DIETARY PROBIOTICS AND ITS EFFECT ON GROWTH RATE, SURVIVAL RATE, AND FEED CONVERSION RATIO OF *Clarias gariepinus*

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(Naskah diterima: 05 Maret 2023; Revisi final: 04 Maret 2024; Disetujui publikasi: 04 Maret 2024)

ABSTRACT

The applications of probiotics in aquaculture show wide range of potential benefits. If fish consume probiotics in sufficient and accurate amounts, it can have a beneficial impact on the health of catfish. The study aimed to identify the effect of dietary probiotics supplementation of artificial diet at different doses on the African catfish (*Clarias gariepinus*) parameters such as specific growth rate, survival rate, and feed conversion ratio. The experiment was performed at the Faculty of Agriculture, Bosowa University, Makassar, and lasted for 2 months. The experiment employed a completely randomized design (CRD) consisting of four treatments and three replicates. The EM4 probiotics were selected as the probiotic supplement and divided into four different doses including treatment: A (5 mL per 100 g feed), B (10 mL per 100g feed), C (15 mL per 100g feed), and D (control). A total of 120 African catfish fingerlings were fed twice every 07:00 a.m. and 06:00 p.m. with a feeding rate of 5% of the body weight. Sampling was performed every week to record catfish body weight and length. The analysis of variance indicated a significant effect of dietary EM4 probiotics supplementation on specific growth rate and feed conversion ratio of African cathfish. However, the result of the experiment revealed 15 mL per 100 g feed did not promote absolute growth rate and survival rate significantly. This study concluded that supplementation of probiotics at a dose of 15 mL per 100 g feed in diet could improve the growth of catfish.

KEYWORDS: African catfish; artificial diet; EM4 supplementation; growth

ABSTRAK: ***Pemberian Pakan dengan Probiotik dan Pengaruhnya terhadap Laju Pertumbuhan, Kelangsungan Hidup, dan Rasio Konversi Pakan*** *Clarias gariepinus*

*Penerapan probiotik dalam budidaya perikanan menunjukkan berbagai manfaat potensial. Jika ikan mengonsumsi probiotik dalam jumlah yang cukup dan tepat, maka dapat memberikan dampak yang menguntungkan bagi kesehatan ikan lele dumbo. Penelitian ini bertujuan untuk mengidentifikasi pengaruh pemberian probiotik pada pakan buatan dengan dosis berbeda terhadap laju pertumbuhan spesifik, sintasan, dan rasio konversi pakan ikan lele dumbo (**Clarias gariepinus**). Eksperimen dilakukan di Fakultas Pertanian, Universitas Bosowa, Makassar, dan berlangsung selama 2 bulan. Eksperimen menggunakan rancangan*

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acak lengkap (RAL) yang terdiri dari empat perlakuan dan tiga ulangan. Probiotik EM4 dipilih sebagai suplemen probiotik dan dibagi menjadi empat dosis perlakuan yang berbeda meliputi perlakuan A (5 mL per 100 g pakan), perlakuan B (10 mL per 100 g pakan), perlakuan C (15 mL per 100 g pakan), dan perlakuan D (kontrol). Sebanyak 120 ekor benih ikan lele dumbo diberi pakan dua kali setiap pukul 07.00 dan 18.00 dengan dosis pemberian pakan sebanyak 5% dari bobot tubuh ikan lele. Pengambilan sampel dilakukan setiap minggu untuk mencatat bobot dan panjang tubuh ikan lele. Analisis varians menunjukkan pengaruh yang signifikan dari suplementasi probiotik EM4 pada laju pertumbuhan spesifik dan rasio konversi pakan ikan lele dumbo. Namun, hasil percobaan menunjukkan bahwa 15 mL per 100 g pakan tidak meningkatkan laju pertumbuhan mutlak dan sintasan secara signifikan. Penelitian ini menyimpulkan bahwa suplementasi probiotik 15 mL per 100 g pakan dalam pakan dapat meningkatkan pertumbuhan ikan lele dumbo.

KATA KUNCI: ikan lele dumbo; pakan buatan; pertumbuhan; suplementasi EM4

INTRODUCTION

African catfish (*Clarias gariepinus*) is one of the most farmed finfish species globally (Dauda *et al.*, 2018; Food and Agriculture Organization, 2017). African catfish is a common species in aquaculture. This trend has encouraged more research on other species, considering the African catfish growth rate, reasonable market value, resistance, and reproductive performance (Kenoye & Kenoye, 2018). Furthermore, market demand for catfish has also surged significantly over the years due to its delicious taste, relatively low price, and fast growth (Soedibya *et al.*, 2017). Artificial diets used in rearing catfish fingerlings should meet the catfish's specific nutritional requirements (Hadijah, 2017; Hadijah *et al.*, 2020).

Feed is one of the determining factors in catfish farming businesses because it dramatically impacts on the increase of production rate. In contrast, the expensive price of commercial pellets is a significant obstacle among catfish farmers because the average production cost for feed ranges from 60-70% of the total production cost (Alfiko *et al.*, 2022). Intensive catfish farming may frequently result in excessive accumulated leftover feed and metabolic waste in the water. High concentrations of these wastes can potentially contaminate the water and cause death in catfish due to ammonia, nitrate, and nitrite toxicity (Olabi *et al.*, 2023; Piskuła & Astel, 2023). Dietary probiotics supplementation appears as one of the most accurate solutions to boost catfish biomass weight, reduce feed cost production, and improve water quality by overcoming the accumulation of toxic compounds at once (Rostika *et al.*, 2020).

Applying probiotics in aquaculture systems is vital in determining the farming success rate. Fish consuming probiotics in sufficient and accurate quantities can positively contribute to the catfish's health condition. A wide range of microorganisms is employed in aquaculture, including both Gram-positive and Gram-negative bacteria. The microbial species contained in the EM4 probiotics is *Lactobacillus* sp. It can grow optimally at the pH range of 5.5-6.5, at a temperature of 30-37°C, and it reacts against Gram-negative bacteria. Other non-bacterial microorganisms such as bacteriophages, microalgae, and yeasts are also commonly identified as probiotics in aquaculture (Lambo *et al.*, 2021; Manan *et al.*, 2023). Dietary supplementation of probiotics can directly impact the speed of digestion in the fish digestive tract. Microorganisms can play an essential role in stimulating optimal nutrient absorption by enhancing the intestinal flora function of the fish digestive system. Therefore, dietary probiotics supplementation can improve fish's physiological function, particularly food digestion (Carnevali *et al.*, 2017).

Probiotics are supplements of live microbial cells, different from chemical-based supplements. Probiotics control the development and population of harmful microbes. They can stimulate the ideal growing environment for good bacteria (Zorriehzahra *et al.,* 2016). These microbes will eventually dominate and create suitable habitats for live microbial growth. Increasing interest among researchers in the studies concerning the roles of probiotics in aquaculture demonstrates a wide range of potential benefits these microbes can promote. It also emphasizes their importance in the ongoing innovation of improving farmed fish health. This study presents a novel potential of combining artificial diet and probiotics in African catfish farming. The study aims to identify dietary probiotics supplementation at different doses on an artificial diet and its effect on specific growth rate, survival rate, and feed conversion ratio of catfish.

MATERIALS AND METHODS

Experimental Design

The study employed a completely randomized design (CRD) consisting of four treatments and three replicates. The treatment doses included treatment A (5 mL per 100 g feed), treatment B (10 mL per 100 g feed), treatment C (15 mL per 100 g feed), and treatment D (control).

Test Diet

The procedure started with the test diet preparation. The composition of the test diet consisted of some stuffs as mentioned in Table 1.

These stuffs were mixed, pelleted (crumble size), and sundried for ± seven days. EM4 probiotic was sprayed on the prepared diet.

Experimental Procedure

The experiment was conducted for 2 months at the Fisheries Laboratory, Faculty of Agriculture, Bosowa University. Healthy catfish fingerlings from the hatchery were acclimated for three days. We maintained of healthy experminetal fish by this parameters: appearance, activity level, appetite, respiration, body condition, fins, eyes, behavior, and stress response described in Table 2. The fingerlings were transferred into the sterilized experimental plastic container. The containers were 18 cm in height and 8 cm in diameter with 3 liters of water capacity. Twelve cylindrical containers equipped with U pipes were filled with freshwater previously aerated for ± two days. This experiment used as many as 120 catfish fingerlings (1 month-old) with 50-70 mm body length and 2.025 g of average initial body weight. Each container was stocked with ten African catfish fingerlings.

The feeding rate was 5% of the fingerlings' body weight. The fingerlings were fed twice at 07:00 a.m. and 06:00 p.m. daily. Water replacement was performed as much as 50% of total water volume once a week before feeding. Container cleaning was performed twice daily at 06:00 a.m. and 03:00 p.m. Water quality parameters measured during this experiment included the temperature and the pH. Parameters was measured in the morning and evening at 07:00 a.m. and 06:00 p.m. Sampling was performed every week aiming to determine the catfish's body weight and length. All catfish fingerlings in each container were used in body weight and length sampling. The observed water quality parameters in this study included temperature and pH. The water temperature during the study ranged from 27-29°C. The results of pH measurements during the experiment indicated that the pH ranged from 7.7 to 8.5

Table 1. The composition of the test diet

Composition of the test diet	Percentage (%)
Shrimp Cephalothorax Meal	30
Soybean Meal	32
Fine Bran	20
Starch Flour	5
Fish Oil	3
Wheat Pollard	7
Vitamin Supplements	3
Total	100

Table 2. Characteristics of healthy catfish

Parameters	Characteristics
Appearance	Healthy catfish typically have a sleek, shiny appearance with vibrant colors. Their skin should be smooth without any lesions, sores, or abnormal growths.
Activity level	They are generally active and responsive, swimming around their environment with agility. Lethargy or sluggish behavior can indicate health issues.
Appetite	Healthy catfish have a good appetite and eagerly consume food when offered. A sudden loss of appetite could be a sign of illness.
Respiration	Normal breathing patterns involve steady gill movements without any signs of labored breathing or gasping for air.
Body condition	They should have a well-rounded body shape without any signs of emaciation or bloating. A healthy catfish will have a firm and muscular body
Fins	Their fins should be intact, without any tears or damage. Fin deterioration can indicate poor water quality or disease.
Eyes	Clear and bright eyes are a sign of good health. Cloudy or sunken eyes could be a sign of infection or poor water conditions.
Behavior	Healthy catfish exhibit normal behavior, interacting with other fish in the tank or pond without aggression or isolation.
Stress response	When handled or approached, healthy catfish may show some stress response, but they should quickly recover and resume normal behavior once the stressor is removed.

Observed Parameters

The observed parameters encompassed the absolute growth rate (AGR), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR). The parameters are calculated using the following formula:

The absolute growth rate is calculated using the following formula (1) ((De Silva & Anderson, 1994):

$$AGR = Wt\text{-}Wo \quad \ldots\ldots (1)$$

Where; W_t is the final weight (g), and W_o is the initial weight during the experiment (g).

The specific growth rate was observed

every seven days by recording the latest weight of the experimental catfish and calculated using the formula (2) designed by De Silva and Anderson (1994), as follows:

$$SGR = \frac{(\ln(W_2) - \ln(W_2))}{(t_2 - t_1)} \times 100\% \quad \text{.....(2)}$$

Where SGR is the specific growth rate (%) W_1, is the initial weight (g), W_2 is the final weight (g), and t is the period (days).

The survival rate is calculated using the following formula (3) (Effendie, 1979);

$$SR = \frac{N_t}{N_o} \times 100\% \quad \text{.....(3)}$$

Where SR is the survival rate (%), N_t is the number of fish stocked (ind), and N_o is the number of fish that survived (ind).

The feed conversion ratio (FCR) is a comparison between the amount of consumed feed (g) and the total weight gain (g). It can be calculated using the following formula (4) (Effendie, 1979):

$$FCR = \frac{F}{W_t - W_o} \times 100\% \quad \text{.....(4)}$$

Where FCR is the feed conversion ratio, F is the total feed fed (g), W_t is the final weight (g), and W_o is the initial weight during the experiment (g).

Data Analysis

The data obtained from the calculation results for each parameter were analyzed using analysis of variance (ANOVA) with the Statistical Tool for Agricultural Research (STAR) Software (STAR Version 2.0.1) from IRRI. Subsequently, a follow-up analysis using the least significant difference (LSD) test was conducted in case any significant differences were identified among the treatments.

RESULTS AND DISCUSSION

Absolute Growth Rate

The results of dietary EM4 probiotics supplementation at different doses on AGR of African catfish is presented in the following Figure 1. The analysis of variance (ANOVA) showed that probiotic supplementation in each treatment (figure 1) did not significantly affected the AGR of African catfish, which ranged from 4.50 g to 5.34 g during the experiment. However, as we can see the lowest and the highest AGR value appeared in control treatment and treatment C (15 mL per 100 g feed), respectively. The results also emphasize that the percentage of feed and probiotics was sufficient, and catfish could survive in treatment conditions. Dietary EM4 probiotics supplementation in treatment 15 mL per 100 g feed generated the highest AGR value. This also correlates to the most optimal survival rate from treatment C, accounting for 90%.

Studies have demonstrated that the use of probiotics in diets on African catfish can enhance the growth performance of African catfish by increasing their growth rate, hence leading to improved growth performance in this particular type of fish (Abdul-Malik *et al.,* 2023; Sohel *et al.,* 2023). The presence of good bacteria that helped catfish digestion contributed to this. Water quality parameters were still within the tolerable or acceptable threshold for catfish survival (Kurniawan *et al.*, 2022). Additionally, another study demonstrated that the supplementation of probiotics to artificial catfish diet resulted in a notable enhancement in growth performance (Fitriana *et al.*, 2024). The use of probiotics as a first feed from African catfish juvenile has been shown to improve survival, growth rate, and feed utilization efficiency (Adedokun & Dina, 2023). These findings indicate that adding probiotics into the diet of African catfish can enhance their growth.

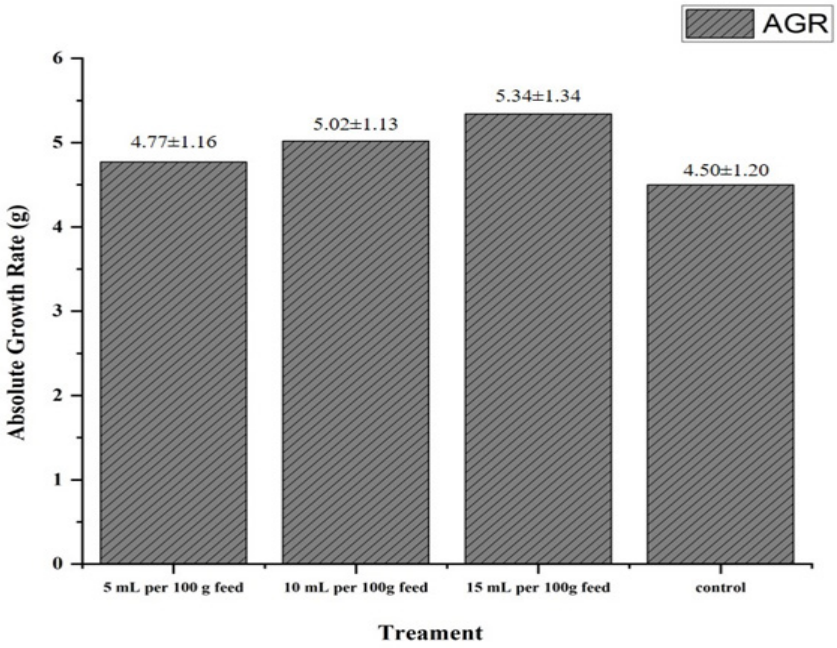

Figure 1. Absolute growth rate of African catfish supplemented with EM4 probiotics at different doses (means ± standard deviation)

Specific Growth Rate

The results of dietary EM4 probiotics supplementation at different doses on SGR of African catfish is presented in the following Figure 2. The analysis of variance (ANOVA) showed that probiotic supplementation in each treatment significantly affected the SGR of African catfish, which ranged from 0.12% to 0.15% within 4 weeks in experiment. The treatment A (5 mL per 100 g feed) and treatment B (10 mL per 100 g feed) did not showed significant different while treatment C (15 mL per 100 g feed), and treatment D (control) showed significant different. as we can see the lowest and the highest SGR value appeared in control treatment and treatment C (15 mL per 100 g feed), respectively. It indicated that the African catfish successfully absorb nutrients and energy supplied from the feed to grow.

Treatment C or dietary EM4 probiotics at 15 mL per 100 g feed, appeared as the most effective treatment, reaching an SGR value of 0.15%. This confirmed that dietary probiotic supplementation at 15 mL per 100 g feed for African catfish fingerlings resulted in a good SGR response (Rostika *et al.*, 2020). Such good result in SGR is supported by the good bacteria population that enters and colonizes catfish's digestive tract. Probiotics are living bacteria that, when given in sufficient quantities, provide health advantages to the host. Probiotics have been investigated for their ability to improve growth performance and overall health in African catfish (Genç *et al.*, 2020). Some studies reported that supplementation dietary with probiotics can increase SGR of African catfish (Aini *et al.*, 2024; Mansour *et al.*, 2024). Furthermore, the use of probiotics was found to improve feed utilization, body indices, and protein efficiency rate in African catfish (Lawal *et al.*, 2019)

Bacteria collected from aquatic and land animal intestines are commonly used as probiotics in aquaculture. By supplementing aquaculture systems with probiotics derived from diverse sources, farmers aim to optimize the health and performance of their fish stocks while reducing the reliance on antibiotics and other chemical additives. However, it is essential to carefully select and evaluate probiotic strains to ensure their safety and effectiveness in specific aquaculture settings (Yousuf *et al.*, 2023). Furthermore, these

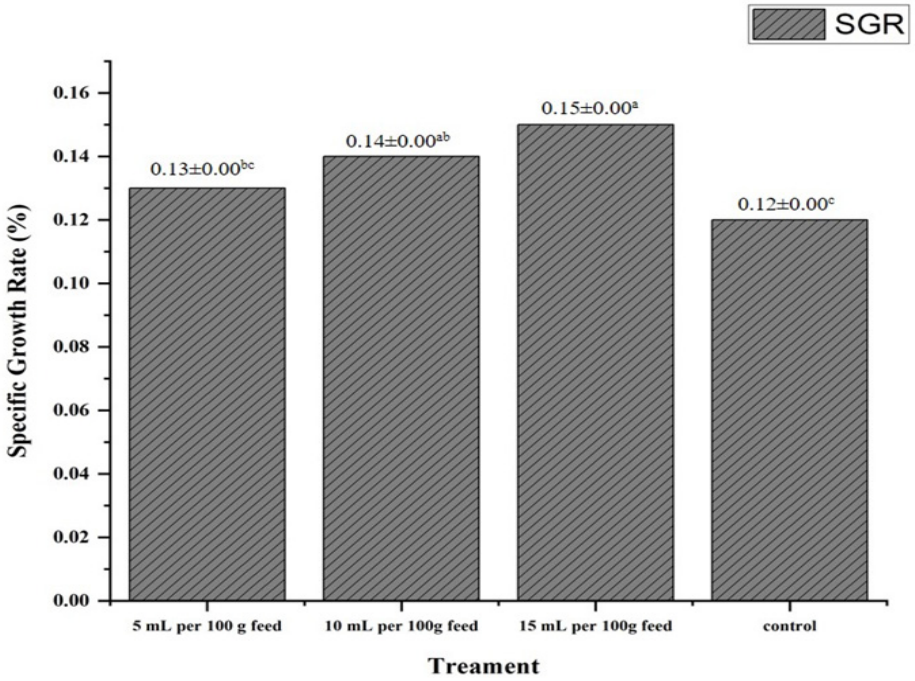

Figure 2. Specific growth rate of African catfish supplemented with EM4 probiotics at different doses (means ± standard deviation; means with different superscript letters are significantly different. Least significant difference test level of significance = 5%.* significant)

bacteria in the fish digestive tract secrete digestive enzymes such as proteases and amylases, which can improve feed digestion. Microbial activity in the digestive system will change rapidly if other microbial populations enter the digestive system through feed or water and eventually will change the balance of the intestinal microbial population inside the digestive tract (Sarojini *et al.*, 2020).

Survival Rate

The results of dietary EM4 probiotics supplementation at different doses on SR of African catfish is presented in the following Figure 3. The analysis of variance (ANOVA) showed that probiotic supplementation in each treatment did not significantly affected the SR of African catfish, which ranged from 85% to 90% during the experiment. However, as we can see the lowest and the highest SR value appeared in control treatment and treatment A (5 mL per 100 g feed) and treatment C (15 mL per 100 g feed), respectively.

Research has revealed that the survival rate was increased in fish fed diets with probiotics, indicating a positive impact on the survival of African catfish (Abdul-Malik *et al.*, 2023; Fitriana *et al.*, 2024). Probiotics dietary can augment survival rates, optimize feeding behavior, boost immune response, and promote productivity. The addition of probiotics to fish diets has been associated with increased survival rates, potentially due to factors such as improved nutrient digestibility, enhanced immunity and production of antimicrobials that help combat pathogens through various mechanisms that improve overall health and disease resistance (Coulibaly *et al.*, 2023; Mirzabekyan *et al.*, 2023; Yao *et al.*, 2020)

The survival rates of fish can vary substantially depending on the nature of their diet and the specific species of fish being fed. Additional research on the growth features, survival rates, and body composition of fish has revealed that feeding rates have a significant impact on survival rates. In general, higher feeding rates tend to result in higher survival rates (El-Hack *et al.*, 2022)

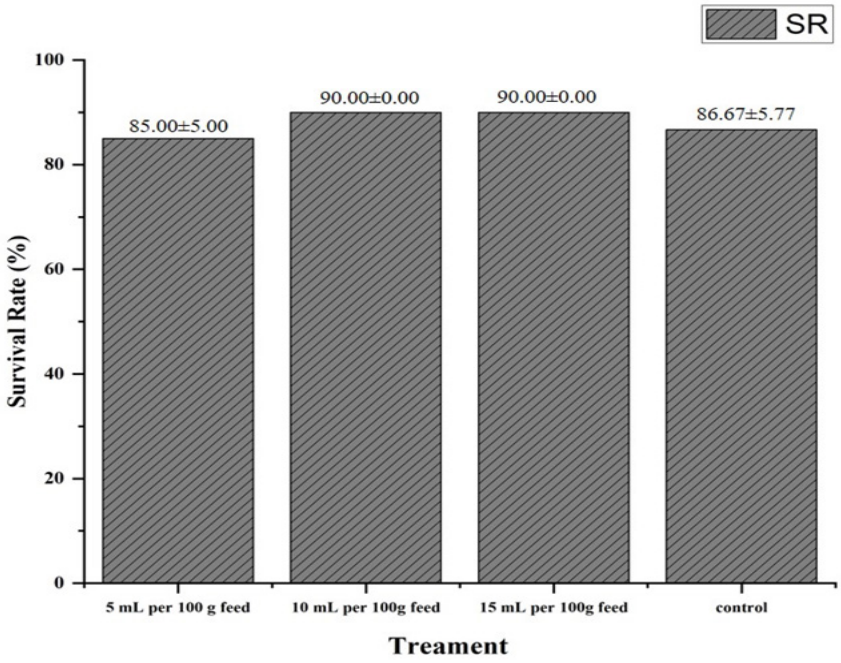

Figure 3. Survival rate of African catfish supplemented with EM4 probiotics at different doses (means ± standard deviation)

Feed Conversion Ratio

The results of dietary EM4 probiotics supplementation at different doses on FCR of African catfish is presented in the following Figure 4. The analysis of variance (ANOVA) showed that probiotic supplementation in each treatment significantly affected the FCR of African catfish, which ranged from 1.55 to 2.33 within 4 weeks in experiment. The treatment A (5 mL per 100 g feed) and treatment B (10 mL per 100 g feed) did not show significant different, while among control, treatment A (5 mL per 100 g feed) or treatment B (10 mL per 100 g feed) and treatment C (15 mL per 100 g feed) showed significant different. As we can see the lowest and the highest FCR value appeared in treatment C (15 mL per 100 g feed) and control, respectively. Dietary probiotics in treatment C produced the lowest FCR compared to all other treatments. It implies that dietary probiotics supplementation at 15 mL per 100 g feed promoted the best nutrient absorption, which contributes to fish optimal growth, feed conversion ratio, protein efficiency ratio, and survival rate. Studies have demonstrated that adding probiotics to fish diets can enhance the feed conversion ratio (FCR), resulting in enhanced growth performance. Research has shown that diets containing probiotics have a beneficial impact on the growth, feed efficiency, and survival of fish. Commercial probiotics can enhance weight gain, feed conversion ratio, and overall health status of fish. These findings emphasize the important role of probiotics in aquaculture by improving nutrient utilization and promoting better growth efficiency in fish (Sîrbu *et al.*, 2022; Xia *et al.*, 2020).

The FCR is considered in the high category. Anee (2021) reported that probiotic supplementation affected fermentation speed and the nutrient absorption rate in the fish digestive tract. Response from control is still within an acceptable FCR for catfish farming. However, low feed nutrient absorption or high FCR were highly affected by digestion activity without supplemented probiotics intervention. Energy absorption for fish growth could be better.

Feed conversion is the ratio between feed intake and weight gain that determines the effectiveness of both feed intake and weight gain at particular time intervals (Besson *et al.*,

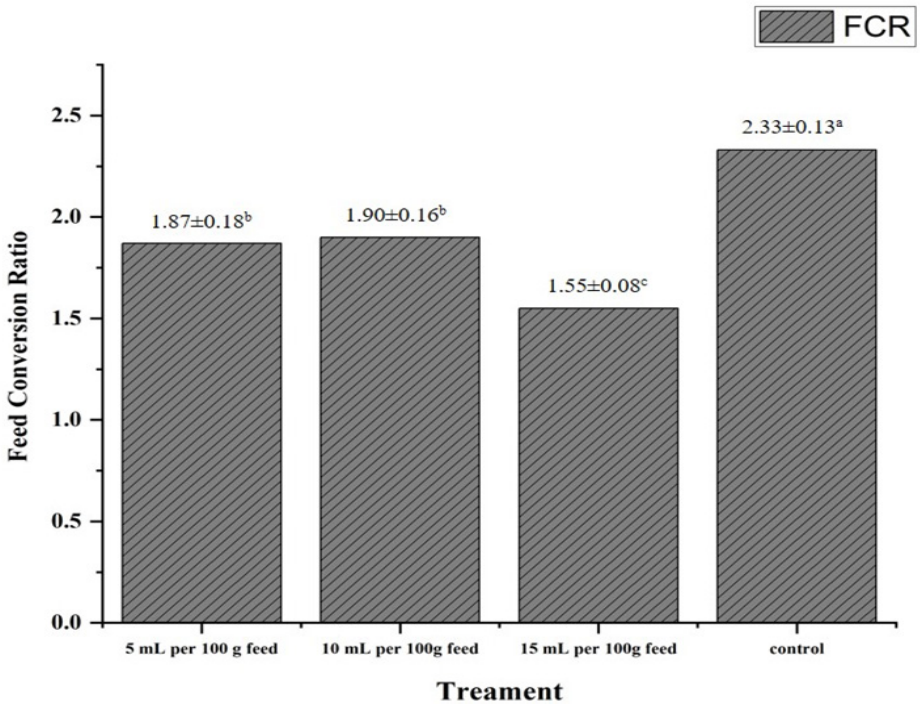

Figure 4. Feed conversion ratio of African catfish supplemented with EM4 probiotics at different doses (means ± standard deviation; means with different superscript letters are significantly different. Least significant difference test level of significance = 5%.* significant)

2020; Wahyudi *et al.*, 2021). The scale of the feed conversion ratio represents the achieved food efficiency. Lower FCR implies better feed quality, resulting in maximized energy supplies absorbed by African catfish for growth. It also implies that the African catfish can have an increasing growth rate with less feed quantity. Feed conversion ratio can be affected by factors such as stocking density, individual weight, age, water temperature, and feeding quality, quantity, and frequency.

CONCLUSION

It can be concluded that dietary EM4 probiotics in the African catfish artificial diet significantly affected specific growth rate and feed conversion ratio of African catfish and did not significantly affect absolute growth rate and survival rate of African catfish. Dietary EM4 probiotics at 15 mL per 100 g feed appeared as the most optimal treatment generating the best African catfish growth and feed efficiency compared to all other treatments.

ACKNOWLEDGEMENTS

The authors would like express their gratitude thank to Yayasan Aksa Mahmud for funding this research and providing research equipment and tools, including the Laboratory facility. The authors would like also to thank to laboratory assistant and all other researchers contributing to this research.

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