediation by breaking down nitrogenous waste products. These bacteria contribute to processes such as nitrification and denitrification, which help reduce toxic ammonia levels and improve overall water quality **(El-Kady *et al.*, 2022)**. This suggests that probiotics not only contribute to fish growth and health but also improve the sustainability of the cultivation system by maintaining better water quality.

Table 1. Range of water quality values during the research period

Treatments	Parameters			
	Temperature (ºC)	pH	DO ($mg.L^{-1}$)	Ammonia ($mg.L^{-1}$)
A	22-25	6,78-7,15	4,2-7,2	0-0,25
B	22-25	6,78-7,15	4,2-7,2	0-0,25
C	22-25	6,78-7,15	4,2-7,2	0-0,25
D	22-25	6,78-7,15	4,2-7,2	0-0,25
E	22-25	6,78-7,15	4,2-7,2	0-0,25
Optimum Range	23 – 27 **(Muflikha *et al.*, 2008)**	4 - 7 **(BPBAT Mandiangin, 2014)**	0,2 - 8,6 **(BPBAT Mandiangin, 2014)**	<0,02 **(BPBAT Mandiangin, 2014)**

The results of the study suggest that the bucket system used for the snakehead fish cultivation, in combination with water spinach plants, played a crucial role in maintaining water quality. The relatively stable water quality observed during the research indicates that the system effectively manages waste produced by the fish, thanks to the biofiltration function of the plants.

Water spinach, as part of an aquaponics setup, can absorb and utilize the nutrients and waste products from the fish, such as ammonia and nitrates, which helps reduce their concentrations in the water. This system helps keep the water quality in optimal conditions for fish growth and survival, as well as minimizing the risk of toxic buildups. **Wicaksana *et al.* (2015)** also found that the presence of plants in aquaponic systems can result in lower levels of ammonia, nitrite, and nitrate compared to conventional ponds without plants, further supporting the effectiveness of this approach.

The application of aquaponics is a sustainable solution for maintaining water quality in intensive fish farming systems. By incorporating plants in the cultivation process, waste produced by the fish is not only filtered from the water but also utilized as fertilizer for the plants, creating a closed-loop system where nutrients are recycled. This minimizes waste discharge into public waters and reduces environmental impact **(Tanody *et al.*, 2023)**.

The results highlight the potential of combining fish farming with plant cultivation in bucket systems, offering an environmentally friendly, efficient, and sustainable approach to aquaculture.

CONCLUSION

The results of this study suggest that the addition of probiotics to the feed of the snakehead fish raised in the bucket system had a significant impact on their growth parameters, including absolute length growth, absolute weight growth, specific growth rate, feed conversion ratio (FCR), and feed efficiency. The findings also indicate that the different probiotic doses (5-20ml.kg-1) did not produce significantly different effects on the growth parameters, suggesting that the lower dose of 5ml.kg-1 was already sufficient to achieve optimal results.

The lack of significant difference in fish survival between the control group and the probiotic treatments further emphasizes the primary benefit of probiotics being their positive impact on growth rather than survival. This implies that probiotics can enhance the fish's ability to utilize feed more efficiently, which results in improved growth, but survival remains relatively stable under the tested conditions.

The conclusion that a 5ml.kg-1 dose of probiotics is adequate for achieving the maximum beneficial effect is important for practical applications. By using a lower dose, fish farmers can reduce costs without sacrificing performance, as higher doses did not result in additional growth benefits. This makes the 5ml.kg-1 dosage an optimal and cost-effective choice for the snakehead fish cultivation in the bucket system.

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