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*Research article*

# Effects of Selected Probiotics on Water Quality and Growth Performance of Nile Tilapia Cultured in a Recirculating Water System.

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**Abstract:** This study used selected probiotics (*Bacillus subtilis* HW3B and *Lactiplantibacillus plantarum* DW5L) in combination with a recirculating water system to evaluate the effects of the selected probiotics on Nile tilapia growth and water quality in the culture system. The experiment was conducted in aquaria (45 x 60 x 45 cm for each aquarium with 150 liters of water and a 40-liter internal filtration system). Both selected probiotic bacteria were tested in raising 15 fish, 2-3-inch in each Nile tilapia in aquaria with a recirculating water system for 10 weeks by consisting of 4 experimental sets with 5 replications each, consisting of T1 (control set – no microbial products added), T2 (microbial inoculum PM.1, a product of Department of Fisheries as positive control), T3 (mixed selected probiotic strains HW3B and DW5L at 1:1 by volume), and T4 (only *B. subtilis* HW3B). It was found that T3 significantly controlled BOD and TSS ($P < 0.05$) better than the control set. T2 was the most effective to control total ammonia, nitrite, nitrate, and total phosphorus, followed by T3, with significant differences. The number of detected bacteria fluctuated with the level of BOD in the water. A significant increase of total bacteria found in T 1 and T4 sets ($P < 0.05$), while *Bacillus* spp. found in all inoculated sets ($P < 0.05$). A remarkable increase of lactic acid bacteria (LAB) was observed only in T3 set ($P < 0.05$). It can be concluded that the use of T3 (the mixed probiotics HW3B and DW5L set) in combination with the recirculating water system can reduce the amount of waste in the water almost as effectively as the positive control (T2) and significantly better than the control set (T1). Moreover, T3 showed a better tilapia growth performance on final fish body weight and average daily length values compared with the T1 set ($P < 0.05$).

**Keywords:** Probiotics; Nile tilapia; Recirculating aquaculture system

## 1. Introduction

In Thailand, Nile tilapia is the second most popular culture after shrimp and is number one in all fish farming [1]. It is an important source of protein for Thai people, while generates a large income for farmers. Therefore, tilapia farming has increased rapidly according to the demand of consumers. As tilapia

farming has been raised in high density with a lot of feeding giving an adverse effect on the water quality to cause the environment deteriorated and eventually caused epidemics in tilapia farming [2]. From these problems, farmers normally use chemicals and antibiotics to solve the problem [2], but resulted in drug resistance of pathogens and residues in fish meat. It has the possibility to cause problems with consumer health; and this makes it more difficult to export fish [3].

Recently, cultivation of tilapia in a recirculating water system and the use of probiotics is one solution in this regard. Because fish cultured in a recirculating water system reduces the risk of introducing pathogens from outside into the system. It can also control water quality in the system [4]. Additionally, probiotics can help to enhance the growth of fish. It also helps to resist pathogens and decompose waste in the rearing system for better water quality [5]. Raising aquatic animals in a recirculating water system can help to raise aquatic animals at higher densities because the system will filter waste from the water all the time by filtering large waste through a physical filter and treat nitrogenous waste with microorganisms in biological filters using nitrogen transformation through the nitrogen cycle [6]. However, preparing a recirculating water system for raising aquatic animals still has limitations, such as the time required for biofilm formation, and it cannot guarantee that the desired bacteria will develop in the filtration system.

In this study, two types of bacteria that passed all probiotic properties tests, *Bacillus subtilis* HW3B and a lactic acid bacteria (LAB) *Lactiplantibacillus plantarum* DW5L, from our previous work [7] were used as probiotics for Nile tilapia cultivation by combination with a recirculating water system to evaluate the effects of both selected probiotics on water quality and Nile tilapia growth.

## 2. Materials and Methods

### 2.1 Preparation of bio-fermented water containing probiotic bacteria

Two bacterial strains (*B. subtilis* HW3B and *L. plantarum* DW5L) from tilapia pond and fish body in Songkhla Province were obtained based on their probiotic properties (assessments of antagonistic effects against *Aeromonas hydrophila* and *Streptococcus agalactiae*, nutrient digestion, cell surface hydrophobicity, and resistance to acids and bile salts) [7]. Both probiotics were prepared by culturing strains DW5L and HW3B in MRS and NB broths, each containing 1% NaCl, and incubated at 30 °C for 24 h. Each probiotic at 10 ml was inoculated into 500 ml molasses sugar, 500 g finely ground tilapia food, and 1,000 ml distilled water to make bio-fermented water. The ratio of bio-fermented water consisted of NaCl:probiotic:molasses:tilapia food: distilled water equal to 1:1:50:50:100. The bio-fermented water formula was modified to increase the number of probiotics, according to the preparation of PM.1 for use as seedlings [8]. The strongest aeration was provided at the level that created the least foam with the sand nozzles in the *B. subtilis* HW3B for 36 h. However, no need for aeration in *L. plantarum* DW5L as a facultative anaerobe. After 36 h incubation, bio-fermented water formulae were stored at 4 °C until used. Before fish cultivation and during cultivation, total bacteria, LAB and *Bacillus* species in each formula were counted on solid media using NA for total bacteria and *Bacillus* species, and MRS for LAB. However, water samples were heated at 80 °C for 10 min for counting *Bacillus* species.

### 2.2 Preparation of a recirculating water system

Nile tilapia were raised in a recirculating water system consisting of a glass aquarium size 45x60x45 cm containing 150 liters of freshwater and a 40-liter filter system equipped inside the aquarium. The glass fiber was used as a physical filter and filter mat fibers was cut to fit the filter tank cavity as the adhesive material for the biological filter. The air lift system was used to circulate water through the filtration system at a rate of 600 liters/h. The aeration was provided with 2 sand nozzles on opposite sides of the aquarium (Figure 1). Ammonium chloride ($NH_4Cl$) solution with a concentration of 2 mg per liter was added to rearing water to stimulate the growth of nitrifying bacteria (adapted from [9]). The readiness of the culture system was checked by observing the formation of a biofilm layer and all ammonia was converted to nitrate.

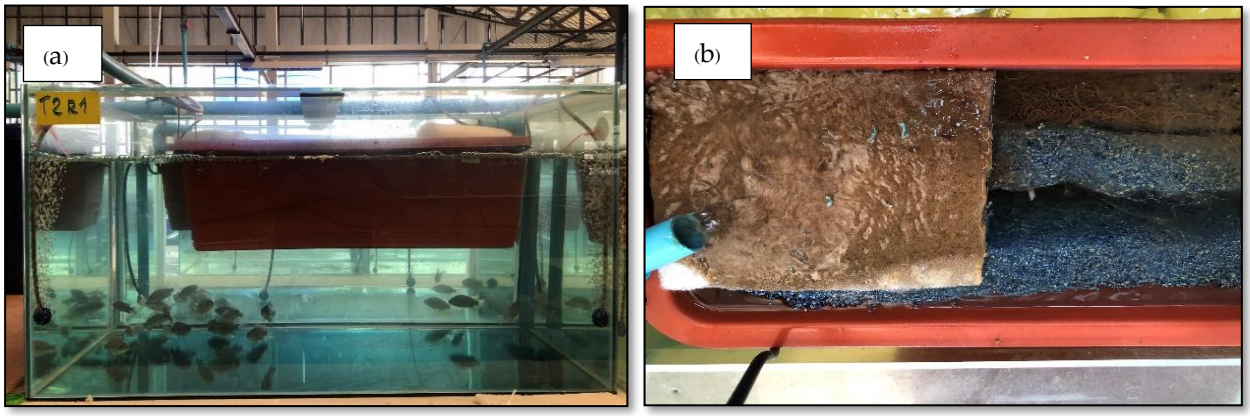


**Figure 1.** The aquarium size 45x60x45 cm containing a total of 150 liters of water and used a 40 liters filter system inside the aquarium (a) and the formation of a biofilm layer in the filter system (b).

### 2.3 Fish cultivation and growth performance evaluation

The experiment was conducted using a completely randomized design (CRD). It was composed of 4 experimental sets with 5 replications each, consisting of set 1 (T1) - control set (no added microbial product served as recirculating water system alone), set 2 (T2) - adding microbial inoculum PM.1 at 10 ml/m$^3$ (mixed cultures of *B. subtilis*, *B. megaterium*, and *B. licheniformis* [8]), set 3 (T3) - adding mixed probiotics at 10 ml/m$^3$ from each 5 ml of bio-fermented water of *B. subtilis* HW3B and *L. plantarum* DW5L, and the fourth set (T4) with 10 ml/m$^3$ from bio-fermented water of *B. subtilis* HW3B. Experimental design, no set used only strain DW5L was included because the primary purpose was to help control water quality in the recirculating water system. Normally, LAB do not grow well outside the fish as *Bacillus* species do. Therefore, the experimental set used a combination of LAB and *Bacillus* to enhance each other's functions both in the water and inside the fish. Each experimental set raised 2-3 inches Nile Tilapia, 15 fish/aquarium, and used 2 air stone aerators/aquarium with air lift 600 l/h. for the recirculating system. The experiment was conducted for 10 weeks. The fish were fed with a commercial feed for herbivorous fish containing 18% protein at 9 and 17 o'clock and fed until full. The weight and length of the fish were recorded every 2 weeks and the weight of the fish feed consumed was also recorded. Use the fish's weight and length data to calculate its growth performance. It consists of the following parameters: weight gain (WG), length gain (LG), average daily gain (ADG) and average daily length (ADL) from formulae of Jantrarotai *et al.* [10], feed conversion ratio (FCR) from Dupree and Sneed's method [11], survival rate (SR) from Nankervis *et al.* [12] and yield per volume. Each parameter is calculated as follows.

Weight gain (WG, g) = final weight (g) – initial weight (g)

Average daily gain (ADG, g/day) = weight gain (g) ÷ culture period (days)

Length gain (LG, cm) = final length (cm) – initial length (cm)

Average daily length (ADL, cm/day) = Length gain (cm) ÷ culture period (days)

Feed conversion ratio (FCR) = food consumed (g) ÷ weight gain (g)

Yield/m$^3$ (g) = (Sum of harvested fish weight (g) × 1000 (l)) ÷ volume of water in fish aquarium (l)

Survival rate (SR, %) = (Number of fish harvested ÷ Number of fish stocked) × 100

### 2.4 Water quality analysis

Water samples were collected from a glass cabinet at a depth in the center of the cabinet between 8:00-9:00 a.m. and analyzed for total ammonia, nitrite, nitrate, phosphate, dissolved oxygen (DO), biochemical oxygen demand (BOD), and total suspended solids (TSS) using the methods of APHA *et al.* [13]. pH was measured using a pH meter and temperature was measured by a thermometer. All parameters were measured

on the initial day and every 7 days of the experiment for 10 weeks, except for BOD that was measured on the first day and every 14 days of the experiment. In addition, total bacterial count, LAB and *Bacillus* spp. were monitored every 14 days.

### 2.5 Statistical analysis

All data were analyzed for statistical differences in variables by One Way Analysis of Variances ( ANOVA) and comparing differences in means of variables using Duncan's multiple range test at a significance level of $P < 0.05$. Results are given as mean of five replicates ± standard deviation (SD) otherwise stated. R programme (version 3.5.2) was used to analyze the above statistics.

## 3. Results and Discussion

### 3.1 Preparation of probiotic bacteria to use as seedlings

Three bio-fermented water formulae (PM.1, HW3B+DW5L, HW3B), were prepared as previously described to use as seedlings in an experiment raising Nile tilapia in aquaria. The characteristics and color of each bio-fermented water were brown, similar to the color of molasses, as shown in Figure 2. After adding each bio-fermented water formula into rearing water by following the experimental design with 4 treatments, three bacterial parameters: total bacteria, *Bacillus* species, and LAB were enumerated to evaluate significant differeance or not for total bacteria and *Bacillus* populations among all inoculated sets. To count bacterial parameters, all water samples were collected before fish farming from T1 (no added microbial product), T2 (PM.1 bio-fermented water from the Department of Fisheries), T3 (bio-fermented water mixed with LAB and *Bacillus:* Mix), and T4 (bio-fermented water with only *B. subtilis* HW3B: *Bacillus*). The results are shown in Table 1. Total bacteria and *Bacillus* counts were significantly higher ($P < 0.05$) in all inouclation sets (T2, T3 and T4) than unionculated T1. T3 had a significant LAB count compared with other groups ($P < 0.05$).

Among the water samples from the recirculating fish farming system before fish cultivation, the control set (T1) had a significant lower populations than of all bacterial counts ($P < 0.05$). This was due to no addition of microbial product. Among three sets of adding bio-fermented water experiments (T2, T3, T4) found no sinificant difference ($P > 0.05$) for total bacteria and *Bacillus* counts: *B. subtilis* HW3B in (T3 and T4) and *Bacillus* strains (*B. subtilis, B. megaterium,* and *B. Licheniformis*) in T2 that used a formula from the Department of Fisheries [8]. This points that it was possible to evaluate ability of *Bacillus* sp. from T2 set and our selected probiotics in T3 and T4 sets. Interestingly, T3 had a higher significance number of LAB than other treatments, this was due to the addition of *L. plantarum* DW5L.

**Table 1.** Numbers (cfu/ml) of total bacteria, *Bacillus* spp., and lactic acid bacteria in each experimental set before fish cultivation

| Treatment | Total Bacteria | *Bacillus* spp. | Lactic acid bacteria |
|---|---|---|---|
| T1 (Water) | $5.6 \times 10^2 \pm 9.7 \times 10^{1a}$ | $2.1 \times 10^2 \pm 8.3 \times 10^{1a}$ | $0.00^a$ |
| T2 (PM.1) | $1.9 \times 10^4 \pm 1.2 \times 10^{3b}$ | $1.5 \times 10^4 \pm 1.3 \times 10^{3b}$ | $9.3 \pm 0.9^b$ |
| T3 (Mix) | $3.9 \times 10^3 \pm 8.6 \times 10^{1b}$ | $1.1 \times 10^4 \pm 1.5 \times 10^{3b}$ | $3.7 \times 10^1 \pm 0.4^c$ |
| T4 *(Bacillus)* | $5.3 \times 10^3 \pm 5.2 \times 10^{2b}$ | $1.0 \times 10^4 \pm 1.3 \times 10^{3b}$ | $4.1 \pm 0.1^b$ |

Data are given as mean of triplicate ± SD. The mean values in the same column with different superscripts are significantly different ($P < 0.05$).

### 3.2 The effect of probiotic bacteria on water quality in tilapia culture

From the analysis of water quality in each experimental set over 10 weeks to study the effect of probiotics on water quality, it was found that the temperature fluctuation in all treatments tended to have the same pattern. The water temperature on the initial day of the experiment was significantly different ($P < 0.05$). However, after that temperatures were not significantly different throughout the rest of the experiment and ranged 24.74 - 29.68 $^oC$ (Figure 3a).

The pH values throughout the experiment did not differ significantly ($P > 0.05$), except for the 5th week. The pH initially decreased to 5.87 - 6.29 in the 4th week, which was not suitable for fish farming. Therefore, 2 grams of dolomite per aquarium were added in the 5th week, resulting in a gradual increase in pH values, which remained at an appropriate level throughout the experiment (Figure 3b).

The DO content in each treatment was not significantly different during the first 2 weeks, but started to differ significantly ($P < 0.05$) from the 3rd week until the end of the experiment. All treatment sets showed continuously decreasing DO values, reaching their lowest levels in the 10th week. The lowest value was 3.65 mg/l for T3, while the highest was 4.74 mg/l for T1, the control set (Figure 3c).

BOD tended to gradually increased over time in every experimental set. The BOD value of the T3 experimental set was the lowest throughout the experiment and was significantly different from the other experimental sets ($P < 0.05$), measuring 19.47 mg/l in week 10. In contrast, the control set T1 had the highest BOD value at 28.23 mg/l in week 10 (Figure 3d).

Similarly, TSS values tended to increase with the duration of the fish rearing period. Treatments T3 and T2 had similarly low values, which were significantly ($P < 0.05$) lower than those of the control treatments T1 and T4, except for weeks 4, 5, 7 and 10 (Figure 3e).

In a 10-week study on the effect of probiotics on water quality, it was noted that pH values decreased gradually during the first 4 weeks in every experimental set. This decrease was due to the accumulation of organic matter from food and excrement of fish. The bacteria in the water and the biofilter system broke down the organic matter, released carbon dioxide gas, which reacted with the water to form carbonic acid [14], resulting in a lower pH. To maintain the pH level of the water and prevent it from becoming too low and harmful to the fish, dolomite was added. Dolomite, being a type of limestone, reacts slowly [15], which causes the pH to gradually increase over time. Consequently, the pH gradually rose until the 10th week.

From Figure 3., water temperature was low during the first 3 weeks due to influence of rain to cause a lower ambient temperature and then gradually increased throughout the experiment. This rise in temperature positively affected both the bacteria and fish cultures, leading to better growth, as the higher temperature accelerated cellular reactions [14]. This was consistent with the decreasing DO values throughout the experiment, which resulted from increased oxygen consumption by the larger fish and greater use by bacteria decomposing organic matter. The rise in organic matter is reflected in the increasing biochemical oxygen demand (BOD) values throughout the culture. The T3 experimental set had the lowest BOD value because it was most effective to decompose organic matter, followed by T2 from the Department of Fisheries. This was also consistent with the TSS content, which, like the BOD value, increased throughout the experiment. T3 and T2 had the lowest TSS values, respectively. Similarly, the study by Said *et al*. [16] found that the application of probiotics from the *Bacillus* group in Nile tilapia culture resulted in significantly ($P < 0.05$) lower DO values compared with the experimental set without probiotics, and it also led to higher fish growth. Moreover, the use of *Lactobacillus plantarum* in the study of giant freshwater prawns (*Macrobrachium rosenbergii*) was found to result in lower DO levels and pH compared with the control [17].


**Figure 2**. Characteristics and color of each bio-fermented water to use as seedlings

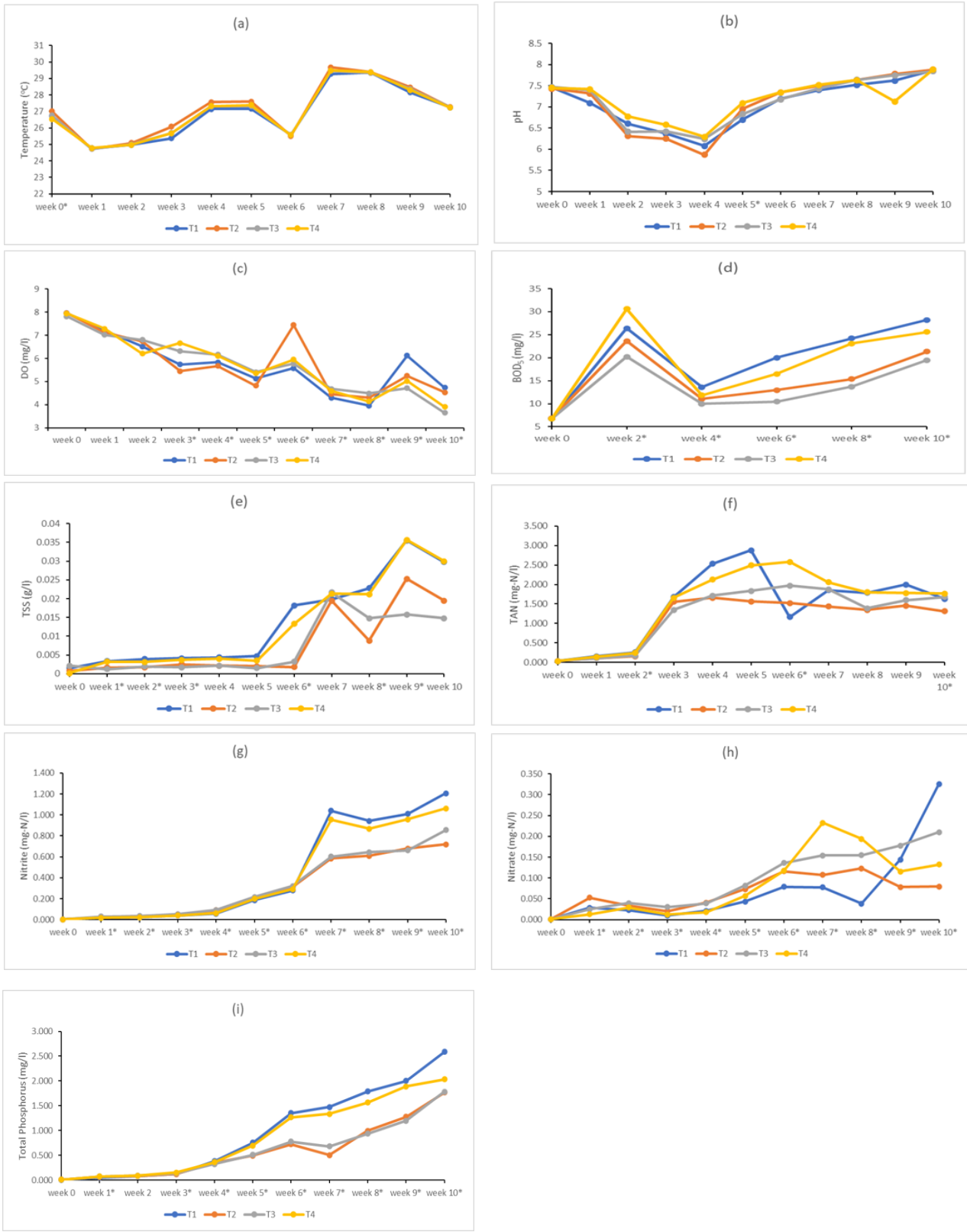


**Figure 3.** Change of water quality parameters: temperature (a), pH (b), dissolved oxygen (c), biochemical oxygen demand (d), total suspended solids (e), total ammonia (f), nitrite (g), nitrate (h) and total phosphorus (i) in each experimental set during 10 weeks of Nile tilapia culture. * There was a significantly different ($P < 0.05$) in that week.

The total ammonia content in all treatment sets tended to increase gradually during the first 2 weeks of the experiment, then rose rapidly, reaching its highest levels in the 5th and 6th weeks, before remaining constant throughout the rest of the experiment. The control set T1 had the highest ammonia content, followed by T4 with the second highest. The treatment set that controlled ammonia content the best ($P < 0.05$) was T2 (PM.1 from Department of Fisheries), with T3 (a mixed probiotic set) being the second most effective (Figure 3f).

The nitrite content gradually increased with the rearing period and were significantly different ($P < 0.05$). From weeks 2 to 7, T1 and T4 had higher values than T2 and T3. From weeks 8 to 10, all experimental sets showed significant differences ($P < 0.05$), with T1, the control, having the highest nitrite value, followed by T4, T3, and T2, respectively (Figure 3g).

In terms of nitrate, every experimental set showed a gradual increase. Nitrate content in all experimental sets increased faster than in T1, the control set, and were significantly different ($P < 0.05$) from the 6th week onwards. For T1, nitrate content rose significantly in the 9th and 10th weeks. At the end of the experiment, all experimental sets had significantly different values ($P < 0.05$), with T2 having the lowest nitrate content and T1 having the highest content (Figure 3h).

The total phosphorus content in all treatments gradually increased over the experimental period, with all treatments showing significant differences ($P < 0.05$) during weeks 5 to 9. By week 10, T2 and T3 had the lowest total phosphorus content, which were not statistically different from each other ($P > 0.05$), but were significantly different from T1 and T4 (Figure 3i).

Total ammonia values increased rapidly after 2 weeks of fish culture and remained at the same level from week 6 to week 10. T2, from the Department of Fisheries, which included three *Bacillus* species (*B. subtilis*, *B. megaterium* and *B. Licheniformis*) was the most effective in controlling ammonia, nitrite and nitrate levels, followed by T3, which used mixed probiotics *B. subtilis* HW3B and *L. plantarum* DW5L, as the second best. In line with what Detsch and Stülke [18], *B. subtilis* utilizes glutamine as primary nitrogen source. In the absence of glutamine, other nitrogen sources, such as ammonium, can be utilized. Like the study by Zokaeifar *et al*. [19]., which used *B. subtilis* strains L10 and G1 in white shrimp (*Litopenaeus vannamei*) larvae, it was found that *B. subtilis* could significantly reduce ammonia, nitrite and nitrate levels ($P < 0.05$) compared with the control in both in *vitro* and in *vivo* studies. This was also consistent with the study by Said *et al*. [16] which investigated raising Nile tilapia with mint in an aquaponic system. The experimental set using the probiotic *Bacillus* spp. was able to control ammonia and nitrite levels significantly better than the experimental set that did not use them.

Similar to nitrogenous species such as ammonia, nitrite and nitrate, *Bacillus* spp. can utilize and reduce phosphorus levels in water. The results showed that T2 and T3 were significantly more effective ($P < 0.05$) in reducing total phosphorus compared with the control. In line with the findings of Lalloo *et al*. [20] which reported that *B. subtilis*, *B. cereus* and *B. licheniformis* can reduce phosphate ion content in aquaculture. Furthermore, the study by Wang *et al*. [21] found that the use of probiotic *Bacillus* can reduce phosphorus levels in ponds of white shrimp (*Penaeus vannamei*) compared with the control set.

In case of T3, which used *B. subtilis* HW3B in combination with *L. plantarum* DW5L, the results in controlling water quality were better than in T4, which used *B. subtilis* HW3B alone. This improvement may be due to the indirect effects of *L. plantarum* DW5L, which can help stimulate the fish's immune system, making them healthier and improving their ability to digest and absorb food. However, in all experimental sets we did not check bacterial populations in digestive tract of fish as only bacterial populations in water cultivation were counted. It should be noted that there were some evidences to support our results in this present study that *L. plantarum* can help improve the digestion and nutrient absorption efficiency in fish such as coho salmon [22] and Nile tilapia [23]. Enhanced nutrient utilization leads to more efficient growth of the aquatic animals, resulting in better conversion of food into body mass. This, in turn, reduces leftover food and excrement, leading to improved water quality [14]. Overall results based on water quality indicate that probiotics, *B. subtilis* HW3B and *L. plantarum* DW5L, were able to act like a combination of *B. subtilis*, *B. megaterium*, and *B. licheniformis* in PM.1.

### 3.3 The effect of selected probiotics on bacterial population in tilapia culture water

The number of bacteria in the tilapia culture water in each experimental set was examined every 2 weeks for the parameters of total bacteria, *Bacillus* spp. and LAB. It was found that the total bacterial counts gradually increased with the culture period. T2 and T3 reached their highest counts in week 8, then decreased by week 10. In contrast, T1 and T4 continued to increase significantly by week 10, with T1 having the highest total bacterial counts, followed by T4. These counts were significantly different ($P < 0.05$) from those in T2 and T3 (Figure 4a).

The number of *Bacillus* spp. in T1, the control, had very little growth throughout the experimental period, while T2, T3 and T4 increased rapidly from weeks 4 to 10. All experimental treatments were significantly different from T1 ($P < 0.05$). In week 10, T2 (PM.1 from the Department of Fisheries) also had significantly different *Bacillus* spp. counts compared to T3 and T4 (Figure 4b).

The number of LAB in T1 was null throughout the experiment. T2 and T4 detected some LAB from the 4th week onwards, while T3 showed a gradual increase in LAB from the 2nd week, with a rapid increase from the 6th week onwards. T3 was significantly different ($P < 0.05$) from the other experimental sets in week 8 and 10 (Figure 4c).

The number of total bacteria was similar in all experimental sets during the early stages of fish culture. However, it gradually varied according to the amount of organic matter as observed from the BOD values. As stated by Olutiola *et al*. [24], "the total bacterial count and coliform density were directly related to the BOD values, but inversely related to the DO". This is because, in the water and sediment, there are many groups of bacteria that decompose organic matter, with the heterotrophic bacteria being particularly important as they require organic matter as an energy source for growth [14]. By the last week, T2 and T3 had significantly different total bacteria counts compared with T1 and T4 due to the varying amounts of organic matter.

The *Bacillus* group was initially less detected during the first 4 weeks of fish culture due to the limited organic matter available as a source of carbon and nitrogen. However, after this period, all experimental sets that included probiotics exhibited a rapid increase in *Bacillus* counts, which was significantly different from the control set (T1). This increase was attributed to the *Bacillus* strains in the T2 set from the Department of Fisheries, which included 3 species: *B. subtilis*, *B. megaterium*, and *B. licheniformis* [8]. These strains were tested by the Department of Fisheries and found to thrive under aquaculture conditions. For T3 and T4, the *Bacillus* strain was selected from the water in the broodstock pond of Nile tilapia in Songkhla Province, which also resulted in good growth. Additionally, in the preparation of all 3 experimental sets, molasses was added weekly as a carbon source, and the Nile tilapia feed, which has a relatively high protein content, served as a nitrogen source. This led to a better *Bacillus* growth compared with the control set that did not include these additives. Consistent with the study by Schneider *et al*. [25], molasses was used in combination with a recirculating drum filter system in African catfish culture. The use of molasses provided an additional carbon source, which resulted in the rapid growth of heterotrophic bacteria and a significant reduction in total ammonia content compared to the control.

Throughout the experiment, no LAB were found in the water of the control set (T1). This may be because the fish culture water had high oxygen levels and a neutral pH, which are not suitable for the growth of LAB. On the other hand, some LAB were found in the experimental sets that did not provide LAB, namely T2 and T4, because biological fermentation, which was added weekly, used molasses that contained LAB. Thungkao and Roeancharoen [26] conducted a study on "The occurrence and identification of LAB in molasses-based ethanol production plants in Thailand". They found that LAB were present in all molasses samples. In T3, which included *L. plantarum* DW5L, LAB were found throughout the experiment, and their numbers were significantly higher than other experimental sets, particularly after the 6th week. This was because the fish culture water had accumulated more organic matter and had been inoculated weekly. *L. plantarum* can thrive in such oxygenated conditions. In line with Fu and Mathews [27], it was stated that "*L. plantarum* is a facultative bacterium and can utilize oxygen as an electron acceptor for cell growth and product metabolism".

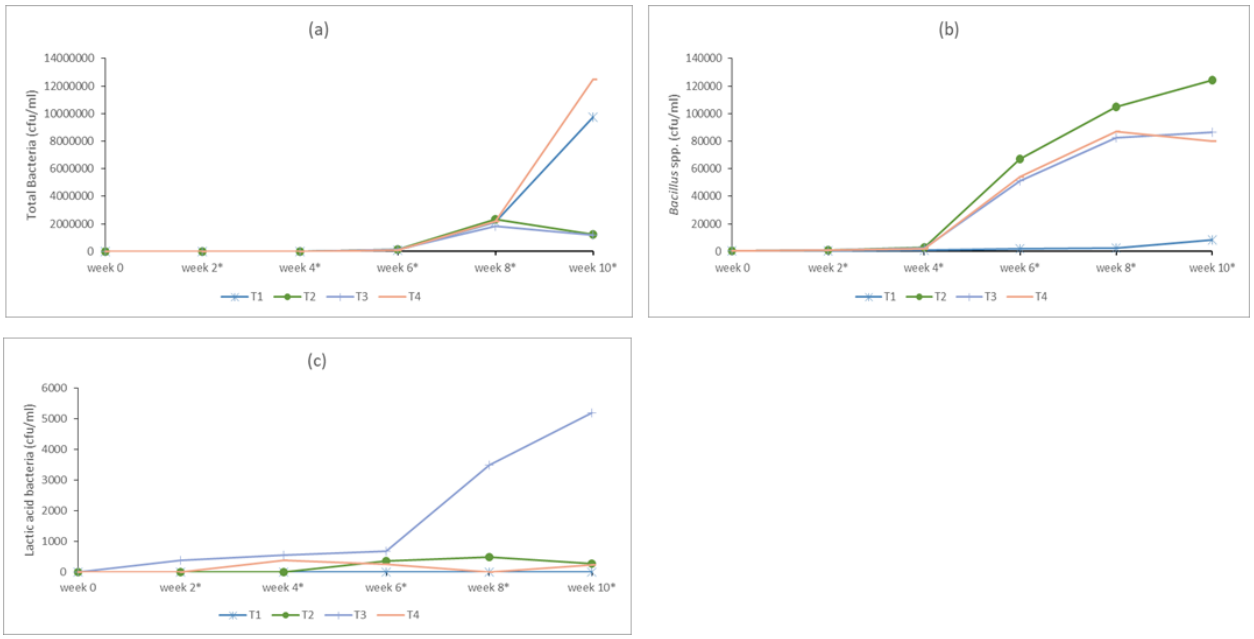


**Figure 4.** Change of the number of total bacteria (a), *Bacillus* spp. (b) and lactic acid bacteria count (c) in water of each experimental set during 10 weeks of Nile tilapia culture. * There was a significant difference ($P < 0.05$) that week.

### 3.4 The effect of selected probiotics on tilapia growth

The efficiency of the selected probiotics, T3 (Mix *B. subtilis* HW3B + *L. plantarum* DW5) and T4 (*B. subtilis* HW3B), compared with T1 (the control set) and T2 (the set using Probiotic PM. 1 of the Department of Fisheries) is shown in Table 2. It was found that after raising fish for 10 weeks, the experimental set using mixed probiotics in T3 set had a value of FBW and ADL significantly higher than the control set ($P < 0.05$), but the value of WG, LG, ADG, FCR and Yield/$m^3$ was not significantly higher than the control set ($P > 0.05$). The PM.1 set exhibited the second highest effectiveness,with a FBW significantly higher than that of the control set. Nevertheless survival rates, of all of them were not significantly different from each other. Fish growth performance (Table 2) suggests that both selected probiotics in T3 and three *Bacillus* species in T2 were effective to promote fish growth.

T3 (mixed *B. subtilis* HW3B and *L. plantarum* DW5L) set was the most effective in promoting the growth of tilapia as a significantly higher of FBW and ADL than the control set ($P < 0.05$), This might be that benefit of the probiotics enhancing nutrient digestion, leading to better nutrient absorption by the fish and resulting in the highest FWB and ADL in the experiment. As previously described, bacterial counts in fish digestive tract were not counted; thereby, they should be counted to make sure for the role of probiotics used, particularly for *L. plantarum* DW5L. It should be mentioned that several reports are in accordance with our study. Hamdan *et al*. [28]they mixed 0.5 and 1.0% *L. plantarum* probiotics in juvenile tilapia diets resulted in significant improvements in FBW, WG, feed intake and FCR of the fish ($P < 0.05$). Obviously, addition of *L. plantarum* probiotic affects fish growth. This may be because probiotics help balance the microbial flora in the gut, resists germs and stimulates nutrient absorption [29]. And from a study in rainbow trout by Merrifield *et al*. [30], it was found that *B. subtilis* also improved immune response and growth, including affected the intestinal microorganisms as well. In addition, the experiment of Bisht *et al*. [31] using selected *B. subtilis* from the digestive tract of common carp to raise common carp, resulted in a better growth of fish in terms of specific growth rate (SGR), feed conversion ratio (FCR) and feed conversion efficiency (FCE). The PM.1 set achieved the second-highest FBW, following the mixed probiotic set. This is because the PM.1 set from the Department of Fisheries contained a combination of three *Bacillus* species: *B. subtilis*, *B. megaterium* and *B. licheniformis*. In contrast, the control set exhibited the lowest growth performance, as it relied on natural bacterial growth without inoculation, leading to a mix of beneficial and potentially harmful bacteria.

This present study on the basis of bacteril counts (total bacteria, *Bacillus* spp. and LAB) found no significant differentce for amounts of total bacteria and *Bacillus* spp. in sets of T2 and T3 (Table 1) points out that both selected probiotics in T3 set had ability to maintain water quality and promote fish growth in the same level with 3 species *Bacillus* in T2 set (Figure 3 and Table 2). This indicates the role of *L. plantarum* DW5L could support ability of *B. subtilis* HW3B to act like a combination of *B. subtilis*, *B. megaterium* and *B. licheniformis* in PM.1.

**Table 2**. Growth performance and survival rate of Nile tilapia raised in a recirculating water system with selected probiotic bacteria for 10 weeks

| Parameter | Control | PM.1 | *B. subtilis* HW3B + *L. plantarum* DW5L | *B. subtilis* HW3B |
|---|---|---|---|---|
| IBW | 7.45 ± 0.65$^{a}$ | 7.41 ± 0.36$^{a}$ | 7.52 ± 0.07$^{a}$ | 7.33 ± 0.18$^{a}$ |
| FBW (g) | 43.11 ± 2.15$^{a}$ | 48.13 ± 1.98$^{b}$ | 50.53 ± 4.36$^{b}$ | 44.82 ± 1.28$^{ab}$ |
| WG (g) | 35.66± 2.71$^{a}$ | 40.72 ± 1.62$^{a}$ | 43.02 ± 4.33$^{a}$ | 37.49 ± 1.41$^{a}$ |
| LG (cm) | 5.59± 0.52$^{a}$ | 5.67 ± 0.29$^{a}$ | 6.28 ± 0.69$^{a}$ | 5.88 ± 0.31$^{a}$ |
| ADG (g/day) | 0.505 ± 0.034$^{a}$ | 0.575 ± 0.029$^{a}$ | 0.617 ± 0.064$^{a}$ | 0.538 ± 0.017$^{a}$ |
| ADL (mm/day) | 0.075 ± 0.003$^{a}$ | 0.083 ± 0.004$^{ab}$ | 0.090 ± 0.005$^{b}$ | 0.086 ± 0.002$^{b}$ |
| FCR | 2.09 ± 0.25$^{a}$ | 1.91± 0.09$^{a}$ | 1.88 ± 0.20$^{a}$ | 1.84 ± 0.27$^{a}$ |
| Yield/m$^{3}$ (g) | 1978.89 ± 315.94$^{a}$ | 2153.02 ± 196.44$^{a}$ | 2155.07 ± 57.86$^{a}$ | 2409.87 ± 465.85$^{a}$ |
| SR (%) | 91.11 ± 3.85$^{a}$ | 82.22 ± 3.85$^{a}$ | 84.44 ± 15.40$^{a}$ | 91.11 ± 10.18$^{a}$ |

Data are given as mean of triplicate ± SD. The mean values in the same row with different superscripts are significantly different ($P < 0.05$).

IBW=initial body weight, FBW=final body weight, WG=weight gain, LG=length gain, ADG=average daily gain, ADL=average daily length, FCR=feed conversion ratio, SR=survival rate

## 4. Conclusions

This study proved that the use of probiotics in combination with a recirculating water system for Nile tilapia culture is more effective in controlling water quality than using a recirculating water system alone without adding seedling. The use of three species of *Bacillus* in combination by the Department of Fisheries showed good results in treating nitrogen in the form of ammonia, nitrite and nitrate. However, the combination of *Bacillus subtilis* HW3B with *Lactiplantibacillus plantarum* DW5L was more effective in reducing BOD and total suspended solids, while also reducing organic nitrogen to a similar extent. Both probiotics were used for raising Nile tilapia in a recirculation water system for 10 weeks. Overall results demonstrated that two promising probiotic strains HW3B and DW5L gave a similar result in promoting the growth of tilapia compared to the popular PM. 1, but more effective than the control without probiotics. Further study such as formulation of the probiotic product, shelf life of the product and on farm trial needs to be undertaken to ensure the success of our probiotic application in fish culture.

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