Decoupled FLOCponics systems as an alternative approach to reduce the protein level of tilapia juveniles’ diet in integrated agri-aquaculture production

Sara M. Pinho\textsuperscript{a,b,1}, Jéssica P. Lima\textsuperscript{a,1}, Luiz H. David\textsuperscript{a}, Magdiel S. Oliveira\textsuperscript{c}, Simon Goddek\textsuperscript{b}, Dalton J. Carneiro\textsuperscript{a,c}, Karel J. Keesman\textsuperscript{b,*}, Maria Célia Portella\textsuperscript{a,*}

\textsuperscript{a}Universidade Estadual Paulista (Unesp), Centro de Aquicultura da Unesp, Via de acesso Prof. Paulo Donato Castellane, s/n, Jaboticabal, SP, CEP 14884-900, Brazil.
\textsuperscript{b}Mathematical and Statistical Methods (Biometrics), Wageningen University, P.O. Box 16, 6700 AA, Wageningen, the Netherlands
\textsuperscript{c}Universidade Estadual Paulista “Júlio de Mesquita Filho”, Faculdade de Ciências Agrárias e Veterinárias Campus Jaboticabal, 14884-900 Jaboticabal, SP, Brazil

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\textbf{A B S T R A C T}

Decoupled FLOCponics (DFP) is a promising aquaponics approach which takes advantage of the nutritional benefits of biofloc technology (BFT). Enabling the use of less protein in the fish diets is one of the benefits of BFT. The effect of the reduction of protein content, and consequently the input of nitrogen, on fish and plant production in DFP systems has not yet been investigated. This study was designed to investigate and evaluate the production of lettuce and tilapia juveniles in a DFP system using different levels of crude protein (CP) in the fish diets. The zootechnical performance of tilapia juveniles and lettuce growth in the DFP system were evaluated, using different diets containing 24, 28, 32, and 36\% CP. Fish production in DFP systems was compared to those reared in traditional decoupled aquaponics systems (DAPS) and in biofloc-based systems (BFT), both fed with 32\% CP diet. The experimental period of tilapia juvenile production lasted 56 days. Lettuce production in two cycles was also performed in DFP systems with different CP levels and their growth was compared to those in DAPS and hydroponics systems, as control treatments. In Cycle 1, the seedling phase was evaluated in a 14-day trial. In Cycle 2, the final production phase was performed for 21 days until harvest. The physical-chemical parameters of the water were monitored in the aquaculture and hydroponic subsystems. High mortality of fish occurred in DFP-36 in the middle of the experiment, thus this treatment was discontinued. The results showed that tilapia reared in DFP and fed with 24 and 28\% CP (DFP-24 and DFP-28) grew similarly to those in DAPS fed with 32\% CP diet. Fish in DFP-32 and BFT fed with 32\% CP diet grew similarly and above the other treatments. Additionally, plant growth results showed no differences in both cycles among all treatments. With respect to water parameters, even though more nutrients were inputted in the treatments with high CP content in the aquaculture subsystem, the mean values of nitrogen compounds and orthophosphate were similar in all treatments. For water parameters in the hydroponics subsystems, only the mean values of pH in Cycle 1 were statistically different in the plant treatments. The results obtained in this study indicate that using less CP in fish diets to produce lettuce and tilapia juveniles is technically possible and feasible in a decoupled FLOCponics system.

1. Introduction

Integrated agri-aquaculture aquaponics systems typically combine a recirculating aquaculture system (RAS) with soilless plant production in hydroponics, based on sharing and reusing nutrients and water (Lennard and Goddek, 2019). Aquaponics has become widespread in aquaculture as a way to increase the efficiency of water and feed use and consequently reduce the discharge of nutrient-rich effluents (Joyce et al., 2019; Yep and Zheng, 2019). Commonly, between 21 and 30\% of the dry matter and 40 to 47\% of the nitrogen (N) content in the feed are retained in the biomass of tilapia, and most of the inputted nutrients are discharged into the environment (Verdegem, 2013). The discharge of
nutrient-rich effluent might result in pollution and eutrophication of waterbodies (Joyce et al., 2019). These problems can be minimized by directing the aquaculture effluents to nourish plants in aquaponics (Enduta et al., 2012; Turcios and Papenbrock, 2014).

Most aquaponics systems are run in coupled setups, in which water and nutrients continuously flow through the aquaculture and hydroponics subsystems (Palm et al., 2019; Abusin and Mandikiana, 2020). However, a trade-off between the required water quality and required environmental conditions in the respective subsystems has been reported as an issue in coupled aquaponics (Goddek et al., 2019). Decoupled layouts have been proposed to solve this trade-off by separating each subsystem component with a unidirectional flow from the aquaculture to the hydroponics subsystem. This allows for meeting the requirements of all subsystems and achieving high productivity of both fish and plants (Kloas et al., 2015; Goddek et al., 2016a; Monsees et al., 2017). In addition to evaluating different layouts, different aquaponics approaches have recently been tested, as well, seeking to improve the sustainable character of food production (Kotzen et al., 2019).

FLOCponics is a term proposed by Pinho et al. (2021) as an offshoot of aquaponics in which RAS is replaced by a system based on biofloc technology (BFT). BFT aims to manage the water quality in aquaculture systems without the need for costly mechanical and biological filters or for high volumes of water exchange (Emerenciano et al., 2017; Dauda, 2020). The growth of specific microbial communities that recycle the nitrogenous waste is directly fomented in the aquaculture tanks (Verdegem and Bosma, 2009; Grab et al., 2012; Emerenciano et al., 2013), by providing strong aeration and water movement. Besides that, an external carbon source is added to increase the carbon-nitrogen ratio of the water and to promote the growth of biofloc microbiota (Browdy et al., 2012; Avnimelech, 2015). As a result, extra macro- and micro-nutrients are added to the water via the carbon source (Becerril-Cortés et al., 2018; da Rocha et al., 2018). Additionally, lower nutrient loss by minimal solids or sludge removal can be linked to BFT when compared to RAS. The higher accumulation of nutrients in FLOCponics water compared to aquaponics using RAS could directly influence plant nutrition. However, the results presented to date have not reached any consensus on the benefit of using BFT effluents for plant growth in FLOCponics systems. Recent studies show positive effects of BFT on lettuce growth (Pinho et al., 2017; Lenz et al., 2018; da Rocha et al., 2018), whereas others have observed the opposite effects (Rahman, 2010; Fimbres-Acedo et al., 2020; Pinho et al., 2021). The negative result of plant production in FLOCponics was in general related to nutrient imbalances, high water pH, and the presence of bioflocs in the plant roots. The presence of bioflocs probably affected the breathing process and the absorption of nutrients by the plants (Rahman, 2010; Rakocy, 2012; Fimbres-Acedo et al., 2020; Pinho et al., 2021). In most of the studies, the FLOCponics systems were run in one loop instead of decoupled layouts (Pinho et al., 2017, 2021; Lenz et al., 2018; da Rocha et al., 2018). Thus, only sub-optimal conditions for plant growth were achieved.

With respect to the effect of FLOCponics on tilapia production, increased zootechnical performance should be expected when comparing it to conventional aquaponics. This is because the BFT microorganisms are a constant and nutrient-rich source of natural food for the fish (Emerenciano et al., 2015; Bossier and Ekasari, 2017; Martínez-Córdova et al., 2017; Becerril-Cortés et al., 2018), resulting in better fish weight gain, survival and feed conversion rate when compared to RAS production (Pinho et al., 2017; Long et al., 2019).

The consumption of bioflocs by tilapia juveniles makes it possible to adapt nutritional strategies. For instance, alternative protein ingredients can be used instead of the conventional high-cost fish meal and soybean meal (Sousa et al., 2019; Freccia et al., 2020; Tobin et al., 2020), or the use of diets with low protein content (Azim and Little, 2008; Mansour and Esteban, 2017; Sgnaoulis et al., 2020). Mansour and Esteban (2017) showed that even with a reduction of 30% to 20% of the dietary protein in tilapia reared in BFT, the fish grew more significantly than those cultured in a clear-water system and fed with 30% protein. To date, whether this nutritional benefit of BFT also occurs in FLOCponics has not yet been reported. Evidence of a negative effect of integration with hydroponics on the benefits of using BFT was found (Pinheiro et al., 2017, 2020; Pinho et al., 2017; Lenz et al., 2018). In these studies low volumes of bioflocs were reported for coupled FLOCponics systems, indicating low availability of natural food. Such a low volume of bioflocs occurred because of the need to limit the quantity of solids in the whole system in order to enable plant production (Barbosa, 2017; Pinho et al., 2017). By individualizing each subsystem in a decoupled layout, proper management of bioflocs in the fish tanks can be carried out. Given the optimal biofloc volume for fish growth in the fish tanks, subsequently optimal nutritional strategies for plant production can be explored.

Developing technologies that allow the reduction of the amount of protein in tilapia diet, without undermining the system yields, benefits the aquaculture sector in both economic and environmental terms (Bossier and Ekasari, 2017; Hisano et al., 2020). This is mainly because the use of low dietary protein may result in: (i) lower feed cost since protein is the most expensive nutrient in fish diets (Jotobi et al., 2014; Hisano et al., 2020); (ii) lower use of fish meal and, on a large scale, minimizing the overexploitation of natural fish stocks (Deutscht et al., 2007); and (iii) decreased input of N and, depending on the production system, less discharge of N into the surrounding environment (Hari et al., 2006; Lazzari and Baldisserotto, 2008). This last consequence of reducing the amount of protein may also influence plant production in the integrated system. The effect of using less CP in the fish diet on plant growth must still be understood. It is important to note that the amount of dietary protein required by fish depends on the employed system and the production phase (Neto and Ostrenskey, 2015; da Silva et al., 2018). For instance, tilapia in the nursery phase (1 to 30 g) usually require high dietary CP to ensure optimal growth when they are young and, consequently, to promote rapid growth until harvest. In systems with minimal natural food available, such as in RAS and cages, the recommended CP for tilapia juveniles varies between 30 and 40% (Hafehd, 1999; Neto and Ostrenskey, 2015), whereas 28% CP has been suggested as enough to achieve high growth performance in BFT (da Silva et al., 2018).

Consequently, decoupled FLOCponics (DFP) seems to be an alternative approach to take advantage of the nutritional benefits of BFT in integrated agri-aquaculture systems and thus reduce the amount of protein in the diets of tilapia juveniles. Additionally, testing different CP levels in this new system is necessary to indicate the optimal input of N to meet both plant and fish nutritional needs. The aim of the study was, therefore, to investigate and evaluate the production of lettuce and tilapia juveniles in a decoupled FLOCponics (DFP) system using different levels of crude protein (CP) in the fish diets. For this, the zootechnical performance of tilapias in DFP systems receiving diets with 24, 28, 32 and 36% CP were compared to those reared in a traditional decoupled aquaponics system (DAPS) and in BFT, both fed with a 32% CP diet. Two cycles of lettuce production were also performed in DFP systems with the different CP levels. Their growth was compared to those in DAPS and traditional hydroponics systems as control treatments. In addition, the physical-chemical parameters of the water were monitored in the aquaculture and hydroponic subsystems.

2. Material and methods

The experiment was carried out in a 100 m² aquaponics greenhouse at the Aquaculture Center of São Paulo State University (Caunesp) in Jaboticabal, São Paulo, Brazil, under the authorization of the Committee on Ethics in Animal Use (CEUA FCAV/Unesp – Protocol No. 001123-20). The greenhouse was covered with a 1.5 mm plastic liner. In addition, a shading net that reduces the luminosity by 40% was put onto the greenhouse. The plastic on the sides and shading net on the top of the greenhouse were movable. The plastic was used as complete coverage only on days when the internal temperature of the greenhouse was below 28 °C and during the night, and the shading net on sunny days.
The water used to fill the tanks and replace the loss by evaporation came from an artesian well.

2.1. Experimental design and diets

A completely randomized experiment was designed to evaluate the production of tilapia juveniles (Oreochromis niloticus) and lettuce (Lactuca sativa) under different production techniques or subjected to diets with different crude protein (CP) contents. In total, seven treatments were tested: one treatment was a tilapia culture in BFT without integration with lettuce production (BFT); the second was a lettuce hydroponic treatment (HP); and the other five were tilapia culture integrated with lettuce production (BFT); the second was a lettuce hydroponic system (HPS); and the other four treatments (BFT and DAPS) were tested: one treatment was a traditional decoupled aquaponics system (DAPS) and the other four were DFP systems using different levels of CP. In the two fish control treatments (BFT and DAPS), diets with 32% CP were used, while in the other DFPs the following levels were tested: 24%, 28%, 32%, and 36% CP (Fig. 1). There were three replications of each fish treatment and six of each plant treatment. From this, the effluent of one aquaculture subsystem was used to nourish two plant tanks. The experiment lasted 56 days. In this period, two cycles of lettuce production and one of tilapia juveniles were performed.

2.1.1. Diets

Ingredients usually used in Brazilian commercial feed industries were selected and their nutrient and energy contents were analyzed at the Laboratory of Animal Nutrition (LANA) of the Faculty of Agricultural and Veterinary Sciences (FCAV-Unesp, Jaboticabal-SP). After that, four diets for tilapia juveniles were formulated to contain different levels of crude protein according to the tested treatments (Table 1). The diets presentation in the diet formulation was based on their availability and nutritional value. The choice of these ingredients and their representation in the diet formulation was based on their availability and quality in Brazil. The diets were formulated to meet some nutritional requirements of tilapia juvenile, i.e., a minimum of 35, 5.5, 17.8, and 4 g kg⁻¹ of ether extract, methionine, lysine, and phosphorus, respectively, and a maximum of 60 and 80 g kg⁻¹ of crude fiber and ash, respectively (Furuya, 2010; NRC, 2011). Diet ingredients were finely ground and sieved in 0.9 mm mesh and 0.5 to 4 mm feed pellets were processed at the Feed Manufacturing Facility of the FCAV-Unesp.

Table 1 Formulas and composition of test diets.

| Ingredient (g kg⁻¹) | Diet |
|---------------------|------|
| Fish meal a | 24 CP | 28 CP | 32 CP | 36 CP |
| Poultry by-product meal b | | | |
| Feather meal | | | |
| Soybean meal d | | | |
| Corn (grain) c | 124.8 | 118.5 | 93.1 | 67.0 |
| Wheat meal b | 124.8 | 118.5 | 93.1 | 67.0 |
| Rice meal d | 124.8 | 118.5 | 93.1 | 67.0 |
| Broken rice b | 124.8 | 118.5 | 93.1 | 67.0 |
| Soy oil i | 37.4 | 28.7 | 27.0 | 28.1 |
| Limestone | 9.7 | 8.1 | 6.5 | 5.0 |
| Dicalcium phosphate m | 20.0 | 19.0 | 18.3 | 17.5 |
| Antioxidant (BHT) d | 0.5 | 0.5 | 0.5 | 0.5 |
| Methionine p | 1.0 | 0.3 | 0.0 | 0.0 |
| Lysine q | 3.2 | 0.0 | 0.0 | 0.0 |
| Salt | 5.0 | 5.0 | 5.0 | 5.0 |
| Total | 1000.0 | 1000.0 | 1000.0 | 1000.0 |

131.6; Crude energy (Mj Kg⁻¹): 201.0. Dietary calcium and phosphorus were set at 3:2:1 for poultry by-product meal, fish meal, and feather meal, respectively, and a maximum of 60 and 80 g kg⁻¹ of crude fiber and ash, respectively. After that, four diets for tilapia juveniles (Fig. 1). Diagram Schematic illustration of the experiment design. Three replicates of each fish treatment and six of each plant treatment were run. CP: crude protein.
2.2. Systems description

The greenhouse hosted individual aquaponics systems run in decoupled mode with unidirectional flow from the aquaculture to the hydroponics subsystems. In each replicate the effluent from the aquaculture subsystem was supplied to two hydroponics subsystems. Thus, in total 18 aquaculture subsystems and 36 hydroponics subsystems were run. The configuration of the aquaculture subsystems differed from each other according to the aquaculture technology employed (Fig. 2). The aquaculture subsystem of the DAPS treatment was run as a recirculating aquaculture system (RAS) and consisted of a circular fish tank (380 L), a radial flow settler (RFS; 100 L), a bag filter (68 μm, 5 L), and a moving bed bioreactor (MBBR; 180 L, containing plastic bio balls with a specific surface area of ~1000 m² m⁻³). When operated as DAPS, the water was recirculated between the aquaculture units using a pump (1000 L min⁻¹) and submerged in the MBBR. The configuration described above was applied in three identical and independent aquaculture subsystems for the DAPS treatment, whereas 15 identical and independent aquaculture subsystems of the biofloc-based treatments were run. These biofloc-based subsystems consisted of a circular fish tank (380 L) and a RFS (100 L).

In contrast to DAPS, in the BFT or DFP treatments the water remained in the fish tank and, in DFPs, was periodically directed to the RFS for the collection of the supernatant for plant nutrition (Fig. 2). The plant nutrition management is detailed below, in subsection 2.4.1. The sedimented organic matter (sludge) from the RFS-DAPS was removed weekly. In the hydroponics subsystem, 36 individualized production units in a deep-water culture (DWC) mode named as plant tanks (PTs) were used, totaling 6 PTs for each treatment. The surface of each PT was immersed in the MBBR. The configuration described above was applied in three identical and independent aquaculture subsystems for the DAPS treatment, whereas 15 identical and independent aquaculture subsystems of the biofloc-based treatments were run. These biofloc-based subsystems consisted of a circular fish tank (380 L) and a RFS (100 L).

2.3. Tilapia juveniles production

Nile tilapia (Oreochromis niloticus) juveniles were purchased from a commercial hatchery (AQUABEL®) and acclimatized for 7 days after arriving at Caunesp. In all treatments, 114 masculinized juveniles (1.42 ± 0.03 g) were stocked, totaling an initial biomass of 0.43 kg m⁻³ and density of 300 fish m⁻³ in each fish tank. After 4 weeks of culture, the number of fish per tank was managed in order to readjust the densities. Thus, 70 fish per tank were kept, resulting in a density of 184 fish m⁻³ and a calculated biomass of 3.6 kg m⁻³.

During the 56-day trial, the fish were hand-fed with the test diets four times a day at 08:30, 11:00, 14:30 and 18:00 h. The amount of feed was calculated based on the percentage of body weight recommended by a commercial feed industry (Raguife®), ranging from 12 to 5% according to the average fish weight. A sample of at least 20% of the total number of fish in each tank was weighed weekly to adjust the amount of feed in all treatments.

At the end of the experiment, all tilapia juveniles were counted and weighed and 15 fish from each tank were individually measured. Final individual body weight (g), total fish length (cm), weight gain (g), yield (kg m⁻³), specific growth rate (SGR), feed conversion ratio (FCR) and survival (%) were assessed. Under the assumption of exponential growth, SGR is defined as: $\text{SGR} = (\ln(W_t) - \ln(W_0)) / (t_f - t_i) \times 100\% \text{ day}^{-1}$, where $t_i$ and $t_f$ are initial and final time; $W_0$ and $W_t$ are the initial and final body weight. Zootechnical performance data and/or fish body composition were used to calculate the protein-use efficiency indices as follows: protein efficiency ratio (PER = mean weight gain/mean crude
protein intake), protein productive value (PPV = [(CP x Wf) - (CP0 x W0)]/crude protein intake x 100%) (%), and crude protein on weight gain (CPwg = [(CP x Wf) - (CP0 x W0)]/mean weight gain x 100%) (%), where CP0 and CPf are the initial and final crude protein content; W0 and Wf are the initial and final body weight.

The protein content of the diets and fish body were determined to evaluate protein use efficiency. For this, three different samples were taken: (i) 30 individuals from the initial fish population; (ii) 10 from each repetition per treatment at the end of the experiment; and (iii) 15 g of each diet. The fish were anesthetized and euthanized. Subsequently, the whole bodies were weighed, packed and frozen at –20 °C for later analysis. The frozen fish were ground, homogenized in a meat grinder (C.A.F, model 22S) and lyophilized (Freeze Dryer Edwards, model Pirani 501). The lyophilized matter was used to determine the percentage of dry matter and crude protein (Lecò Nitrogen/Protein in Organic Samples, model FP528), according to the methodology of A.O.A.C., Association of Official Analytical Chemists (2000). The same analyses were applied to determine the composition of the diets. All analyses of the proximal composition of the tilapia tissue were made in duplicate.

2.4. Lettuce production

Two trials of butter lettuce (Lactuca sativa) production in different phases were carried out. In Cycle 1, the seeding phase was evaluated in a 14-day trial. For this, hydroponic seedlings at 7 days after sowing (d.a.s) and 0.59 ± 0.08 g were grown until 21 d.a.s. In Cycle 2, the final production phase was performed, in which new hydroponic seedlings at 21 d.a.s. and 2.04 ± 0.57 g were planted and cultivated for 21 days until harvest. In both cycles, 8 plants were distributed in each hydroponics subsystem with a density of 19 plants m⁻². The weight (roots and shoot) of four lettuces in all plant tanks were recorded once a week. These four lettuces per tank were selected randomly at the beginning of the trials and the same were weighed weekly throughout each trial.

At the end of Cycle 1, all plants were weighed and the following growth parameters were evaluated: leaf and root height (cm), total wet weight (g), total dry weight (g), number of leaves per plant (–), productivity (g m⁻²) and specific growth rate. At the end of Cycle 2, the following growth parameters were evaluated for seven lettuces from each plant tank: leaf and root heights (cm), wet leaf and root weights (g), dry leaf weight (g), number of leaves per plant (–), and productivity (g m⁻²). Also, in both cycles, a visual analysis was applied to identify the non-marketable plants. Plants that contained up to 33% of abnormalities on the leaf surface, i.e., with a yellowish color, burns or wrinkles, were considered non-marketable (methodology adapted from Pinho et al. (2017)). For the control of plant pests, twelve traps (ColorTrap, Isca®, Brazil) were distributed through the greenhouse. A visual scan of the presence of plant pests or diseases was performed daily on all plants and no sign of them was seen during the trials.

2.4.1. Lettuce nutrition

Prior to the beginning of both trials, 50% of the total volume of each plant tank was filled with water from the aquaculture subsystem and, for the remaining 50%, artesian well water was used. The tanks of HP treatment were only filled with artesian well water. In the DAPS subsystem, the water was collected from the upper-middle portion of the MBBR of the DAPS aquaculture subsystem. In the DFPs treatment, the water of each DFP fish tank underwent a decantation and filtration process before being directed to the plant tanks. This means that the water was pumped into the RFS and remained there for 20 min until the biofloc particles were decanted. After that, the RFS supernatant was directed to a bag filter (68 µm) and then to the PTs. The initial volume of water taken from each aquaculture subsystem, which was used to supply two PTs (2 x 30 L each), was replaced with artesian well water. Between the two cycles of plant production, all the PTs were emptied, cleaned and the aforementioned procedures for filling the PTs were repeated. After filling the PTs, the electrical conductivity (EC) was measured in each PT and a complete commercial fertilizer (Dripool Folhasol®, concentrated 100 times) was added until the EC reached 1.2 mS cm⁻¹ in Cycle 1 and 1.7 mS cm⁻¹ in Cycle 2. The commercial fertilizer was composed of 22.5% N, 9% P, 30% K, 4% Mg, 18.5% Ca, 6% S, 0.15% Fe, 0.085% Zn, 0.05% Mn, 0.015% Cu, 0.004% Mo, and 0.003% B, as informed by the manufacturer. The volumes of fertilizer solution (Vfs) needed to reach these ECs were calculated using the following equation, derived by the authors: Vfs = (ECs - ECfp) x Vfp/ECf; ECs is the electrical conductivity of the concentrated fertilizer solution; ECfp is the electrical conductivity standardized for each plant cycle; Vfp is the registered electrical conductivity in the plant tank; and Vfp is volume of water in the plant tank. When the EC in all PTs was stable, the seedlings were planted.

For plant nutrition during the experiment, water from each aquaculture subsystem or well water (HP treatment) was added manually to the PTs, at the proportion of 2% of the initial volume of the PT since it was the estimated volume of water evaporation in the PTs. In the DFP systems, the biofloc decantation and filtration procedures were always carried out and the decanted bioflocs returned to the fish tanks, except for samples collected in the beginning, middle and end of the experiment. In both plant cycles, the commercial fertilizer solution was added according to the equation above and only if the registered EC values were below the expected ranges. In Cycle 1, the EC was maintained between 1.1 and 1.3 mS cm⁻¹ and a unidirectional water flow between fish and plant tanks occurred once a day on alternate days. In Cycle 2, the EC was between 1.6 and 1.8 mS cm⁻¹ and a unidirectional water flow occurred once a day, six days per week. Aiming to maintain the pH in the PTs at between 5.5 and 6.5, diluted phosphoric acid (1:1) was added when the pH exceeded 6.5.

2.5. Environmental conditions and physical-chemical parameters of the water

Temperature and relative humidity were monitored daily at 11 am at five points, one outside and four inside the aquaponics greenhouse. The water quality parameters such as settleable solids (volume of biofloc by Imhoff cones), pH, electrical conductivity (EC), total dissolved solids (TDS), temperature and dissolved oxygen (Horiba U-526G) were monitored daily in all fish tanks. Concentrations of total ammonia nitrogen, nitrite, nitrate, orthophosphate, and alkalinity in all fish tanks were measured once a week (Koroleff, 1976; Golterman et al., 1978; Mack-ereth et al., 1978). In all PTs, temperature, EC, and pH were monitored daily. The presence or absence of solids in the PTs was also checked daily.

2.6. Statistical analysis

Once the premises of normality (Shapiro-Wilk’s test) and homogeneity of variances (Levene’s test) were fulfilled, water quality, zootechnical performance, protein-use efficiency, and lettuce growth performance data in each plant stage were analyzed by means of one-way ANOVA. For all data related to fish production and water quality in the aquaculture subsystems, three replicates were considered. The productive data of plants and water quality in the hydroponic subsystems were evaluated with four replications per treatment. Significant differences among the treatments were detected using Tukey’s test. All data were analyzed at 5% significance level. Descriptive statistics were also used for water quality parameters in the aquaculture and hydroponics subsystems.

3. Results

Relative humidity and temperature were similar inside and outside the greenhouse (Fig. 3). However, they varied widely during the course of the experiment. Mean relative humidity was 42.3 ± 13.8 and 44.1 ± 14.7% inside and outside, respectively, with minimum values of 22.2
Table 2 shows the descriptive statistics of the water quality parameters in the aquaculture subsystems. DO, pH, EC, and TDS were the values that significantly differed among the treatments. For all these parameters, mean values in DFP-32 and BFT were always statistically similar (p > 0.05), as well as in DFP-24 compared to DAPS values. The variations of nitrogenous compounds and orthophosphate over the experiment are presented in Fig. 4. In all biofloc-based treatments (BFT and DFPs), accumulation of settleable solids in the fish tanks was observed during the experiment. The mean values of ammonia nitrogen varied widely without a clear pattern throughout the experiment, mainly in DFP-28. For nitrite, there is also a notable variation. The nitrite concentration in the DAPS treatment shows contrasting behavior compared to the nitrite concentrations in the DFP and BFT treatments. For nitrate and orthophosphate, a tendency to decrease and accumulate, respectively, was found. For the water quality parameters in the hydroponics subsystems, the descriptive statistics are presented in Table 3.

Only the mean values of pH in Cycle 1 were statistically different (p < 0.05) in the plant treatments. No solids were seen in the plant tanks during the plant cycles. The commercial fertilizer solution was only added in the first two days of each plant cycle regardless of the treatment, i.e., there was no need to add fertilizer during the cycles since the registered EC values were not below the expected ranges. The total volume of fertilizer for each plant tank in Cycles 1 and 2 were, respectively, 316.2 and 366.2 mL in HP, 221.4 and 318.9 mL in DAPS, 208.1 and 314.9 mL in DFP-32, 209.8 and 315.9 mL in DFP-28, and 213.6 and 322.0 mL in DFP-24.

The zootechnical performance and protein-use efficiency of tilapia juveniles are presented in Table 4. Survival, feed conversion ratio, and lengths were similar in all treatments (p > 0.05). The other zootechnical parameters were similar for BFT and DFP-32 means, and both treatments resulted in statistically higher growth performance (p < 0.05) compared to DFP-24, DFP-28, and DAPS. The protein-use efficiency parameters also significantly differed among the treatments. In general, these parameters were significantly higher in the systems that used bioflocs, mainly in DFP-24, compared to DAPS. Table 5 displays the results of lettuce growth parameters in Cycle 1.
Regardless of the plant growth phase, no significant differences were found among the treatments for all parameters. The marketable plants represented 83% of the seedlings produced in all treatments in Cycle 1, whereas in Cycle 2, 100% of the harvested lettuce could be traded. Figs. 5 and 6 show the lettuce growth curves. The growth trend lines were represented, according to the highest $R^2$ achieved, by a polynomial regression in Cycle 1 (Fig. 5) and an exponential regression in Cycle 2 (Fig. 6).

4. Discussion

FLOCponics systems were run in a decoupled layout with the aim of enabling proper management of each subsystem; thus, taking advantage of the nutritional benefits of the biofloc-based culture to produce tilapia juveniles and lettuce. The findings of this study suggest that some critical points usually associated with FLOCponics systems were addressed by individualizing the aquaculture and hydroponic subsystems. For instance, the difficulty of maintaining a low concentration of solids in the hydroponics subsystems and, at the same time, providing a sufficient amount of bioflocs in the fish tanks (i.e. higher than 5 mL L$^{-1}$, Hargreaves, 2013), has been reported as an issue of coupled FP systems (Lenz et al., 2018; Kotzen et al., 2019; Pickens et al., 2020; Pinho et al., 2021). Another challenge of coupled FP is regulating the water pH within the appropriate range for fish, bioflocs and plant growth (Lenz et al., 2018; Kotzen et al., 2019; Pickens et al., 2020; Pinho et al., 2021).

Table 3

| Parameter       | DFP - 24 | DFP - 28 | DFP - 32 | DAPS - 32 | HP       | p-value $^*$ |
|-----------------|----------|----------|----------|-----------|----------|-------------|
| Cycle 1 - Seedling production phase |           |          |          |           |          |             |
| Temperature ($^\circ$C) | 23.74 ± 0.29 | 23.81 ± 0.22 | 23.83 ± 0.16 | 23.75 ± 0.32 | 23.78 ± 0.26 | 0.623     |
| Min - Max       | 23.33 ± 24.21 | 23.41 ± 24.03 | 23.59 ± 24.02 | 23.35 ± 24.25 | 23.40 ± 24.03 |           |
| Electrical conductivity (mS cm$^{-1}$) | 1.27 ± 0.04 | 1.31 ± 0.02 | 1.34 ± 0.02 | 1.31 ± 0.06 | 1.39 ± 0.14 | 0.344     |
| Min - Max       | 1.18 ± 1.35 | 1.26 ± 1.40 | 1.30 ± 1.42 | 1.21 ± 1.42 | 1.06 ± 1.53 |           |
| pH              | 6.77 ± 0.13b | 6.93 ± 0.10b | 6.93 ± 0.14b | 6.59 ± 0.22b | 6.73 ± 0.27b | 0.009     |
| Cycle 2 - Final production phase |           |          |          |           |          |             |
| Temperature ($^\circ$C) | 23.44 ± 0.22 | 23.51 ± 0.21 | 23.47 ± 0.22 | 23.49 ± 0.19 | 23.43 ± 0.23 | 0.699     |
| Min - Max       | 23.14 ± 23.70 | 23.16 ± 23.76 | 23.19 ± 23.78 | 23.27 ± 23.79 | 23.20 ± 23.71 |           |
| Electrical conductivity (mS cm$^{-1}$) | 1.72 ± 0.14 | 1.67 ± 0.05 | 1.67 ± 0.07 | 1.70 ± 0.03 | 1.66 ± 0.06 | 0.700     |
| Min - Max       | 1.45 ± 1.98 | 1.52 ± 1.88 | 1.46 ± 1.97 | 1.42 ± 1.91 | 1.51 ± 1.85 |           |
| pH              | 6.64 ± 0.24 | 6.55 ± 0.10 | 6.66 ± 0.13 | 6.51 ± 0.05 | 6.46 ± 0.08 | 0.079     |
|                 | 6.34 ± 7.05 | 6.44 ± 6.71 | 6.48 ± 6.80 | 6.64 ± 6.58 | 6.36 ± 6.57 | 0.657     |

$^*$ Means followed by different letters in the same line indicate statistical differences (one-way ANOVA at 5% significance level). DFP: decoupled FLOCponics system. DAPS: decoupled aquaponics system. HP: hydroponics control system.
by Imhoff cone), the other results for water quality in the aquaculture subsystems or from the extra commercial fertilizer (Tyson et al., 2012; Pinheiro et al., 2020) or to regulate the C:N ratio (Browdy et al., 2012; Pinheiro et al., 2020) or to recover the nutrients from the BFT effluents by plants.

In the hydroponic subsystems, except for the pH values in the DAPS treatments and the highest values were recorded in the DFP treatments. For the nitrogenous compounds and orthophosphate concentrations, it is recommended to test the methodology adopted for regulating the C:N ratio (Browdy et al., 2012; Pinheiro et al., 2020) or to remove and reuse the solids (Fimbres-Acedo et al., 2020) in FP systems.

The differences found for the mean values of DO, pH, EC and TDS in the aquaculture subsystem were a result of the different input of N and carbon source (molasses) in each treatment. The input of the carbon source seems to be the main factor in these results, since in the DAPS the mean values of pH, EC and TDS were distinct from those recorded in the BFT and DPF-32, even though all of them received the diet with 32% crude protein. For the nitrogenous compounds and orthophosphate results (Fig. 4), it is hard to conclude whether or how the dietary protein or integration with plant production affected the variation of these nutrients during the experiment. Further studies with a focus on the nutrient flows between the BFT and hydroponic subsystems and the carrying capacity of DFP systems are still required to understand the efficiency of recovering nutrients from the BFT effluents by plants.

In the hydroponic subsystems, except for the pH values in Cycle 1, the other parameters of water quality remained within the expected ranges in both cycles. The pH values should remain between 5.5 and 6.5 to enable higher bioavailability of nutrients, whether they come from the aquaculture subsystems or from the extra commercial fertilizer (Tyson et al., 2004). In Cycle 1, the pH was above the recommended range in all treatments and the highest values were recorded in the DFP treatments. Phosphoric acid was added in the plant tanks to regulate the pH when it
Another factor that interfered with the pH was the buffering in biofloc-based cultures. Despite these issues with pH, they exerted no negative effects on the growth of lettuce seedlings (Table 5).

As expected, the results for tilapia growth demonstrated that the well-known benefits of BFT for juvenile nutrition are also found in the DFP systems. Tilapia juveniles fed with 32% CP and grown on both biofloc-based treatments (BFT and DFP-32) grew 22.7% more than those in DAPS also fed with 32% CP. Luo et al. (2014), Long et al. (2015), and Hisano et al. (2019) showed the same tendency of improved zootechnical performance for tilapia grown in BFT compared to RAS, although both were fed with the same amount of CP. They indicated the uptake of the microbial bioflocs as a complementary feed by tilapia as the main reason for these results. Not finding differences in FCR among the treatments is somewhat surprising, since better feed conversion is usually related to biofloc-based culture compared to RAS (Azim and Little, 2008; Long et al., 2015). Nevertheless, the results of PER (3.83), PPV (55.82%), and CPwg (14.65%) show the highest efficiency in using the dietary protein in the fish produced in the biofloc-based system (mainly DFP-24) compared to DAPS (32% CP) with 2.93, 40.11% and 13.70%, respectively. These results suggest that even in an integrated system the in situ food present in biofloc-based systems is used by tilapia juveniles to complement their dietary protein needs. The similar results for tilapia growth in DAPS and in DFP fed with 8% lower CP (DFP-24) reinforce this statement.

The zootechnical results of tilapia fed with lower CP suggest positive economic and environmental implications of DFP. Since protein is usually the most expensive component in the diets (Jatobá et al., 2014; Hisano et al., 2020), the use of lower CP levels will result in lower feed costs. Furthermore, the reduced need for filters in the DFP system compared to DAPS also indicates that FLOCponics might bring economic advantages for the producers. From an environmental point of view, the dependence on feeds is an aquaculture issue (David et al., 2020). Reducing the dietary CP level may mitigate the negative impact of feed on aquaculture sustainability due to the lower need for protein-rich ingredients and lower concentration of N excreted into the natural environment.

With respect to the question of whether DFP might enable lettuce production in comparable yields to DAPS and traditional hydroponics,
this study found no differences among the treatments for the growth parameters in the seedling (Cycle 1) and final production phase (Cycle 2). Interestingly, to achieve these similar yields, less commercial fertilizer was required in the DFP-32 compared to the other treatments. In Cycle 1, the volume of fertilizer added to a DFP-32 plant tank was approximately 51.9% and 6.4% lower than in HP and DAPS, respectively. In Cycle 2, these differences between DFP-32 to HP and DAPS dropped to 16.3% and 1.3%, respectively. Another important finding was that reducing the amount of N in the fish diet in DFP systems did not affect lettuce growth in either cycle. The higher volume of fertilizer added to the DFP-24 and DFP-28 compared to DFP-32 seems to have compensated for the reduction in the amount of N. Nevertheless, in both treatments, the volumes of fertilizer were lower or similar to those added to the DAPS plant tanks.

The curves for lettuce growth presented in Figs. 5 and 6 may be used to predict production in the hydroponic subsystem according to the experimental conditions employed. A notable finding from these curves is the tendency of the seedlings’ growth rate in Cycle 1 to decrease. Possible explanations for this decrease might be that the nutritional management employed in this study seemed to have facilitated lettuce production in both cycles and in all treatments. In spite of this, knowing the specific nutrients that need to be supplemented in the hydroponics subsystems, based on the profile of nutrients in the BFT effluents and on the requirements of the crop, might result in less fertilization dependence, and possibly even greater plant production. For the purpose of developing a specific fertilizer scheme for DFP systems, efforts to constantly characterize the profile of macro- and micro-nutrients in BFT water and to adjust the formulations of the fertilizer according to the dynamics of the BFT will be needed.
For instance, EC of 1.7 mS.cm⁻¹, as in Cycle 2, instead of 1.2 mS.cm⁻¹ and a higher frequency of water flow between fish and plant tanks could have led to exponential growth. Regardless of this growth behavior, the seedlings weighed approximately 14 g at the end of Cycle 1, while the seedlings of the same age (21 d.a.s) purchased from a commercial hydroponic producer for Cycle 2 weighed only 2 g. This difference between the final weight obtained in Cycle 1 in all treatments compared to that achieved by the commercial producer indicates that the management used in this study was more suitable for seedling production than that commonly used in local farms. It should be noted that the present study was carried out under passively controlled climatic conditions, and the results of lettuce growth are directly related to the environmental conditions shown in Fig. 3.

The findings of this study may be useful for producers who already apply the concepts of BFT and seek to increase the sustainable character of their farms. Transforming a BFT farm into a decoupled FLOCponics farm may result in an increased variety of marketable products and a reduction of the overall cost per kg of food produced. Moreover, the integration of BFT and hydroponic production in a decoupled layout seems to allow the reuse of nutrients from the feed and the reduction of the amount of dietary protein, thus minimizing the environmental impacts of aquaculture production. Certainly, several research questions have yet to be answered in order to develop and consolidate decoupled FLOCponics systems and also to make it an option for those who produce in hydroponics or conventional aquaponics systems. Some examples for further investigation have been mentioned throughout this paper, such as: (i) in-depth understanding of the nutrient flows and the carrying capacity of each subsystem; (ii) development of a specific fertilizer to be applied in the hydroponics subsystem taking into account the nutrients available in the BFT effluent; and (iii) alternative solutions for minimizing the accumulation of solids in the aquaculture subsystem, whether by regulating the C:N ratio or by collecting the solids and reusing it for another purpose. The reuse of the solids through a mineralization process seems to be a promising option given the substantial amount of nutrients in the BFT solids (Blanchard et al., 2020; Fimbres-Acedo et al., 2020). Positive impacts of using the effluent of a RAS-sludge mineralization as extra fertilizer for plant production in decoupled aquaponics has been reported (Goddek et al., 2016b), and this might also be the case for mineralized bioflocs. In addition, economic and sustainability assessments which consider the local climate, market, and target species must be performed to measure the final applicability of the proposed system.

5. Conclusions

The results obtained in this study indicate that decoupled FLOCponics (DFP) is a promising technology to produce lettuce and tilapia juveniles using less CP in the fish diets when compared to traditional decoupled aquaponics. Tilapia cultured in DFP and fed with 24 and 28% CP grew similarly to those in DAPS fed with 32% CP diet, suggesting that even after the integration with hydroponics the consumption of microbial bioflocs led to an 8% reduction in the amount of CP. The non-interference of the integration with plant production on the nutritional benefit of BFT was also corroborated by the results of fish growth in DFP-32 and BFT fed with 32% CP diet. Both grew similarly and above the other treatments. Additionally, plant growth results showed no differences in both production cycles (seedling and final growth) among all treatments. Less volume of commercial fertilizer was required in DFP-32, followed by DFP-28, DFP-24, DAPS, and HP.

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Ethical approval

All applicable institutional guidelines for the care and use of animals were followed by the authors. The experiment was carried out under the authorization of the Committee on Ethics in Animal Use of the Faculty of Agrarian and Veterinarian Sciences of the São Paulo State University (CEUA FCAV/Unesp – Protocol No. 001123/20).

Authorship statement

We declare that all authors have contributed significantly and confirm that all of them agree with the content of this manuscript. We also declare that the authors have no conflicts of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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