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Growth, length-weight relationship and condition factor of Nile tilapia (*Oreochromis niloticus* L.) fed with probiotic bacterium (*Bacillus amyloliquifaciens*) supplemented diet

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Abstract
In this study, the growth, survival, length-weight relationship and condition factor of Nile tilapia fingerlings fed with *Bacillus amyloliquifaciens*-supplemented diet were evaluated to determine the best dosage of probiotic bacterium incorporation in feeds (T1 = 10 mL/kg, T2 = 30 mL/kg and T3 = 50 mL/kg). The fingerlings were reared for 45 days in concrete circular tanks. Basic water quality parameters were also monitored.

After 45 days of rearing, the final average weights of Nile tilapia fingerlings provided with *B. amyloliquifaciens*-supplemented diet (T1 = 12.370±4.630 g; T2 = 12.410±45.050 g; T3 = 12.560±5.520 g) were significantly higher as compared with control (9.010±4.580 g); fingerlings fed with the highest bacterium supplementation resulted to highest final weight. Tilapia fingerlings fed with supplemented diet (T1 = 0.232±0.100 g; T2 = 0.234±0.110 g; T3 = 0.236±0.120 g) had significantly higher AGR as compared with control (0.157±0.100 g); the best AGR was acquired using Treatment 3. The same result was observed at SGR wherein treatments (T1 = 3.970±0.879%; T2 = 3.920±1.051%; T3 = 3.930±1.059%) were significantly higher than the control (3.150±1.112%); the highest SGR was recorded in Treatment 1. There was no significant difference observed between control (84.670±5.030%) and treatments (T1 = 88.000±10.580%; T2 = 86.000±3.460%; T3 = 82.000±12.170%) in terms of per cent survival; the highest per cent survival was recorded in Treatment 1.

In the LW relationship, value of exponent b provides information on fish growth. Except T2 which was positive allometric growth, all of them (Control, Treatment 1, Treatment 3) were classified as negative allometric. Negative allometric means that the organism grows slender, so it gains length faster than weight; while positive allometric means that the organism becomes stouter as it increases in length, so it gains weight faster than length. The LW relationship equation of control was $W = 0.184\ L^{2.9115}$, $W = 0.202\ L^{2.8612}$ for Treatment 1, $W = 0.168\ L^{3.0524}$ for Treatment 2 and $W = 0.180\ L^{2.9771}$ for Treatment 3. The highest condition factor was recorded in Treatment 1 (K = 2.764±1.545) and this was significantly higher as compared to control (1.994±0.947), Treatment 2 (1.624±0.341) and Treatment 3 (1.959±0.280).

Keywords: probiotics, *Bacillus amyloliquifaciens*, length-weight relationship, condition factor, growth

1. Introduction
Aquaculture is the fastest growing food-producing sector in the world at an average rate of 8.9% per year since 1970, compared with only 1.2% in capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period [1]. One of the most cultured fish in the Philippines is tilapia (*Oreochromis niloticus*) because of its fast growth and palatability. The growth and nutrition of the cultured fish in aquaculture sector is limited by diseases caused by bacteria, fungi, viruses and parasites. Therefore, the need for alternative techniques is increasing and the contribution of probiotics may be considered [2].

With the growing claim for environmentally pleasant aquaculture, the application of non-antibiotic eco-friendly agents such as probiotic is considered as one of the most significant tool for health management in the field of aquaculture [2]. Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut [2].

The main objective of using probiotics is to re-establish a favorable association between beneficial and harmful microorganisms that make up the microbiota of the intestine of the fish. Probiotics are capable of retaining the healthy intestinal condition. One of the most expected consequences of using probiotic bacteria is the direct effect of probiotic on the growth performance of fish either by direct increment in nutrition uptake or providing nutrients [2].

The most commonly used probiotics in aquaculture are *Bacillus* sp., *Lactobacillus* sp. and *Saccharomyces* sp.3. In a study conducted by Selim and Reda [3], *B. amyloliquefaciens* improves growth, intestinal villous heights, goblet cell and intra-epithelial lymphocyte (IEL) numbers, and gastrointestinal bacterial populations. The supplementation of tilapia diets with *B. amyloliquefaciens* appeared to enhance *in vitro* serum bactericidal activities against *Aeromonas hydrophila*. The antibacterial effect of serum is known for its capability to inhibit the growth and multiplication of pathogenic microorganisms [3].

The general objective of this study was to evaluate the growth and condition factor of Nile tilapia (*O. niloticus*) fed with probiotic bacterium (*B. amyloquifaciens*) supplemented diet. Specifically, the study aimed to: (1) compare the growth, length-weight relationship and condition factor of probiotic-fed and non-probiotic-fed tilapia and; (2) determine the best dosage of probiotic bacterium incorporation in feeds to the growth and survival rate of the tilapia fingerlings

2. Materials and Method
2.1. Preparation of bacterial suspension
Two to three colonies of 24 hours *B. amyloliquifaciens* were suspended in Trypticase Soy Broth (TSB) and incubated at 37 °C for 1 to 2 hours. The bacterial suspension was adjusted

to 0.5 McFarland turbidity standards to come up with 10^8 cells/mL. The bacterial suspension was made daily.

2.2. Preparation of *B. amyloliquifaciens*-supplemented diet
The study employed one control and three treatments with three replications: Control = commercial feeds only; Treatment 1 = 10 mL bacterial suspension + 1 kg commercial feeds; Treatment 2 = 30 mL bacterial suspension + 1 kg commercial feeds and; Treatment 3 = 50 mL bacterial suspension + 1 kg commercial feeds. The bacterial suspension was sprayed in the feeds in a daily basis.

2.3. Rearing and feeding of tilapia fingerlings
BFAR GET-Excel fingerlings, size #14 were conditioned for three days in aquaria. The average initial length and bulk weight of the fingerlings was determined. Each circular tank was stocked with 50 pieces fingerlings. The fingerlings were fed based upon the control and treatments described above at 10% feeding rate and four times feeding frequency. The fingerlings were reared in 45 days.

2.4. Water quality monitoring
Temperature, pH and dissolved oxygen were monitored twice a day (9:00 to 10:00 am and 2:00 to 3:00 pm) using multi-parameter equipment.

2.5. Computation of survival rate, specific growth rate and absolute growth rate
Individual length and weight of fish after 45 days were determined. The number of surviving individuals was recorded also. Data on specific growth rate (SGR), absolute growth rate (AGR) and survival rate was computed using the following formula:

$$\textbf{Survival rate} = \left(\frac{\text{total number of fingerlings survived}}{\text{total number of fingerlings stocked}}\right) \times 100 \qquad (1)$$

$$\textbf{Specific growth rate (SGR)} = \frac{[(\text{In (final weight) - In (initial weight)})]}{\text{time interval (days)}} \times 100 \qquad (2)$$

$$\textbf{Absolute growth rate(AGR)} = \frac{\text{Final weight-Initial weight}}{\text{Number of days}} \qquad (3)$$

2.6. Length-weight relationship
The length and weight of fish after 45 days were tabulated. The relationship between total length (L, cm) and wet weight (W, g) was calculated by power regression $W = e^a \times L^b$ (a = intercept of the regression curve and b = regression coefficient). The degree of association between L and W was calculated by the relation coefficient (r^2). In the length-weigh relationship, value of exponent b provides information on fish growth. When b = 3, the increase in weight is isometric. The increase of weight is positive allometric if b > 3, while the increase of weight is negative allometric if b < 3[4].

2.7. Condition Factor
The condition factor (k) was calculated from the relationship, $K = 100W/L^b$, where W = weight (g), L = total length (cm) and b = regression coefficient[5].

2.8. Statistical analysis
Statistical difference in survival rate, absolute growth rate, specific growth rate, relation coefficient, condition factor and water quality were determined using one-way analysis of variance. Tukey's Test was used for the multiple comparisons of means.

3. Results and Discussion
3.1. Growth and survival of Nile tilapia fingerlings (*Oreochromis niloticus*) fed with *Bacillus amyloliquifaciens*-supplemented diet
Growth is a never-ending process as long as an organism is alive, and it is influenced by many intrinsic factors like genetic and hormonal, and extrinsic factors like temperature and availability of food. There is no standard growth model applicable to every living thing, because it varies from species to species and from different stages of life [6, 7]. Fishes have two growth patterns: determinate in short-lived warmer climate, and indeterminate in long-lived colder climate [6]. Growth rate, especially in aquaculture, is an important manifestation of production yield [8]. Therefore, if the probiotic bacterium used in this study is efficient, it will be reflected on better growth and survival of the different treatment groups.

Table 1 shows that the average initial weights of tilapia fingerlings used in the control and probiotic supplemented treatments were the same (1.93 g), thus, statistically not significant when compared with one another (p>0.05). After the fingerlings were reared for 45 days in outdoor concrete tanks, Table 1 shows that the final average weights of fingerlings provided with *B. amyloliquifaciens*-supplemented diet (T1 = 12.370±4.630 g; T2 = 12.410±45.050 g; T3 = 12.560±5.520 g) were significantly higher as compared with control (9.010±4.580 g) (p<0.05). Fingerlings fed with the highest bacterium supplementation (T3 = 50 mL bacterial suspension + 1 kg commercial feeds) resulted to highest final weight when compared to the control and the rest of treatments.

To elaborate more the effect of *B. amyloliquifaciens*-supplemented diet on the growth of the experimental fish, different ways to describe fish growth are computed in this study such as AGR and SGR [9, 7]. The AGR is often used because of its simplicity in expressing the amount of weight gained per day, as shown in the equation mentioned earlier, therefore, it is widely accepted on reporting growth [9, 7]. Nile tilapia fingerlings fed with supplemented diet (T1 = 0.232±0.100 g; T2 = 0.234±0.110 g; T3 = 0.236±0.120 g) had significantly higher AGR as compared with control (0.157±0.100 g) (p<0.05); the best AGR was acquired using Treatment 3 (Table 1). On the other hand, SGR is derived by multiplying instantaneous growth rate to 100 so that it could reflect the percent of weight gain per day, which is useful in short-term and nutrition studies [9, 7]. Same result was observed at SGR wherein probiotic-supplemented treatments (T1 = 3.970±0.879%; T2 = 3.920±1.051%; T3 = 3.930±1.059%) were significantly higher than the control (3.150±1.112%); the highest SGR was recorded in Treatment 1 (10 mL bacterial suspension + 1 kg commercial feeds) (Table 1).

Survival rate was calculated by dividing the number of surviving fingerlings by the total number of fish stocked. There was no significant difference observed between control (84.670±5.030%) and probiotic-supplemented treatments (T1 = 88.000±10.580%; T2 = 86.000±3.460%; T3 = 82.000±12.170%) in terms of per cent survival.

Table 1: Growth and survival of Nile tilapia fingerlings (*Oreochromis niloticus*) fed with *Bacillus amyloliquifaciens*-supplemented diet

	Initial Weight (g)	Final Weight (g)	SGR (%)	AGR	Survival (%)
Control	1.930±0.040[a]	9.010±4.580[b]	3.150±1.112[b]	0.157±0.100[b]	84.670±5.030[a]
T1	1.930±0.030[a]	12.370±4.630[a]	3.970±0.879[a]	0.232±0.100[a]	88.000±10.580[a]
T2	1.930±0.020[a]	12.410±5.050[a]	3.920±1.051[a]	0.234±0.110[a]	86.000±3.460[a]
T3	1.930±0.030[a]	12.560±5.520[a]	3.930±1.059[a]	0.236±0.120[a]	82.000±12.170[a]

Note: Control = commercial feeds only; T1 = 10 mL bacterial suspension + 1 kg commercial feeds; T2 = 30 mL bacterial suspension + 1 kg commercial feeds; T3 = 50 mL bacterial suspension + 1 kg commercial feeds
*Means in a column with different superscripts were significantly different with each other at $p<0.05$

Several species of *Bacillus*, such as *B. subtilis*, *B. pumilus* and *B. licheniformis* are commonly used in other studies as probiotic which increases the growth rate and final body weight, while studies regarding *B. amyloliquifaciens* remain inadequate [10, 11, 12, 13, 3]. In the study of Silva *et al*. [14], the addition of *B. amyloliquifaciens* in the diet had no significant effect in the growth of common carp (*Cyprinus carpio*) but it resulted to histological improvement on the height of intestinal villi, therefore increasing the efficiency of nutrient absorption. Nile tilapia fed with *B. amyloliquifaciens* supplementation has resulted to increase growth performance and feed utilization efficiency by increasing the height of intestinal villi, as well as the general improvement of immune and stress response of the fish as manifested on the enhanced hematological characteristics[11]. In the study of Ridha and Azad[15], treatment with *B. amyloliquifaciens* has higher feed conversion ratio, increase in body weight in terms of SGR and immunological parameters compared to control group and treatment group with *Lactobacillus* sp. According to Selim and Reda [3], with supplementation of *B. amyloliquifaciens* showed enhancement of the production of bactericidal serum *in vitro* against *A. hydrophila*, increase in the height of intestinal villi, as well as the number of goblet cells and intra-epithelial lymphocyte.

3.2. Length-Weight Relationship and Condition Factor of Nile Tilapia Fingerlings (*Oreochromis niloticus*) Fed with *Bacillus amyloliquifaciens*-Supplemented Diet

Length and weight (LW) are two parameters often used to evaluate different data especially in fish biology; therefore, the analysis involving length and weight became a staple in different studies. However, there are two things that can be yielded from length and weight, and this will be defined and differentiated to avoid confusion [16]. First is the relationship between length and weight such that the value of one can be calculated given that the value of the other is known. Such relationship is often expressed mathematically as $W = aL^b$. With this, any given value of length, which can be measured faster than weight, can yield the value of weight. Transforming the said exponential equation to logarithmic equation will yield $\log W = \log a + b \log L$, which can be plotted as a linear equation of slope-intercept form [16, 7]. Second is the degree of variation from the given weight and the anticipated length of the fish, as indication of general health. It is calculated by finding the ratio between the observed weight and the anticipated observed length. Mathematically, it is expressed as $K = \frac{100W}{L^b}$. Condition factor (K) therefore, can be interpreted as differences in fatness, abundance of food and gonad development [16].

Given the results from Table 2, all of the groups showed very strong association of L-W as reflected on the values of r, which ranged from 0.9585 to 0.9821. Highest and lowest L-W association was obtained in fish fed with T2 (0.9821) and T1 (0.9585), respectively (Table 2). On the other hand, the value of b ranged from 2.8612 to 3.0524, with Treatment 2 (3.0524) having the highest value, followed by Treatment 3 (2.9771), Control (2.9115) and Treatment 1 (2.8612) (Table 2). In the LW relationship, value of exponent b provides information on fish growth: b <3 is said to be negative allometric, b = 3 is isometric and b >3 is positive allometric [17]. Isometric growth means that as the organism grows, there is no change on the body shape, and that the weight is increasing as the cube of length. Negative allometric means that the organism grows slender, so it gains length faster than weight; while positive allometric means that the organism becomes stouter as it increases in length, so it gains weight faster than length [18, 19]. Except T2 which was positive allometric growth, all of them (Control, Treatment 1 and Treatment 3) were classified as negative allometric because the value of their b is less than 3.

The LW relationship equation of control (Figure 1) was $W = 0.184 L^{2.9115}$, $W = 0.202 L^{2.8612}$ for Treatment 1 (Figure 2), $W = 0.168 L^{3.0524}$ for Treatment 2 (Figure 3) and $W = 0.180 L^{2.9771}$ for Treatment 3 (Figure 4). This LW equation is needed to understand the relationship of length of the fish to its given weight and to understand the relationship of weight of the fish to its given length.

Condition factor of control and probiotic supplemented treatment are shown in Table 2. The highest condition factor was recorded in Treatment 1 (K = 2.764±1.545) and this was significantly higher as compared to Control (1.994±0.947), Treatment 2 (1.624±0.341) and Treatment 3 (1.959±0.280).

Table 2: Equation parameters of length-weight relationship and condition factor of Nile tilapia fingerlings (*O. niloticus*) fed with *Bacillus amyloliquifaciens*-supplemented diet

	r^2	r	a	b	LWR Equation	K
Control	0.9612	0.9804	-1.6931	2.9115	$W = 0.184 L^{2.9115}$	1.994±0.947[bc]
T1	0.9188	0.9585	-1.5986	2.8612	$W = 0.202 L^{2.8612}$	2.764±1.545[a]
T2	0.9647	0.9821	-1.7864	3.0524	$W = 0.168 L^{3.0524}$	1.624±0.341[d]
T3	0.9474	0.9733	-1.7134	2.9771	$W = 0.180 L^{2.9771}$	1.959±0.280[b]

Note: Control = commercial feeds only; T1 = 10 mL bacterial suspension + 1 kg commercial feeds; T2 = 30 mL bacterial suspension + 1 kg commercial feeds; T3 = 50 mL bacterial suspension + 1 kg commercial feeds *Means in a column with different superscripts were significantly different with each other at $p<0.05$

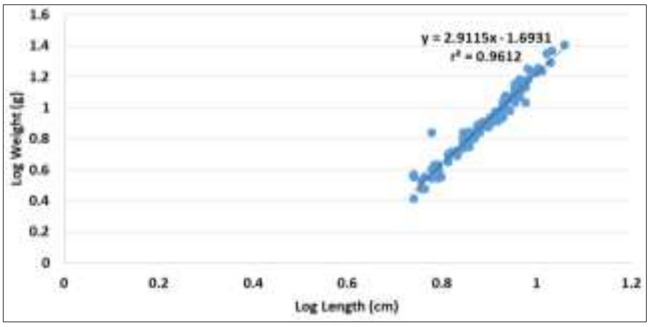

Fig 1: Length-weight equation graph of Nile tilapia fingerlings (*O. niloticus*) fed with commercial diet (Control)

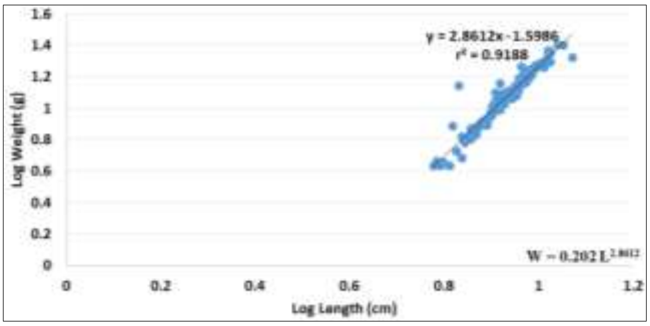

Fig 2: Length-weight equation graph of Nile tilapia fingerlings (*O. niloticus*) fed with *Bacillus amyloliquifaciens*-supplemented diet (T1 = 10 mL bacterial suspension + 1 kg commercial feeds)

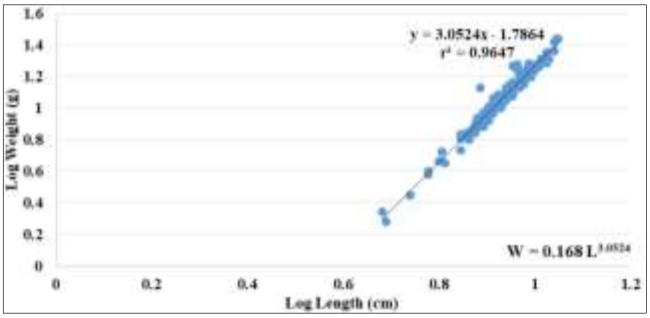

Fig 3: Length-weight equation graph of Nile tilapia fingerlings (*O. niloticus*) fed with *Bacillus amyloliquifaciens*-supplemented diet (T2 = 30 mL bacterial suspension + 1 kg commercial feeds)

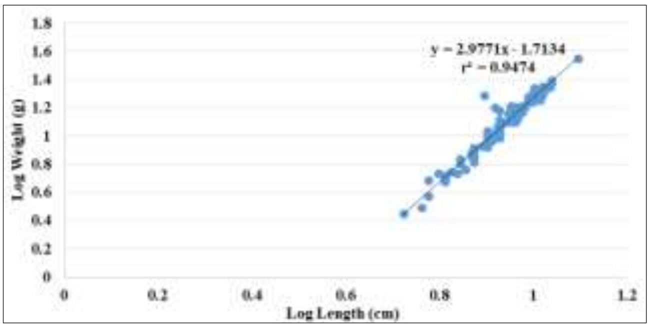

Fig 4: Length-weight equation graph of Nile tilapia fingerlings (*O. niloticus*) fed with *Bacillus amyloliquifaciens*-supplemented diet (T3 = 50 mL bacterial suspension + 1 kg commercial feeds)

3.3. Water quality parameters

As shown in Table 3, the addition of *B. amyloliquifaciens* in the diet (Treatments 1 to 3) had no significant effect on the quality of the water when compared with the control ($p>0.05$). The data shows that temperature ranged from 29.12 to 29.74 °C was considered suitable for tilapia culture [20]. However, DO readings ranged from 3.90 to 4.13 mg/L, wherein the desired level is 4 to 6 mg/L[20]. It appeared that experimental unit provided with the bacterium had higher but not significant average DO readings (T1 = 4.10±0.44 mg/L; T2 = 4.03±0.15 mg/L; T3 = 4.13±0.76 mg/L) as compared with the control (3.90±0.30 mg/L). The pH's of the water ranged from 6.67 to 6.73 which were under the optimum range of 6.5 to 9.0 [20].

Table 3: Monitored water quality parameters of the experimental set-ups

	Temperature (°C)	DO (mg/L)	pH
Control	29.74±0.54^a	3.90±0.30^a	6.67±0.04^a
T1	29.62±0.49^a	4.10±0.44^a	6.67±0.11^a
T2	29.12±0.66 ^{a}	4.03±0.15^a	6.68±0.04^a
T3	29.41±0.54 ^{a}	4.13±0.76^a	6.73±0.06^a

Note: Control = commercial feeds only; T1 = 10 mL bacterial suspension + 1 kg commercial feeds; T2 = 30 mL bacterial suspension + 1 kg commercial feeds; T3 = 50 mL bacterial suspension + 1 kg commercial feeds

*Means in a column with different superscripts were significantly different with each other at $p<0.05$

4. Conclusion

It was proven in this study that *B. amyloliquifaciens* supplementation in feeds had significant effect on the growth (final weight, SGR, AGR) and condition factor of Nile tilapia fingerlings fed for 45 days in outdoor concrete tanks. If the cost and labor of producing the supplemented feeds will be considered, the lowest dose which was 10 mL bacterium per 1 kg commercial feeds (T1) was the most recommended because the results on gain in weight, SGR and AGR were comparable on higher doses. Also, this treatment resulted to significantly higher condition factor as compared to control and rest of the treatments.

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