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Effect of probiotic *Bacillus* spp.-supplemented feed on the growth, length-weight relationship, and condition factor of Nile tilapia (*Oreochromis niloticus*)

Jaypee S. Samson^{1,2*} 

¹College of Fisheries, Central Luzon State University, Science City of Muñoz 3120, Nueva Ecija, Philippines

²Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz 3120, Nueva Ecija, Philippines

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ABSTRACT

This study was carried out to evaluate the effect of two probiotic *Bacillus* spp. (RM10 and BFAR9) on the growth, length-weight relationship (LWR), and condition factor (k) of Nile tilapia (*Oreochromis niloticus*). For this, one hundred thirty-five fingerlings (1.12±0.08 g weight and 1.26±0.15 cm length) were divided into three groups (Control, RM10, and BFAR9) and distributed into nine circular concrete tanks. The fish were fed with commercial (control) and *Bacillus* spp. supplemented diets at 5% of body weight for 56 days. The results of the study revealed better ($P<0.05$) growth concerning average body weight (ABW - 17.12±0.71g), specific growth rate (SGR - 4.89±0.22 g·day⁻¹), absolute growth (AG - 16.02±0.78 g), and feed conversion ratio (FCR - 1.31±0.09) in the group fed with *Bacillus* sp. RM10 as compared to the control (ABW- 13.25±2.34g; SGR - 4.41±0.17g·day⁻¹; AG - 12.13±2.25g; FCR - 1.62±0.11). The LWR in all experimental treatments showed a significant correlation ($P<0.05$) with an R² value of 0.988, 0.966, and 0.979 for Control, RM10, and BFAR9, respectively. The k value revealed that all treatments are in good condition as k value is greater than 1 (1.913, 2.038, and 1.896 for control, RM10, and BFAR9 respectively). The result of the current study revealed that application of *Bacillus* sp. RM10 improves the growth and feed utilization in Nile tilapia.

* Corresponding author

E-mail: samsonjaypee@clsu.edu.ph

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1 Introduction

Aquaculture is playing an important role in increasing fish production and helping to meet the growing demand of the global population for fishery products. One of the most popular aquaculture species are the tilapias (*Oreochromis* spp.), which is the worldwide second most farmed fish (Prabu et al. 2019). These species are native to Africa and the Middle East and were introduced into over 90 countries for aquaculture and fisheries (Watanabe et al. 2002; Prabu et al. 2019). The global production of tilapia in 2015 was estimated at 5,576,800 MT (Prabu et al., 2019). In the Philippines, tilapia is the second most farmed fish with an estimated production of 277,006 MT in 2018, which contributes to 12.02% of the total aquaculture production of the country (Bureau of Fisheries and Aquatic Resources 2018). Tilapia production has quadrupled over the past decade and continued to increase global production rapidly due to its suitability for aquaculture, stable market prices, and the increasing demand for food fish as a result of the growing population (Prabu et al. 2019). However, this intensification led to the proliferation of antibiotic-resistant microorganisms as a result of the improper use of chemotherapeutic agents to improve the growth and disease resistance of the fish. As an alternative, recent studies explored the use of beneficial bacteria or probiotics to encourage more eco-friendly aquaculture (Balcazar et al. 2006). *Bacillus* spp. are widely used as a probiotic microorganism for aquatic animals to promote growth performances, disease resistance, and better immune response (Balcazar et al., 2006). These bacteria are suitable probiotic candidates since they are generally non-pathogenic and non-toxic when fed to fish, can survive under harsh environmental conditions, and produce antimicrobial substances as compared to other probiotics (Kuebutornye et al. 2019).

The need for sustainable aquaculture has encouraged exploration into the use of probiotics on aquatic organisms. The original interest was focused on their use as a growth promoter and to improve the health of animals; however, new extents have been found, such as their effect on reproduction or stress tolerance, although this requires a more scientific development (Cruz et al. 2012). The information on probiotics has increased in the past years and currently, it has been established that they have an antimicrobial effect on their host organism (Cruz et al. 2012). Probiotic bacteria enact their antimicrobial effect by amending the intestinal microbiota of their host through secretion of antibacterial substances (bacteriocins and organic acids), prevention of pathogen adhesion to the intestine, competing for nutrients essential for pathogen survival, and production of antitoxin effect (Cruz et al. 2012). Furthermore, probiotics are also capable of modifying the immune system, regulating the allergic response of the body, and reducing the proliferation of cancer in mammals. As a result, when probiotics are provided in a certain concentration these are positively affecting the host's health (Myers 2007).

Previous studies also reported a significant effect of *Bacillus* spp. on growth, disease resistance, and hematological parameters of Nile tilapia (Soltan et al. 2016; Sutthi et al. 2018; Elsbagh et al. 2018; Kuebutornye et al. 2020; Won et al. 2020; Ghalwash et al. 2021) and other aquatic species (Hauville et al. 2016; Munir et al. 2016; da Paixão et al. 2017; Kong et al. 2017; Wang et al. 2017; Amoah et al. 2021; Saravanan et al. 2021). In this present study, the effect of two previously isolated *Bacillus* spp. on the growth performance, length-weight relationship, and condition factor of Nile tilapia was evaluated.

2 Materials and Methods

2.1 Preparation of Bacteria and Experimental Diets

Two probiotic strains viz., BFAR9 and RM10 of *Bacillus* spp. with GenBank accession numbers: MH919302 and MH919308, isolated from African nightcrawler earthworm (*Eudrilus eugeniae*) were used in this study (Samson et al., 2020). Overnight cultures of the probiotic strains were suspended in a 250 mL nutrient broth and incubated at 37 °C for 24 hours. The suspensions were aseptically transferred into sterile test tubes, pelleted, washed, and suspended in Phosphate-buffered Solution (PBS). The bacterial density was adjusted using the McFarland standard. Dilutions were carried out using PBS to have a similar bacterial density (McFarland, 1907).

Experimental diets were prepared using a commercial feed (31% crude protein, 5% lipid, 8% crude fiber, and 12% crude ash) as the basal diet (control). The probiotic-treated diets were prepared to contain a single strain of *Bacillus* spp. at 10^8 CFU·g⁻¹ of feed. The selected isolates were sprayed on the basal diet and air-dried for 2 hours before storing at 4°C.

2.2 Experimental Fish and Set-up

One hundred thirty-five *O. niloticus* fingerlings (FaST strain) were obtained from the Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Experimental fish were allowed to acclimatize for 7 days before the experiment. The fingerlings were randomly distributed into 9 circular concrete tanks (200-L capacity) and divided into 3 experimental groups (three replicates per group). Each group consists of 45 fish, which had an initial weight and length of 1.12 ± 0.08 g and 1.26 ± 0.15 cm, respectively. Prepared diets were given thrice daily (8:00, 12:00, and 16:00), initially at 5% of fish body weight per day for 56 days. Sampling was done biweekly to measure the length and weight of the fish and adjust the feeding ration. The water quality parameters such as temperature, dissolved oxygen, and pH were measured daily using the YSI multiparameter (YSI 556 - YSI Incorporation, Yellow Spring, USA).

2.3 Data Collection

At the end of the feeding trial, all fish were measured and weighed. The specific growth rate (SGR), absolute growth (AG), feed conversion ratio (FCR), survival rate (SR) and condition factor (k) were measured as follows (Bagenal 1978; Fulton 1904):

$$\text{SGR} = \frac{(\ln [\text{final weight}] - \ln [\text{initial weight}])}{(\text{time interval in days})} \times 100$$

$$\text{AG} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{FCR} = \frac{\text{Amount of feed given (g)}}{\text{Weight gain (g)}}$$

$$\text{SR (\%)} = \frac{\text{No. of individuals at the end of the experiment}}{\text{No of individuals at the beginning of the study}} \times 100$$

$$k = 100 \times \frac{\text{Weight (g)}}{\text{Length (cm)}^3}$$

2.4 Length-weight relationship

The length and weight of fish were tabulated and analyzed after 56 days. The relationship between the length (L, cm) and weight (W, g) was calculated by regression equation (Keys 1928)

$$W = \alpha \times L^b$$

Here α represents the antilog of the intercept of the regression curve, and b is the regression coefficient. The degree of association between L and W was calculated by the correlation coefficient (r). In the length-weight relationship, the value of exponent b provides information on fish growth; when $b = 3$, the increase in weight is isometric, while $b > 3$ means increase of weight is positive

allometric, and if $b < 3$, the increase of weight is negative allometric (Tesch 1968).

2.5 Statistical Analyses

Results were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at a significant level of $P < 0.05$ using R Statistics software. Results are presented as means \pm SD. The LWR was calculated using linear regression analysis in Microsoft Excel using the log-transformed data of weight and length.

3 Results

3.1 Growth Performance and Survival

The data on growth performance, survival, and condition factor of experimental fish are presented in Table 1. Results of the study revealed that the initial length and weight measurements of the fish had no significant difference among the groups. Further, no significant differences between the two probiotic-fed groups in terms of length and weight; however, significantly higher ($P < 0.05$) ABW (17.12 g), SGR (4.89 g·day⁻¹), and AG (16.02 g) was observed from *Bacillus* sp. RM10-fed group compared to the control group (ABW = 13.25 g, SGR = 4.41 g·day⁻¹ and AG = 12.13 g). On the other hand, no significant difference was observed in the length of the fish among all treatments. Furthermore, in terms of feed efficiency, the results of the current study showed significantly lower FCR in RM10 (1.31) compared to the control (1.62). The survival rate of the experimental fish also revealed that there are no significant differences in all treatments. After 56 days of culture, all treatments have ≥ 80 % survival. The water quality parameters recorded during the experiment are temperature 26.7 ± 0.8 °C, pH 7.8 ± 0.2 , and dissolved oxygen level 4.65 ± 1.16 ppm.

Table 1 Growth performance, feed utilization, survival, and condition factor of Nile tilapia fed with probiotic *Bacillus* spp.

PARAMETERS	TREATMENT		
	Control	RM10	BFAR9
Initial Body Length (IBL) (cm)	3.89 \pm 0.44 ^a	3.91 \pm 0.19 ^a	3.82 \pm 0.19 ^a
Initial Body Weight (IBW) (g)	1.11 \pm 0.10 ^a	1.11 \pm 0.11 ^a	1.14 \pm 0.09 ^a
Final Body Length (FBL) (cm)	8.83 \pm 0.53 ^a	9.44 \pm 0.24 ^a	9.18 \pm 0.16 ^a
Final Body Weight (FBW) (g)	13.25 \pm 2.34 ^b	17.12 \pm 0.71 ^a	16.02 \pm 0.28 ^{ab}
Feed Conversion Ratio (FCR)	1.62 \pm 0.11 ^a	1.31 \pm 0.09 ^b	1.54 \pm 0.15 ^{ab}
Specific Growth Rate (SGR) (g day ⁻¹)	4.41 \pm 0.17 ^b	4.89 \pm 0.22 ^a	4.71 \pm 0.11 ^{ab}
Absolute Growth (AG) (g)	12.13 \pm 2.25 ^b	16.02 \pm 0.78 ^a	14.85 \pm 0.20 ^{ab}
Survival Rate (SR) (%)	83.33 \pm 15.28 ^a	80.00 \pm 10.00 ^a	90.00 \pm 17.32 ^a
Condition Factor (k)	1.91 \pm 0.02 ^a	2.04 \pm 0.11 ^a	1.90 \pm 0.08 ^a

Data are given as mean \pm SD (n=3). The mean values in the same row with different superscript letters are significantly different at $P < 0.05$.

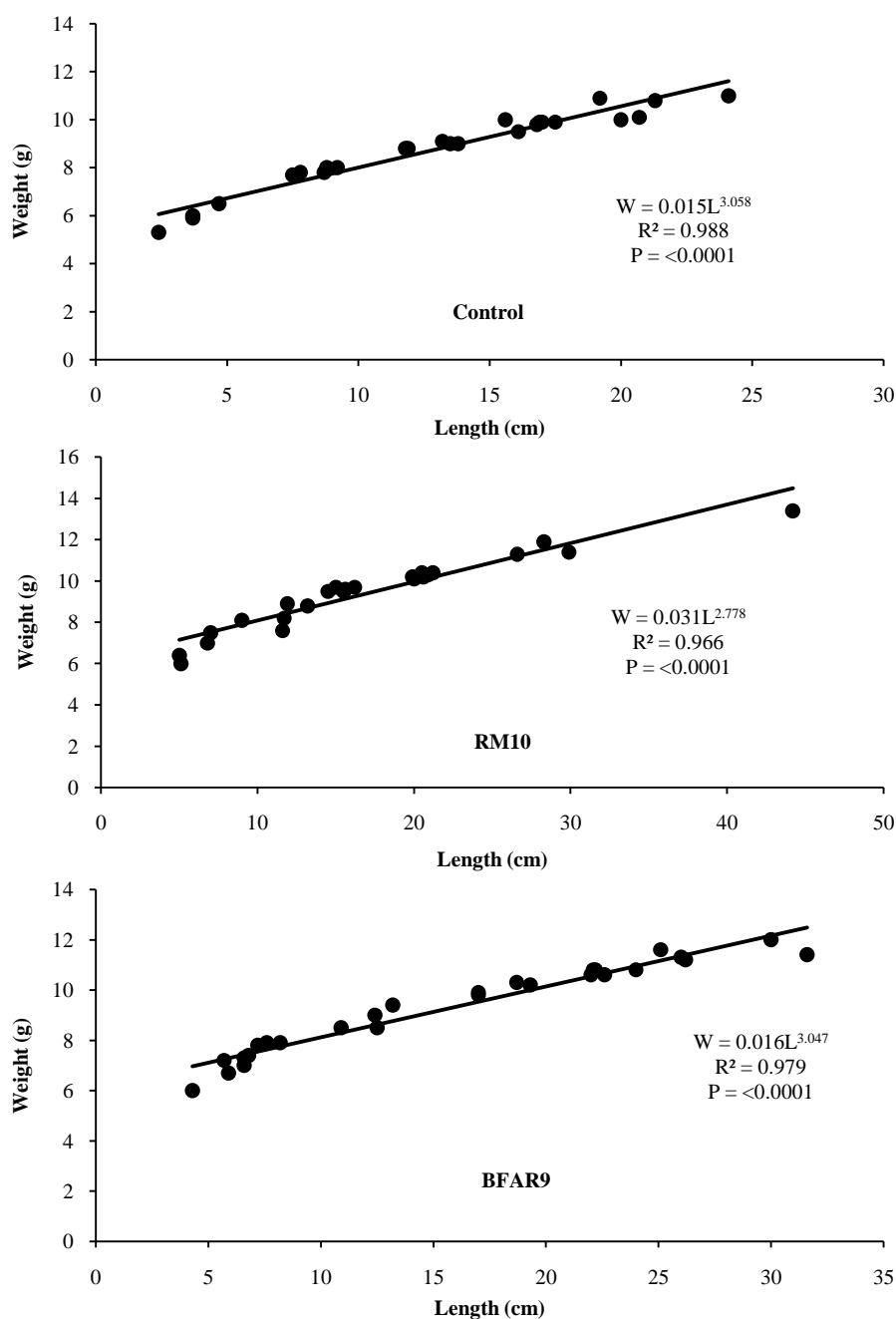


Figure 1 Logarithmic regression of weight and length data of Nile tilapia fed with probiotic *Bacillus* spp.

3.2 Length-weight Relationship and Condition Factor

The length-weight relationship of the non-probiotic-fed and probiotic-fed groups was evaluated after the feeding trial. The logarithmic regression of LWR and coefficient of determination values (R^2) are presented in Figure 1. Results showed a significant correlation ($P < 0.05$) between the length and the weight among all experimental groups with an R^2 value of 0.988, 0.966, and 0.979

for Control, RM10, and BFAR9, respectively. Moreover, the value of slope (b) in BFAR9 (3.047) and Control (3.058) is close to 3, except for RM10 (2.778). No significant differences were reported in the condition factor on all treatment groups. However, the condition factor revealed that the experimental fish in all groups are in good condition as demonstrated by the k-value. All groups that have k-value is greater than 1 considered as good one (Control = 1.913, RM10 = 2.038, and BFAR9 = 1.896).

4 Discussion and Conclusions

The applications of probiotics in aquaculture have already been reported for several years. The increasing demand for an eco-friendlier approach in combating the emerging diseases in aquaculture and intensifying of production has turned the industry to explore the use of these microorganisms. Probiotic application has already been proven to increase aquaculture production by improving the growth and survival of the cultured species. In the present study, the application of the probiotic *Bacillus* spp. improved the growth performance of the fish. Higher ABW and growth parameters (AG and SGR) were observed in the experimental group fed with *Bacillus* spp. RM10. Previous research studies have already reported the beneficial effect of *Bacillus* probiotics application on fish growth. Similar findings were reported in Nile tilapia (Apún-Molina et al. 2009; Zhou et al. 2009; Ridha and Azad 2012; Soltan et al. 2016; Sutthi et al. 2018; Opiyo et al. 2019; Rahman 2019;), white shrimp, (*Litopenaeus vannamei*) (Nimrat et al. 2012; Zokaeifar et al. 2012), rainbow trout (*Oncorhynchus mykiss*), and African catfish (*Clarias gariepinus*). Previous studies showed the colonization ability of *Bacillus* spp. in the gut of the fish. This ability aids in imposing the beneficial effects of probiotic bacteria through stimulation and production of digestive enzymes, enhanced organic acid production, reducing the antinutritional factors of feed ingredients, and collectively maintaining a healthy gut; thus, improving the digestion and nutrient absorption of the host. This is demonstrated by the lower FCR value of the group fed with *Bacillus* sp. RM10, which showed that the probiotic application improved the feed utilization of the fish. On the other hand, the application of *Bacillus* sp. BFAR9 did not result in any significant improvement on Nile tilapia growth. Similar findings were observed in tambaqui (*Colossoma macropomum*) (da Paixão et al. 2017), gilthead sea bream (*Sparus aurata*) (Ariğ et al. 2013), and Nile tilapia (Shelby et al. 2006; Silva et al. 2015). Although growth improvement is not evident in these reports, a significant increase in the villi height of the intestine (Silva et al. 2015) and digestive enzyme activities (Ariğ et al. 2013) revealed that the application of *Bacillus* probiotics have a positive effect on the digestion of the fish. Therefore, the effect of probiotic bacteria can be possibly observed in other aspects of the host, such as enhanced immunity, digestion, pathogen prevention, stress tolerance, and reproduction. Furthermore, it is also possible that the effect of a certain strain of probiotics varies in every species of fish. Similarly, the probiotic effect of *Bacillus* spp. might be due to differences in each strain or species of bacteria.

In the present study, the effect of *Bacillus* probiotics application in the LWR and condition factor of Nile tilapia was evaluated. The results showed a significant correlation between the length and weight of the fish. In the LWR, the value of slope (b) provides

information on fish growth, $b < 3$ is said to be negative allometric, $b = 3$ is isometric and $b > 3$ is positive allometric. Isometric growth pattern (*i.e.*, the proportional increase in length and weight that gives fish ideal shapes) was observed in BFAR9 (3.047) and control (3.058) as indicated by their slope value, which is close to the value of ideal growth ($b = 3$), as suggested by Froese (2006). Although experimental groups fed with *Bacillus* spp. RM10 demonstrated a lower slope value (2.778) than the ideal growth value, it is still within the acceptable range of 2.5–3.5 which was estimated by Froese (2006). Furthermore, the condition factor in all treatments showed that the experimental fish are in good condition ($k \geq 1$). This condition factor is widely used in fisheries and general fish biology studies. It is calculated from the relationship between the weight of a fish and its length, to describe the condition of that individual. Previous studies have utilized this parameter to evaluate the overall condition of aquatic organisms as affected by several factors and for fish stock assessment purposes. The present study demonstrated the beneficial effect of the probiotic *Bacillus* spp. application in the growth of Nile tilapia. Further intensive investigations are required to evaluate the role of these microorganisms in disease resistance, stress tolerance, and fish reproduction.

In conclusion, this study revealed that supplementation of *Bacillus* sp. RM10 in the diet of Nile tilapia improves the growth performance and feed utilization of the fish. Therefore, based on the result of this study, the application of *Bacillus* sp. RM10 in the diet can increase the production and lessen the feed requirements of Nile tilapia.

Abbreviations

Average Body Weight (ABW), Specific Growth Rate (SGR), Absolute Growth (AG), Survival Rate (SR) and Feed Conversion Ratio (FCR)

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Conflict of Interest

The author declares no conflict of interest.

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