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Preparation of Fish Silage From Fish Waste Using Lactic Acid Bacteria and Its Effect on Growth of Nile Tilapia (*Oreochromis niloticus*) and Basil (*Ocimum basilicum*) in an Aquaponics System

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ABSTRACT

The use of fish waste to produce silage is a practical approach to decrease its discharge into the environment and obtain a liquid organic fertilizer (LOF) for aquaponics (AP) system. The aim of this study was to investigate the elemental composition of fish silage produced from rainbow trout (*Oncorhynchus mykiss*) waste using two species of lactic acid bacteria (LAB), including *Lactobacillus casei* and *Lactobacillus plantarum* and the impacts of adding the best biological fish silage (BFS) in terms of its elemental contents as a water additive on growth performance of Nile tilapia (*Oreochromis niloticus*) and basil (*Ocimum basilicum*) plant in a coupled AP system. The results showed that that calcium, magnesium, phosphorus, iron, zinc, and manganese concentrations significantly increased during 21 days of microbial fermentation of fish waste in the control and the LAB groups ($p<0.05$). The concentrations of magnesium, iron, manganese, and zinc in the LAB groups were significantly higher than the control ($p<0.05$). Nile tilapia (*O. niloticus*) fish and basil (*O. basilicum*) plant were cultured in two AP systems without and with adding BFS obtained from rainbow trout waste using *L. plantarum*, referred to AP system (control) and AP-BFS, respectively. The results showed that the growth performance parameters of Nile tilapia fish, including the final weight, weight gain rate (WGR), average daily gain (ADG), specific growth rate (SGR), and food conversion ratio (FCR) improved in the AP-BFS system by 12.22%, 23.89%, 20%, 16.26%, and 19.86% compared to the AP group ($p<0.05$). The dry matter and crude protein contents of whole-body fish increased significantly in the AP-BFS system compared to the AP group ($p<0.05$). The morphological and biomass indices of basil plants, including total plant fresh weight, leaf, shoot and root fresh weight, shoot height, root length, and number of leaves, nodes, and buds per plant in the AP-BFS system were significantly higher than the AP group ($p<0.05$). The phosphorus, potassium, and iron contents of plant tissues were significantly higher in the AP-BFS than the AP system ($p<0.05$). Nitrate concentration was significantly lower in the AP-BFS system than the AP system ($p<0.05$). In conclusion, the use of LAB for production of fish silage improved nutrient recovery from rainbow trout waste. Adding BFS to the AP system improved the growth performance of Nile tilapia fish and basil plants.

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1 | Introduction

Food security and water scarcity have emerged as two critical challenges in the world in recent years [1]. It is estimated that food production will need to increase by 50% by 2050 to meet the food requirements of an additional 2 billion people [2, 3]. Sustainable food production is considered as an alternative approach to minimize the loss of water and nutrients required for agriculture production [4, 5]. Aquaponics (AP), a system that integrates recirculating aquaculture system (RAS) and hydroponics is an innovative approach to meet the demand for sustainable food production [6]. AP, a land-based system is independent of natural water and soil [7]. It can allow simultaneous production of fish and fresh vegetables in places that are not suitable for agriculture such as urban centers [8].

AP system consists of fish, microorganisms, and plants and their interrelations are very complex [9, 10]). In an AP, nutrients needed by plants are provided through fish feed and water. Fish feed is a significant source of nitrogen, potassium, phosphorous, iron, zinc, and manganese and tap water contains elements such as calcium, magnesium, sodium, sulfur, and copper [11]. In this system, total ammonia nitrogen (TAN) generated from fish metabolism, feces and un-consumed feed is transferred to nitrate (NO_3^-) for the plant by nitrifying bacteria through nitrification process in the biofilter [12]. Fish sludge (feces+un-consumed feed) contains many of the nutrients needed by plants, but these nutrients are in solid and insoluble form and plants are unable to uptake them from the water [7]. Various studies have shown that dissolved nutrients in aquaculture effluent are not sufficient for plants in an AP system [13–15]. Maneepong [16] reported that potassium (K) and iron (Fe) concentrations were not sufficient for growth of Water Convolvulus (*Ipomoea aquatica*) and Tokyo Bekana (*Brassica rapa*) plants and these nutrients should be added regularly to water of AP system. Adding mineral nutrients such as phosphorus, calcium, potassium, iron, manganese, and molybdenum to AP, which increase the cost, are required to achieve maximum plant growth [6, 11]. Chemical fertilizers are usually expensive, difficult to access, and often made using environmentally harmful methods [17]. To compensate for nutrient deficiencies in AP systems, replacing chemical-based nutrient solutions with organic fertilizers made out of animal and plant waste materials such as fish waste can be considered as a sustainable approach.

Globally, the total fisheries and aquaculture production, and fish consumption per capita have shown a considerable increase in recent years. As a result, the amount of fish waste also has increased in the world [18]. Fish waste including viscera, bones, scales, and skin make up to 30%–80% of the fish body weight [19]. Fish waste management is of great concern for fish-processing industry from both an environmental and economic point of view [20]. Fish waste offer a good source of valuable nutrients whose composition is reported to be 49.22%–57.92% protein, 7.16%–19.10% lipid, and 21.79%–30.16% ash based on dry matter [21]. Esteban et al. [22] reported that fish waste contained 5.8% Ca, 2.1% P, 0.7% K, 6% Na, and 0.2% Mg, based on dry matter. By-products of fish waste like fish hydrolyzed solution [23], fish waste extract [24], and liquid fish silage (LFS) [25] have used as organic fertilizer for improving the growth and yield of different plants.

Fish silage is a liquid product obtained by the liquefaction of whole fish or a mixture of different parts of fish carried out by the endogenous enzymatic activities and the lower pH value [26, 27]. Biological fish silage (BFS) can be prepared by fermentation process using lactic acid bacteria (LAB) with adding the carbohydrate source to fish waste [28]. The LAB inhibits the growth of spoilage microflora though production of different compounds like organic acids, bacteriocins, and hydrogen peroxide [29]. Fish silage contains valuable nutrients, including peptides, hydrolyzed protein, free amino acids, unsaturated fatty acids, minerals, and vitamins [30] that can be used as a suitable liquid organic fertilizer (LOF). Karim et al. [25] reported that LFS made by fermentation of fish waste contained 1.84% N, 0.41% K, 0.50% P, 0.84% Ca, and 0.36% Mg, and use of the LFS at 5.0%–10.0% concentration produced similar yields and quality of pakchoy (*Brassica rapa* L. subsp. *chinensis*) as plant fertilized by commercial fertilizer.

However, limited research has been conducted on impact of different species of LAB on elemental composition of fish silage, and also usage of silage as a LOF in AP systems. Therefore, in the present study, fish silage was produced through fermentation process using two species of LAB. Then, the impacts of adding the best BFS in terms of its elemental contents as a water additive on growth performance of Nile tilapia (*Oreochromis niloticus*) and basil (*Ocimum basilicum*) plant in a coupled AP system were investigated. According to the results of the first experiment, the *L. plantarum* was chosen to produce the BFS for using in the AP systems, because the iron content of the BFS produced with *L. plantarum* was significantly higher than that produced with *L. casei*. The concentration of other elements was same in the BFS produced with both LAB. In the present study, rainbow trout (*Oncorhynchus mykiss*) waste was used to produce silage because this fish is the most important aquaculture species in Iran, and more than 25% of its global production is carried out in this country [31].

2 | Materials and Methods

2.1 | First Experiment

2.1.1 | Proximate and Elemental Analysis of Fish Waste

In this study, 20 rainbow trout (*O. mykiss*) fish with average weight of 1.3 ± 0.34 kg were obtained from a rainbow trout farm (Mashhad, Iran) and transferred to laboratory on ice. The body surface of fish was rinsed with clean water, the scales were removed, and the fish were gutted and filleted. The head, bones, viscera, and skin were used as waste. The fish waste was ground several times using a meat grinder until it became a uniform paste, and stored at −20°C for use in the first and second experiments. A small amount of the paste was dried at 50°C for 72 h in an oven to measure its dry matter, ash, and elemental contents. The ash content was determined through placing the dried samples in a muffle furnace (Exciton Co., 1200°C model, Iran) at 550°C for 6 h [32]. The elemental contents (Ca, Mg, Na, K, P, Fe, Zn, Mn, and Cu) of digested samples were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro Acros, Germany). To digest sample, the dried sample (0.5 g) was mixed with HNO_3 (3 mL), HCL (9 mL), and H_2O_2 (2 mL) in a 100 mL glass volumetric flask. The solution was kept

at room temperature for 24 h and then heated at 95°C for 2 h. The digested sample was filtered using a 0.45 μm filter paper (Cellulose Acetate Membrane Filter; MFCA047045) and diluted to 25 mL using ultra-pure water (Milli-Q water).

2.1.2 | Production of BFS

In this study, the effects of two species of LAB, including *Lactobacillus plantarum* (PTTC 1896) and *Lactobacillus casei* (PTTC 1608) on the quality of biological silage were investigated from point of view of its elemental content. The experimental groups were as follows: (1) Biological silage without adding LAB (Control), (2) biological silage with adding *Lactobacillus plantarum*, and (3) biological silage with adding *Lactobacillus casei*.

The LAB were purchased as lyophilized ampoules from Persian Type Culture Collection (PTCC; Tehran, Iran). The *L. Plantarum* and *L. casei* were cultured separately in MRS broth (Merck, Germany) and incubated at 37°C and 30°C for 72 h in an incubator, respectively. Then, the bacteria were harvested by centrifugation (7000 rpm) at room temperature for 10 min and washing three times with phosphate-buffered saline (PSB). The bacterial suspension was diluted 1:40 using phosphate buffer. The colony-forming unit (CFU/mL) of the diluted bacteria was determined based on McFarland standards-based spectrophotometry method using a UV–Vis spectrophotometry (HACH DR-4000, USA) at wavelength of 500 nm. An absorption of approximately of 0.4 corresponded to 4 McFarland standard (12×10^8 CFU/mL) [33]. Each of the LAB was used to prepare biological silage at a count of 1.2×10^9 CFU/mL.

To produce biological silage, nine glass vessels with a capacity of 1 L were prepared and filled with 500 g of paste-like fish waste. Then, sugarcane molasses as carbohydrate source (15% w/w), distilled water (30% v/w), and butylated hydroxyltoluene (BHT; 39 mg) as antioxidant were added to paste-like fish waste in each glass vessel. The glass vessels were kept in an autoclave (121°C) for 20 min to be sterilized. After cooling and reducing the temperature of the vessels to room temperature, the *lactobacillus* bacterium (5% v/w) was added to paste-like fish waste in each glass vessel in each vessel. The vessels without adding the LAB were considered as control. Then, the lids of the vessels were tightly closed and kept in a shaker incubator (180 rpm) at 30°C for 21 days under anaerobic condition. The mixture of each vessel was stirred three times daily with a sterilized glass rod. The temperature and pH of vessels were measured daily using a portable multi-meter (AZ-8603 model). This experiment was performed in three replications.

A sample of the mixture was taken from each vessel and centrifuged at 10,000 rpm for 20 min. The supernatant oil of each sample was removed and the remaining liquid was used for further analysis. The electrical conductivity (EC) of obtained liquid was measured using a conductivity meter (JENWAY 4510) every week. The dry matter, ash, and elemental contents of obtained liquid were also determined at the beginning and end of the experiment based on the method described in section 2.1.1.

2.2 | Second Experiment

2.2.1 | Experimental Groups

Six pilot-scale AP systems were designed and installed to evaluate the impacts of BFS as a water additive on growth performance of Nile tilapia (*O. niloticus*) and basil (*O. basilicum*) plant, and water quality in an 8-week experiment. Two experimental groups in triplicate included: (1) AP system without adding fish silage, referred to AP (control) and (2) AP system with adding BFS, referred to AP-BFS.

2.2.2 | AP System Design

The AP systems were installed in a greenhouse at Ferdowsi University of Mashhad (Mashhad City, Iran). The components of an AP system shown in Figure 1 are as follows: (1) Rectangular polyethylene fish tank (100 cm × 50 cm × 30 cm) filled with 120 L of dechlorinated tap water. (2) Radial flow settler (RFS; 50 cm × 38 cm; 50 L) filled with sponge filter foam and aquarium filter pad. (3) Moving bed bioreactor (MBBR; 50 cm × 40 cm; 60 L) 50% filled with plastic media (25 mm × 12 mm; 1000 m^2/m^3). (4) Three PVC channels (120 cm × 9 cm) with seven holes each one as hydroponic

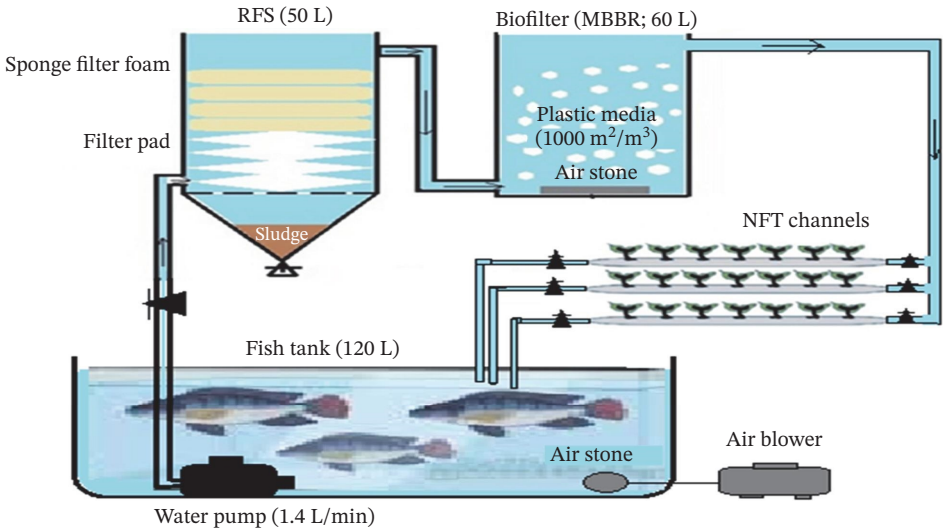


FIGURE 1 | Schematic figure of the coupled aquaponics system for cultivation of Nile tilapia (*Oreochromis niloticus*) fish and basil (*Ocimum basilicum*) plant. RFS, radial flow settler; MBBR, moving bed bioreactor; NFT, nutrient film technique.

unit. (5) Twenty-one hydroponic net pots to culture plants. (6) Water submersible pump (Huangjunda, China) to circulate water. (7) Water pipes. (8) Central air blower (LD 004 H21 R12 model, China). (9) Aeration tubes and air stones. (10) Controlled valves.

In each AP system, the water from the bottom of fish tank was pumped (1.4 L/min) to the bottom of the RFS using a submersible pump. Some of particles of un-consumed feed and feces settled at the bottom of the RFS, while others were trapped between the filter pad and sponge filter foam. The supernatant water flowed from the RFS to the MBBR via the upper outlet. The plastic media in the MBBR were suspended by aeration and water flow from the bottom. The hydroponic unit was the nutrient film technique (NFT) and the treated water flowed from the MBBR to the three parallel PVC channels by gravity. The water flow in each channel was controlled via a valve. Finally, the water returned to the fish tank by gravity.

2.2.3 | Preparation of BFS

In the second experiment, the fish silage was added to the water of three AP systems. According to the results of the first experiment, the *L. plantarum* was chosen to produce fish silage using rainbow trout (*O. mykiss*) waste in a pilot-scale setting for 21 days in triplicate. In this order, three vessels with capacity of 10 L were placed inside a polyethylene tank (1 m × 0.5 m × 0.4 m) filled with water and equipped with a 300 W aquarium heater as a water bath. Each vessel, filled with 3.5 kg of paste-like fish waste, 1 L of distilled water (30% v/w), 525 g of sugarcane molasses (15% w/w), and BFT (455 mg; 0.01%) as antioxidant. The distilled water, fish waste, and molasses were sterilized at 121°C for 20 min using an autoclave before adding to the vessels. Then, 175 mL of the *L. plantarum* (PTTC 1896) with density of 1.2×10^9 CFU/mL was added to each vessel and the lids of the vessels were tightly closed. The temperature of vessels was set at 30°C during the 21-day experiment. The mixture of each vessel was stirred three times daily with a sterilized glass rod. The pH of the mixture was monitored every day using a portable multi-meter (Water Proof Meter 7200 model). Samples were collected from each vessel at days 0, 7, 14, and 21 of the experiment to measure the elemental content of the silage based on the method described in section 2.1.1. The crude protein and crude lipid contents of silage were also measured based on the Kjeldahl method (Nitrogen content × 6.25) and the Soxhlet method, respectively [32]. The prepared silage was kept at 4°C until adding to the AP systems.

2.2.4 | Fish and Plant Culture

In this study, 480 fingerling Nile tilapia (*O. niloticus*) fish with initial weight of 5–8 g were purchased from a tilapia farm (Mashhad City, Razavi Khorasan province, Iran). The fish were kept in two 500-L intex tanks for 2 weeks before stocking in the AP system. The fish were fed on extruded tilapia feed (Faradaneh Aquafeed Manufacturing Co., Iran) (Table 1) with pellet size of 2.0 ± 0.2 mm three times a day at a rate of 4% body weight. Simultaneously, the six AP systems were filled with tap water and launched. To accelerate the growth of chemoautotrophic bacteria involved in nitrification process in the MBBR, each of ammonium chloride (NH_4Cl) and sodium nitrite ($NaNO_2$) salts was added to water once every 3 days at a concentration of 5 mg/L [34] for 2 weeks. To start the experiment, 80 fish with average weight of 9.13 ± 0.41 g were randomly stocked in the fish tank of each AP system. The fish were fed with commercial tilapia feed three times a day (8:00; 12:00, and 16:00) at a rate of 4% of the body weight for 8 weeks. After 2 weeks, 126 seedlings of basil (*O. basilicum*) plant with an average weight of 0.89 ± 0.12 g were transplanted in the Net Pots filled with perlite–cocopeat mixture (70:30). Then, twenty-one net pots were placed inside the holes of NFT channels of each AP system, and the plants were cultured for 6 weeks. The prepared fish silage was filtered using a bag filter (10 L; 80 μm) and obtained liquid phase was diluted with distilled water (1:10). The diluted fish silage (500 mL; 0.2% v/v of total water volume of AP) was added to the hydroponic units of the AP-BFS group once every 3 days. Daily, 10% of the water in each AP system was replaced with freshwater.

2.2.5 | Water Quality Assessment

Water quality parameters of the fish tanks, including temperature and dissolved oxygen were measured every day using a portable multi-meter (Water Proof Meter 7200 model) and their changes were recorded as 27 ± 1.5°C and 6.4 ± 0.2 mg/L during the experiment, respectively. To determine other water quality parameters, sampling from each fish tank was performed every 2 weeks. The nitrate (NO_3^-), TAN, and phosphate (PO_4^{3-}) concentrations were measured according to the Hach Cadmium Reduction, Hach Ammonia-Nessler, and Hach Molybdovanadate methods using a spectrophotometer (DR 5000TM model) at wavelength of 500, 425, and 430 nm, respectively. The pH and EC of samples were determined using a portable multi-meter (Water Proof Meter 7200 model). The elemental content of samples including, Mg, Ca, K, Na, P, Fe, Cu, Zn, and Mn, was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro Acros, Germany).

2.2.6 | Fish Growth Performance

At the end of the experiment, the fish were fasted for 24 and then anesthetized with clove oil (15 mg/L) to measure their weight and length. The survival rate and growth performance indices

TABLE 1 | Proximate composition of extruded tilapia feed.

Chemical composition	Content (%)
Crude protein	39
Crude lipid	7
Crude fiber	1.5
Ash	9
Moisture	7
Phosphorus	1
Vitamins	
Vit C	500 mg/kg
Vit E	240 mg/kg
Vit D3	2400 IU
Vit A	9600 IU

were calculated using the following formulas:

$$\text{Weight gain rate (WGR; \%)} = [(\text{Final body weight (g)} - \text{Initial body weight (g)})/\text{Initial body weight (g)}] \times 100,$$

$$\text{Specific growth rate (SGR; \% body weight day}^{-1}) = [(\text{Ln final body weight} - \text{Ln initial body weight})/\text{Time (day)}] \times 100,$$

$$\text{Average daily gain (ADG, g)} = \text{Final body weight (g)} - \text{Initial body weight (g)}/\text{Time (day)},$$

$$\text{Feed conversion ratio (FCR)} = \text{Total feed consumed (g)}/\text{Body weight gain (g)},$$

$$\text{Condition factor (CF; g/cm}^3) = [\text{Final body weight (g)}/\text{Final length}^3\text{ (cm)}] \times 100,$$

$$\text{Survival rate (\%)} = (\text{Final number of fish}/\text{Initial number of fish}) \times 100.$$

2.2.7 | Plant Growth

The growth parameters of the basil (*O. basilicum*) plants were measured after 6 weeks of the cultivation period. All plants were harvested from each hydroponic unit and fresh weight of plant, fresh weight of leave, shoot, and root, height of shoot and root, leave surface, number of leaves, nodes, and buds were determined.

2.2.8 | Proximate and Elemental Analysis of Whole-Body Fish and Plant

At the end of the experiment, the three fish were randomly sampled from each fish tank and their skin surfaces were washed with distilled water. The fish were ground several times using a meat grinder until became a uniform paste, and kept at −20°C until use. The dry matter, ash, and elemental contents of the fish and the basil plant were measured based on the methods described in the section 2.1.1.

2.3 | Statistical Analysis

Data were statistically analyzed using SPSS software (version 22), and showed as mean ± SD. The Kolmogorov–Smirnov test was employed to assess the normality distribution of the data. One-way analysis of variance (ANOVA) followed by Duncan's New Multiple Ranges test was performed to determine the significant differences between means of more than two samples. The significant difference between means of two samples was determined using Independent-Samples *T* Test and Paired-Samples *T* Test. ANOVA with repeated measures test followed by Paired-Samples *T* Test was applied to examine the significant differences between the means at different times of each experimental group. Significant difference between means was determined at $p < 0.05$.

3 | Results

3.1 | First Experiment

3.1.1 | Characterization of Fish Waste

The proximate composition and elemental contents of rainbow trout (*O. mykiss*) waste used for production of the fish silage were shown in Table 2.

3.1.2 | Nutrient Contents of BFS

The dry matter and ash contents of biological silage produced from fish waste without and with adding LAB showed no significant differences ($p > 0.05$) (Table 3).

The pH of BFS decreased during the first week in all groups ($p < 0.05$), but its value had no significant change until day 21 ($p > 0.05$). The pH of BFS was lower in the *L. casei* group than the control on day 7 and 14 ($p < 0.05$). On day 21, the pH of BFS was lower in the LAB groups than the control ($p < 0.05$) (Figure 2a). The EC of BFS showed an increasing trend from day 0 to day 21 in all groups ($p < 0.05$). On day 21, the EC of BFS was higher in the control group than the LAB groups ($p < 0.05$) (Figure 2b).

TABLE 2 | Proximate composition and elemental content of rainbow trout (*Oncorhynchus mykiss*) waste (mean ± SD, $n = 3$).

Proximate composition of fish waste (%)	
Dry matter	38.55 ± 3.47
Ash	3.57 ± 0.28
Elemental analysis of fish waste (mg/kg dry weight)	
K	20.29 ± 1.91
Na	69.41 ± 5.12
P	31.24 ± 6.31
Ca	62.26 ± 7.10
Mg	1.48 ± 0.39
Fe	0.44 ± 0.029
Zn	0.29 ± 0.025
Cu	0.14 ± 0.002
Mn	0.018 ± 0.002

TABLE 3 | Proximate composition of biological fish silage produced from rainbow trout (*Oncorhynchus mykiss*) waste with adding lactic acid bacteria (LAB) after 3 weeks (mean ± SD, $n = 3$).

	Experimental groups		
Parameters	**Control**	***Lactobacillus plantarum***	***Lactobacillus casei***
Dry matter (%)	30.45 ± 1.79[a]	29.92 ± 1.35[a]	29.60 ± 1.01[a]
Ash (%)	2.68 ± 0.37[a]	2.59 ± 0.35[a]	2.50 ± 0.57[a]

Note: Data assigned with different letters in the same raw are significantly different (ANOVA, $p < 0.05$).

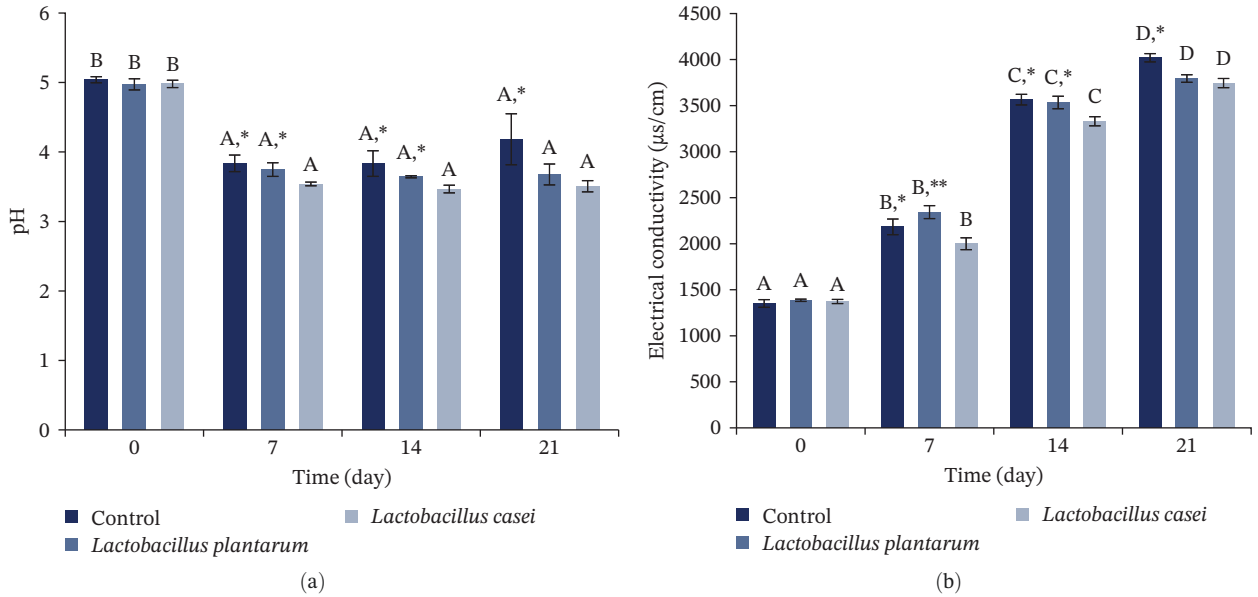


FIGURE 2 | Fluctuations in pH (a) and electrical conductivity (EC) (b) in different groups of biological fish silage produced from rainbow trout (*Oncorhynchus mykiss*) waste during different days of the experiment (mean ± SD; $n = 3$). Bars assigned with an asterisk (*) in each time show significant difference among groups (ANOVA, $p < 0.05$). Bars with different capital letters in each group are significantly different (NOVA with repeated measures test followed by paired-samples T test, $p < 0.05$).

Fluctuations in macro-element concentrations in different groups of the BFS during 21 days are presented in Figure 3. The macro-element (Ca, Mg, P) concentrations increased significantly from day 0 to day 21 in all experimental groups ($p < 0.05$). On day 21, the Mg concentration was higher in the *L. plantarum* and *L. casei* groups than the control ($p < 0.05$). In contrast, the Ca and P concentrations showed no significant differences between the experimental groups ($p > 0.05$) (Figure 3a,b,e). The K concentration had no significant difference between the experimental groups on different days ($p > 0.05$) (Figure 3c). The Na concentration showed an increasing trend from day 0 to 21 in the control group, while its concentration decreased in the LAB groups ($p < 0.05$). On day 21, the Na concentration was lower in the LAB groups than the control ($p < 0.05$) (Figure 3d).

Figure 4 shows the variations of micro-element concentrations in different groups of the BFS during 21 days. The macro-element (Fe, Mn, Zn) concentrations increased significantly in all groups during 21 days of the experiment ($p < 0.05$). On day 21, the Fe concentration was higher in the LAB groups than the control and the highest concentration was recorded in the *L. plantarum* group ($p < 0.05$). On day 21, the Zn and Mn concentrations increased in the LAB groups compared to the control ($p < 0.05$) (Figure 4a–c). The Cu concentration had a decreasing trend from day 0 to day 21 in the control group ($p < 0.05$), while its concentration in the LAB groups did not change significantly during 21 days ($p > 0.05$). On day 21, the Cu concentration was higher in the LAB groups than the control group (Figure 4d).

3.2 | Second Experiment

3.2.1 | Nutrient Contents of BFS Used in AP System

The variations of elemental contents of BFS during different days of fermentation process using *L. plantarum* were shown in Table 4. The concentrations of P, Ca, Mg, Zn, and Mn increased from day 0 to day 14 ($p < 0.05$), but their concentrations had no significant changes until day 21 ($p > 0.05$). The Fe concentration increased significantly during 3 weeks ($p < 0.05$). The Cu concentration decreased from day 0 to day14 ($p < 0.05$), while its concentration showed no significant change until day 21 ($p > 0.05$). No significant changes were observed in the Na and K concentration during 3 weeks ($p > 0.05$).

Table 5 shows the soluble nutrient contents of BFS used as a LOF in coupled AP systems. The pH and EC of BFS were measured as 3.53 ± 0.16 and 3.81 ± 0.06 ms/cm, respectively.

3.2.2 | Water Quality of AP System

3.2.2.1 | BFS Supplementation Stabilizes Water Phosphorus and Enhances Micronutrient Availability. Figure 5 shows the variations of macro and micro-element concentrations in the water of AP and AP-BFS systems during 8 weeks of cultivation period. The Ca concentration showed no significant differences in both AP systems during the experiment ($p > 0.05$) (Figure 5a). The Mg, Na, K, and Cu concentrations decreased significantly in both AP systems from day 0 to day 56 ($p < 0.05$). There were no significant differences in concentrations of these elements between two AP systems ($p > 0.05$) (Figure 5b–d,i). The P concentration decreased in the AP system ($p < 0.05$), but its concentration showed no significant change in the AP-BFS during 56 days of the experiment ($p > 0.05$). The P concentration was significantly higher in the AP-BFS system than the AP system on days 42 and 56 ($p < 0.05$) (Figure 5e). In both AP systems, the Fe and Mn concentrations showed an increasing trend significantly from day 0 to day 56 ($p < 0.05$). The Fe concentration increased in the AP-BFS system compared to the AP system on day 42, but its concentration decreased in the

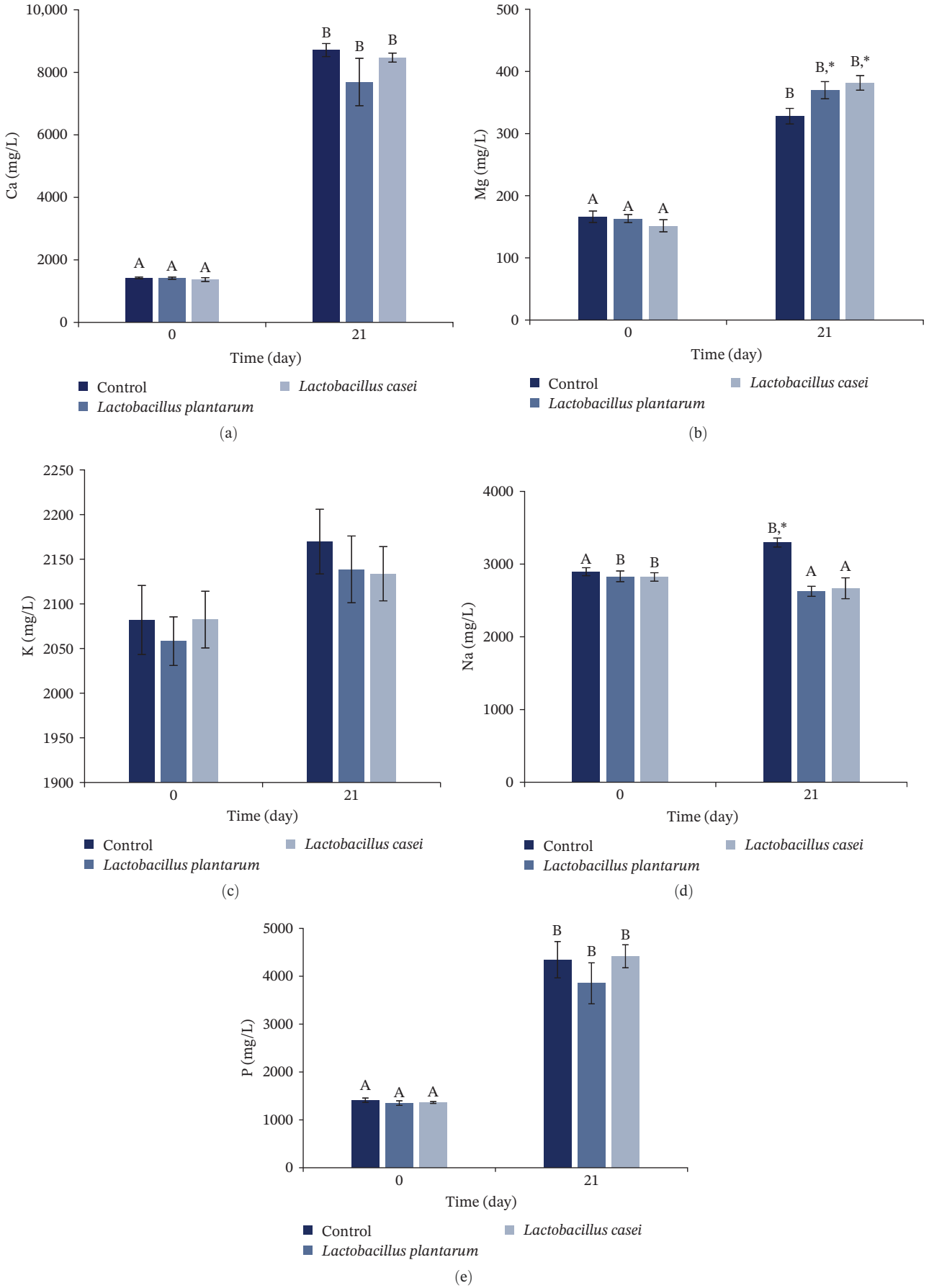


FIGURE 3 | Variations of calcium (a), magnesium (b), potassium (c), sodium (d), and phosphorous (e) concentrations in different groups of biological fish silage produced from rainbow trout (*Oncorhynchus mykiss*) waste during different days of the experiment (mean ± SD; $n = 3$). Bars

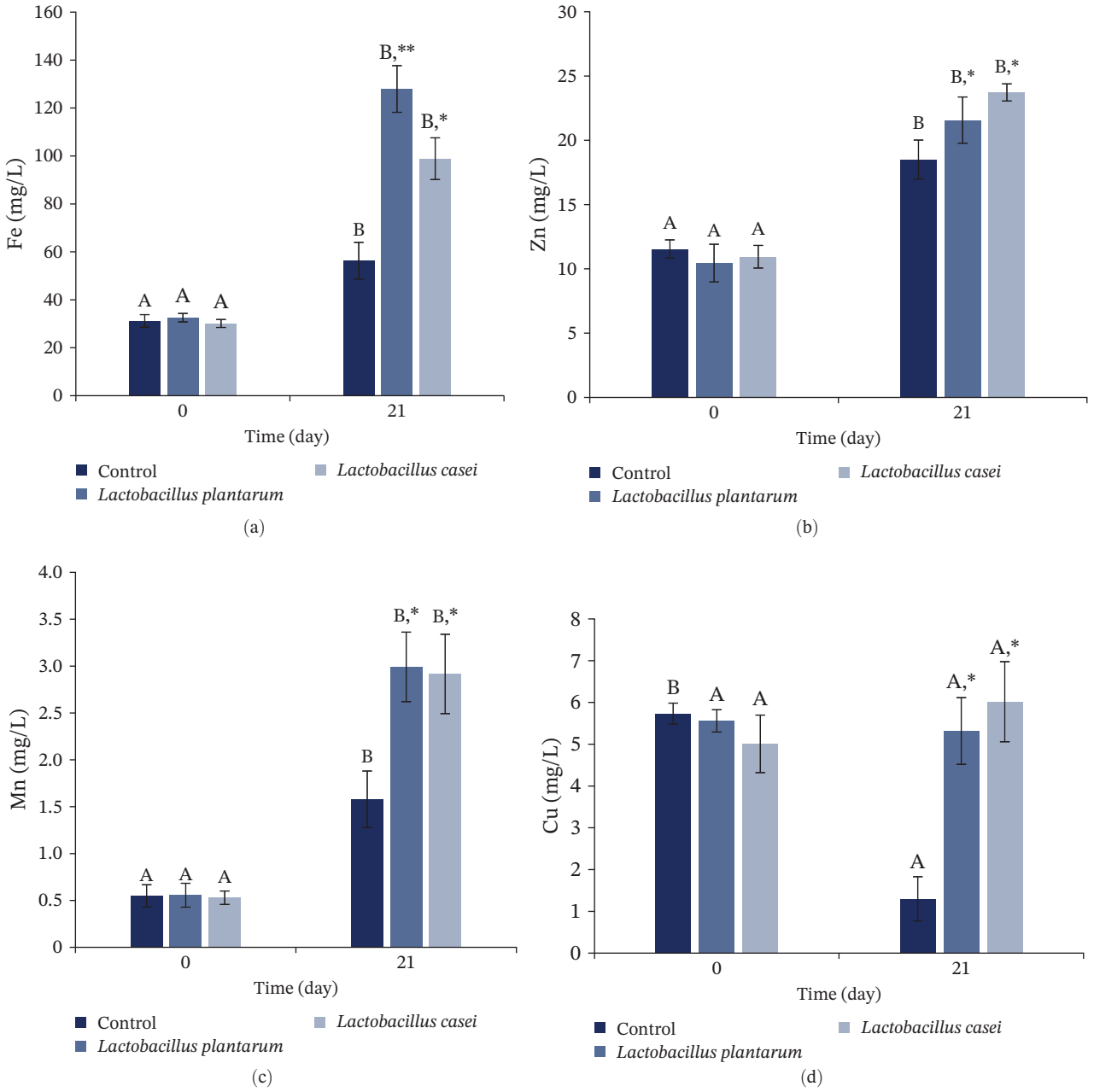


FIGURE 4 | Fluctuations in iron (a), zinc (b), manganese (c), and copper (d) concentrations in different groups of biological fish silage produced from rainbow trout (*Oncorhynchus mykiss*) waste during different days of the experiment (mean ± SD; $n = 3$). Bars assigned with an asterisk (*) in each time show significant difference among groups (ANOVA, $p < 0.05$). Bars with different capital letters in each group are significantly different (paired-samples *T* test, $p < 0.05$).

AP-BFS system compared to the AP system on day 56 ($p < 0.05$) (Figure 5f). On day 56, the Mn concentration was higher in the AP-BFS system than the AP system ($p < 0.05$) (Figure 5h). The Zn concentration had no significant change in the AP system during the experiment ($p > 0.05$). In contrast, its concentration in the AP-BFS system increased from day 0 to day 28 and, then decreased until day 56 ($p < 0.05$). On day 42, the Zn concentration was higher in the AP-BFS system than the AP system, but its concentration was lower in the AP-BFS system than the AP system on day 56 ($p < 0.05$) (Figure 5g).

3.2.2.2 | Effect of BFS Supplementation on Nitrogen and Phosphate Content. The TAN concentration had an increasing trend in both AP systems during 8 weeks ($p < 0.05$). The TAN concentration showed no significant differences between two systems on different days except day 28 ($p > 0.05$). The TAN concentration was lower in the AP-BFS system than the AP system on day 28 ($p < 0.05$) (Figure 6a). The nitrate concentration increased significantly from day 0 to 14, but its value decreased until day 56 in both AP systems ($p < 0.05$). The nitrate concentration was lower in the AP-BFS system than the AP system on days

assigned with an asterisk (*) in each time show significant difference among groups (ANOVA, $p < 0.05$). Bars with different capital letters in each group are significantly different (paired-samples *T* test, $p < 0.05$).

TABLE 4 | Elemental contents of biological fish silage produced from rainbow trout (*Oncorhynchus mykiss*) waste using *Lactobacillus plantarum* during different days of the experiment (mean ± SD; $n = 3$).

	Time (day)			
Element (mg/L)	**0**	**7**	**14**	**21**
P	1378.20 ± 373.3^{a}	2875.45 ± 344.13^{b}	5700.28 ± 308.95^{c}	6337.33 ± 432.20^{c}
Ca	1841.90 ± 95.42^{a}	5079.95 ± 205.74^{b}	9783.93 ± 462.43^{c}	11187.53 ± 549.25^{c}
Mg	249.90 ± 13.30^{a}	390.13 ± 38.76^{b}	733.96 ± 30.73^{c}	791.91 ± 62.84^{c}
Na	3270.13 ± 137.06	3406.94 ± 89.06	3641.17 ± 48.11	3659.75 ± 97.70
K	4294.92 ± 46.77	4297.80 ± 66.98	4319.24 ± 145.03	4559.27 ± 112.06
Fe	55.26 ± 2.56^{a}	85.08 ± 5.67^{b}	135.10 ± 8.47^{c}	155.91 ± 9.89^{d}
Zn	14.53 ± 1.10^{a}	17.71 ± 2.43^{a}	22.50 ± 1.75^{b}	25.41 ± 2.42^{b}
Mn	1.0 ± 0.20^{a}	1.6 ± 0.25^{b}	2.7 ± 0.35^{c}	2.7 ± 0.41^{c}
Cu	6.13 ± 0.802^{b}	4.36 ± 0.540^{b}	2.54 ± 0.351^{a}	2.63 ± 0.251^{a}

Note: Values shown with different letters in each row are significantly different (ANOVA with repeated measures test followed by paired-samples *T* test, $p < 0.05$).

TABLE 5 | Nutrient contents (wet weight basis) of biological fish silage used as liquid organic fertilizer in a coupled aquaponics system (mean ± SD; $n = 3$).

Nutrients (%)	Crude protein	Crude lipid	N	P	K	Na	Ca	Mg	Fe	Zn	Cu	Mn
	9.81 ± 0.31	3.23 ± 0.4	1.56 ± 0.5	0.63 ± 0.04	0.45 ± 0.01	0.36 ± 0.009	1.11 ± 0.05	0.08 ± 0.006	0.01 ± 0.0009	0.0025 ± 0.0002	0.0002 ± 0.00002	0.0002 ± 0.00002

28, 42, and 56 ($p < 0.05$) (Figure 6b). In both AP systems, the phosphate concentration increased from day 42 to 56 ($p < 0.05$). On day 42, the phosphate concentration was higher in the AP-BFS system than the AP system ($p < 0.05$) (Figure 6c).

3.2.2.3 | Effect of BFS Supplementation on pH and EC.

The pH of water changed between 7.01–7.27 and 6.87–7.15 in the AP and the AP-BFS systems during 8 weeks, respectively. The pH was lower in the AP-BFS system than the AP system on days 14, 28, and 42 ($p < 0.05$) (Figure 6d). In both systems, the EC value showed an increasing trend from day 0 to 14, and then its value decreased until day 56 ($p < 0.05$). In the AP system, the EC increased from day 42 to 56 ($p < 0.05$). The EC value was lower in the AP-BFS system than the AP system on days 28, 42, and 56 ($p < 0.05$) (Figure 6e).

3.2.3 | Fish in AP System

The growth performance indices and survival rate of Nile tilapia (*O. niloticus*) fish cultured in two AP systems for 56 days were shown in Table 6. The final body weight, weight gain rate (WGR), average daily gain (ADG), and specific growth rate (SGR) indices increased in the AP-BFS system in comparison to the AP system ($p < 0.05$). The food conversion ratio (FCR) was lower in the AP-BFS system than the AP system ($p < 0.05$). The final body length, condition factor (CF) indices, and survival rate of fish showed no significant differences between two AP systems ($p > 0.05$).

The crude protein and dry matter contents of whole-body fish reared in the AP-BFS system increased compared to that in the AP system ($p < 0.05$). There were no significant differences in the crude lipid, ash, macro and micro-element contents of whole-body fish between two AP systems ($p > 0.05$) (Table 7).

3.2.4 | Plant in AP System

Table 8 shows the growth performance parameters of the basil (*O. basilicum*) plant during different days of cultivation period in two groups of AP systems. In both AP groups, all growth parameters of the basil plant increased significantly from day 0 to day 42 ($p < 0.05$). The growth parameter values were significantly higher in the AP-BFS system than the AP system on days 21 and 42 ($p < 0.05$).

Table 9 shows the macro and micro-element contents of the basil plant cultivated in two AP systems. The P and Fe contents were higher in the AP-BFS system than the AP system ($p < 0.05$). The Ca, Mg, Zn, and Cu contents in the AP system increased compared to the AP-BFS system ($p < 0.05$). There were no significant differences in the Na, K, and Mn contents between two AP systems ($p > 0.05$).

4 | Discussion

One of the fundamental problems in traditional AP systems is the lack of soluble nutrients for plant growth, and chemical fertilizers

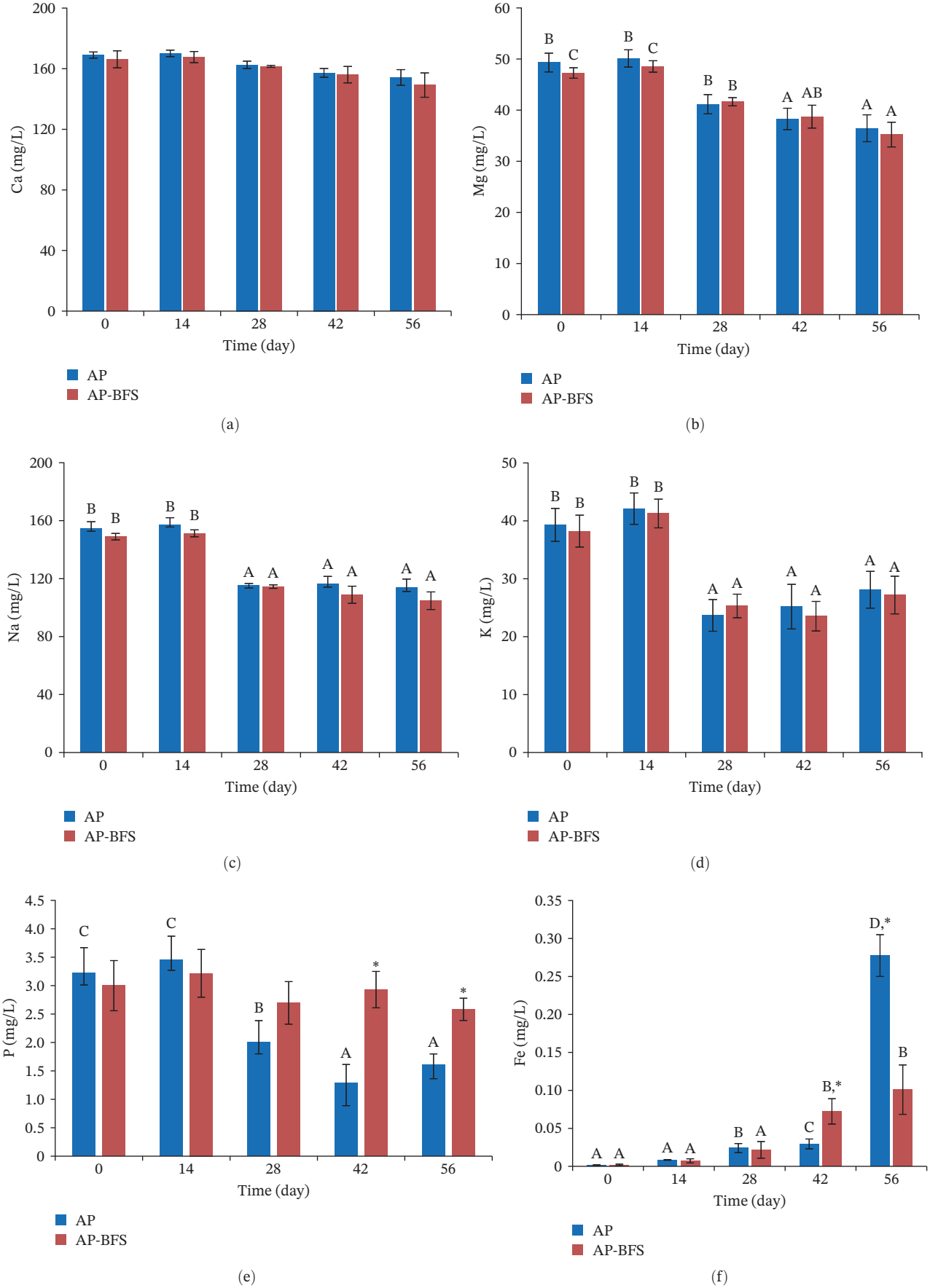


FIGURE 5 | (Continued)

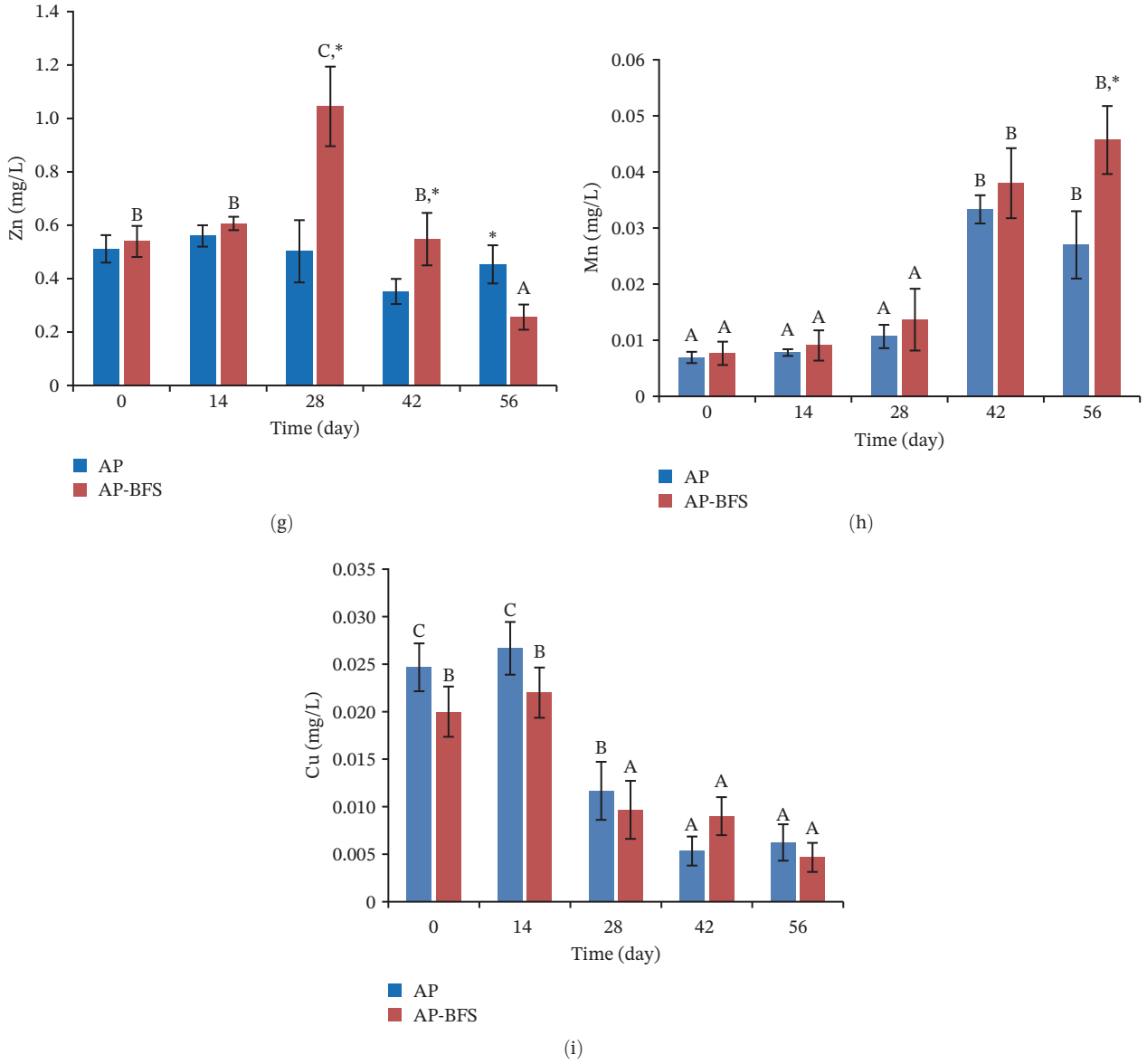


FIGURE 5 | Variations of calcium (Ca) (a), magnesium (Mg) (b), sodium (Na) (c), potassium (K) (d), phosphorus (P), (e), iron (Fe) (f), zinc (Zn) (g), manganese (Mn) (h), and copper (Cu) (i) concentrations of water in AP and AP-BFS systems during different days of the experiment (mean ± SD; $n = 3$). Bars assigned with an asterisk (*) in each time show significant difference between two aquaponics systems (independent-samples T test, $p < 0.05$). Bars with different capital letters in each aquaponic system are significantly different (ANOVA with repeated measures test followed by paired-samples T test, $p < 0.05$). AP, aquaponic; AP-BFS, aquaponic-biological fish silage.

are usually used to resolve this problem, which is usually expensive and harmful for the environment. The administration of LOF obtained from animal waste like fish waste, can be considered as a cost-effective and environmentally friendly approach. Fish waste is an abundant source of minerals, protein, and lipid [21] that are necessary for plant growth. Viles et al. [35] found that dry matter and ash contents of visceral tissues and head of rainbow trout (*O. mykiss*) fish varied from 45.28%–50.27%, 0.83%–1.28%, 32.63%–35.13%, and 3.23%–3.96%, respectively. The dry matter and ash contents of rainbow trout waste during the catching season ranged between 36.53%–47.69% and 2.15%–3.16%, respectively [36]. In the current study, the dry matter (38.55%) and ash (3.57%) contents of rainbow trout waste were almost similar to other studies. Low ash content of fish waste is an advantage for silage production, as high ash content can increase pH, weaken the activity of lactobacillus bacteria, and ultimately reduce fermentation efficiency [37]. The presence of relatively high humidity (more than 60%) is also considered, from the perspective of the fermentation process, to provide a suitable environment for the growth of beneficial bacteria; however, these conditions require hygiene and careful temperature control to prevent the growth of spoilage bacteria.

The pH of silage decreased significantly from 4.9 ± 0.036 to 3.7 ± 0.15 during the first week of fermentation in different experimental groups, remaining stable until the end of the process. After 3 weeks of fermentation, the pH of silage in the presence of LAB was significantly lower than the control. Tropea et al. [20] reported that the pH of silage obtained from fish waste using *Lactobacillus reuteri* became stable at 3.5 after 4 days. Reducing the pH of fermented silage below 4 due to the production of lactic acid by *Lactobacillus* genus promotes silage conservation by

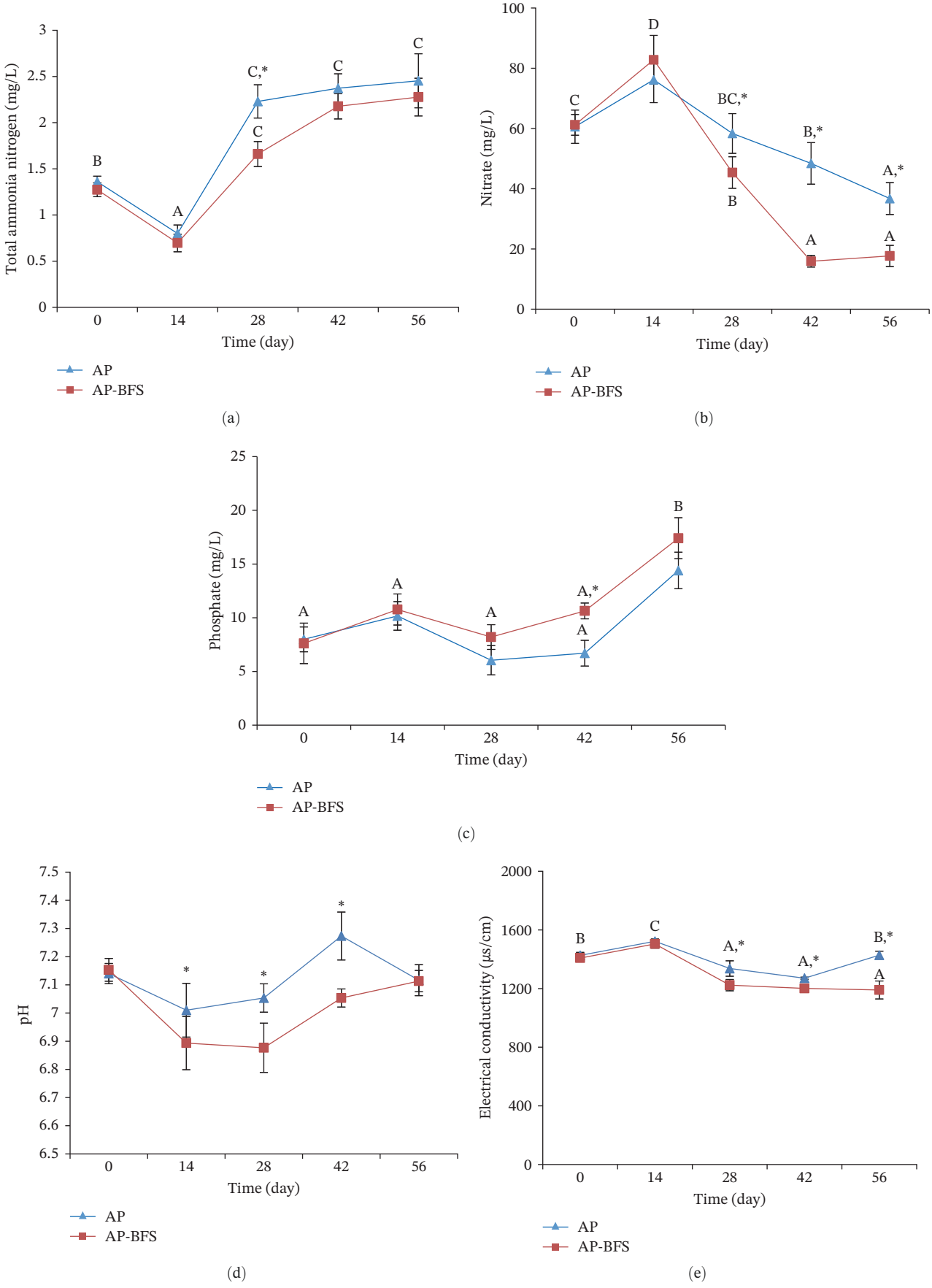


FIGURE 6 | Variations of total ammonia nitrogen (TAN) (a), nitrate (NO_3^-) (b), phosphate (PO_4^{3-}) (c), pH (d), and electrical conductivity (EC) (e) concentrations of water in AP and AP-BFS systems during different days of the experiment (mean ± SD; $n = 3$). Bars assigned with an asterisk (*) in each time show significant difference between aquaponics systems (independent-samples T test, $p < 0.05$). Bars with different capital letters in each aquaponic

TABLE 6 | Growth performance and survival rate (%) of Nile tilapia (*Oreochromis niloticus*) reared in two groups of aquaponics systems for 8 weeks (mean ± SD; $n = 3$).

	Experimental groups	
Parameters	**AP (control)**	**AP-BFS**
Initial weight (g)	9.23 ± 0.44	9.04 ± 0.39
Initial length (cm)	8.68 ± 0.29	8.58 ± 0.12
Final weight (g)	23.40 ± 0.14[a]	26.26 ± 1.37[b]
Final length (cm)	12.24 ± 0.61[a]	12.15 ± 0.22[a]
WGR (%)	153.69 ± 11.30[a]	190.41 ± 9.11[b]
SGR (%BW day^{-1})	1.66 ± 0.0.079[a]	1.93 ± 0.054[b]
ADG (g)	0.25 ± 0.006[a]	0.30 ± 0.019[b]
FCR	1.46 ± 0.10[b]	1.17 ± 0.054[a]
CF (g cm^{-3})	1.28 ± 0.18	1.46 ± 0.063
Survival rate (%)	100 ± 0.00	100 ± 0.00

Note: Data with different letters in same lines are significantly different (Independent-Samples *T* Test, $p < 0.05$).
Abbreviations: ADG, average daily gain; AP, aquaponic; AP-BFS, aquaponic-biological fish silage; CF, condition factor; FCR, food conversion ratio; SGR, specific growth rate; WGR, weight gain rate.

TABLE 7 | Whole-body proximate composition and macro and micro-element contents of Nile tilapia (*Oreochromis niloticus*) cultured in two groups of aquaponic systems for 8 weeks (mean ± SD; $n = 3$).

	Experimental groups	
Parameter	**AP (control)**	**AP-BFS**
Whole-body proximate composition (%)		
Crude protein	11.43 ± 0.34[a]	14.52 ± 1.02[b]
Crude lipid	8.27 ± 0.43	8.02 ± 1.24
Dry matter	26.17 ± 0.65[a]	27.88 ± 0.79[b]
Ash	3.12 ± 0.39	3.77 ± 0.10
Macro-element contents (g/kg dry weight)		
P	8.75 ± 1.08	8.22 ± 1.10
Ca	13.10 ± 2.73	11.40 ± 2.27
Mg	0.15 ± 0.032	0.14 ± 0.034
Na	7.02 ± 0.38	6.76 ± 0.64
K	10.98 ± 1.17	10.87 ± 1.11
Micro-element contents (mg/kg dry weight)		
Fe	39.40 ± 8.86	36.15 ± 7.92
Zn	34.49 ± 5.34	36.40 ± 7.81
Mn	0.29 ± 0.015	0.31 ± 0.024
Cu	7.29 ± 0.94	7.06 ± 1.74

Note: Data with different letters in each row are significantly different (Independent-Samples *T* Test, $p < 0.05$).
Abbreviations: AP, aquaponic; AP-BFS, aquaponic-biological fish silage.

inhibiting the growth of pathogenic and spoilage microorganisms [38, 39], and activates enzymes in the viscera responsible for protein hydrolysis [28].

The fermentation of fish waste using LAB had no significant effect on dry matter and ash contents of the final product. In the present study, the ash content of biological silage produced with whole fish waste ranged from 2.50% to 2.68%. Santana et al. [28] also reported that ash content of fish fermented silage using different carbohydrate sources ranged between 1.7% and 5.3%. The EC of the BFS increased by 2.97, 2.73, and 2.73 times after 3 weeks of fermentation process in the control, *L. plantarum*, and *L. casei* groups, respectively. The remarkable increase in EC of BFS confirms digestion of whole fish waste through acidification and release of minerals. The EC of silage in LAB groups was lower than the control probably due to partial mineral utilization, especially sodium (Na), by the bacteria. The sodium concentration decreased in LAB groups during 21 days of fermentation process, while its concentration increased in the control group. Zhang et al. [40] evaluated the nutrient consumption patterns of *Lactobacillus fermentum* mediated by sodium lactate. They found that the most consumed metal ions (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Zn^{2+}, Mn^{2+}, Fe^{2+}) by *L. fermentum* was Na^{+} (16326.17–25678.66 nmol log CFU^{-1}) at different sodium lactate concentrations. Reducing the sodium concentration of silage can be considered a positive point, because excessive sodium concentration arrests plant growth due to changes in cellular biochemistry [41, 42]. The examination of fluctuations in other mineral concentrations during fermentation process showed that Mg, Ca, P, Fe, Mn, and Zn concentrations significantly increased in all experimental groups. The calcium concentration of the BFS increased by 5.92, 5.44, and 6.19 times after 3 weeks of fermentation process in the control, *L. plantarum*, and *L. casei* groups, respectively. It seems that bacteria destroy organic and inorganic structures containing calcium, such as hydroxyapatite in the bone by producing organic acids and specific enzymes, thereby facilitating the release of calcium from these compounds [43, 44]. The increase in elemental contents of the BFS in the control group is probably due to the presence and function of natural microorganisms present in fish waste. The adding LAB to fish waste resulted in greater increases in Mg, Fe, Zn, and Mn concentrations compared to control group. These results revealed that the impact of LAB on the release of micro-elements from fish waste was greater than the release of macro-elements during fermentation process. Both LAB had same effect on elemental composition of the BFS, except iron (Fe). The concentration of Fe increased by 293.17% and 228.31% at the end of fermentation process compared to zero time in the *L. plantarum* and *L. case* groups, respectively. Therefore, the BFS was produced using *L. plantarum* for adding as LOF to AP systems. The crude protein and crude lipid contents of the BFS were 32.78% ± 1.03% and 10.78% ± 1.33% in dry matter, respectively. The protein content of liquid fish fertilizers is higher

system are significantly different (ANOVA with repeated measures test followed by paired-samples *T* test, $p < 0.05$). AP, aquaponic; AP-BFS, aquaponic-biological fish silage.

TABLE 8 | Growth performance of basil plant (*Ocimum basilicum*) during different days of cultivation period in two aquaponics systems (mean ± SD, $n = 3$).

	Initial (Day 0)		Day 21		Day 42	
Parameter	**AP (control)**	**AP-BFS**	**AP (control)**	**AP-BFS**	**AP (control)**	**AP-BFS**
Total fresh weight (g)	0.88 ± 0.09^{a}	0.90 ± 0.15^{a}	—	—	77.40 ± 2.48^{b}	176.55 ± 9.65^{c}
Fresh weight (g plant^{-1})	0.059 ± 0.006^{a}	0.060 ± 0.010^{a}	—	—	1.96 ± 0.11^{b}	4.95 ± 0.37^{c}
Leave fresh weight (g plant^{-1})	0.026 ± 0.001^{a}	0.024 ± 0.001^{a}	—	—	$0.68 \pm 0.0.68^{b}$	1.76 ± 0.19^{c}
Shoot fresh weight (g plant^{-1})	0.012 ± 0.002^{a}	0.016 ± 0.001^{a}	—	—	0.69 ± 0.045^{b}	1.92 ± 0.16^{c}
Root fresh weight (g plant^{-1})	0.013 ± 0.001^{a}	0.014 ± 0.002^{a}	—	—	0.24 ± 0.070^{b}	1.03 ± 0.13^{c}
Root height (cm)	5.97 ± 0.53^{a}	6.11 ± 0.48^{a}	—	—	11.24 ± 0.39^{b}	12.37 ± 0.40^{c}
Leave surface (cm^2)	0.35 ± 0.039^{a}	0.37 ± 0.045^{a}	5.85 ± 0.97^{b}	9.53 ± 1.68^{c}	5.51 ± 1.28^{b}	14.74 ± 1.91^{d}
Shoot height (cm)	3.24 ± 0.13^{a}	3.25 ± 0.06^{a}	9.26 ± 0.38^{b}	12.71 ± 0.42^{c}	16.64 ± 0.52^{d}	28.03 ± 0.40^{e}
Number of leaves/plant	3.45 ± 0.23^{a}	3.60 ± 0.43^{a}	7.36 ± 0.36^{b}	8.84 ± 0.20^{c}	11.65 ± 0.17^{d}	23.72 ± 1.71^{e}
Number of nodes/plant	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}	2.68 ± 0.18^{b}	3.40 ± 0.13^{c}	4.45 ± 0.32^{d}	6.62 ± 0.43^{e}
Number of buds/plant	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$0.78 \pm 0.21a^{b}$	0.95 ± 0.34^{b}	3.25 ± 0.35^{c}	6.64 ± 0.72^{d}

Note: Data with different letters in same lines are significantly different (ANOVA, $p < 0.05$).
Abbreviations: AP, aquaponic; AP-BFS, aquaponic-biological fish silage.

TABLE 9 | Macro and micro-element contents of basil (*Ocimum basilicum*) plant cultivated in two aquaponic systems for 6 weeks (mean ± SD; $n = 3$).

	Experimental groups	
Element	**AP (control)**	**AP-BFS**
Macro-element contents (g/kg dry weight)		
P	5.74 ± 0.44^{a}	7.18 ± 0.58^{b}
Ca	36.12 ± 2.02^{b}	28.48 ± 1.03^{a}
Mg	11.71 ± 1.27^{b}	8.07 ± 0.66^{a}
Na	13.39 ± 0.67	12.68 ± 0.62
K	27.15 ± 1.45^{a}	31.07 ± 1.84^{b}
Micro-element contents (mg/kg dry weight)		
Fe	266.01 ± 37.45^{a}	349.14 ± 28.01^{b}
Zn	201.90 ± 11.10^{b}	97.00 ± 13.28^{a}
Mn	10.09 ± 0.98	8.16 ± 0.46
Cu	8.13 ± 0.28^{b}	4.86 ± 0.38^{a}

Note: Values assigned with different letters in each row are significantly different (Independent-Samples T Test, $p < 0.05$).
Abbreviations: AP, aquaponic; AP-BFS, aquaponic-biological fish silage.

than industrial fertilizers [45]. Tropea et al. [20] showed that the protein and lipid contents of fermented fish by-product of *Dicentrarchus labrax* using *Lactobacillus reuteri* and *Saccharomyces cerevisiae* increased slowly from 11.68% ± 0.48% to 48.55% ± 1.15% and from 13.74% ± 0.72% to 15.25% ± 0.8% in dry matter by 96 h. They confirmed that the final fermented product represents adequate protein and lipid contents for using in aquaculture feeds. In contrast, Santana et al. [28] reported that fermented tambaqui (*Colossoma macropomum*) viscera silage characterized by low protein (<20%) and high fat (around 60%) contents in dry matter. The high content of lipid in silage makes it less suitable for use in feed. The nutritional value of fish silage depends on the chemical composition of the raw material [46]. In the present study, the BFS used as LOF in AP systems contain 1.56% ± 0.5% nitrogen, 0.45% ± 0.01% potassium, 0.63% ± 0.04% phosphorus, 0.36% ± 0.009% sodium, 1.11% ± 0.05% calcium, and 0.08% ± 0.006% magnesium. The macro-elemental composition of LOF was approximately similar to those reported by Blatt and Sanford [47], Palani et al. [48], and Karim et al. [25].

The BFS was added as a LOF to water of AP systems for 6 weeks. The BFS had no detrimental impact on survival rate of Nile tilapia (*O. niloticus*), with a survival rate of 100%. The investigation of growth performance of Nile tilapia fish cultured in the AP system with adding the BFS showed that the final weight, WGR, ADG, SGR, and FCR parameters improved by 12.22%, 23.89%, 20%, 16.26%, and 19.86% in comparison to fish cultured in the AP system without adding the BFS, respectively. These findings can be attributed to the positive impacts of valuable nutrients like protein, lipid, free amino acids, low molecular weight peptides, omega-3 fatty acids, and minerals in the BFS on growth performance of fish. To the best of our knowledge, there is no information on impact of fish silage as a water additive on growth performance of fish. Putra et al. [49] reported that feeding grouper (*Epinephelus coioides*) fish with diets supplemented with BFS (1 and 3 g/kg) improved the SGR and WGR values and the best FCR was found in fish fed 1 g/kg silage. The fish silage can increase the nutritional value of the feeds through hydrolysis of protein content and also improve the FCR value by meeting the nutritional need and appetites of fish. Khan et al. [50] reported that growth performance and feed utilization of Pangas catfish (*Pangasianodon hypophthalmus*) fry enhanced with 100% substituting fish meal with fish silage. They attributed the better growth rate of fish to the presence of higher amount of

free amino acids and active hydrolytic enzymes in fish silage. In contrast, substituting fish meal with dried fermented fish silage (FFS) more than 25% in diet of Nile tilapia (*O. niloticus*) fry had detrimental effect on growth rate and feed utilization. The indispensable amino acid content of FFS did not cover the requirements of Nile tilapia from these amino acids [51]. In the present study, the Nile tilapia fish fed with commercial feed contained 39% crude protein and adding the BFS with 9.81% ± 0.31% crude protein (wet weight basis) to water can probably meet some of the fish's amino acid needs. The growth-enhancing effect of BFS may also be related to the presence of *L. plantarum* in silage. Abbas et al. [52] found that dietary administration of *L. plantarum* as probiotic significantly increased the body weight and length of Olive flounder (*Paralichthys olivaceus*), as well as enhanced feed conversion efficiency. Huang et al. [53] reported that water additive of *Lactobacillus* sp. and *Bacillus* sp. combination enhanced growth rate of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus polyphekadion*). The probiotics beneficially influence on growth and health of host through secretion of digestive enzymes, modulation of the immune system positively, and improvement of water quality [53]. Further studies are needed to investigate the effect of using BFS as a water additive on intestinal microflora, digestive enzyme activity, and nutrient digestibility in different fish species.

Exposure of fish to sub-lethal concentrations of ammonia could decrease feed intake [54] and subsequently decrease the growth performance [55, 56]. In the present study, the TAN concentration was significantly lower in the AP-BFS system than the AP system during different days of the experiment. However, it was not statistically significant except on day 28. Improved growth rate of barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) co-cultured with peppermint (*Mentha* × *piperita* L.) was attributed to improved water quality of AP system [57]. The used BFS in AP systems contained enough amounts of macro and micro-elements, which likely had a positive impact on growth performance of Nile tilapia fish. Abdel-Warith [58] reported that the growth performance and whole-body crude protein content of Nile tilapia (*O. niloticus*) cultured in earthen ponds received chicken manure and artificial feed improved compared to that cultured in ponds received only artificial feed without any fertilization. Fish silage can increase protein, lipid, and crud fiber in animal flesh [59]. In the current study, the whole-body dry matter and crude protein contents of Nile tilapia increased with adding the BFS to AP system.

Basil (*Ocimum* sp.) is a commercially important herb because of its proper nutritional, medicinal, and antimicrobial values and wide range of industrial applications [60]. The sweet basil (*Ocimum basilicum*) is one of the most popular plants grown in APsystems [60, 61], because of its rapid growth, high fresh market value, and a compact growth habit [60]. In the present study, the growth and morphological traits of basil plants increased remarkably in both AP systems after 6 weeks of the cultivation period. Some nutrients such potassium (K), calcium (Ca), and iron (Fe) are commonly deficient in AP system, which can led to a reduction in crop yield [62, 63]. Therefore, it is recommended to prevent nutrient deficiencies and thus optimize the production of the plant in this system. In this study, the BFS was added to the water to meet nutrient requirements of the basil plants and enhance its yield. The fresh weight and the total fresh weight of harvested plants were 2.52 times and 2.28 times higher in the AP-BFS system than the AP system, respectively. Fahlivi [45] found that the liquid fertilizer obtained from fish viscera had enough nutrients for growing onion and garlic plants as it produced higher yields, compared to industrial fertilizer. Karim et al. [25] reported that the LFS contains 1.84% ± 0.38% of nitrogen, 0.41% ± 0.05% of potassium, and 0.50% ± 0.09% of phosphorus was very effective for plant growth. The elemental analysis of BFS used in the present study showed approximately same macro-elemental composition. Hepsibha and Geetha [64] reported that the micro-element (i.e., Fe, Zn, Cu, Mn) contents of fermented fish waste as an organic fertilizer satisfy the requirements of the plants for their healthy growth. They found a positive correlation between the level of micro-elements and the nodulation and root length of *Vigna radiate*. In the present study, the number of nodules per plant and root length of the basils increased by 433% and 10% in the AP-BFS system compared to the AP system, respectively. Amino acids as fundamental units of protein serve key roles in plants, including protein synthesis, metabolic and growth regulation, nutrient transport, improvement of stress tolerance, and structural support [65, 66]. Amino acids as growth-promoting substances have potential to increase biomass yield of sweet basil (*O. basilicum*) [67]. Jumar et al. [68] reported that the application of fish amino acid derived from fermentation of fish waste with dose of 1 mL/L increased the height of rice plants by 5.5% and N-uptake by 9.6% in rice leaves. The application of BFS with 9.81% ± 0.31% crude protein content in the AP system likely had a growth-promoting effect on the basil plants, although its amino acid profile was not determined.

The elemental analysis of basil plant showed that the phosphorus (P), potassium (K), and iron (Fe) contents were higher in the AP-BFS system than the AP system. These findings indicated that the application of BFS probably improved the uptake of these elements by basil plants from water. Ndzingane et al. [69] reported that the use of organic fertilizers increased P and K contents in leaves of sweet basil (*O. basilicum*). Seeda et al. [70] also found that the use of organic fertilizers increased the iron (Fe) content of plants cultivated in APand hydroponics systems. The calcium and magnesium contents of the basil tissues decreased in the AP-BFS system compared to the AP system, which is probably due to increased uptake and accumulation of potassium ions in plant tissues. The antagonist effect of potassium on the calcium and magnesium contents has reported in the literature [71]. The iron content of the basil tissues in the AP-BFS system increased significantly compared to the AP system, while the zinc and copper contents deceased. Increased iron content can lead to saturation of transporters and reduced uptake of other micronutrients. Erenoğlu [72] found that iron deficiency in wheat plant increased zinc uptake, so that zinc content was higher in iron-deficient plants than in iron-sufficient plants. Several studies have confirmed zinc content of plant has an antagonism state with phosphorus and excessive accumulation of phosphorus causes zinc deficiency in plant [73, 74]. In the present study, the increase in phosphorus content of the basil tissues probably caused a decrease in zinc uptake by plant in the AP-BFS system. The pH of water in AP system influences on nutrient availability for the plants. The uptake of macro-elements (i.e., N, P, Ca, K, S, and Mg) by plants takes place across a wide range of pH, while micro-elements (particularly Fe) uptake frequently occurs between a pH of 5.5–6.0 [75]. The adding BFS with pH of 3.53 ± 0.16 to the aquaonics system decreased the pH compared to the AP

system without BFS on days 14, 28, and 42. The better growth performance of basil plants in the AP-BFS system may be attributed to lower pH and subsequently higher nutrients uptake by the plants. EC represents the amount of soluble nutrients in a growth medium [17]. The optimal EC for basil hydroponic production is 1600 μS/cm [76]. In this study, the EC values ranged between 1270.66–1522.50 μS/cm and 1190.33–1504.33 μS/cm during 56 days of the experiment in the AP system and the AP-BFS system, respectively. Despite the addition of BFS, the EC of water was significantly lower in the AP-BFS system than the AP system, which could indicate better mineral uptake by the basil plants.

The TAN concentration decreased during the first 2 weeks of cultivation, while the nitrate concentration increased at the same time in both AP systems, indicating the occurrence of the nitrification process. The increase in TAN concentration up to day 56 is most likely due to increased feeding and consequently higher ammonia excretion by the fish or the conversion of organic nitrogen present in feces and feed residues to ammonium. The nitrate concentration decreased by 51.77% and 78.64% from day 14, coinciding with the introduction of the basil plants, to day 56 in the AP and AP-BFS systems, respectively. This result reveals higher uptake of nitrate by the basil plants from water in the AP-BFS system. Nitrate is directly absorbed by the roots, where it metabolized or transported to the aerial parts of the plant [4, 77]. Knaus et al. [78] observed that the height of basil plant rises when the uptake of nitrate increases. In the present study, the shoot height of basil in the AP-BFS system, where nitrate uptake was higher, was 68.44% higher than the AP system.

The phosphorus concentration of water decreased from day 14, coinciding with the introduction of the basil plants, to day 56 in the AP system. In contrast, its concentration showed no significant change, despite the adding BFS to the water in the AP-BFS system. These results indicate the phosphorus uptake by the basil plants from the water. Previous studies have reported that phosphorus concentrations often decrease over time in AP system and its deficiency can limit plant growth [62]. Therefore, using LOF like BFS can prevent phosphorus deficiency in AP systems. The phosphate concentration of water did not change significantly in both AP systems, despite the adding BFS and fish feed to the water until day 42, which was probably due to the simultaneous occurrence of the processes of converting organic and inorganic phosphorus into phosphate by phosphate-solubilizing microorganisms (PSM) and the phosphate uptake by the basil plants. The phosphate concentration increased significantly during the last 2 weeks of the experiment in both AP systems, which was probably due to a decrease in its uptake by the basil plants or an increase in the conversion of phosphorus to phosphate.

The calcium concentration of the water showed no significant change during the 8-week experiment in both AP systems. The stability of calcium concentration could be due to its reduced uptake by the plants compared to other nutrients or its tendency to precipitate. Rafiee and Saad [79] found that calcium concentration remains relatively stable in AP systems. In contrast, the magnesium, sodium, and potassium concentrations decreased significantly in both AP systems. This trend could be due to the uptake of these macro-nutrients by the basil plants or chemical precipitation, especially magnesium ions. The iron (Fe) and manganese (Mn) concentrations of water increased during the 8-week experiment in both AP systems. The increase in these micronutrients could be due to their release from various sources like fish silage, feces, and un-eaten food. Han et al. [80] attributed the increases in Fe and Mn concentrations to the solubilization of phosphate bound to these micronutrients by phosphate-solubilizing bacteria (PSB) [80]. In the last 2 weeks of the experiment, the iron concentration was significantly lower in the AP-BFS system than the AP system. This result indicates the uptake of iron from the fish silage by the basil plants. The zinc (Zn) concentration of water increased with the adding BFS until day 28 and then showed a decreasing trend. The significant decrease in zinc concentration in the last 4 weeks of the experiment indicates the zinc uptake by the basil plants in the AP-BFS system. It has been found that adequate supply of zinc can promote root development and ultimately increase plant growth [81, 82]. Optimal copper concentration maintains metabolic activities and increases basil plant growth [83]. In the current study, the copper (Cu) concentration of water showed a decreasing trend with the introduction of basil seedlings until the end of the experiment in both AP systems. The decrease in the Cu concentration is probably due to its uptake by the basil plants.

5 | Conclusion

The calcium, magnesium, phosphorus, iron, zinc, and manganese concentrations significantly increased during 21 days of microbial fermentation of fish waste in the control and LAB groups. The production of fish silage using *L. plantarum*, and *L. casei* led to a greater increase in magnesium, iron, zinc, and manganese ions compared to the control group. The adding BFS produced from rainbow trout waste using *L. plantarum* to the water of AP systems improved the growth performance of Nile tilapia (*O. niloticus*) and basil plant (*Ocimum basilicum*). The dry matter and crude protein contents of whole-body fish increased in the AP-BFS system compared to the AP system. The phosphorus, potassium, and iron contents of plant tissues were higher in the AP-BFS system than the AP system. Nitrate concentration was lower in the AP-BFS system than the AP system. In conclusion, fermentation of fish waste using the LAB can be considered as an effective method to produce BFS as a LOF. The use of BFS can reduce the nutritional needs of plants and the cost of using chemical fertilizers and solutions in an AP system. However, the method and amount of its usage require further studies.

Author Contributions

Rezvan Bastchi: investigation, methodology, and data curation. **Mehrdad Sarkheil:** investigation, conceptualization, topic organization, data analysis, and writing – original draft preparation. **Davar Shahsavani:** investigation, conceptualization, and writing – review and editing. **Masoomeh Mehraban Sangatash:** conceptualization, methodology, and writing – review and editing.

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Disclosure

All authors have read and agreed to the published version of the manuscript.

Ethics Statement

The guidelines of Ferdowsi University of Mashhad (FUM) (IR.UM.REC.1402.177) were applied for fish care and handling.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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