

THE EFFECT OF ADDITION OF PROBIOTIC CYCLIC NITROGEN BACTERIA ON AMMONIA ACCUMULATION IN NILE NILE FISH (*Oreochromis niloticus*)

Pengaruh Penambahan Probiotik Nitrogen Cyclic Bacteria Terhadap Akumulasi Amonia pada Budidaya Ikan Nila (*Oreochromis niloticus*)

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(Received May 1st 2026; Accepted June 3rd 2026)

ABSTRACT

Intensive aquaculture of Nile tilapia (*Oreochromis niloticus*) frequently faces water quality deterioration problems due to ammonia accumulation from feed residues and fish metabolism. This study aimed to determine the effect and optimal dose of Nitrogen Cyclic Bacteria (NCB) probiotic on ammonia accumulation in Nile tilapia aquaculture. An experimental method with a Completely Randomized Design (CRD) consisting of 5 treatments and 3 replications was used, namely control (without probiotic) and probiotic addition at doses of 0.5, 1, 1.5, and 2 ml/L for 40 days. Parameters observed included growth, survival rate, and water quality, particularly ammonia. The results showed that probiotic addition significantly affected ($p < 0.05$) fish growth, with the best dose at treatment D (1.5 ml/L), producing the highest absolute growth of 6.67 ± 0.32 g, relative growth of 46.70%, and daily specific growth rate of 0.96%/day. Survival rate was not significantly different among treatments ($p > 0.05$), but tended to be higher in probiotic treatments compared to control. Ammonia content was not significantly different among treatments, but all values remained within the safe range (< 0.1 ppm), with lower tendency at the highest dose (treatment E, 0.055 ppm). It was concluded that NCB probiotic can improve Nile tilapia growth and help maintain water quality, although its effect on ammonia reduction was not statistically significant.

Keywords: Ammonia, Nile Tilapia Aquaculture, Nitrification, Nitrogen Cyclic Bacteria, Probiotic

ABSTRAK

Budidaya ikan nila (*Oreochromis niloticus*) secara intensif kerap menghadapi masalah penurunan kualitas air akibat akumulasi amonia dari sisa pakan dan metabolisme ikan. Penelitian ini bertujuan untuk mengetahui pengaruh dan dosis optimal probiotik Nitrogen Cyclic Bacteria (NCB) terhadap akumulasi amonia pada budidaya ikan nila. Metode yang digunakan adalah eksperimen dengan Rancangan Acak Lengkap (RAL) terdiri atas 5 perlakuan dan 3 ulangan, yaitu kontrol (tanpa probiotik), serta penambahan probiotik dengan dosis 0,5;

1; 1,5; dan 2 ml/L, selama 40 hari. Parameter yang diamati meliputi pertumbuhan, kelangsungan hidup, dan kualitas air, khususnya amonia. Hasil penelitian menunjukkan bahwa penambahan probiotik berpengaruh nyata ($p < 0,05$) terhadap pertumbuhan ikan, dengan dosis terbaik pada perlakuan D (1,5 ml/L) yang menghasilkan pertumbuhan mutlak tertinggi sebesar $6,67 \pm 0,32$ g, pertumbuhan relatif 46,70%, dan laju pertumbuhan spesifik harian 0,96%/hari. Tingkat kelangsungan hidup tidak berbeda nyata antar perlakuan ($p > 0,05$), namun cenderung lebih tinggi pada perlakuan probiotik dibandingkan kontrol. Kandungan amonia tidak berbeda nyata antar perlakuan, tetapi seluruh nilai masih berada dalam kisaran aman ($< 0,1$ ppm), dengan kecenderungan lebih rendah pada dosis tertinggi (perlakuan E, 0,055 ppm). Disimpulkan bahwa probiotik NCB mampu meningkatkan pertumbuhan ikan nila dan membantu menjaga kualitas air, meskipun pengaruhnya terhadap penurunan amonia tidak signifikan secara statistik.

Kata Kunci: Amonia, Budidaya Ikan Nila, Nitrifikasi, Nitrogen Cyclic Bacteria, Probiotik

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is an important commodity in the freshwater fish industry and has high economic value. Tilapia are known for their easy reproduction, rapid growth, and ability to thrive in intensive aquaculture systems. However, intensive aquaculture often faces problems with declining water quality, particularly the accumulation of ammonia (NH₃), nitrite (NO₂), and other parameters critical to fish life (Pratiwi *et al.*, 2016).

In aquaculture systems without water exchange, the concentration of wastes such as ammonia, nitrite, and CO₂ increases rapidly and is toxic to cultivated organisms. Fish can excrete 80-90% of ammonia through osmoregulation, while feces and urine contribute 10-20% of total ammonia-nitrogen (TAN) (Setijaningsih & Suryaningrum, 2015). Decomposing feed residue and feces can deteriorate water quality and trigger mass die-offs, as has occurred in Lake Maninjau since 1997, causing billions of rupiah in annual losses.

One solution to address this problem is the use of probiotics containing nitrifying bacteria, specifically *Nitrosomonas* sp. and *Nitrobacter* sp. *Nitrosomonas* sp. bacteria oxidize ammonia to nitrite, while *Nitrobacter* sp. oxidize nitrite to nitrate, which is relatively safe for fish (Hapisha, 2021). Nitrogen Cyclic Bacteria (NCB) probiotics containing these two bacteria are thought to help reduce ammonia accumulation and increase tilapia growth.

Research on the administration of NCB probiotics on ammonia accumulation in tilapia aquaculture has not been widely reported. Therefore, this study aims to determine the effect and optimal dosage of NCB probiotics on ammonia levels and tilapia growth parameters.

RESEARCH METHODS

Place and Time

This research was conducted for 40 days, from August 28 to October 8, 2023, at the Fish Production Laboratory, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram.

Tools and Materials

The tools used include an aerator, stationery, a DO meter, a camera, a 45x30x30 cm container, a ruler, a pH meter, a scoop, a spectrophotometer, a thermometer, and a digital scale. The materials used consist of fresh water as a cultivation medium, 8-10 cm tilapia seeds (approximately 150 fish), commercial feed Hi Pro Vite 781 with a protein content of 31-33%, and probiotic NCB.

Research Methods and Design

The research used an experimental method with a Completely Randomized Design (CRD) consisting of 5 treatments and 3 replications, as follows:

Treatment A : Control (without NCB probiotics)

Treatment B : Addition of NCB probiotics 0.5 ml/L

Treatment C : Addition of NCB probiotics 1 ml/L

Treatment D : Addition of NCB probiotics 1.5 ml/L

Treatment E : Addition of NCB probiotics 2 ml/L

Research Procedures

Fifteen maintenance tanks (according to treatment and replication) were cleaned and dried before use. 20 liters of filtered and aerated well water were added to each tank. NCB probiotics were administered once at the beginning of the maintenance period, according to the dosage for each treatment. Ten tilapia fry, acclimatized for 2-3 days, were stocked per tank. Feed was provided three times daily (8:00 AM, 12:00 PM, and 4:00 PM WITA) at a dose of 5% of the fish's body weight. Water changes were not performed during maintenance, but water filtration was performed every three days and siphoning was performed daily.

Test Parameters

The parameters observed included: (1) Absolute growth ($W_m = W_t - W_o$); (2) Relative growth ($h = (W_t - W_o) / W_o \times 100\%$); (3) Daily specific growth rate ($SGR = ((W_t / W_o)^{(1/t)} - 1) \times 100\%$); (4) Survival rate ($SR = N_t / N_0 \times 100\%$); and (5) Water quality including ammonia levels (measured 3 times: day 0, 20, and 40 using a spectrophotometer), temperature, pH, and DO (measured every 7 days).

Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level. If the results showed a significant difference, the Least Significant Difference (LSD) test was performed at the 5% level.

RESULT

Absolute Growth

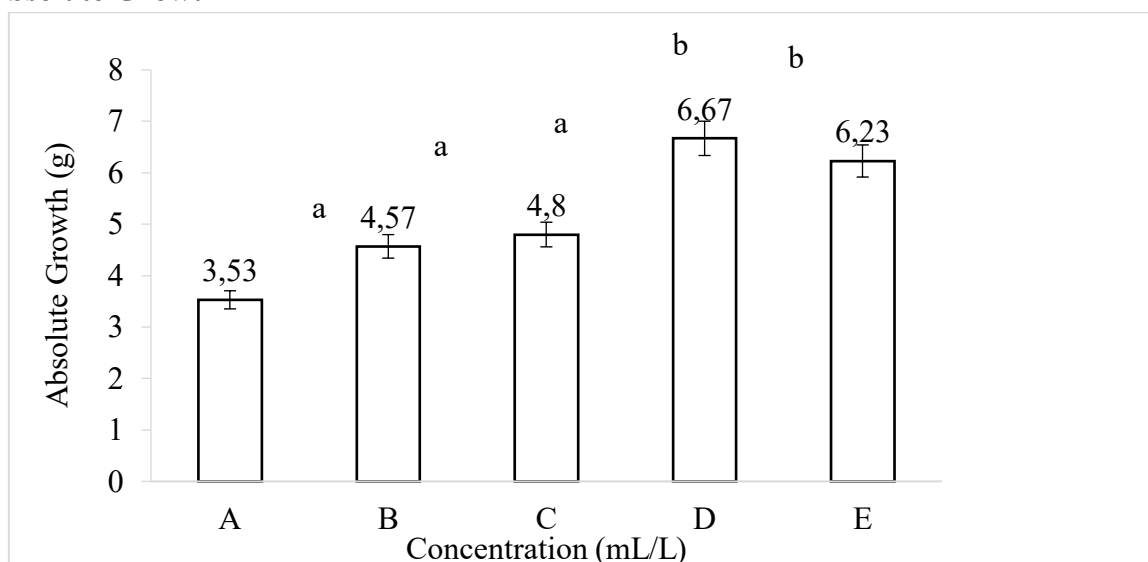


Figure 1. Average absolute growth of tilapia in each treatment

* Significantly different ($p < 0.05$). Different letter notations indicate significant differences in the 5% BNT test (BNT = 1.440).

Relative Growth

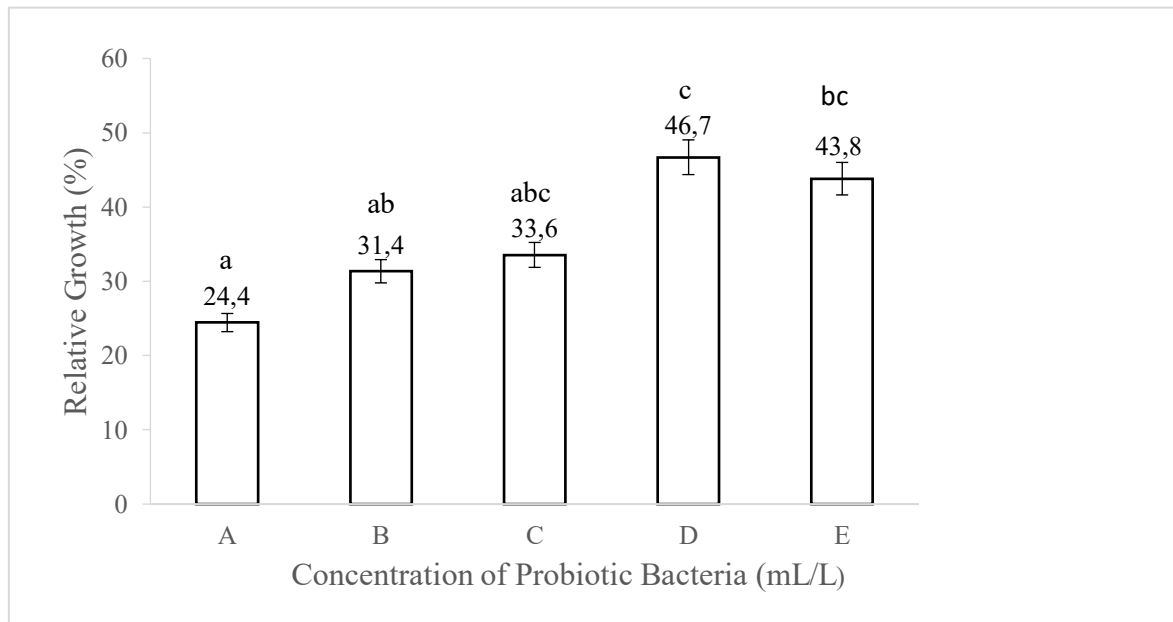


Figure 2. Average relative growth of tilapia in each treatment

* Significantly different ($p < 0.05$). Different letter notations indicate significant differences in the 5% BNT test.

Daily Specific Growth Rate (SGR)

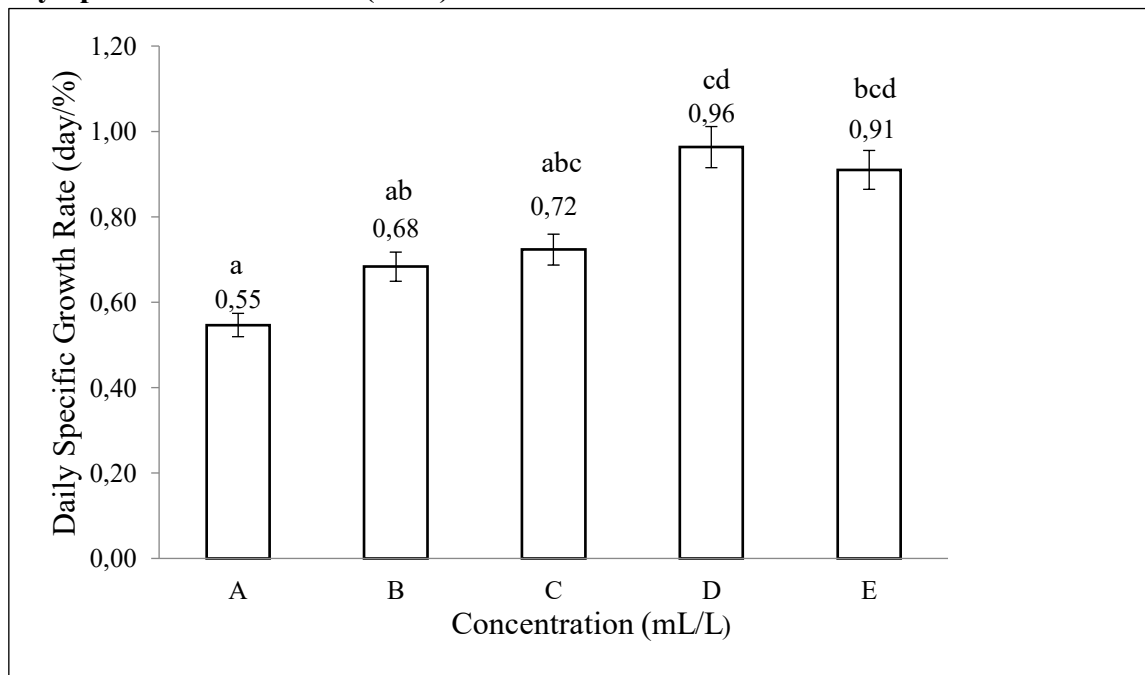


Figure 3. Average daily specific growth rate (SGR) of tilapia in each treatment

* Significantly different ($p < 0.05$). Different letter notations indicate significant differences in the 5% BNT test (BNT = 0.250)

Survival Rate (SR)

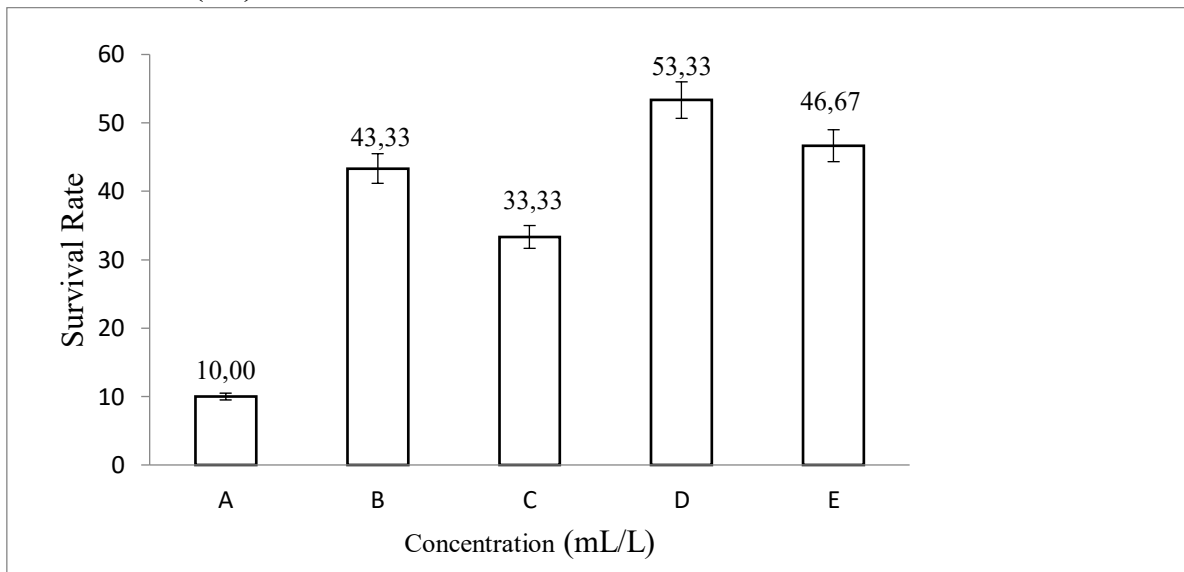


Figure 4. Average survival rate (SR) of tilapia in each treatment

Ammonia Content

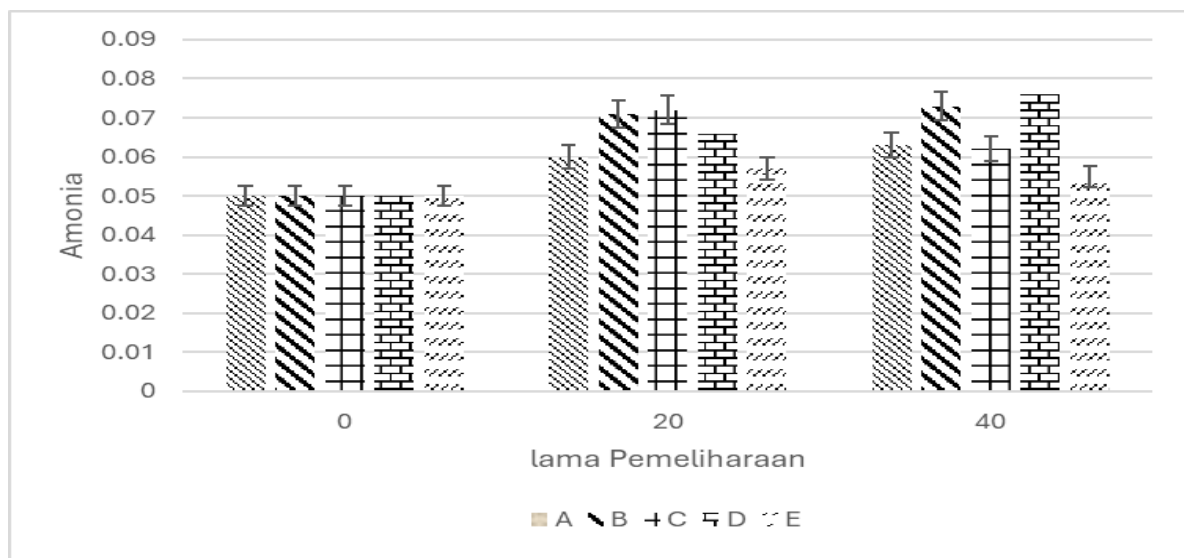


Figure 5. Ammonia content (ppm) in tilapia maintenance media

*not significantly different ($p > 0.05$). The ammonia tolerance threshold for tilapia is < 0.1 ppm (SNI 7550:2009)

Other Water Quality Parameters

Table 1. Water quality parameters during tilapia maintenance

Parameter	A	B	C	D	E	Literature Standards
Temperature (°C)	28,0-30,2	28,6-30,2	28,5-30,5	28,3-30,2	28,5-30,2	25-32
DO (mg/L)	5,8-6,2	5,8-6,3	5,8-6,3	5,8-6,5	5,7-6,3	> 5

pH	5,66-7,63	5,91-7,66	5,81-7,50	6,12-7,66	5,76-7,63	6,0-8,5
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DISCUSSION

Absolute Growth

Treatment D (1.5 ml/L) produced the highest absolute growth (6.67 ± 0.32 g), significantly different from the control (3.53 ± 1.01 g). This indicates that the concentration of NCB probiotics in treatment D is able to increase the efficiency of feed utilization optimally. Nurrafa & Al (2025) reported that administering the best commercial probiotics to tilapia resulted in an absolute weight gain of 7.4 g, proving that selecting the right type and dose of probiotics significantly contributed to high absolute growth. The mechanism of increasing absolute growth through probiotics is closely related to improving the condition of the microbial ecosystem in the fish's digestive tract. Probiotics can increase the number of intestinal microflora, thereby increasing the activity of digestive enzymes, which ultimately increases the digestibility and absorption of nutrients from feed. Hasan *et al.* (2023) stated that lactic acid bacteria in the fish intestine are able to balance the microbes in the digestive tract, thereby increasing feed digestibility through an enzymatic series that lowers pH and stimulates the production of endogenous enzymes. The lower absolute growth in the control group indicates that the natural microflora in the digestive tract of tilapia without probiotic supplementation is not optimal enough to support maximum feed digestion. Patang (2022) confirmed that the administration of probiotics in the culture medium consistently had a positive impact on fish weight accumulation compared to the control group.

Relative Growth

Treatment D produced the highest relative growth ($46.70 \pm 1.16\%$), significantly different from the control ($24.44 \pm 8.33\%$). This high value indicates that a dose of 1.5 ml/L is the optimal dose to support the growth of tilapia. The mechanism of action of probiotics in increasing fish growth is thought to be through three pathways: balancing the intestinal microflora and increasing digestive enzyme activity; enhancing the fish's immune system; and improving water quality through bioremediation and ammonia degradation. This is in line with Pascu (2022) who stated that probiotics not only increase growth but also improve the overall physiological condition of fish. The lower relative growth value of treatment E compared to treatment D, despite the higher dose, indicates an optimum dose effect. Administering probiotics exceeding the optimal dose can cause ecological imbalance in the intestinal microbiota, where excessive bacterial populations actually compete with the host and reduce digestive efficiency. According to Panase (2023), differences in growth responses to probiotics can be caused by differences in probiotic activity, interactions of beneficial bacteria in the intestine, the amount of product added, species composition, viability, type of feed, and duration of administration.

Daily Specific Growth Rate (SGR)

Treatment D produced the highest SGR ($0.96 \pm 0.02\%/day$), significantly different from the control ($0.55 \pm 0.17\%/day$). This SGR value is still within the normal range for tilapia from the nursery to the grow-out phase. Sartika & Setyono (2022) stated that probiotics can improve fish health because the bacteria they contain produce protease, lipase, and amylase enzymes that play a role in the digestion of proteins, fats, and carbohydrates. This increase in substrate digestibility ultimately increases the amount of nutrients absorbed into the blood, including energy for growth. The lower SGR value in the control indicates that without probiotic supplementation, the tilapia's digestive tract has not reached its optimal capacity. In treatment E, which has a higher dose than treatment D, the SGR value was actually slightly lower. This

is consistent with the findings of Sartika (2022) who stated that high bacterial counts can trigger competition for nutrients between bacteria and fish, thereby reducing growth at the highest dose.

Survival Rate (SR)

Although not statistically significant ($p > 0.05$), the significant difference in SR values between the probiotic treatment and control groups (10.00%) indicates a positive role of probiotics in tilapia survival. The low SR in the control group is thought to be related to the absence of beneficial microorganisms in the media, making water quality more susceptible to degradation due to the accumulation of fish metabolic waste. Putra & Utomo (2021) stated that administering probiotics to tilapia cultivation can increase fish survival descriptively, although not always showing statistically significant differences at certain densities. Probiotic bacteria can improve water quality through the bioremediation process by degrading organic matter, reducing ammonia levels, and stabilizing pH and dissolved oxygen values. Munisa *et al.* (2022) stated that administering probiotics to tilapia cultivation media significantly reduced the concentration of total ammonia nitrogen (TAN), which was positively correlated with an increase in SR values at the end of the culture period. The high variation in SR between replicates also indicates that other factors such as maintenance management and individual fish conditions influence survival.

Ammonia Content

Ammonia content in all treatments showed an increasing pattern with increasing maintenance period. At the beginning of maintenance (day 0), all treatments had a uniform ammonia content (0.050 ppm). This condition is normal considering that at the beginning of maintenance there was no significant accumulation of fish metabolic waste. Over time, ammonia content increased on days 20 and 40, reflecting the accumulation of nitrogen excretion products from fish metabolic activity and the decomposition of organic matter from leftover feed. Treatment E consistently showed a tendency for lower ammonia content compared to the other treatments. This indicates that a dose of 2 ml/L is more effective in supporting the activity of nitrogen-degrading bacteria. *Nitrosomonas* sp. bacteria contained in the probiotic NCB oxidize NH_3 to NO_2^- , then *Nitrobacter* sp. further oxidize it to NO_3^- which is relatively safe for fish. Widanarni *et al.* (2012) confirmed that the autotrophic bacteria in probiotics are able to utilize leftover feed and fish feces and then oxidize the ammonia content in the water bed. The lack of statistically significant differences is likely due to the ammonia concentrations in all treatments, which were initially very low and relatively homogeneous. Furthermore, the implementation of routine water filtration and siphoning management helped to equalize ammonia levels across all experimental units. According to Kordi & Tancung (2007), water exchange is the dominant factor in controlling ammonia accumulation in intensive cultivation systems. Overall, ammonia levels throughout the study remained below the tolerance threshold for tilapia (< 0.1 ppm) according to SNI 7550:2009, thus categorizing the water quality as safe for tilapia survival.

Other Water Quality Parameters

The water temperature during cultivation (28.0-30.5°C) was within the optimal range for tilapia (25-32°C). There was no significant difference in the temperature range between treatments, indicating that the addition of probiotics did not impact the temperature of the culture medium. According to Muhsoni (2021), in tropical fish, increasing water temperature accelerates the metabolic rate, thus increasing feed requirements to support biological activity. The dissolved oxygen content (5.7-6.5 mg/L) was above the minimum threshold required for intensive tilapia cultivation (> 5 mg/L). The observed DO range reflects that the implemented

aeration system was functioning well and was able to meet the oxygen needs of both tilapia and the active probiotic microorganisms in the medium (Telaumbanua, 2025). The pH value (5.66-7.66) was mostly within the optimal range for tilapia cultivation (6.0-8.5), although there were several measurements slightly below the lower limit of the optimal range, particularly in the control (5.66). The pH fluctuations that occurred were within the tolerance limits of tilapia and were not permanent, so they are not expected to have a significant impact. Descriptively, treatment D showed a higher minimum pH value (6.12) compared to the control (5.66), indicating the role of probiotics in maintaining pH stability through bacterial metabolic activity.

CONCLUSION

The dose of NCB probiotics that had the best effect on the growth of tilapia (*Oreochromis niloticus*) was treatment D (1.5 ml/L), with the highest absolute growth of 6.67 ± 0.32 g, relative growth of 46.70%, and daily specific growth rate of 0.96%/day. The administration of NCB probiotics had no significant effect ($p > 0.05$) on the ammonia content in the rearing media, either on the 20th or 40th day; however, the ammonia levels in all treatments were still within the tolerable range of tilapia (< 0.1 ppm), with the lowest trend in treatment E (2 ml/L) at 0.055 ppm. NCB probiotics were proven to be able to increase the growth of tilapia and help maintain water quality, although its effect on reducing ammonia was not statistically significant.

ACKNOWLEDGEMENT

The author would like to express his deepest gratitude to Mr. Alis Mukhlis, S.Pi., M.Si. and Mrs. Dr. Laily Fitriani Mulyani, S.Pi., M.P. as supervisors who have provided guidance and support during the research and writing of this article. He also thanks the Fish Production Laboratory of the Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram, for the facilities provided during the research.

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