

Effects of Protein Skimmer Inclusion in an Integrated Aquaponic System Between the Red Tilapia Hybrid (*Florida strain*) and Mint (*Mentha Spicata*)

Tasneem. E. Ali¹, Osama. A. Kadour¹, Mohamed. M. Said²

¹Department of Engineering Science, Faculty of Fish Resources, Suez University, Egypt

²Department of Aquaculture, Faculty of Fish Resources, Suez University, Egypt

*Corresponding author: msaid226@yahoo.com

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ABSTRACT

This study determined how the addition of a protein skimmer in an aquaponic system affected the red tilapia performance, water quality, feed utilization, body proximate composition, and mint growth performance. The experiment consisted of two treatments, one of which was a control system (non-PS), and the other was supported by a protein skimmer (PS). Experimental systems were stocked with 60 fish per m³. The dissolved oxygen (mg/ l) was significantly higher ($P < 0.05$) 7.20 ± 0.14 in the PS treated group compared to the control treatment (6.40 ± 0.32). Both ammonia and nitrite decreased significantly in PS with values of 0.17 ± 0.02 and 0.002 ± 0.001 , respectively. Salinity was significantly lower in PS (0.66 ± 0.06) than in non-PS (1.11 ± 0.11). Weight gain and specific growth rate were significantly higher in PS (109.97 ± 10.43 and 2.65 ± 0.11 , respectively) than in non-PS (76 ± 5.38 and 2.27 ± 0.08 , respectively). Survival rate % decreased significantly in non-PS than in PS (73.66 ± 1.85 and 85.74 ± 1.95 , respectively). The FCR was significantly lower in the PS (0.53 ± 0.01) than in the non-PS (0.84 ± 0.02). The protein efficiency ratio was higher in PS (0.11 ± 0.02). The plant fresh weight, plant height, the number of leaves, fresh biomass, fresh cut biomass, and dry cut biomass were significantly ($P < 0.05$) higher with the addition of PS than the non-PS control group. Mint root length was significantly ($P < 0.05$) higher in non-PS. The two treatments didn't differ significantly in fish body moisture and protein contents. Significantly lower fish body ash content (14.34 ± 0.55) was found in PS treatment than in non-PS treatment (18.94 ± 0.7). The total chlorophyll content, chlorophyll (a), and chlorophyll (b) of mint were significantly higher in PS (50.79 ± 2.04 , 32.65 ± 1.36 , and 19.52 ± 1.97 , respectively) than in the non-PS. The addition of protein skimmer to aquaponic systems caused several advantages in water quality, fish growth performance, and plant growth performance.

INTRODUCTION

Aquaculture plays an increasingly important role in the global food chain, providing human consumption with high-quality and affordable aquatic foods. While global harvest of capture fisheries has remained stable at around 90 million tonnes since the 1990s, aquaculture production must expand responsibly with consideration for the environment, farm animal welfare, human rights, and providing decent work for those

participants. It is already the world's primary source of aquatic food and is expected to remain so as the world's human population grows. (FAO, 2020).

Although aquaculture has been practiced in Egypt for many years, modern management methods have recently been applied to increase production (Shaalan *et al.*, 2018). The sector has developed dramatically in Egypt over the past 20 years as a result of the paradigm shift from conventional extensive to semi-intensive and intensive aquaculture systems (FAO, 2020).

The combination of fish culture and soilless plant culture is known as aquaponics. The overarching objective is to increase sustainability by using the nutrient waste of the fish farming system's as a nutrient to grow plants and produce two different kinds of marketable products. The idea is that there will be enough synergy between aquaculture and plant culture to make the combination of the two viable economically. Combining the two systems will make management more difficult and place restrictions on both systems. The fish feces from the feed input supply vital nutrients to the plants. The plants clean the water by removing ammonia, nitrites, nitrates, phosphates, potassium, and organic carbon, which can then be given back to the fish. Although root systems provide an abundance of surface area for bacteria to remove BOD from water, removal of BOD by roots using oxygen can decrease the concentration of dissolved oxygen (DO) in the root zone. This might restrict plant growth, health, or selection. Many systems do not require a separate biofilter due to the biological filtering provided by the plants and their surroundings (Colt *et al.*, 2021).

There are three types of aquaponic systems: (1) The coupled aquaponic system, water flows from the fish to parts of the plant and back again. (2) An uncoupled aquaponic system, systems for fish and plants are different, independent hydraulic loops. Although water continues to flow from the fish to the plant beds, it does not return to the plants. (3) The flow-through aquaponic system has been utilized with coldwater fish. Although this system needed pumping, other applications might rely on gravity flow (Colt *et al.*, 2021).

In the development of the aquaponic system, add a protein skimmer to improve the circulation of water, protein skimming, on the other hand, is used in aquariums to remove undesirable organics from the tank water, which is beneficial (Feldman *et al.*, 2009). The addition of protein skimmer to aquaponic systems could be examined in order to maximize the performance of those systems. This study was conducted to investigate different effects of protein skimmer inclusion in an integrated aquaponic system between red tilapia hybrid and mint.

MATERIALS AND METHODS

1. Study area

This experiment was conducted in the aquaponics unit, Faculty of Fish Resources, Suez University, Suez Government, Egypt. The experiment lasted from June 10 to September 10, 2021.

1.1. Experimental design

The experiment was conducted using aquaponic systems. The experiment consisted of two treatments including a control system (non-PS) and the other treatment (PS) supported by a protein skimmer where each of them included one replicate. The two treatments consisted of 60 fish and 120 seedlings of mint. Two systems are typical in the general design, water cycle, and aeration system for 24h. It is controlled by the human factor and thermal unit.

1.2. Experimental system

Each system is similar to the other in terms of design, flow rate, and aeration. Each system contains aquaculture and hydroponic systems. Hydroponic is a raft system with pots of plants. Water flows from the fish tank (1000 cubic liter plastic tank) by PVC pipes (38cm long, 2 inch diameter) to the mechanical filter (200 liter cylindrical plastic tank) and then to the biological filter (200 liter cylindrical plastic tank). Afterward, the water was transferred from the biological filter to the plant tank (400-liter rectangular plastic tank) by PVC pipes (360cm long, 2 inches diameter). The dimensions of the plant tank were 300 x 150 x 75 (length x width x height), respectively. The biofilter contained bio ball media (Model: D1-10-8) with a specific surface area of $600\text{m}^2/\text{m}^3$ and material HDPE. Subsequently, the water was transferred from the plant tank to the sump tank 50-liter cylindrical plastic tank through a pipe of 108cm long and 2 inches in diameter. Finally, water was transferred from the sump tank to the fish tank by the submersible pump with 230 volts and 130L/ min drainage, as shown in Fig. (1).

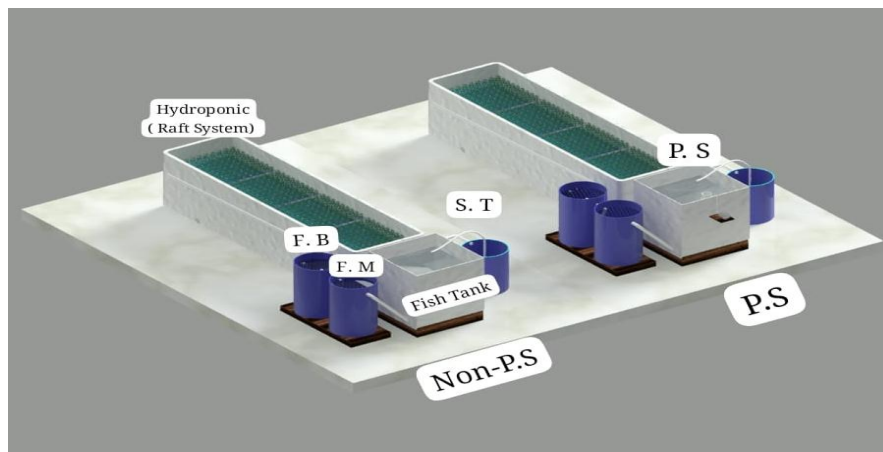


Fig. 1. Experimental design in aquaponic systems. Non-PS: Aquaponic system control, PS: Aquaponic system with protein skimmer

1.3. Protein skimmer management

1.3.1. Protein skimmer design

The protein skimmer was designed and drawn in AutoCAD based on the selection of the type of protein skimmer. The used type of PS in the experiment was a counter-

current skimmer which contained a single column with separate water and air inputs, and air was forced from the bottom of the column, while water flowed from the top, as displayed in Fig. (2).

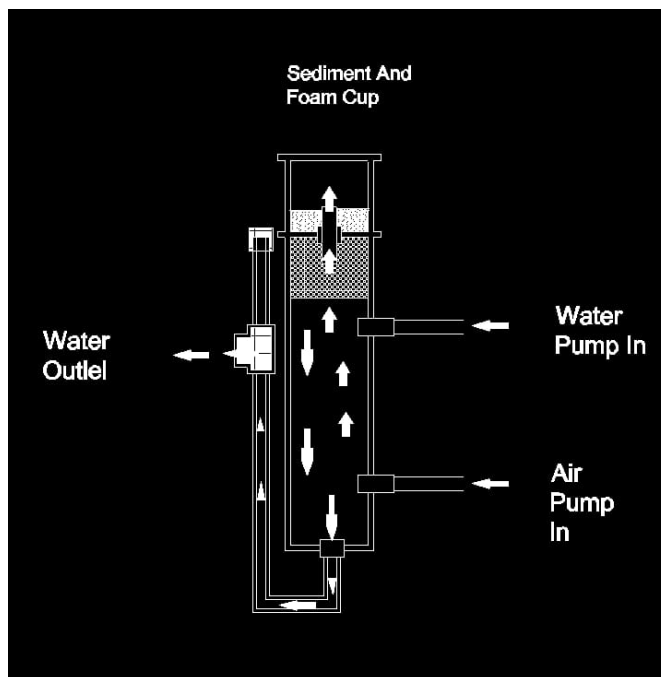


Fig. 2. Design of the experimental protein skimmer

1.3.2. Protein skimmer manufacture

Protein skimmer was manufactured manually with PVC materials (White tube and 3 plugs (4 inches), tube and 2 elbow (2inches), T-shaped elbow and plug (2 inches), 2 male/female adaptors (2 inches), male/ female adaptor and tube (1 inch) and other materials used to collect parts of device such as (CPVC Cement-Thread seal tape-saw-welding machine) (Fig. 3). Other devices were used in protein skimmer such as air blower (Model: BOYU ACQ-003, 50W, 50L/MIN, 220V and 50Hz) and water pump (Model: ONE SHOT DL-2500, 90W, 2200L/H, 220V and 50Hz).



Fig. 3. The contents of the protein skimmer showing: (A): Protein skimmer body, (B): Water pump, (C): Air stone, (D): Device holder, (E): Foam cup and plug, and (F): Air pump

1.4. Combined system formation

The red tilapia fish (*Florida strain*) were obtained from the hatchery of the General Authority of Fish Resources Development (GAFRD) located at 21km on the highway of Alexandria, Egypt. Experimental fish were transferred into oxygenated plastic bags and were acclimated in the system for 2 weeks. The fish were acclimated to 2ppt. The experimented fish were received in 1L³ plastic tank for 7 days and then were distributed on 2 plastic tanks (1000 liter per each) at stocking density 60 fish/ m³ with an initial weight of 39.65± 0.59g for non-PS, and 40.2± 0.55g for PS. The initial length was 10.87± 0.52cm for non-PS, and 11.12± 1.08cm for PS.

Mint (*Mentha Spicata*) seedlings used in this experiment were obtained from El-Fayrouz arboretum, Geser El-Suez, Cairo, Egypt. The number of plants was 120 seedlings for each system. The initial stem length and root length were 9.38± 0.75cm and 9.78± 2.12cm, respectively. The number of initial leaves was 6± 0.7. Plant seedlings were grown in floating rafts in 7cm diameter plastic cups with small holes in the bottom, each cup containing a medium of gravels and peat moss.

Aquaponic systems stocked with 60 fish/ m³ were planted with 120 seedlings. The fish-to-plant ratio was 1:2 (Shete *et al.*, 2015).

1.5. Experimental management

The experimental system included an air blower 1.5KW (Vortex® MODEL: HG-1500) that compress air to the experimental tanks through a pipe (86cm long, 1.5inch diameter) for 24h.

The diet was given three times a day using commercial fish feed containing 30% crude protein (CP) of grand aqua, as shown in Fig. (4). The feed ratio was 1.5% of total fish biomass. Feed quantity was modified every 15 days by taking random samples and calculating the new total fish biomass.

Water quality parameters were measured weekly to ensure proper water quality for the experimental fish. Dissolved oxygen (DO) and temperature were measured using HANNA portable DO meter, salinity was measured using TDS water quality, the pH level was recorded with a HANNA Microprocessor pH meter, the temperature was measured using a thermometer, ammonia assayed with Lovibond MD 100 ammonia test kit and nitrite were measured with other kits.

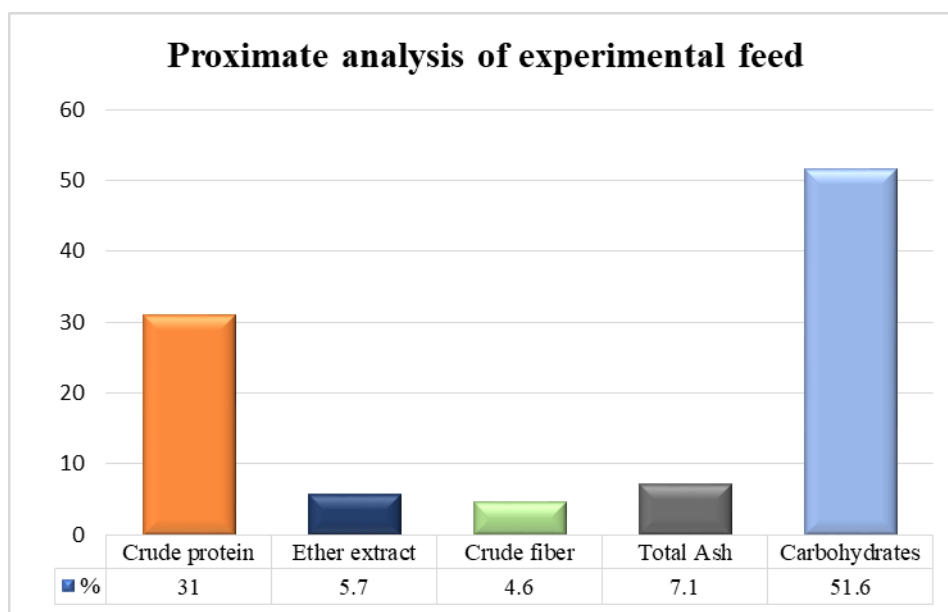


Fig. 4. Proximate analysis of experimental feed on dry matter

1.6. Measurements

1.6.1. Water quality

Water quality was compared between the different experimental treatments with the regular monitoring of dissolved oxygen, pH, salinity, ammonia, and nitrite.

1.6.2. Growth performance of the red tilapia

Growth performance was evaluated through the following parameters: Weight gain (WG) = $W_t - W_0$. Where, W_t = final weight, W_0 = initial weight (Goda *et al.*, 2007). The specific growth rate (SGR (% day⁻¹)) = $(\ln W_t - \ln W_0) / t \times 100$. Where, Ln= Logarithm natural, t = rearing duration by days (Goda *et al.*, 2007). Length gain

(LG) = $L_t - L_0$. Where, L_t = final length, L_0 = initial length (Panase & Mengumphan, 2015). Feed conversion ratio (FCR) = Feed intake/ weight gain, and the feed efficiency (FE %) = $(1/ \text{FCR}) \times 100$ (Goda *et al.*, 2007). Protein efficiency ratio (PER) = wet weight gain (g)/ total protein intake (g) (Ayisi *et al.*, 2017). Condition Factor (CF) = total weight/ standard length³ (Leitritz & Lewis, 1980). At the end of the experiment, all fish in each tank were netted and counted for survival rate determination (Ayisi *et al.*, 2017). Survival rate = (Number of fish at the end of the study/ number of fish initially stocked) *100.

1.6.3. Growth performance of mint

Plant samples of each system were used to measure the number of leaves/ plants, plant height, and root length at the end of the experiment (Shete *et al.*, 2013). Mint was harvested once at the end of the experiment (Shete *et al.*, 2013). At harvest, fresh biomass, fresh cut biomass, and dry biomass were measured as mint (Knaus *et al.*, 2020).

1.6.4. Fish body proximate analysis

At the end of the experiment, ten fish were sampled from each rearing tank and frozen at -20°C in polyethylene bags for further chemical analyses. Accurately, dry matter, protein, fat, and ash contents of whole fish were determined following the methods described by AOAC (1990). Moisture content was estimated by heating samples in an oven at 85°C till constant weight and calculating weight loss. Nitrogen content was measured using a Kjeldahl apparatus and crude protein was estimated by multiplying nitrogen content by 6.25. Total lipids content was determined by ether extraction (60- 80) for 16h and ash content was determined by combusting samples in a muffle furnace at 550°C for 2h.

1.6.5. Plant body proximate analysis

The chemical composition of investigated leaves (moisture, crude fiber, crude protein, and crude lipid content) was determined, according to the method outlined by AOAC (2012).

1.6.6. Chlorophyll concentration analysis

Extraction of chlorophyll (Arnon, 1949), one gram of finally cut fresh leaves was taken and ground with 20- 40ml of 80% acetone. Then, it was centrifuged at 5000-10000rpm for 5min. The absorbance of the solution and the solvent (acetone) blank were measured by spectrophotometer (Model: T60 UV-visible spectrophotometer) from PG instruments at wavelengths of 645 and 663nm. The chlorophyll content was calculated by using the equations that is outlined by Porra (2002).

1.7. Statistical analysis

Data were analyzed using SPSS version 22. (2014). One-way ANOVA and T-test were applied. Duncan's test was used to determine the significance of differences between treatments. The significance was tested at 0.05 levels. All data are represented as the mean \pm SE.

RESULTS AND DISCUSSION

1. Water quality

The physical and chemical water parameters including water temperature, dissolved oxygen (DO), salinity, pH, ammonia (NH₃), and nitrite (NO₂) in the experimental units are presented in Table (1).

The analysis of the physicochemical water parameters in the aquaculture units during the study showed differences between the two treatments (non-PS, PS) and the cup of protein skimmer showed in Table (1). Water temperature and pH were not significantly different between all treatments: non-PS (30.64± 0.57°C), PS (30.14± 0.51°C), cup (30± 0.53°C) and non-PS (7.79± 0.13), PS (7.8± 0.12), cup (7.51± 0.09), respectively. The dissolved oxygen showed no significant difference between PS and the cup (7.2± 0.14, 6.88± 0.35mg/ L), respectively, while significant differences were observed between non-PS, both PS and cup were obtained. The cup showed significantly highest values in ammonia (0.21± 0.01mg/ L), followed by non-PS (0.2± 0mg/ L) and then PS (0.17± 0.02mg/ L). The non-PS showed significantly highest values in nitrite (0.11± 0.03mg/ L), followed by cup (0.04± 0.02mg/ L) then PS (0.002± 0.001mg/ L). The non-PS showed significantly highest values in salinity (1.11± 0.11ppt), without observing any significant differences between PS (0.66± 0.06ppt) and cup (0.66± 0.08ppt).

One of the most essential elements of water quality that contributes to the sustainability of the aquaculture sector is dissolved oxygen (**Missaghi *et al.*, 2017**). According to several researches, when aquaculture aquatic species are grown in the proper range of dissolved oxygen, their growth rate is the fastest and their efficiency is the highest (**Ren *et al.*, 2018**). According to pertinent research, too low DO level may greatly hinder the proper growth of aquatic species and, in extreme situations, even result in widespread mortality (**Fijani *et al.*, 2019**). However, sustained high DO levels can cause fish to develop air bubble illness, which is especially risky to fish eggs and juvenile fish (**Wei *et al.*, 2019a, 2019b**).

The dissolved oxygen value was shown to be higher significantly in PS than in non-PS in this study. **Rahman *et al.* (2012)** found that the protein skimmer affected water quality, and dissolved oxygen concentrations increased by 7%. The skimmer and aeration diffuser were turned on for 60 minutes, it showed that the initial DO value of the skimmer was 6.15mg/ l, which was higher than that of aeration (4.80mg/ l). The DO value continued to drop to 5.00mg/ l on the skimmer and 4.85 on the aeration (**Susanto *et al.*, 2021**).

Table 1. Water quality parameters in recirculating aquaponic systems between the red tilapia and mint with and without protein skimmer

Water quality parameter	Experimental Treatment		
	Non-PS	PS	Cup
Water temperature (C°)	30.64 ± 0.57	30.14 ± 0.51	30 ± 0.53
DO (mg/ L)	6.4±0.32 ^b	7.2±0.14 ^a	6.88±0.35 ^a
pH	7.79 ± 0.13	7.84 ± 0.12	7.51 ± 0.09
Ammonia (mg/ L)	0.2 ± 0 ^b	0.17 ± 0.02 ^c	0.21± 0.01 ^a
Nitrite (mg/ L)	0.11 ± 0.03 ^a	0.002±0.001 ^c	0.04 ± 0.02 ^b
Salinity (ppt)	1.11±0.11 ^a	0.66± 0.06 ^b	0.66± 0.08 ^b

A probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

One of the most important factors for the survival of the fish, microorganisms, and plants in an aquaponic system is pH. The pH of the water is important for effective plant growth and development in soilless culture systems, as well as for ensuring nutrient availability and enabling optimal nutrient uptake by plants. The pH of the aquaponic system should be maintained around 7 to ensure the success of the nitrification process. An acceptable range of pH allows the tilapia fish to grow without negatively affecting their health, and the tilapia growth performance has increased significantly (**Said *et al.*, 2022**). A pH below 6.5 can interfere with the nitrification process, which will cause the aquaponic system to malfunction (**Goddek *et al.*, 2015**; **Yıldız & Bekcan, 2017**). A pH range between 9 and 11 will cause slow growth for fish, and when pH is above 11, consider the alkaline death point (**Boyd, 1998**; **Boyd, 2017**).

The pH range in this study was 7.51- 7.84 which was suggested by **Goddek *et al.* (2015)** and **Yıldız and Bekcan (2017)**. **Espinosa *et al.* (2016)** through their experiment using herbaceous plants as a biological filter for aquaponics systems, produced water quality parameters such as pH values in aquaponic systems and showed a pH range between 7.4- 7.9. **Shete *et al.* (2017)** showed that the value of pH was ranged between 7.0- 7.5 in the aquaponics mint production system. **Knaus *et al.* (2020)** reported that water quality parameters of extensive and intensive aquaponic systems where pH that ranged between 7.2- 6.6, respectively and, this result showed similar to the value of pH parameter in current study. All these studies confirmed that the aquaponic systems had the ability to keep pH balanced in an acceptable range.

According to **Hargreaves and Tucker (2004)** and **Zaki *et al.* (2020)**, the accumulation of feces and N in the form of NH_3 and NO_2 will negatively affect the water quality. In the aquaponic system, ammonia is a result of fish excretion via the gills and the decomposition of uneaten feed (**Eck *et al.*, 2019**). High ammonia levels slow fish growth, decrease survival, and cause many physiological dysfunctions in fish (**Anantharaja *et al.*, 2017**; **Yildiz *et al.*, 2017**). High non-ionized ammonium–nitrogen levels cause slow growth and tissue damage in fish (**Francis-Floyd *et al.*, 2009**). In a balanced aquaponic system, fish are rarely affected by ammonium toxicity. The concentration of nitrite also should be kept as low as possible to avoid it reaching a toxic level. This affects the fish's ability to transport oxygen via blood (**Yildiz *et al.*, 2017**). According to **Hargreaves and Tucker (2004)** and **Zaki *et al.* (2020)**, the accumulation of nitrogen in the form of NH_3 , NO_2 and feces will have a negative impact on the water quality.

In the current study, the result of ammonia concentration in non-PS was significantly higher than PS which decreased until the end of the experiment. Due to the presence of organic matter in the protein skimmer's cup, ammonia concentration showed similar to non-PS. In the same trend of this study, **Rahman *et al.* (2012)** reported that the protein skimmer decreased TAN concentration by 31%, and NO_2 concentration by 35%. In contrast to our findings, **Espinosa *et al.* (2016)** through their experiment produced NO_2 ranging between 0.01 to 0.29mg/ L. **Shete *et al.* (2017)** determined that the ammonia concentration in the water between different treatments revealed that it was 2:2.5mg/ L and the nitrite range was 0.01- 0.10mg/ L, which was higher than what is determined in our study. **Knaus *et al.* (2020)** reported values of 0.29- 0.48mg/ L nitrite for extensive and intensive systems, respectively, which were higher than the values of the system with protein skimmer (PS).

Rahman *et al.* (2012) conducted an experiment in two small-scale recirculating systems (with and without a protein skimmer) and discovered that the protein skimmer significantly influenced salinity concentration but had no impact on water temperature. Water quality also varied significantly with a decrease in salinity by using a protein skimmer over the 87-day culture period and the present study showed the same results. A protein skimmer removes organic matter from water before it degrades into nitrogenous waste, hence improving water quality.

3.2. Growth performance of the red tilapia

Growth performance parameters of the red tilapia significantly differ between non-PS (aquaponic system without protein skimmer), and PS (aquaponic system with protein skimmer), as shown in Table (2), including final weight, weight gain, final length, total biomass, survival rate, SGR, and ADWG.

Table 2. Growth performance parameters of the red tilapia in recirculating aquaponic systems between red tilapia and mint with (PS) and without (Non-PS) protein skimmer

Growth parameter	Experimental treatment	
	Non-PS	PS
Initial weight (g)	39.65 ± 0.59	40.2 ± 0.55
Final weight (g)	92.5 ± 5.38 ^b	125.37 ± 10.43 ^a
WG (g)	52.75 ± 0.32 ^b	85.17 ± 0.55 ^a
Initial length (cm)	10.87 ± 0.52	11.12 ± 1.08
Final length (cm)	14.5 ± 0.45 ^b	16.28 ± 0.56 ^a
LG (cm)	3.63 ± 0.52 ^b	5.16 ± 0.57 ^a
Initial CF	6.35 ± 0.89	5.25 ± 1.01
Final CF	4.99 ± 0.2	4.50 ± 0.1
Total biomass (kg)	4.07 ± 0.01 ^b	6.52 ± 0.02 ^a
Survival rate (%)	73.33 ± 1.85 ^b	86.67 ± 1.95 ^a
SGR (%/day)	0.91 ± 0.01 ^b	1.22 ± 0.02 ^a
ADWG (g)	0.57 ± 0.07 ^b	0.92 ± 0.01 ^a

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

At the end of the experiment, it was revealed that there was a significant difference in growth parameters between the two treatments, as displayed in Table (2). The highest WG was obtained in PS (85.17g) compared with non-PS (52.75g). The LG (cm) values were higher at PS with (5.16cm) than at non-PS with (3.63cm). Concerning the SGR%/day, the higher SGR% was found in PS with 1.22% than SGR% in non-PS with 0.91%. ADWG was higher in PS (0.92g) than in non-PS (0.57g).

The survival rate (SR %) of the red tilapia (Table 2) was affected by using a protein skimmer, and the results showed a significant difference between PS and non-PS. The survival rate was significantly higher in PS (86.67%) than in non-PS (73.33%).

Rahman et al. (2012) conducted an experiment in two small-scale recirculating systems (with and without protein skimmer) and found that abalone survived 88.30% with PS and 86.70% without PS. **Michael et al. (2017)** carried an experiment about the effect of different dietary levels of commercial wood charcoal on the red tilapia hybrid and reported that the survival rate was 82% in the control. Using a protein skimmer slightly improved the survival rate of the red tilapia than the reviewed results.

According to **Rahman et al. (2012)**, abalone consumed more feed and had considerably greater growth rates (higher shell growth) in the PS system than in the non-PS system. The abalone feed composition in the PS system was 23% higher than in the non-PS system. Shell growth and SGR in weight of abalone were 66- 79% greater in the PS system than in the non-PS system, respectively. These results are in agreement with the results of the current study.

In the current study, using protein skimmer didn't negatively impact on the growth of the red tilapia (ADWG 0.92g and SGR 1.22%), which is consistent with Ng *et al.* (2007), who conducted a feeding trial on RAS between the Nile tilapia and the red tilapia with two percentages of protein. When using a diet of 25% protein for the red tilapia, ADWG was 0.49g, and SGR was 1.86%, while using the diet of 35% protein for the red tilapia ADWG and SGR were 0.44g and 1.74%, respectively. There were significant differences in the results of fish growth between the PS treatment and the non-PS treatment based on the variation in water quality between the two treatments. Water quality impacts the general condition of cultured organisms since it determines their health and growth circumstances. Water quality is thus an important consideration when planning for high aquaculture productivity (Mallya, 2007).

Table 3. Feed utilization of the red tilapia in recirculating aquaponic systems between red tilapia and mint with (PS) and without (Non-PS) protein skimmer

Experimental treatment	Feed utilization and body conformation			
	Feed intake(g)	FE (%)	FCR	PER
Non-PS	6345	36.63±0.12 ^b	2.73±0.02 ^a	1.22 ± 0.01 ^b
PS	6345	69.93±0.42 ^a	1.43±0.02 ^b	2.33 ± 0.02 ^a

A probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

The feed conversion ratio (FCR) is the amount of feed (kg) required to produce 1 kilogram of fish which means the lowest ratio of FCR is better than the highest ratio, the results in this study showed the lowest FCR in PS (1.43) with significantly difference between the two treatments (Table 3). There were significant differences between the two treatments in protein efficiency ratio (PER) which was in non-PS (1.22) and PS (2.33) and feed efficiency (FE) with non-PS was 36.63% and 69.93% for PS.

In the study by Rahman *et al.* (2012), abalone had a much better FCR in the PS system than in the non-PS system. FCR reduced by 38% in the system with PS compared to the system without PS.

Xiao *et al.* (2020) conducted an experiment on a recirculation filtration system and found that the FCR was 2.84 and the PER was 1.39 after 60 days of feeding hybrid tilapia. Genc *et al.* (2007) reported that the feed conversion ratio of the red tilapia was 1.35 after 80 days of the experiment, while the protein efficiency ratio was 1.75 for the control. Similar results regarding feed utilization of the red tilapia in a closed system were reviewed. Water quality enhances as a result of the protein skimmer, and FE and PER increase, but FCR decreases as fish feeding improves and fish profit from the protein in the feed.

3.3 Mint growth performance

Mint performance difference between non-PS (aquaponic system without protein skimmer), and PS (aquaponic system with protein skimmer) (Table 4).

In the present study, mint was grown in a gravel medium of the aquaponic system and was supplied with fish wastewater. At the end of the experiment, treatment PS showed significantly higher growth, as shown in Fig. (5). Total mint yield after the harvest varied significantly among treatment and control. Maximum yield was obtained in treatment PS. Plant height after the harvest was significantly higher in the treatment PS with ($93 \pm 4.88\text{cm}$). Fresh biomass, fresh cut biomass, and dry biomass were all significantly higher in PS than in non-PS with values of $283.8 \pm 35.14\text{g}$, $124.4 \pm 25.76\text{g}$, $67.2 \pm 2.49\text{g}$, $38.6 \pm 3.31\text{g}$, and $10.01 \pm 1.2\text{g}$, $5.59 \pm 1.02\text{g}$, respectively. Root length was significantly higher in the non-PS treatment ($43 \pm 7.55\text{cm}$) than in PS ($32.6 \pm 3.35\text{cm}$).



Fig. 5. The difference in growth of mint between non-PS and PS showing: (A): Non-PS at the beginning of the experiment, (B): Non-PS at the end of the experiment, (C): PS at the beginning of the experiment, and (D): PS at the end of the experiment

Table 4. Growth performance parameters of mint in recirculating aquaponic systems between red tilapia and mint with (PS) and without (Non-PS) protein skimmer

Parameter	Treatment	
	Non-PS	PS
No. of leaves/ plant	514 ± 81.23 ^b	1009.4±44.19 ^a
Plant height (cm)	57 ±2.72 ^b	93 ±4.88 ^a
Root length (cm)	43 ±7.55 ^a	32.6 ± 3.35 ^b
Fresh biomass (g/plant)	124.4 ±25.76 ^b	283.8 ± 35.14 ^a
Fresh cut biomass (g/plant)	38.6 ±3.31 ^b	67.2 ±2.49 ^a
Dry cut biomass (g/plant)	5.59 ±1.02 ^b	10.01 ±1.2 ^a

Probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

Knaus *et al.* (2020) reported plant height (51.0± 16.9cm), fresh biomass (190.7± 105.6g/ plant), and cut fresh biomass (31.8± 13.8g/ plant) from an experiment carried out in aquaponic with a total production area of 1000m² (the hydroponics unit 600m²) over a period of 70 days. These results were compatible, while all plant parameters in the unit supplied with the protein skimmer were higher. **Shete *et al.* (2017)** conducted an experiment for 2 months at the aquaponics system and reported the plant height of the floating raft and control at 17.1cm, and 17.6cm, respectively, at the final harvest. **Espinosa *et al.* (2016)** reported production of 647g/ m² from the experiment of mint (*Mentha spicata*) conducted inside a greenhouse consisting of three independent aquaponics systems for 90 days, which was lower than determined in the PS treatment in our study. **Laribi *et al.* (2023)** conducted a hydroponic experiment and measured the shoot length and root length in control (untreated plants), which were 17.61 and 15.90cm/ plant, respectively. The results of plant height, fresh biomass, fresh cut biomass, and dry cut biomass in PS were all higher than in non-PS in this current study and many previous studies in aquaponics systems. These due to that water quality is the most abundant material in a growing plant. The necessity of water quality is readily apparent and has many functions in the plant, since it is a major component of the plant body and an essential solvent in which mineral nutrients are dissolved and translocated from the roots to the apex of the plant body, according to **Kumar *et al.* (2019)**.

3.4. Proximate body composition of the red tilapia

The results of the chemical composition of the whole fish body on dry weight are represented in Table (5). The moisture content was significantly different with the highest

value found in the start with 76.01% followed by PS and non-PS with 61.04 and 60.24%, respectively. The results showed that the dry matter of the chemical analysis of fish was significantly different between non-PS, PS and start with the highest values in non-PS with 39.76%, PS with 38.96%, and the lowest value in start was 23.99%. Protein contents were not significantly different but were higher at start with 41.1% followed by non-PS and PS with 38.76 and 38.69%, respectively. Fat content was highest in start with 25.84% followed by PS and non-PS with 24.46 and 21.26, respectively. The ash content percentages were significantly higher in non-PS than PS and start, with the highest ash percentage (18.94%) found in non-PS followed by PS and start with 14.34 and 13.18%, respectively. Carbohydrate content was higher in PS (22.49%) followed by non-PS and start with 20.31 and 18.64%, respectively, as shown in Table (5).

Many studies investigated the chemical composition of the fish body (red tilapia) in a recirculation aquaponics system and the reported results were in agreement with the results of PS of the current study (38.69% protein, 24.46% fat, 14.34% ash, and 22.49% carbohydrate). **Ng et al. (2007)** evaluated the performance of red tilapia diets containing two levels of protein in a RAS system over a period of 10 weeks. The results showed that the diet with 25% protein had 71.6% moisture, 16.4% protein, 7.6% lipid, and 4.3% ash on a wet weight basis. In comparison, the diet with 35% protein had 71.2% moisture, 17.1% protein, 6.3% lipid, and 4.6% ash. **Xiao et al. (2020)** conducted an experiment in a recirculation filtration system on growth performance, body composition, and biochemical parameters in the plasma of juvenile hybrid tilapia (*Oreochromis niloticus*, *Oreochromis aureus*) with 29% protein diet while the results revealed 71.28% moisture, 16.08% protein, 6.99% lipid and 4.7% ash on a wet weight basis. **Genc et al. (2007)** conducted the feeding trial of hybrid tilapia in a closed aquaculture system and the results revealed 76.69% moisture, 21.49% protein, 1.35% lipid, and 1.36% ash on a dry weight basis with the diet of 42% protein. All previous results confirmed the positive effects of adding a protein skimmer to the aquaponics system.

Table 5. Proximate chemical composition of the red tilapia in recirculating aquaponic systems between red tilapia and mint with (PS) and without (Non-PS) protein skimmer

Proximate analysis		Experimental treatment		
		Start	Non-PS	PS
	Moisture (%)	76.01± 1.63 ^a	60.24 ± 4.19 ^b	61.04 ± 5.04 ^b
	Dry matter (%)	23.99 ± 1.35 ^b	39.76 ± 0.98 ^a	38.96 ± 1.22 ^a
On dry matter %	Protein (%)	41.1 ± 1.49	38.76 ± 0.71	38.69 ± 1.08
	Fat (%)	25.84 ± 1.25 ^a	21.26 ± 2.57 ^b	24.46 ± 1.99 ^a
	Ash (%)	13.18 ± 1.39 ^b	18.94 ± 0.7 ^a	14.34 ± 0.55 ^b
	Carbohydrate (%)	18.64 ± 1.98 ^b	20.31 ± 1.49 ^a	22.49 ± 1.92 ^a

A probability value (*P*) of less than 0.05 was used to indicate statistically significant differences •

3.5. Proximate body composition of mint

Proximate chemical composition of mint between non-PS (aquaponic system without protein skimmer), and PS (aquaponic system with protein skimmer) (Table 6).

Table 6. Proximate chemical composition of mint in recirculating aquaponic systems between the red tilapia and mint with (PS) and without (Non-PS) protein skimmer

Proximate analysis		Experimental treatment	
		Non-PS	PS
	Moisture (%)	86.67 ± 0.37	85.26 ± 1.19
	Dry matter (%)	13.33	14.8
On dry matter (%)	Protein (%)	21.34 ± 0.68	21.59 ± 0.39
	Fat (%)	3.17 ± 0.19 ^b	4.19 ± 0.06 ^a
	Ash (%)	13.38 ± 0.25 ^a	11.49 ± 0.28 ^b
	Fiber (%)	36.21 ± 3.93 ^b	42.49 ± 1.99 ^a
	Carbohydrate (%)	25.88 ± 3.39	20.22 ± 2.53

A probability value (*P*) of less than 0.05 was used to indicate statistically significant differences.

The present study showed the proximate analysis of mint planted in two treatments and revealed significant differences between the two treatments. PS showed significantly higher fat and fiber contents with 4.19 and 42.49%, respectively, than non-PS (3.17% and 36.21%, respectively). The non-PS showed significantly higher ash (13.38%) than the PS (11.49%). Moisture was not significantly different between non-PS and PS, in which non-PS had the highest moisture content (86.67%), but PS had the lowest moisture content (85.26%). Protein and carbohydrates were not significantly different between all treatments (non-PS 21.34% and PS 21.59%) and non-PS 25.88% and PS 20.22%, respectively.

Some studies that investigated the chemical composition of mint showed some close values to our findings in the current study. **Scherer *et al.* (2013)** conducted an experiment about the antioxidant and antibacterial activities and composition of Brazilian spearmint (*Mentha spicata L.*). Samples of spearmint (*M. spicata*) were purchased and then analyzed and the results revealed moisture 86%, protein 2.3%, fat 0.4%, ash 1.7%, and carbohydrates 9.6% on wet weight. **Amasaib *et al.* (2013)** mentioned that the proximate composition of dry spearmint was CP 19.25%, EE 2.10%, CF 19.57%, ash 12.82%, and NFE 40.32%.

3.6. Chlorophyll analysis of mint

Chlorophyll analysis of mint between non-PS (aquaponic system without protein skimmer) and PS (aquaponic system with protein skimmer) is shown in Table (7).

Table 7. Types of chlorophyll (% on fresh matter basis) of mint in recirculating aquaponic systems between the red tilapia and mint with PS and without Non-PS protein skimmer.

Experimental treatment	Types of chlorophyll ($\mu\text{g}/\text{ml}$)		
	Total chlorophyll	Chlorophyll a	Chlorophyll b
Non-PS	25.60 ± 5.07^b	18.36 ± 3.38^b	7.24 ± 1.71^b
PS	50.79 ± 2.04^a	32.65 ± 1.36^a	19.52 ± 1.97^a

A probability value (P) of less than 0.05 was used to indicate statistically significant differences.

Chlorophyll was analyzed by the Arnon method. The study showed a significant difference between the two treatments, as PS was higher in total chlorophyll, chlorophyll a, and chlorophyll b ($50.79\mu\text{g}/\text{ml}$, $32.65\mu\text{g}/\text{ml}$, and $19.52\mu\text{g}/\text{ml}$, respectively) than the non-PS ($25.6\mu\text{g}/\text{ml}$, $18.36\mu\text{g}/\text{ml}$, and $7.24\mu\text{g}/\text{ml}$, respectively).

Chrysargyris et al. (2017) conducted greenhouse hydroponic trials and analyzed the content of chlorophylls (Chl a, Chl b, and total Chl). The Chl a content varied from 1.65 to 1.93mg/ g fresh weight, the Chl b content varied from 0.53 to 0.63mg/ g fresh weight and the total Chl content varied from 2.19 to 2.57mg/ g fresh weight. **Laribi et al. (2023)** conducted a hydroponic experiment and measured the total chlorophyll, chlorophyll a, and chlorophyll b in control *Mentha piperita* (untreated plants), which were 337.830, 169.636, and $116.233\mu\text{g}\cdot\text{mg}^{-1}$, respectively. According to **Wen et al. (2019)**, a lack of $\text{NO}_3\text{-N}$ inhibits the production of the chlorophyll precursor porphobilinogen (PBG), thereby impeding chlorophyll synthesis. Protein skimmer purifies water from organic matter and thus reduces turbidity and this allows the bacteria responsible for converting dissolved ammonia into nitrates to work, hence the amount of nutrients for plant growth is available and therefore the percentage of chlorophyll increases.

CONCLUSION

The results of the present study clearly confirm that the inclusion of protein skimmer in an aquaponic system helps in the enhancement of water quality, fish growth, survival rate, and feed utilization of the red tilapia, in addition to improving mint growth and the concentration of chlorophyll.

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