Evaluation of probiotic effects on the growth performance of Nile tilapia (*Oreochromis niloticus)* in a high-density biofloc system.

by

Beatriz Pacheco Nogueira Oliveira

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Approved by

D. Allen Davis, Chair, Professor, School of Fisheries, Aquaculture, and Aquatic Sciences
Timothy J. Bruce, Assistant Professor, School of Fisheries, Aquaculture, and Aquatic Sciences
Luke A. Roy, Associate Extension Professor, School of Fisheries, Aquaculture, and Aquatic Sciences

Abstract

Biofloc technology (BFT) is an aquaculture production system that has gained popularity with tilapia producers. Probiotics provide benefits for the host and/or the environment. Probiotics have been reported in some cases to enhance growth performance, improve water quality, and prevent infections. One of the administration routes for probiotics is to apply them through the feed. When a probiotic is combined with a biofloc system, the production yield may be improved through better fish growth, disease resistance, or enhanced survival. This research aimed to evaluate the growth performance of Nile tilapia *Oreochromis niloticus* fed commercial top-coated probiotics. Two growth trials were conducted, Trial A had fish with a mean initial weight of 71.4 ± 4.4g, and fish in Trial B had a mean initial weight of 5.34 ± 0.42g. Tilapia were offered commercial feed (38 % protein floating tilapia feed, Optimal Aquafeed, Omaha, NE) in both trials, in the first fish feed was top coated with two probiotics, AP193 (*Bacillus* spp.; provided by Dr Mark Liles, Auburn University) final concentration of 1×10^7 CFU g^{-1}, and BiOWISH® Feedbuilder Syn3 (BiOWISH Technologies ® Cincinnati, OH, USA) in a final concentration of 3.6×10^4 CFU g^{-1}. In Trial B, feed was top coated with two different concentrations of BiOWISH® Feedbuilder Syn3 (BiOWISH Technologies, Cincinnati, OH, USA) at the final concentrations of 3.6×10^4 CFU g^{-1} and 7.2×10^4 CFU g^{-1}. The same commercial feed was used as a reference, resulting in 3 experimental treatments with three tank replicates each. The results of both growth trials indicated no significant differences in growth performance (except FCR (feed conversion rate) that showed significant differences in Trial B, where both concentrations of BiOWISH® Feedbuilder Syn3 showed improvement), survival, water quality, solids management, and bacterial composition of water and fecal matter. Even though growth performance results presented no significant differences, results could differ based on the concentration and the route of the probiotic

administration, but most importantly, their impact on the microbial community of the culture water developed in the biofloc system. According to the data collected, testing on a larger scale with different probiotic doses is necessary to achieve an effective dosage to improve tilapia culture.

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1. Introduction

Aquaculture is one of the fastest-growing sectors of animal production in the world (Hagar Dighiesh, 2014; Wally, 2016; Kaleem and Bio Singou Sabi, 2021), with tilapia being one of the most popular and highly cultured farmed fish, having an average production of over 4.5 million tonnes (FAO, 2020). These fish are native to Africa and have been spread to different continents for different reasons, but mainly for food (Halwart et al. 2004). Tilapia production has increased worldwide due to attributes that allow tilapia to be farmed under various conditions. They can be produced in freshwater and brackish water due to their tolerance to different salinity concentrations. They can tolerate low dissolved oxygen and high ammonia concentrations; they are resistant to diseases and stress; have a fast growth rate, and attain early sexual maturity (that can be considered a disadvantage for production and an advantage for hatchery or seed production) (El-Sayed, 2006).

Aquaculture technologies have steadily increased the commercial level production intensity resulting in higher levels of inorganic and organic wastes entering the culture system, potentially increasing diseases outbreaks and deteriorating the environment (Ahmed et al. 2019; Shafique et al. 2021; Naiel et al. 2022). Under intensive rearing conditions, fish are regularly subjected to a diverse spectrum of microorganisms and stress factors that increase their vulnerability to infectious illnesses (Negm et al. 2021; Shafique et al. 2021; Naiel et al. 2022). The potential costs from disease outbreaks in aquaculture have reached billions of dollars annually and have been cited as a danger to the aquaculture industry's profitability (Assefa and Abunna, 2018).

A possible solution to improve fish production is using biofloc technologies (BFT), which are considered environment-friendly aquaculture systems. This culture methodology may have

advantages for increasing the cost/benefit ratio for fish production (Hargreaves, 2013). BFT consists of a macro-aggregation of algae, bacteria, fungi, and nutrients detritus, creating a floc that stays suspended in the water column through heavy aeration and mixing. Therefore, the main principle of a biofloc system is the recycling of the waste in terms of leftover feed and feces within the system while producing bacterial biomass or "biofloc" particles (Khanjani et al. 2021; Khanjani et al. 2022). One of the advantages of biofloc systems, is increased survivals rates, most likely related to reduced stress caused by improved water quality and nutritional substances contained in biofloc (Yu et al., 2023).

The management of BFT systems is based on the carbon (C) and nitrogen (N) ratio, which can be through feed input or different carbon sources, such as molasses or sugar cane bagasse. The role of the C:N ratio is to be used as a source of energy (carbon source) for the heterotrophic bacteria to consume the ammonia present in the system (Hargreaves, 2013). This type of system depends on the equilibrium between high stocking density and the biofloc, with low or no water exchange, thus providing efficient use of water and land (Ray and Mohanty, 2020; Khanjani et al., 2022). Reducing water exchange decreases the entry of pathogens into the system, thereby minimizing the risk of disease outbreaks (Allameh et al., 2021). The microbial assimilation of nutrients results in the transformation of carbon and the control of nitrogen levels in the water. For some fish species, the concentration of microbial protein in the water can serve as a secondary food source, effectively resulting in an upcycling of nutrients (Avnimelech, 2012; Khanjani et al., 2022).

The administration of antibiotics is not only used to treat disease problems but also to help prevent them. However, the extended and improper use of antibiotics can cause pathogens to be resistant (Gatesoupe, 2004; Hai, 2015; Huerta-Rábago et al., 2019; El-Kady et al., 2022). If the

use of antibiotics is to be reduced or eliminated, there is a need to find alternative management strategies to minimize and control disease outbreaks. One management strategy is using probiotics (Kaleem and Bio Singou Sabi, 2021). Gibson et al. (2017) defined probiotics as "live microorganisms that, when administered in sufficient concentrations, can confer beneficial results." The source of probiotics can be exogenous or indigenous, recurring naturally in different hosts (Tuan et al., 2013).

Probiotics work in different ways in aquatic animal organisms, such as "immunomodulation or competitive exclusion," where they act in the gastrointestinal mucosa (GI) (Skjermo et al., 2015; Hai, 2015). According to Standen et al. (2016), the gastrointestinal tract has an essential role due to being the organ where probiotics will establish and perform their effects. The microbial communities found in the GI are most affected by feed and rearing conditions (Giatsis et al. 2015; Dehler et al. 2017; Deng et al. 2022). The bacteria will act in the mucosal barrier, modulating gut microflora and reducing pathogenic bacteria (Sîrbu et al., 2022), resulting in an increase in feed consumption, absorption of nutrients, and increase in immune response (Mugwanya et al., 2022). Thus, they can benefit the host by enhancing growth performance, water quality, immune system response, disease response, and increasing nutrient availability for zooplankton (Merrifield et al., 2010; Hai, 2015).

Probiotics are utilized for several reasons dictated by application and species of choice. Different routes can be applied, such as in the feed (via pellet feed with probiotic or live food with probiotic) or added to the water column. In aquaculture, they are commonly used as feed additives due to most of the probiotics being produced to be incorporated in the feed (Gomes et al. 2009). *Bacillus sp.* is the most common probiotic and is considered effective for enhancing growth and health (Jahangiri and Esteban, 2018; Naiel et al., 2022); they will also modulate the microbial

community within the fish (Li et al., 2022). For instance, Zhou et al. (2010) experimented with tilapia using three probiotics: *Bacillus subtilis, B. coagulans,* and *P. palustris.* They added the probiotics to the water at 1 x 10^7 CFU mL^{-1}. After 40 days, the administration of *Bacillus coagulans* B16, and *Rhodopseudomonas palustris* G06 conferred beneficial results on tilapia growth but not significant effects on water quality parameters. Several studies have demonstrated that *Bacillus* species contribute significantly to the reduction of nitrogenous and phosphorus components in the rearing water in addition to the maintenance of bacterial community structure equilibrium (Yi et al. 2018; Soltani et al. 2019; Li et al. 2022).

Incorporating existing disease control methods as possible alternatives to enhance fish immune response might be an appropriate way to improve the ability of fish to resist disease and, as a result, enhance growth performance and reduce disease outbreaks. Thus, two growth trials were conducted with the following objectives:

1. Evaluate the growth performance and nutrient retention of Nile tilapia offered a commercial feed top coated with one of two probiotics in a biofloc-based culture system.
2. Analyze the effects of probiotics on water quality and microbial communities on fish fecal matter and culture system water.

2. Materials and methods

2.1 Experimental system

Two trials were conducted in a greenhouse-based de-coupled aquaponics system at the E. W. Shell Fisheries Center at Auburn University, Auburn, Alabama, in agreement with the Auburn University animal care policy. The research system consisted of nine 1000-gal (3.8 m^3) cylindrical polypropylene t s connected to two 500 gallons (1.9m^3) reservoir tanks, Aquadyne bead filter (0.2 m^2 media, 0.6 m × 1.1 m; source) and a 0.25-hp circulation pump (Cascade-PerformancePro Pumps, Hillsboro, OR). Each culture tank was equipped with one (120 cm) long diffuser tubing (rubber/polyethylene diffuser hose, Pentair Aquatic Eco-Systems, Inc.) hooked to a standard regenerative blower (1.5-hp, Sweetwater-Aquatic ECOSYSTEMS, Inc.). To control solids, each culture tank was equipped with a 30 gal (113.6 liters) conical settling chamber that received water via a powerhead (Maxi-Jet 110 gph) which returned settled water back to the culture tank. During the acclimation period, the system was used as a common recirculating aquaculture system (RAS) to allow the development and equalization of the biofloc community across all tanks.

2.2 Growth Trial

Trial A: Evaluation of different probiotics

Prior to the start of the experiment, an excess of fish (150 $tank^{-1}$) were stocked and acclimated into nine tanks for 26 days, and the fish were fed with commercial feed twice daily. At the start of the 109-day growth trial, fish were harvested, sorted for uniformity, counted, weighed, and restocked at a density of 120 fish $tank^{-1}$ (mean initial weight 71.43 ± 4.44 g). The dietary treatments were randomly assigned to fish in each tank, and the system switched to individual biofloc systems the day after stocking. A commercial feed (38% protein floating tilapia feed, Optimal Aquafeed®, Omaha, NE) was used throughout the trial. The commercial feed was top-

coated with two different probiotics, AP193 (*Bacillus velezensis*.; provided by Dr. Mark Liles, Auburn University) and BiOWISH® Feedbuilder Syn3 (BiOWISH technologies ® Cincinnati, OH, USA – *B. subtilis*) prior to feeding. Three treatments with three tank replicates were assigned: a control treatment (the commercial feed), a commercial feed top coated with AP193, and a commercial feed top-coated with BiOWISH® Feedbuilder Syn3. The fish were offered experimental diets at 4 % body weight, and the feeding ration was adjusted every four weeks based on fish growth and feeding response. An average of five fish per tank were collected as an initial sample for proximate analysis, and individual weight and length were recorded at the start of the trial.

Trial B: BiOWISH® Feedbuilder Syn3 concentrations evaluation

The system was stocked with fingerlings tilapia ($200 tank^{-1}$) for Trial B and acclimated in 9 tanks for one day. At the start of the growth trial, the fish were counted and weighed (mean initial weight 5.34 ± 0.42 g). Dietary treatments were randomly assigned to fish in each tank, and the system switched to individual biofloc systems after stocking. The trial used two commercial feeds (46% protein tilapia feed and 38 % protein floating feed, Optimal Aquafeed®, Omaha, NE). The commercial feed was top coated with BiOWISH® Feedbuilder Syn3 (BiOWISH Technologies ® Cincinnati, OH, USA – *B. subtilis*) in two different concentrations (BiOWISH X1 - 3.6×10^4 CFU g^{-1} and BiOWISH X2 - 7.2×10^4 CFU g^{-1}). Three treatments with three replicates were assigned to this study as follows: a control treatment (the commercial feed), commercial feed top coated with BiOWISH ® Feedbuilder Syn3 X1, and commercial feed top coated with BiOWISH® Feedbuilder Syn3 X2. The fish were offered experimental diets at 4% body weight, and the feeding ration was adjusted every two weeks based on fish growth and feeding response.

An average of ten fish per tank were collected as an initial sample for proximate analysis, and individual weight and length were recorded at the start of the trial.

2.3 Preparation of probiotic top-coated diets

Trial A: Evaluation of different probiotics

A floating commercial diet (Optimal Aquafeed Tilapia grower-G3 (38% protein), was used throughout the study for all treatments. The diet was sent to the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO) for proximate composition analysis (Table 1). BiOWISH Feedbuilder Syn3 (*Bacillus subtilis)* is a water-soluble probiotic that was used to topcoat the commercial pelleted feed at a manufacturer-recommended concentration of 200 g ton^{-1} of feed, which yields a final concentration of 3.6×10^4 CFU g^{-1}, that were mixed with distilled, water (>2L/T). After the solution preparation, the feed was loaded into a conveying paddle mixer, sprayed with the probiotic solution, and left to air dry until ready to use. For AP193, the commercial spore suspension (1.3×10^{10}) was prepared and top-coated at 8 % (w/v) following the same top-coating procedure as the BiOWISH product to obtain a concentration of $1x10^7$ CFU g^{-1} of feed. Feed was stored at 4 ˚C until use.

Trial B: BiOWISH® Feedbuilder Syn3 concentrations evaluation

In Trial B, two commercial feeds were used. At the beginning of the trial, fish were offered 2mm - 46% protein tilapia feed - Optimal Aquafeed® (Omaha, NE), and in the 4^{th} week, due to fish growth, the feed was increased to 3mm - 38% protein floating feed, Optimal Aquafeed® (Omaha, NE), both were offered throughout the trial for all treatments. Two different concentrations of BiOWISH Feedbuilder Syn3 (*Bacillus subtilis)* were used. For the treatment "BiOWISH X1", the commercial stock was 7.2×10^7 CFU g^{-1} suspended in distilled water, according to the manufacturer's specifications. The solution was sprayed onto the feed (for a final

concentration of 3.6×10^4 CFU g^{-1}) using a conveying paddle mixer and sprayed with the probiotic solution, and then diets were left to air dry until ready to be used. For the BiOWISH X2 treatment,

Table 1. Proximate composition of the commercial diet in the feeding trial.

Parameters	**g/100g as is**
Crude protein*	41.01
Moisture	5.81
Crude Fat	11.64
Fat (acid hydrolysis)	13.75
Crude Fiber	1.79
Ash	6.61
Phosphorus	1.17

*Analysis conducted by the University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA) (Results are expressed on g/100g of feed as is, unless otherwise indicated).

Crude protein= %N x6.25; estimates provided. § non-proteinogenic amino acids. Results are expressed on an "as is" basis unless otherwise indicated.

the commercial stock was 7.2×10^7 CFU $^{g-1}$ suspended in distilled, deionized water and sprayed on the feed for a final 7.2×10^4 CFU g^{-1} concentration.

2.4 Growth performance measures and feed efficiency

During Trial A, an average of 30 fish were sampled every four weeks to follow growth and estimate total tank biomass. The daily ration was adjusted based on growth and feeding response. Following 109 d of culture, fish were counted and weighed to determine the final weight, weight gain, final biomass, survival, and feed conversion ratio (FCR). A bacteria challenge was conducted following both trials to assess the health benefits of the probiotic inclusion in the diet to evaluate the immune response (Padeniya et al. 2023). Five fish per tank were randomly collected, packed in sealed bags, and stored in a freezer (-20 ℃) to determine proximate whole body and mineral composition. For Trial B, an average of 50 fish tank^{-1} were sampled every two weeks for biomass weight, to determine the fish growth, and the feed amount was adjusted according to growth performance and feeding response. At the end of the growth trial (90th day), the same procedures listed above were conducted.

Mean weight (g) = Total weight g of fish / no. of fish in the same tank

Weight gain (g) = W2 - W1

W1 = Initial mean weight & W2 = Final mean weight

*Percent weight gain (WG %) = W2-W1/W1*100*

Survival %: = final fish number/ initial fish number × 100.

Feed conversion ratio (FCR) = (total feed input g / final biomass g) /100.

Apparent Net protein retention (ANPR %) = (final weight × final protein content) - (initial weight × initial protein content) × 100 / protein intake.

2.5 Water analysis, solids management, and sample collection

Throughout both experiments, dissolved oxygen, salinity, and water temperature were measured twice daily using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA) and were maintained within an acceptable range for fish culture. Total ammonia-Nitrogen (TAN) and nitrite-N were measured twice weekly with a YSI 9300 photometer (YSI corporation, Yellow Springs, Ohio, USA). During the study, the system pH was monitored twice weekly with the pHTestr30 (Oakton Instrument, Vernon Hills, IL), and an amount of sodium bicarb ($Ca(OH)_2$) was added as needed. To quantify solids production (effluent) in both trials, each tank was equipped with an independent conical bottom settling chamber. Once a month, settleable solids were discharged, collected into a bucket, and the slurry quantified. A homogenized sub-sample was taken and placed in a large crucible and dried in the oven at 105 ºC overnight. Dry matter was recorded for each of the nine crucibles. To determine ash, 1 g of sample was placed in a small crucible and combusted in a muffle furnace (Thermo Fisher Scientific, Asheville, NC) at 600 ºC for 9 h. The samples were cooled, and ash was quantified. The effluent/solids were quantified as follows:

Solids (Kg) per Liter = amount of dry solids / per liter

Solids (Kg) per feed input = total amount of solids / total feed input

Solids (Kg) per Biomass produced = total amount of solids / total biomass

2.6 Microbial composition for fecal matter and water samples

Samples for gut composition were taken on day 0 and the last day of the trial (Trial A: day 109; Trial B: day 90). On day 0, five fish, and at the end of the trial, five fish from each tank were randomly selected. These fish were euthanized using an overdose of 250 mg L^{-1} of tricaine methane sulfonate (MS-222) buffered to a pH of 7.0-7.5 in culture water. Fish were aseptically dissected,

and the distal intestine close to the anus was separated from the rest of the gut and used for analysis. The gut was gently squeezed to ensure any remaining digesta were removed. The collected gut samples were transferred to cryotubes and submerged in liquid nitrogen before being stored at -80℃ until further analysis of gut microbiome communities. Water samples were also collected on day 0 and at the end of the trial. The water samples were filtered through a 0.45 μm bottle top filter (ThermoScientificTM Nalgene™ Rapid-Flow) using a vacuum filter (Model no. 25228-01 WELCH, Monroe, Louisiana, USA). All the samples were stored at -80℃. Samples were sent for DNA extraction and sequencing and were processed and analyzed with the ZymoBIOMICS® Targeted Sequencing Service (Zymo Research, Irvine, CA).

2.7 Statistical analysis

Data were analyzed using SAS (V9.4, SAS Institute, Cary, NC, USA). Growth performance, solids management (dry matter and ash), proximate whole-body, and mineral composition of fish were subjected to a one-way ANOVA followed by Tukey's multiple comparison tests to evaluate significant differences among treatment means ($p < 0.05$). Water quality parameters were analyzed using one-way ANOVA followed by a time series analysis. The alpha diversity values of water and fecal samples between different treatments after the challenge were analyzed using one-way ANOVA. The bacterial data were analyzed using R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Water Quality and Solids Management

Water quality parameters for Trial A are presented as mean ± SD among treatments as follows: morning and evening dissolved oxygen (7.37 ± 0.64 mg/L, 7.21 ± 1.42 mg/L), temperature (26.84 ± 1.92 ˚C), salinity (0.94 ± 0.44 ppt), pH (6.83 ± 0.66), total ammonia nitrogen (6.58 ± 8.48

mg/L), and nitrite (0.6 ± 0.51 mg/L) (Table 2). Evaluation of the data, pooled across time, pooled by week, or as time series analysis showed no difference (*P>0.05)* between treatments for dissolved oxygen (mg/L), salinity (ppt), pH, and nitrite (mg/L). However, in the case of temperature (˚C), there was a significant difference in the BiOWISH treatment *(P= 0.040)* due to one of the tanks situated close to the exit door of the greenhouse. For total ammonia nitrogen (mg/L), and unionized ammonia (mg/L) ANOVA found a difference, however Tukey's multiple comparison tests did not recognize the statistical difference. Water quality parameters for Trial B (Table 3) are presented as mean ± SD among treatments and include: morning and evening dissolved oxygen (8.05 ± 0.52 mg/L 7.74 ± 0.49 mg/L), temperature (24.71 ± 2.46 ˚C), salinity (1.87 ± 0.39 ppt), pH (7.0 ± 0.6), total ammonia nitrogen (0.35 ± 0.33 mg/L), and nitrite (0.44 ± 1.79 mg/L). Results revealed no significant differences regardless of treatments across time, and by week or time series analysis, in terms of water quality parameters evaluated: (dissolved oxygen (mg/L), salinity (ppt), pH, total ammonia nitrogen (mg/L), and nitrite (mg/L). One-way ANOVA analysis demonstrated no significant differences *(P >0.05)* for either Trial between, total solids (Kg) ash (%), solids per unit of fish (Kg), and solids per unit of feed input (Kg) (Table 4 and 5).

3.2 Growth performance and nutrient retention

Tilapia growth performance response in Trial A after feeding with two different probiotics (*B. velezensis* and *B. subitilis*) for 109 days is presented in Table 4. One-way ANOVA followed by Tukey's multiple comparison tests showed no significant difference *(P>0.05)* across treatments

Table 2. Water quality parameters summarized throughout 109 days of rearing tilapia (*Oreochromis niloticus)* juveniles in a biofloc system, stocked with 120 fishtank^{-1} with mean initial weight 71.43 ± 4.44g, while offered a commercial feed top coated with BiOWISH® Feedbuilder Syn3 and AP193, compared to the commercial diet. Values are presented as the mean ± standard deviation.

	Basal	BiOWISH	AP193	PSE	*P-value*
Morning DO (mg/L)	7.47 ± 0.62	7.41 ± 0.61	7.44 ± 0.67	0.27	0.543
Evening DO (mg/L)	7.23 ± 1.4	7.16 ± 1.47	7.21 ± 1.38	0.40	0.832
Temperature (ºC)	26.64 ± 1.82 [b]	26.90 ± 2.14[a]	26.56 ± 1.76 [b]	0.42	0.040
Ph	6.84 ± 0.28	6.89 ± 0.62	6.95 ± 0.92	0.27	0.795
Salinity (mg/L)	0.96 ± 0.44	1.0 ± 0.46	0.94 ± 0.42	0.20	0.244
TAN (mg/L)	8.56 ± 6.75[a]	8.31 ± 5.26 [a]	4.73 ± 11.39 [a]	2.77	0.044
Unionized ammonia (mg/L)	3.8 ± 4.73 [a]	3.53 ± 4.36 [a]	2.14 ± 3.2 [b]	1.39	0.044
Nitrite (mg/L)	0.58 ± 0.42	0.51 ± 0.67	0.63 ± 0.38	0.17	0.594

PSE=Pooled Standard Error
TAN = Total ammonia nitrogen

Table 3. Water quality parameters summarized throughout 90 days of rearing tilapia (*Oreochromis niloticus)* juveniles in a biofloc system, stocked with 200 fish tank^{-1}, with mean initial weight 5.34 ± 0.42g, and offered feed with two different concentrations of BiOWISH® Feedbuilder Syn3 (BiOWISH technologies ® Cincinnati, OH, USA – *B. subtilis*), compared with the commercial diet. Values are presented as the mean ± standard deviation.

	Commercial	BiOWISH X1	BiOWISH X2	PSE	*P-value*
Morning DO (mg/L)	8.07 ± 0.51	8.04 ± 0.53	8.04 ± 0.5	20.04	0.437
Evening DO (mg/L)	7.77 ± 0.47	7.73 ± 0.49	7.72 ± 0.47	0.29	0.552
Temperature (ºC)	24.8 ± 2.31	24.73 ± 2.37	24.73 ± 2.46	1.46	0.948
Ph	6.87 ± 0.57	7 ± 0.51	7.03 ± 0.45	0.31	0.161
Salinity (mg/L)	1.84 ± 0.22	1.81 ± 0.24	1.89 ± 0.48	0.21	0.295
Total ammonia nitrogen (mg/L)	0.28 ± 0.18	0.36 ± 0.29	0.27 ± 0.23	0.15	0.085
U. ammonia (mg/L)	0.14 ± 0.09	0.18 ± 0.15	0.14 ±0.11	0.15	0.085
Nitrite (mg/L)	0.23 ± 0.22	0.73 ± 2.99	0.29 ± 0.26	1.13	0.281

PSE=Pooled Standard Error
U. ammonia = Unionized ammonia.

Table 4. Response of juvenile tilapia (mean initial weight 71.43 ± 4.44g) reared over a 109-day culture period in individual biofloc type systems, stocked with 120 fish tank^{-1}, while offered a commercial feed or one top coated with BiOWISH® Feedbuilder Syn3, or AP193.

	Basal	BiOWISH	AP193	PSE	*P-value*
Growth Performance					
Final Biomass (kg)	26.46	27.77	26.46	0.668	0.736
Final mean weight (g)	252.9	239.98	236.75	5.360	0.093
Weight gain (g)	179.89	171.11	165.96	6.130	0.278
Weight gain (%)	148.70	149.17	134.53	0.110	0.760
FCR	1.38	1.27	1.44	0.110	0.586
ANPR (%)	30.57	28.45	28.95	0.560	0.658
Survival (%)	89.17	96.67	96.67	4.000	0.373
Discharged solids					
Total Solids (Kg)	217.3	141.79	173.97	18.03	0.299
Solids per unit of fish (kg)	8.14	6.52	5.31	0.68	0.306
Solids per unit of feed input (kg)	7.99	4.97	6.96	0.72	0.294
Ash (%)	82.82	82.94	81.09	0.330	0.101
Whole-body proximate composition					
Moisture (%)	67.27^{b}	67.53^{b}	69.20^{a}	0.200	0.014
Dry matter (%)	32.73^{a}	32.47^{a}	30.80^{b}	0.200	0.014
Protein DW (%)	50.40^{b}	50.40^{b}	55.50^{a}	0.390	0.003
Fat DW (%)	31.03	31.17	32	1.210	0.941
Ash DW (%)	16.33	16.73	12.60	0.670	0.086

PSE=Pooled Standard Error
ANPR = Apparent net protein retention
DW = Dry weight

Table 5. Response of juvenile tilapia (5.34 ± 0.42g) reared over 90 days of culture period in individual biofloc type systems, stocked with 200 fish tank^{-1}, and offered a commercial diet with three (0, 1x, 2x) levels of BiOWISH® Feedbuilder Syn3, top-coated to the diets.

	Basal	BiOWISH X1	BiOWISH X2	PSE	*P-value*
Growth Performance					
Final Biomass (Kg)	7.8	9.16	9.03	0.483	0.291
Final mean weight (g)	53	53	50	2.90	0.796
Weight gain (g)	48	47	45	2.94	0.767
Weight gain (%)	968	887	884	55.27	0.393
FCR	1.12[a]	1.05[b]	1.03[b]	0.01	0.005
ANPR (%)	25.95	23.63	23.26	1.12	0.325
Survival (%)	76	87	90	4.33	0.167
Discharged solids					
Total Solids (Kg)	2,752	2,351	1,317	729.38	0.426
Solids per unit of fish (kg)	370.30	271.00	160.3	91.60	0.371
Solids per unit of feed input (kg)	301.40	274.90	159.3	91.43	0.540
Ash (%)	31.17	28.77	28.51	1.97	0.268
Whole-body proximate composition					
Moisture (%)	72.65	71	72.27	0.1269	0.220
Dry matter (%)	27.35	29	27.73	0.1269	0.220
Protein DW (%)	55.5	52	54.57	0.0712	0.220
Fat DW (%)	26.9	30.23	29.43	0.1134	0.139
Ash DW (%)	12.30	12.03	13.27	0.5373	0.430

PSE=Pooled Standard Error.
ANPR = Apparent net protein retention
DW = Dry weight

in final biomass (26,456-27,767g), weight gain (134.53-149.17%), weight gain (165.96-179.89g), feed conversion rate (1.27-1.44), net protein retention (28.45-30.57 %), and survival (89.17-96.67 %) of tilapia fed with either probiotic and the commercial diet. For Trial B, results revealed a similar trend following 90 days of culture (Table 5), showing no significant differences *(P>0.05)* between treatments, and parameters ranges were as follows: final biomass (920-1130g), weight gain (%) (45.95-57.59%), weight gain (42.39-52.14g), feed conversion rate (1.02-1.13), net protein retention (39.96–51.18%), and survival (67.5-97.5%). In Trial B, FCR (*p=0.0051)* showed significant differences between the basal diet and the addition of the probiotic (BiOWISH) in the diet, regardless of the concentration. Results from whole-body proximate analysis for the first experiment are summarized in Tables 4 and 5, which revealed significant differences between moisture (*P=0.014*), dry matter (*P=0.014*), and protein DW (*P=0.003*). However, fat and ash showed no significant differences *(P>0.05).* Regarding the second experiment, the data analyzed yielded no significant differences *(P>0.05)* between treatments in moisture, dry matter, protein, fat, and ash.

3.3 Microbial composition for fecal matter and water samples

Bacterial V3-V4 16S rRNA gene analysis was performed to characterize the microbial communities associated with biofloc water and fecal matter of fish. After processing and chimera removal, the numbers of DNA sequences per sample ranged from a minimum of 23,328 to a maximum of 64,900 reads from the fecal matter samples, and a minimum of 27,898 to a maximum of 44,587 reads from the water samples in Trial A, and a minimum of 157,552 to a maximum of 242,649 reads from fecal matter and a minimum of 153,483 reads to a maximum of 218,375 reads from water samples in Trial B. The metagenomic analysis showed that the microbial community associated with fecal matter in fish represented by a total of 12 phyla (figure 1) in Trial A and 19

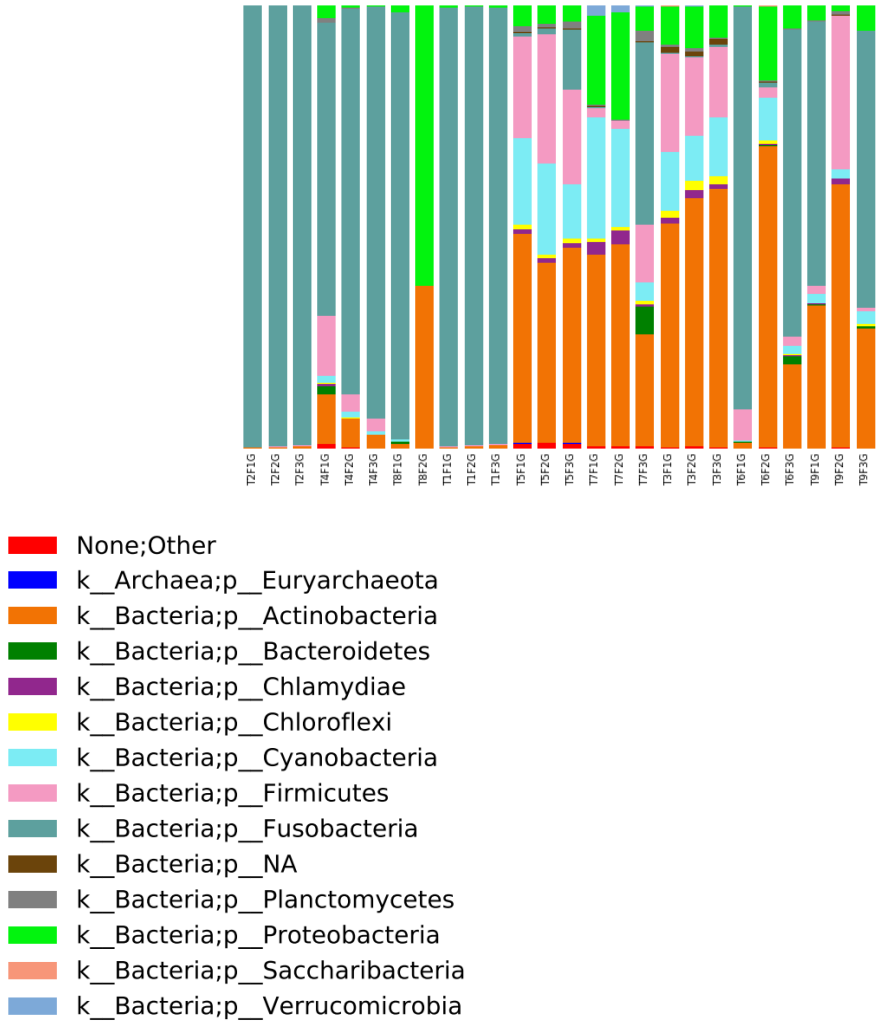

Figure 1. Relative abundance of bacterial phyla presents in the fecal matter of fish in different treatments for tilapia (*O. niloticus)* over a 109-day production period, stocked with 120 fish tank^{-1} with mean initial weight 71.43 ± 4.44 g, while offered a commercial feed top coated with BiOWISH® Feedbuilder Syn3and AP193, compared to a commercial diet.

phyla in Trial B (Figure 2) across all treatments, and the microbial communities associated with biofloc water were represented by a total of 23 phyla in Trial A (Figure 3) and 27 phyla in Trial B (Figure 4) across all treatment groups. In Trial A, the bacteria with the highest relative abundance were fusobacteria and actinobacteria in fecal samples and actinobacteria and proteobacteria in water samples. These phyla were present in all samples of the different treatment groups. The phylum firmicutes, to which the genus *Bacillus* belongs, was also present in the study; nevertheless, these were found with a lower abundance compared to other phyla except the fecal matter of BiOWISH-treated fish. Table 6 shows the highest-ranking phyla in different treatment groups in fecal matter and water samples. In Trial B, the highest relative abundance in fecal matter across all treatment groups was Actinobacteria, and the second highest was Cyanobacteria (Table 7). The phylum firmicutes had higher relative abundances in BiOWISHx1 and BiOWISHx2 than in the commercial feed-treated fish group. A similar trend was also observed in water samples, so Actinobacteria were the most abundant phylum in all treatments. In water samples, proteobacteria was the second most abundant in all treatments (Table 7).

Bacterial alpha diversities of both fecal matter and water were assessed by the observed species and Shannon diversity index. These metrics varied moderately among different treatment groups. In Trial A, out of the three treatments compared in fecal matter, AP193-treated fish contained the highest average of observed species, and the least were found in the commercial feed-treated fish. However, this was not significantly different ($p=0.455$; Figure 5). Contrary to these findings, the highest observed species were contained in water where commercial feed was added, and the lowest in AP193 treated tanks ($p= 0.207$; Figure 6). Table 8 shows the average of observed species and Shannon index values of bacterial communities found in fecal matter and water samples in Trial A. In Trial B, the same analysis was done in both fecal matter and

Table 6. Relative percentage of bacteria families identified in the fecal matter of tilapia (*Oreochromis niloticus)* juveniles cultured in a biofloc system over a 109-day production period, stocked with 120 fish tank^{-1} with mean initial weight 71.43 ± 4.44g while offered a commercial feed top-coated with BiOWISH® Feedbuilder Syn3 and AP193, compared to the commercial diet. Relative frequency of bacterial families identified with the most frequency from each treatment.

Treatment	Rank	Phylum	Composition %
Fecal matter			
Commercial	1	Fusobacteria	80.09
	2	Actinobacteria	7.41
AP193	1	Fusobacteria	39.32
	2	Actinobacteria	27.50
	3	Cyanobacteria	11.82
BiOWISH	1	Actinobacteria	41.29
	2	Firmicutes	11.58
	3	Proteobacteria	6.42
Water sample			
Commercial	1	Actinobacteria	25.33
	2	Proteobacteria	24.93
	3	Bacteroidetes	12.43
AP193	1	Actinobacteria	52.27
	2	Proteobacteria	25.80
	3	Firmicutes	5.13
BiOWISH	1	Actinobacteria	30.13
	2	Proteobacteria	27.97
	3	Cyanobacteria	15.23

Table 7. Relative percentage of bacteria families identified in the fecal matter of tilapia (*Oreochromis niloticus)* juveniles cultured in a biofloc system over a 90-day production period, stocked with 200 fish tank^{-1} with mean initial weight 5.34 ± 0.42g while offered a commercial feed top coated with two different concentrations of BiOWISH® Feedbuilder Syn3 compared to the commercial diet. Relative frequency of bacterial families identified with most frequency from each treatment.

Treatment	Rank	Phylum	Composition %
Fecal matter			
Commercial	1	Actinobacteria	47.00
	2	Cyanobacteria	13.63
	3	Firmicutes	12.30
BiOWISH x1	1	Actinobacteria	46.29
	2	Cyanobacteria	19.55
	3	Firmicutes	11.41
BiOWISH x2	1	Actinobacteria	46.65
	2	Cyanobacteria	21.61
	3	Firmicutes	15.33
Water sample			
Commercial	1	Actinobacteria	26.65
	2	Chloroflexi	19.45
	3	Proteobacteria	19.40
BiOWISH x1	1	Actinobacteria	32.57
	2	Proteobacteria	19.17
	3	Chloroflexi	11.00
BiOWISH x2	1	Actinobacteria	27.93
	2	Proteobacteria	21.07
	3	Chlorofelxi	19.40

Table 8. Average of observed number of species and Shannon diversity index of bacterial communities identified in fecal matter and water for tilapia (*O. niloticus)* over a 109-day production period, stocked with 120 fish tank^{-1} with mean initial weight 71.43 ± 4.44g, while offered a commercial feed top coated with BiOWISH® Feedbuilder Syn3 and AP193, compared to the commercial diet.

Treatment	Shannon	Observed number of species
Fecal matter		
AP193	3.19	70.53
BiOWISH	3.36	58.39
Commercial	1.82	34.19
Water samples		
AP193	4.70	141.72
BiOWISH	5.12	173.87
Commercial	5.28	216.93

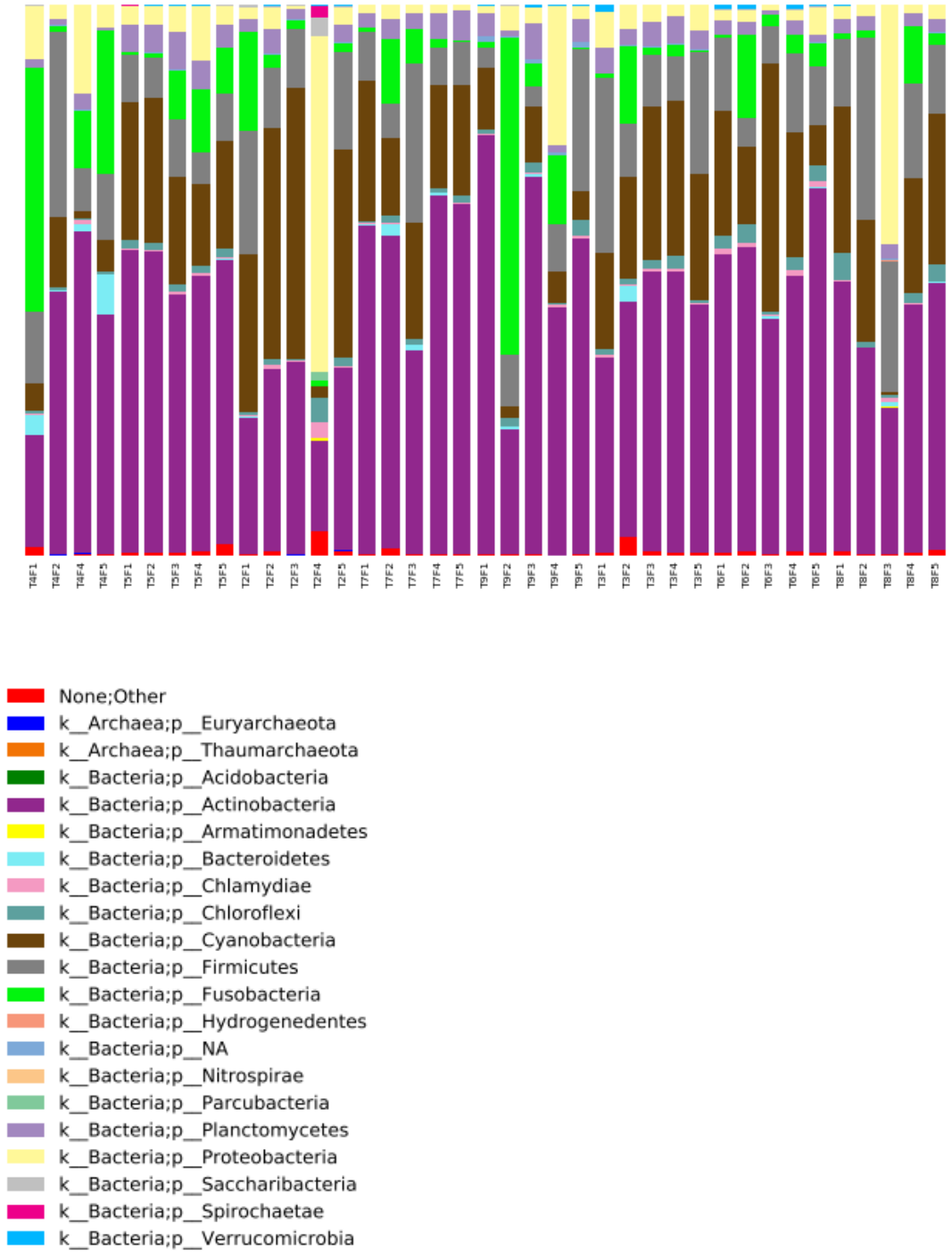

Figure 2. Relative abundance of bacterial phyla presents in fecal matter of fish in different treatments for tilapia (*O. niloticus)* over a 90-day production period, stocked with 200 fish tank^{-1} with mean initial weight 5.34 ± 0.42g, while offered a commercial feed top coated with two concentrations of BiOWISH® Feedbuilder Syn3, compared to a commercial diet.

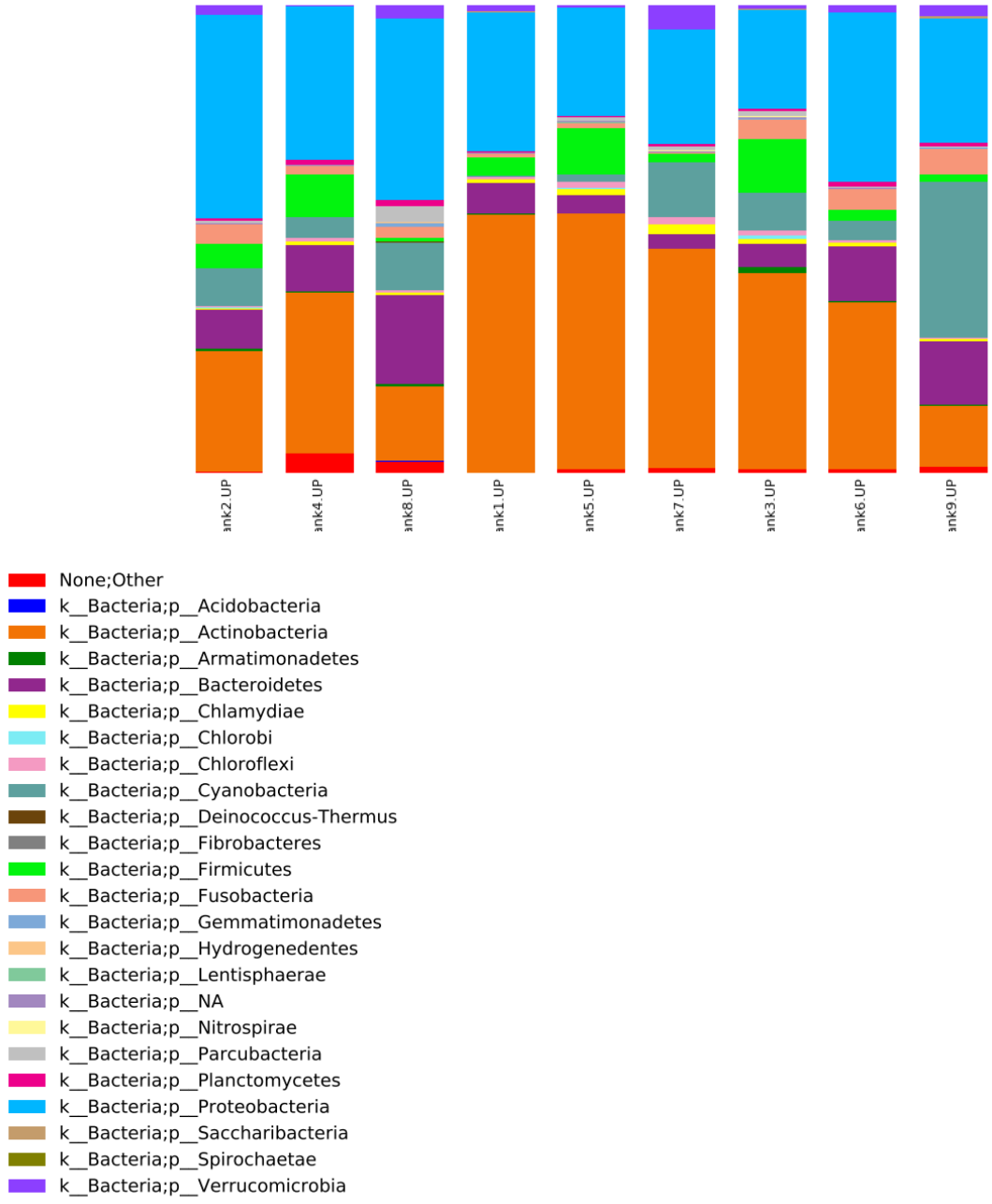

Figure 3. Relative abundance of bacterial phyla presents in water samples of different treatments for tilapia (*O. niloticus)* cultured over a 109-day production period, stocked with120 fish tank^{-1} with mean initial weight 71.43 ± 4.44g, while offered a commercial feed top coated with BiOWISH® Feedbuilder Syn3 and AP193, compared to a commercial diet.

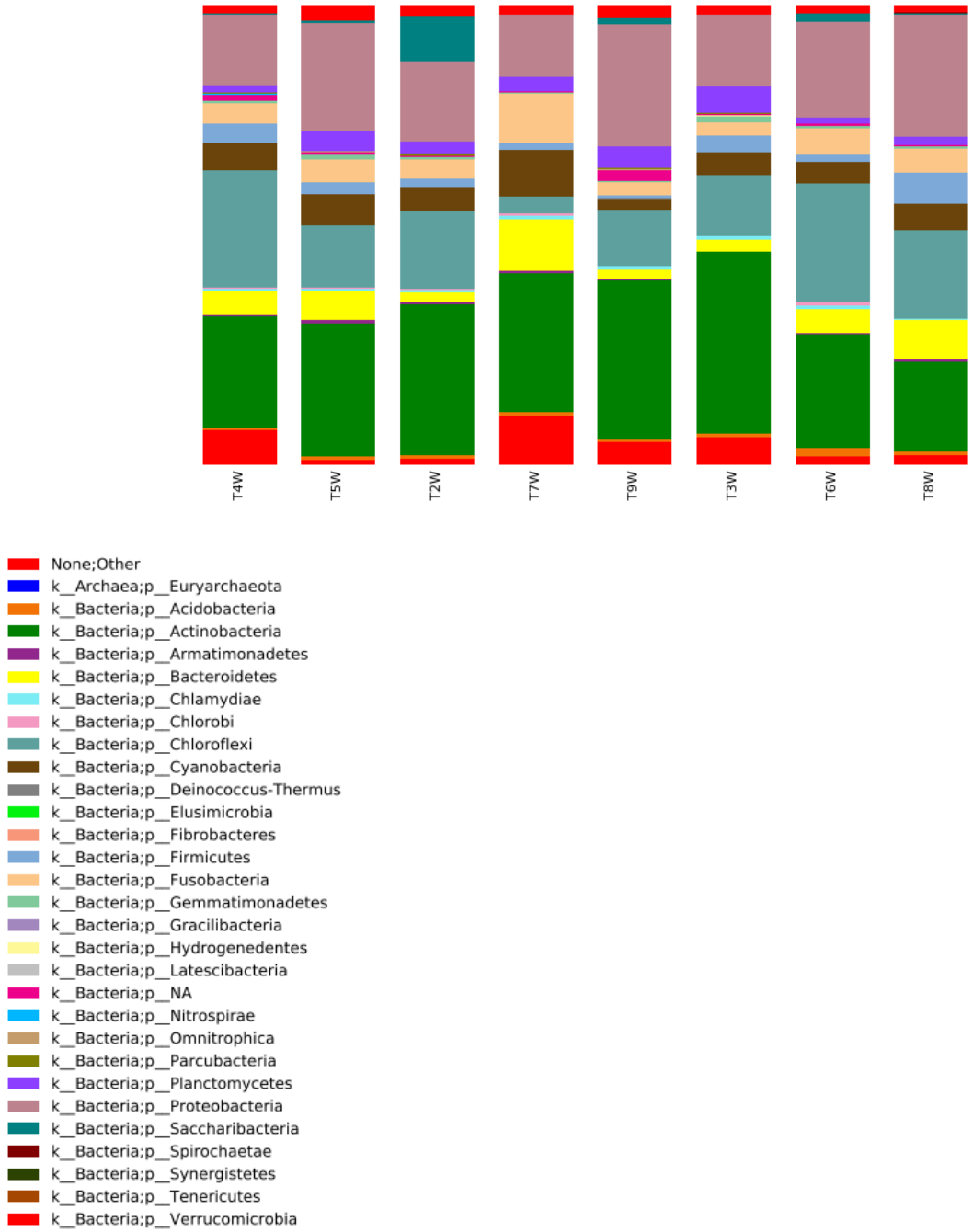

Figure 4. Relative abundance of bacterial phyla presents in water samples of different treatments for tilapia (*O. niloticus)* over a 90-day production period, stocked with 200 fish tank^{-1} with mean initial weight 5.34 ± 0.42g, while offered a commercial feed top coated with two different concentrations of BiOWISH® Feedbuilder Syn3, compared to a commercial diet.

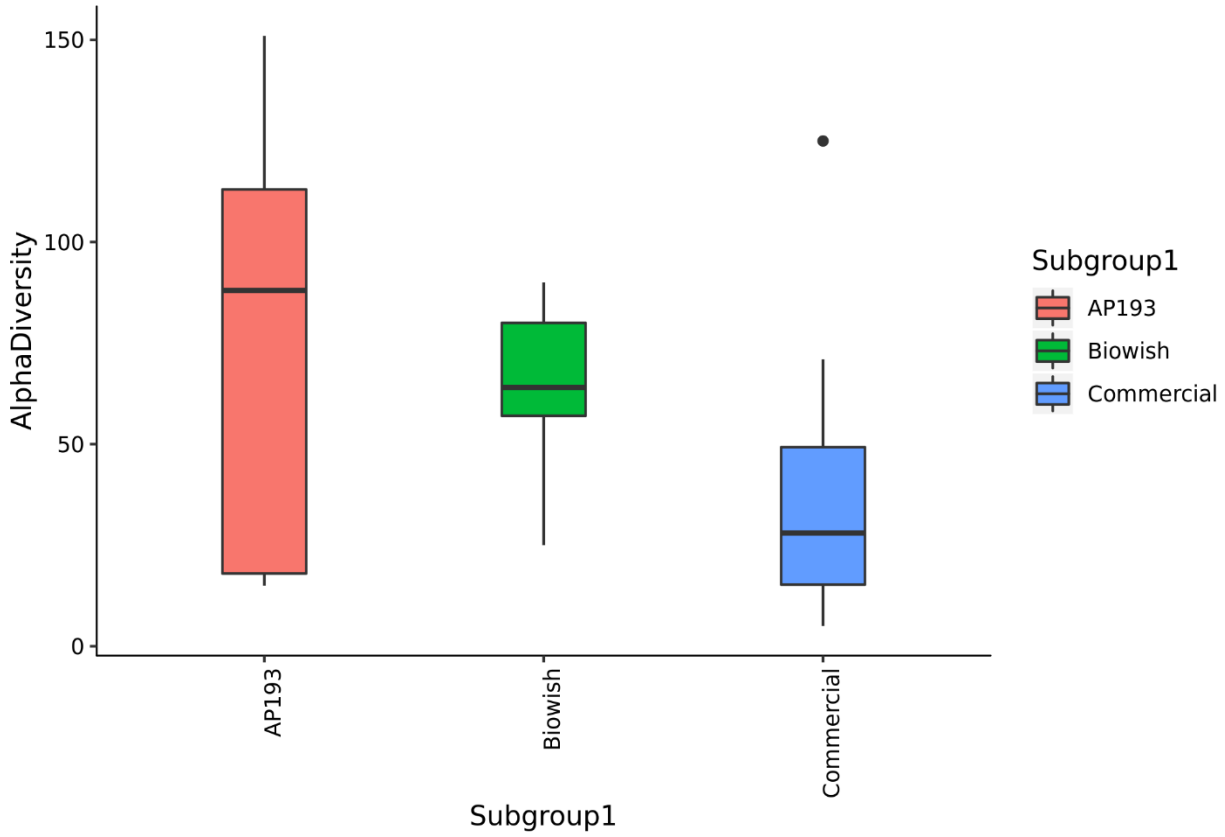

Figure 5. Alpha diversity index of observed species bacterial communities in fecal matter of tilapia (*O. niloticus)* over a 109-day production period, stocked with 120 fish tank^{-1} with mean initial weight 71.43 ± 4.44g, while offered a commercial feed top coated with BiOWISH® Feedbuilder Syn3 and AP193, compared to a commercial diet.

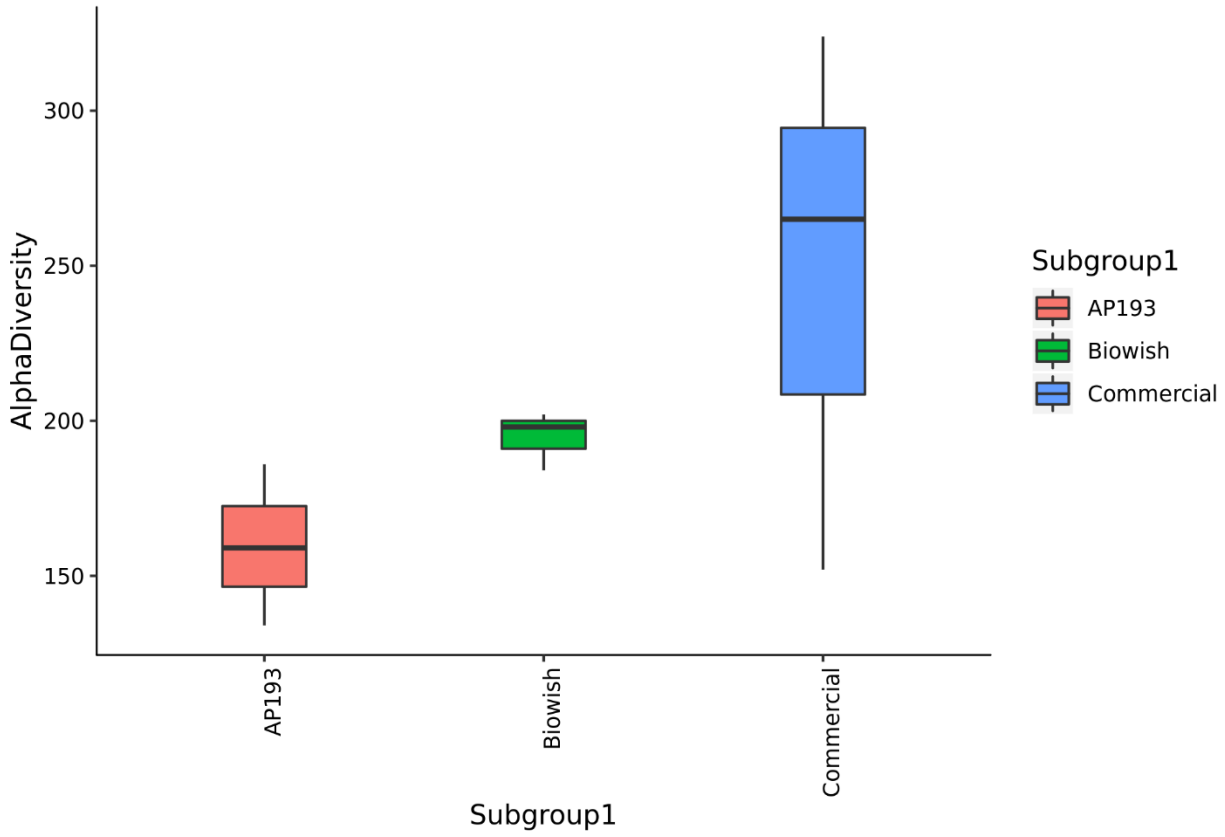

Figure 6. Alpha diversity index of bacterial communities present in water samples of tilapia (*O. niloticus)* over a 109-day production period, stocked with 120 fish tank^{-1} with mean initial weight 71.43 ± 4.44g, while offered a commercial feed top coated with BiOWISH® Feedbuilder Syn3 and AP193, compared to a commercial diet.

water samples. When the observed species were assessed for bacterial alpha diversity of fecal matter, the BiOWISHx2 treatment group contained the highest average, and the lowest was found in BiOWISHx1. The alpha diversity of BiOWISHx1 group was significantly lower than the other two groups (P=0.020; Figure 7). When comparing the Shannon index values, the highest was observed in the commercial feed-treated group, and the lowest was found in the BiOWISHx1, but there were no significant differences ($P= 0.171;$ figure 8). The average of observed species and Shannon index for fecal matter and water samples in Trial B is shown in Table 9.

Table 9. Average of observed number of species and Shannon diversity index of bacterial communities identified in fecal matter and water for tilapia (*O. niloticus)* over a 90-day production period, stocked with 200 fish tank^{-1} with mean initial weight 5.34 ± 0.42g, while offered a commercial feed top coated with two concentrations of BiOWISH® Feedbuilder Syn3 compared to the commercial diet.

Treatment	Shannon	Observed number of species
Fecal matter		
BiOWISH x1	18.16	180.11
BiOWISH x2	20.34	228.63
Commercial	18.59	227.73
Water samples		
BiOWISH x1	5.66	362.26
BiOWISH x2	5.64	404.87
Commercial	5.77	429.25

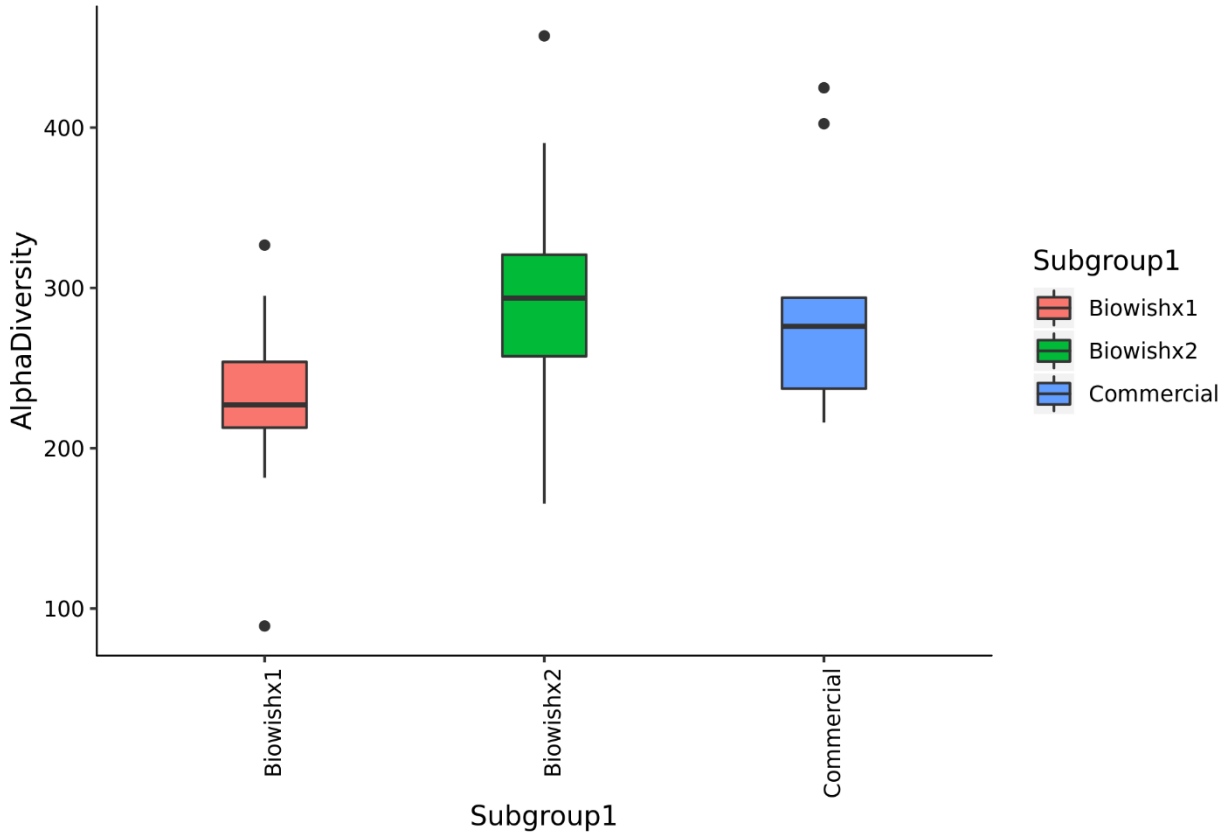

Figure 7. Alpha diversity index of bacterial communities in fecal matter of tilapia (*O. niloticus)* over a 90-day production period, stocked with 200 fish tank^{-1} with mean initial weight 5.34 ± 0.42g, while offered a commercial feed top coated with two different concentrations of BiOWISH® Feedbuilder Syn3, compared to a commercial diet.

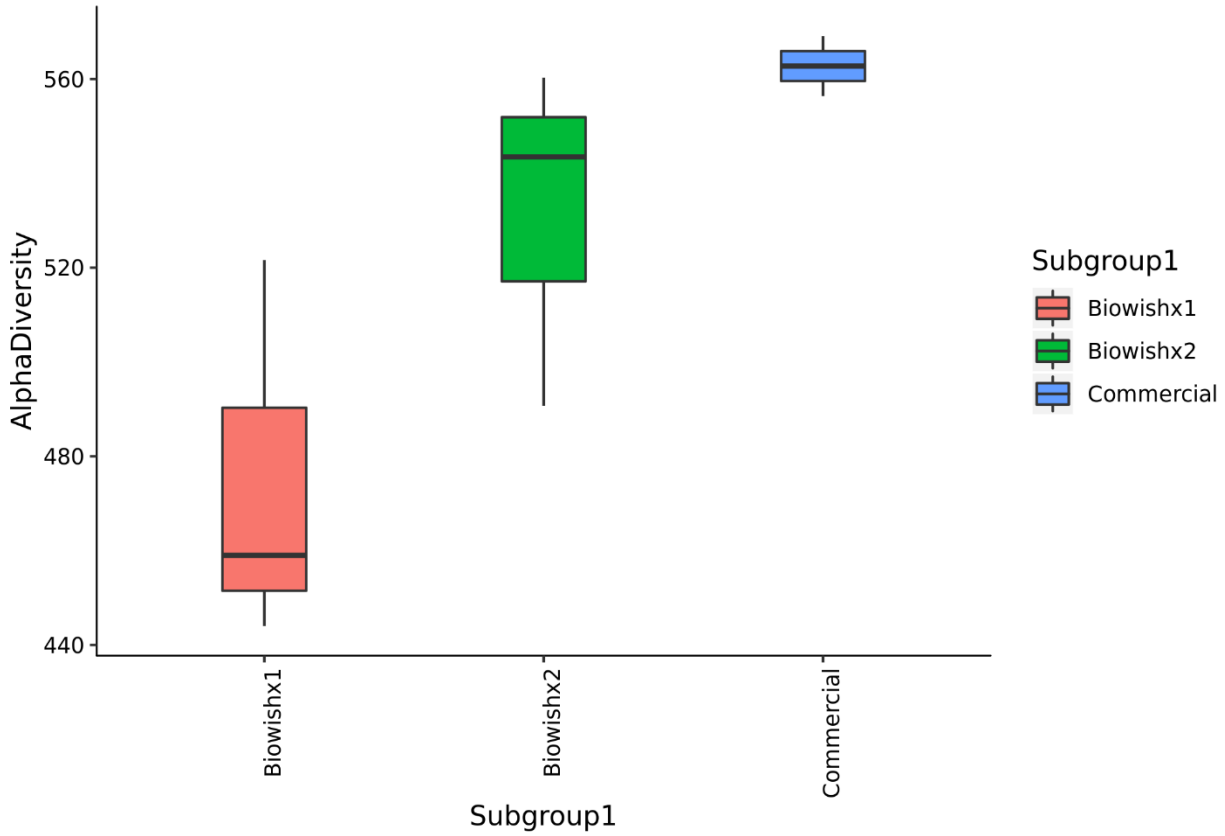

Figure 8. Alpha diversity index of bacterial communities in water samples of tilapia (*O. niloticus)* over a 90-day production period, stocked with 200 fish tank^{-1} with mean initial weight 5.34 ± 0.42g, while offered a commercial feed top coated with two different concentrations of BiOWISH® Feedbuilder Syn3, compared to a commercial diet.

4. Discussion

Biofloc production systems are a breakthrough technology, with a significant impact and contribution to environment-friendly growth of aquaculture. This technology has been considered for tilapia culture due to the limitations of natural environments and the demands to increase productivity through high stocking densities (Khanjani et al. 2022). Studies show that the growth of tilapia in BFT can potentially add to the nutritional intake of the fish, when compared to more traditional culture methods (Nguyen et al. 2021). Thus, the combination of biofloc systems along with probiotics may be a viable method to improve fish culture and decrease disease outbreaks (Zabidi et al. 2021).

Different studies have indicated that using probiotics resulted in increased growth performance, immune response, and improved water quality in aquaculture production systems. However, the use of *Bacillus subtilis* and *B. velezensis* in both trials in this study did not lead to improved growth performance of tilapia under the reported experimental conditions. This is consistent with a study Nguyen et al. (2022), which examined feed top coated with AP193 (*B. velezensis)* and BiOWISH (*B. subtilis)* in juvenile channel catfish (*Ictalurus punctalus*) in an indoor flow through system (pond water). These authors found no significant difference in the growth performance of catfish after a six-week feed trial. The second experiment consisted of an 8-week growth trial, in a flow through setting, using three different concentrations of BiOWISH top coated on commercial diets offered to channel catfish fingerlings. Under these experimental conditions, the data presented the same outcome as the first trial (Nguyen et al. 2022). However, in Trial B of our study, an improvement in FCR was observed for both concentrations of BiOWISH® Feedbuilder Syn3, compared to a commercial diet. Abarike et al. (2018), conducted a growth trial with Nile tilapia at Guangdong Ocean University - Zhanjiang, China. The animals

were offered a combination of *Bacillus subtilis* and *Bacillus licheniformis* (1:1 ratio), with different dose concentrations, for 4 weeks, in a closed system with 30% water exchange daily. At the end of the trial, an improvement was observed in all probiotic treatments for FCR (feed conversion rate), final weight, and weight gain.

Al-Deriny et al. (2020) conducted an aquarium study in with tilapia at Kafrelsheik University, Egypt. In this trial, *Spirulina plantensis* and *Bacillus amyloliquefaciens* were offered to tilapia for 60 days, as well as a combination of both probiotics. These authors found that weight gain was higher in fish fed with *Spirulina* and *Bacillus* or individually. Likewise, Thurlow et al. (2019) carried out a ten-week feeding trial with channel catfish in a flow-through in-pond raceway system, fed with a commercial feed top-coated with *Bacillus velezensis*l. In both aquaria and raceways systems, improvement in growth performance with the probiotic treatment was demonstrated in terms of weight gain. Our results do not align with what was reported in those studies, as no significant difference between probiotic supplementation and the commercial diet was noted. The results observed in growth performance parameters, such as weight gain (%), FCR, and survival (%), nevertheless showed promising results.

The manner in which probiotics can help improve growth performance, also can influence fish body composition by dietary supplementation. According to whole-body composition results of Trial A, significant differences were observed when tilapia were fed a commercial feed top coated with *Bacillus velezensis* (Table 4). A 7-month growth trial with Nile tilapia was conducted at Karatina University - Nairobi, Kenya. The experiment was carried out in 4 earthen ponds, with 7 cages each, with each pond serving as a different treatment. Two different probiotics were offered to the tilapia, including *Saccharomyces cerevisiae*, and *Bacillus subtilis*. The results demonstrated that different levels of *B. subtilis* supplementation presented significant results in

protein, moisture, and ash (Opiyo et al. 2019). In Trial B, different levels of BiOWISH concentration revealed no significant results in whole-body composition. Protein retention in the current study was lower, albeit not statistically significant. Reda and Selim (2015), at Zagazig University - Sharkia, Egypt, performed a 60-day aquarium growth trial with tilapia (*Oreochromis niloticus)*, with 25% water exchange daily. The animals were offered a diet comprised of 39% protein and 10.89% fat and supplemented with *Bacillus amyliquefaciens.* Probiotic supplementation also revealed no significant differences in moisture and ash content and presented lower protein retention (39.4-43.6%).

In aquaculture systems, high stocking densities can be a problem for production due to the concentration of nitrogenous waste products in the system. However, in biofloc systems, bacteria in the floc help control ammonia throughout the nitrification process, algal uptake, and bacterial assimilation. These interactions between bacteria and algae in the biofloc system are complex (Hargreaves, 2013). Using probiotics to help manage water quality, improve feed utilization, and inhibit potential pathogenic microorganisms is a widespread but complex process (Olmos et al., 2011; Yang et al., 2011; Selim and Reda, 2015; Li et al., 2022). The current study showed no significant differences in either trial for most water quality parameters examined. It is worth noting that the concentration of total ammonia nitrogen in Trial A peaked in all treatments during the 7th week to the 11th week, which occurred in parallel with an increase in feed allotment. El-Kady et al. (2022) tested three different commercial probiotics. AquaStar® (a mix of *Bacillus sp., Pediococcus Sp.)*, EM® (a blend of *Rhodopseudomonas spp., Lactobacillus spp., and Saccharomyces spp.),* and MicroPan® Complex (a union of *Bacillus spp.*, and enzymes), administrated through the water body. The study was performed in concrete ponds under natural environment conditions, with Nile tilapia fingerlings. After 60 days, an improvement in water

quality was found due to decreased concentrations of total ammonia nitrogen in the system. The decrease of nitrogen compounds in the rearing water contributed to improved growth performance of the cultured fish.

Not only can probiotics influence water quality, but they also have the potential to degrade organic waste and reduce total solids buildup within the production system. Hence, we also examined discharged solids or effluent. A possible burden of the biofloc system design is the accumulation of solids, which can be harmful to cultured fish and lead to deterioration of water quality, which in turn decreases cultured species and production system performance (Gaona et al. 2011; Schveitzer et al. 2013; Chen et al. 2020; Ekasari et al. 2023). For both trials in the current study, probiotics in the system showed no significant difference in total solids (g) compared to the control treatment. However, there was a higher level of discharged solids in Trial B. Several factors influence waste produced in an intensive system production system such as biofloc. The concentrations of phosphorus and nitrogen compounds, due to excretion, microbe metabolism, and uneaten feed, can eventually present a problem when high levels are reached in the system (Debbarma et al. 2022). Feed is the main source of waste, and the nutrient content, quality, and quantity of feed will impact waste produced from dietary sources (Dauda et al. 2019). Hence, feeding protocol and quantity of feed inputs directly impact the concentration of solids or waste produced. The concentration of solids produced per kg of fish or feed was not significantly different between treatments in either trial. In our work, each trial was initiated with different-sized fish (Trial A: 71.43 ± 4.44g; Trial B: 5.34 ± 0.42g), and different seasons (Trial A was conducted in summer and Trial B in winter). We observed a trend between the amount of feed input and waste production, as the higher feed inputs resulted in higher nutrient loads, poorer water quality, and higher amounts of solids removed from the biofloc system. Effluent management

helps control water quality, and can be optimized to remain within a range most suited for the specific species cultured in the biofloc system. The goal is to maintain levels of suspended solids under the maximum level recommended (Zemor et al. 2019).

In aquatic environments, microorganisms play a crucial role in trophic networks. They facilitate nutrient recirculation and interact with various organisms, making them an integral part of the ecosystem. To comprehend their specific niche and function, it is vital to identify and quantify community members. Metagenomics techniques have become an essential tool for studying non-cultured microorganism communities (Vieites et al. 2008). The findings of the metagenomic analysis conducted in this study demonstrated the microbial community associated with biofloc and when probiotics were added as dietary supplements for the tilapia grown in biofloc systems. In Trial A, Fusobacteria and Actinobacteria were the most abundant taxa within the phyla detected in fish fecal matter (Table 6, Figure 1). These results were similar in the treatment and control groups, indicating that they are typically present in aquatic environments. Apart from this, firmicutes were also the dominating phyla in BiOWISH-treated fish (Table 6). Fusobacteria, the most dominant phyla in the control group, is a group of anaerobic bacteria commonly found in aquatic environments such as biofloc systems. Fusobacteria mainly consisted of *Cetobacterium* spp. in this study. This genus is an obligate gut associate and contributes to fish health, producing vitamin B12 (Tsuchiya et al. 2007). Actinobacteria was the most dominant phyla in probiotic-treated groups and fecal matter of all treatment groups in Trial B (Table 7, Figure 2).

According to the results of (Kathia et al. 2018) and (Abakari et al. 2021), Actinobacteria is the most abundant bacterial species in tilapia raised in biofloc systems. Actinobacteria are typically Gram-positive bacteria and live off of decaying organic matter. They are known for their ability to form extensive networks of thread-like structures called mycelia. In addition to their important

role in nutrient cycling, Actinobacteria are also well-known for their ability to produce a wide variety of antibiotics. The production of antibiotics is thought to be one way that Actinobacteria help to maintain the balance of microbial communities in aquatic ecosystems. By producing these compounds, they can inhibit the growth of harmful bacteria and protect the health of aquatic organisms (Goodfellow and Fiedler, 2010). Apart from this, Actinobacteria help promote floc formation, and their biomass can serve a nutritive function (Liu et al. 2019). Proteobacteria was the second highest prevalent bacterial phyla in water samples across all treatments in Trial B. This phylum has been reported in different studies as it is ubiquitous in aquatic environments (Cardona et al. 2016; Meenakshisundaram et al. 2021). This phylum is also important in nutrient recycling and mineralization of organic matter (Kathia et al. 2018).

One of the primary goals of this study was to examine how the addition of probiotics affected the microbial community in biofloc systems used for tilapia culture. Results revealed that the addition of probiotics did not significantly alter the existing microbial community, though they were more abundant in probiotic-treated groups, nor did they dominate the biofloc system. This could be due to the addition of probiotics as dietary supplements as opposed to addition directly to the culture water. The relative abundance of *Bacillus* spp., which was a component of the two probiotics added, was low compared to other phyla. It should be noted that when utilizing a biofloc culture system, the conditions for producing aquatic organisms are quite different from those of conventional systems. Biofloc systems promote the growth of a diverse range of heterotrophic bacteria through an external carbon source and aeration supply. This can benefit the cultivated species and enable the growth of the most adaptable bacteria to this unique set of culture conditions (Wilen et al. 2008; Ray and Lotz 2014). Some authors have also mentioned similar results upon the addition of probiotics containing *Bacillus spp*. and *Lactobacillus ap*. in biofloc systems which

reared Pacific white shrimp (*Litopenaues vannamei*) and Nile tilapia (de Paiva Maia et al. 2016; Kathia et al. 2018).

The analyzed diversity index in the current work indicates that bacterial diversity (Shannon index) in the fecal matter of fish treated with AP193 was 3.19, and BiOWISH was 3.36. These values were much higher compared to another previous study carried out by Cardona et al. (2016), which reared *Litopenaeus stylirostris* in a biofloc production system. The alpha diversity indices (Shannon and observed species) among the treatments in fecal matter and water samples showed no significant differences in trial A. However, in trial B one of the alpha diversity index (observed species) in BiOWiSHx1 showed significantly lower bacterial diversity than the other two treatments. According to Borges et al. (2021), when the probiotic concentration increases there can be an imbalance in the microbial composition in the guts of fish. This could be the reason why BiOWiSHx2 and commercial diet fed fish had a similar number of observed species compared to BiWiSHx1. The lower observed species in BiOWiSHx1 might be because of the addition of probiotics altering the bacterial diversity, resulting in only a few dominant species. A significant difference was only seen in terms of observed species and not in the Shannon diversity index. So, more studies evaluating bacterial diversity composition on different probiotic dosages should be performed for more precise results. It is important to mention that certain types of bacteria reside in the gut microbiota, and when exposed to water that is rich in carbohydrates, they can proliferate and grow significantly (Rurangwa et al. 2009). This growth occurs without the need for additional external sources of probiotics or other supplements and is instead fueled by the influx of carbon from carbohydrate-rich water. This could be why there were no significant differences in growth performance of tilapia between the probiotic and non-probiotic-treated groups (Dosta et al. 2015).

5. Conclusion

Tilapia production in biofloc production systems is an established and growing culture method used by commercial producers in many regions worldwide. Despite the widespread culture of tilapia using the biofloc production system, little information is available on the efficacy of dietary supplementation of probiotics on growth performance of this species. Based on the observed findings of this and other studies, it can be quite challenging to predict the effects of supplementary probiotics on cultured aquatic species within a biofloc system. To further complicate matters, variability in production systems, husbandry techniques, and management can make comparisons between studies problematics. According to the findings of Trial B, the bacterial composition of fecal matter as indicated by the Shannon index displayed an improvement when administered the recommended concentration of BiOWISH ® Feedbuilder Syn3. However, there were no positive benefits on growth performance of tilapia, water quality, solids management. Thus, large-scale experimentation with various dosages under different production and husbandry conditions is required to establish appropriate protocols for the use of probiotics in biofloc systems to further optimize tilapia production.

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