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Combined effects of surface area of periphyton substrates and stocking density on growth performance, health status, and immune response of Nile tilapia (*Oreochromis niloticus*) produced in cages

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Abstract

A 4-month feeding trial was conducted to investigate the effect of surface area of periphyton substrates (PS) and stocking density (SD) of juvenile Nile tilapia, *Oreochromis niloticus*, on growth, major blood constituents, and some immunity biomarkers. Six treatments (T1–T6) were established in 18 floating cages (1 m^3 water each) fixed in an earthen pond for growing tilapia (1.12 ± 0.10 g), in a 2 × 3 factorial design experiment (2 SD × 3 PS). For T1, T2, and T3, fish were stocked at a rate of 70 m^{-3} with 1, 2, or 3 PS units (area: 0.7, 1.4, and 2.0 m^2, respectively). For T4–T6, fish were stocked at 90 m^{-3}, also with 1, 2, or 3 PS units. All fish were fed a 20% crude protein supplemental diet. The best growth rates, feed efficiency, body composition, immune response, and overall health status were attained for T2 group with 70 fish m^{-3} in the presence of 2 PS units (1.4 m^2) followed by T5 with 90 fish m^{-3} plus 2 PS. Stocking density affected albumin while periphyton substrates impacted cholesterol, triglycerides, and alanine aminotransferase levels in fish serum. Meanwhile, total proteins and glucose-6-phosphate dehydrogenase were not significantly affected. Serum immunoglobulin (IgM and IgG) values were relatively higher in fish reared at 70 m^{-3} than at 90 fish m^{-3} indicating better immunity response. Periphyton biomass (dry matter, DM and ash-free DM) was lower in T2, than in other treatments, whereas periphyton populations belonged mainly to Chlorophyta (52–75%), Cyanobacteria (17–23%), and Charophyta (4–21%). Accordingly, the present study suggests that 70 fish m^{-3} and 2 PS units (surface area of 1.4 m^2) would be an optimal combination for the best growth, health status, and immunity response of juvenile Nile tilapia reared in periphyton-based cage system.

Keywords Nile tilapia · Periphyton · Cage-fish farming · Stocking density · Hematology · Serum biomarkers

Introduction

Successful and sustainable aquaculture depends on economically viable and environmentally friendly feeds, which are a major operational cost in intensive fish farming (Rana et al. 2009; El-Sayed 2020). Therefore, optimizing cost-effectiveness of feed is a major challenge facing aquaculture sustainability, particularly in developing countries (Hasan and New 2013). Tilapia is one of the most important commercially farmed fish species in the world, second only to carps (FAO 2019). Tilapia aquaculture has gained a considerable attention as a consolidated food production sector within the global economics, and more production of tilapia would mean more nutrition and more income for the farmers (El-Sayed 2020). However, the high cost of fish feeds has posed some problems for the expansion of tilapia farming industry. Therefore, attempts have been made to reduce feed costs, mainly by using cheaper alternatives for producing fish diets. Accordingly, new simple applications need to be surveyed for proper exploitation of the existing water bodies, by maximizing the existence of their natural food.

In recent years, periphyton-based aquaculture has been considered to have a real potential to be conducted in pond and cage culture systems (Huchette and Beveridge 2003; Azim et al. 2005; Garcia et al. 2016; An and Anh 2020

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among others). Periphyton has traditionally been used as a natural fish food in many countries, especially in South-East Asia (van Dam et al. 2002). Periphyton is the community of microorganisms that colonize on submerged substrata. In semi-intensive pond aquaculture, periphyton production could be considered an essential complementary food source for cultured fish (Azim et al. 2005). Several studies indicated that periphyton communities, grown on submerged substrata, provide a good source of quality food for farmed fish and increase fish production per unit area (Keshavanath et al. 2004) and also improve water quality (Kosáros et al. 2010).

One of the most common forms of tilapia aquaculture is the production of Nile tilapia in cages (El-Sayed 2020). Tilapia show opportunistic omnivore feeding behavior with a great tendency towards herbivory (Beveridge and Baird 2000) and have morphological adaptations that allow them to feed on phytoplankton and periphyton (Sanderson et al. 1996). Therefore, if natural food is present, the protein content of commercial diet can be reduced (Sakr et al. 2015). A number of studies have indicated that periphyton can be used as a natural food source in cage-farmed tilapia, leading to a significant reduction in added feeds (Milstein et al. 2008; Sakr et al. 2015) and increasing profitability (Huchette and Beveridge 2003; Sakr et al. 2015; Garcia et al. 2016, 2017).

However, the combined effects of stocking density of fish and the surface area of periphyton substrates on fish growth performance, health status, and immunity response have not yet been investigated. Therefore, the present study was carried out to evaluate the effect the surface area of periphyton substrates and fish stocking density on growth performance, health status, and immunity response of juvenile Nile tilapia (*Oreochromis niloticus*) reared in cages system.

Materials and methods

Experimental design

Eighteen cages (1 m^3), made of nylon netting with a mesh size of 0.35 mm, were installed in an earthen pond (0.2 ha, 1.8–2.0 m depth) supplied with fresh water, in El-Max Experimental Fish Farm (15 km west of Alexandria) which belongs to the National Institute of Oceanography and Fisheries (NIOF), Egypt. The cages were fixed by wooden bars, 50 cm above the pond bottom. Ten bamboo poles (each about 60 cm length and 3–4 cm diameter) were used as one unit of periphyton substrate (PS), giving a surface area of 0.7 m^2. Before the initiation of the feeding trial, the periphyton substrates/units were installed under water surface of the experimental pond and left for 1 month to allow the growth and colonization of periphyton communities on them. At start of the trial, the PSs were introduced into the middle of the cages at the proposed numbers (1, 2, or 3 PS per cage), and vertically fixed, parallel to each other, as roped units completely under water surface. A plastic feeding tray (30 × 30 cm) was further hung in each cage at about 20 cm below the water surface where the supplemental feed pellets were supplied.

Fish and farming regime

Two thousands of all-male juvenile Nile tilapia were obtained from a private hatchery, transported to the farming facility and acclimatized to the prevailing conditions in a 24-m^3 cage, installed in the same experimental pond. The fish were fed on a commercial diet (32% CP) for 7 days before the start of the feeding trial. At the end of acclimatization period, fish were distributed in triplicate groups into 18 cages (1 m^3), at two densities 70 and 90 fish m^{-3}. At each density, the cages were provided with either: one, two, or three periphyton substrate units (1 PS, 2 PS, or 3 PS), with a surface area of 0.7, 1.4 or 2.0 m^2, respectively. Fish in each cage were collectively weighed and the average initial body weight (IBW, g) was determined, and overall average IBW for all cages was 1.12 ± 0.10 g $fish^{-1}$.

All fish were fed a supplemental diet containing 20% crude protein (CP), 7% crude lipids and 17.8 MJ kg^{-1} gross energy (Table 1). This low-protein diet was adopted based on a

Table 1 Composition and proximate analyses of the supplementary diet (as fed)

Ingredient	g kg^{-1}
Fish meal (FM)[a]	50
Soybean meal (SBM)[b]	220
Yellow-maize flour (YM)[c]	330
Wheat bran (WB)[c]	240
Rice bran (RB)[c]	110
Fish oil and soybean oil mix (1:1, v/v)	20
Vitamins and minerals premix[d]	20
Di-calcium phosphate	10
Proximate analyses (%DM)	
Crud protein (CP)	21.26
Lipids	6.76
Crude fiber	5.43
Nitrogen-free extract (NFE)[e]	58.76
Ash	7.76
Gross energy (GE, MJ kg^{-1})[f]	17.85

[a] 999 LT (Esbjerg, Denmark, 72% CP)

[b] Hexane-extracted (44% CP)

[c] Local products: YM (7% CP), WF (13% CP)

[d] NRC (2011)

[e] Calculated by difference

[f] Calculated based on 23.6, 39.5 and 17.2 KJ g^{-1} of protein, lipid and carbohydrate, respectively

previous study (Sakr et al. 2015) which indicated that protein content of a supplementary diet for Nile tilapia reared in a periphyton-based system can be reduced to 20% or even lower. The daily feed was offered at 5% of the fish live weight divided into 3 meals during the first 2 months, then reduced to 3% supplied twice a day for another 2 months. Uneaten feed was collected daily, re-dried and weighed, and daily amount of feed consumed was then quantified for each cage. Fifty fish from each cage were sampled and weighed at 15-day intervals, their average weights were recorded, and the daily amount of feed for each cage was readjusted accordingly. At the end of the feeding trial, all fish in each cage were collected and counted, and the average final body weight (FBW, g $fish^{-1}$) for each treatment and fish survival were calculated.

Water quality parameter

Water quality parameters were monitored on a weekly basis throughout the experimental period. Water temperature was recorded with a thermometer at 20 cm depth, dissolved oxygen by using oxygen meter (YSI 56, Yellow Springs, USA) and pH by a pH meter (Orion, USA). During the trial, average values (±SE) of water temperature were 26.6 ± 0.8 °C; salinity, 5.43 ± 0.25 ppt; dissolved oxygen, 7.57 ± 0.25 mg L^{-1}; pH, 7.65 ± 0.23 and ammonia, 0.10 ± 0.007 (mg L^{-1}). All measured water quality parameters were within the acceptable limits for rearing juvenile Nile tilapia (El-Sayed 2020).

Proximate composition analyses

At the end of the trial, five fish from each cage were randomly collected and frozen at − 20 °C for final body composition analyses. Initial body analyses were performed on a pooled sample of 10 fish per treatment, which was weighed and frozen before the study. Proximate analyses of whole body moisture, protein, lipid, and ash were performed according to standard AOAC (2005) methods. Briefly, for moisture content determination, fish samples were dried at 105 °C to constant weight. Protein was measured as nitrogen by a semi-automatic Kjeldahl (N × 6.25; VELP Scientifica, UDK 126, Italy) following acid digestion. Lipid content was measured gravimetrically after Soxhlet extraction with petroleum ether (40–60 °C) as a solvent. Ash content was determined after ignition in a muffle furnace at 550 °C for 6 h. All analyses were conducted in triplicate samples for each parameter.

Data collection and samples analysis

At the termination of the trial, average final body weight (FBW, g $fish^{-1}$) was calculated as mentioned in "Water quality parameter." Individual total fish length (cm) and total weights were further recorded for a 15 fish sample from each cage. Five fish from each cage were randomly collected immediately after capture for blood analyses. The fish were lightly anesthetized using a few drops of clove oil (2–3 drops L^{-1}) and blood samples were collected by cardiac puncture. Blood samples for each cage were pooled either in heparinized glass tubes for blood profile analysis or without heparin for serum assays. Major blood constituents including hemoglobin concentration (Hb), hematocrit (Ht), red blood cells/erythrocytes (RBC), and white blood cells/leukocytes (WBC) counts were all estimated using an automated technical analyzer (Celltac α MEK-6400 J/K, USA), adopting Dacie and Lewis (2006) techniques. Blood samples, without heparin, were left to coagulate at 4 °C prior to centrifugation for 20 min at 3000 rpm to separate serum. Sera were stored at − 80 °C until further analyses. Commercial diagnostic kits (Bio-Merieux, Chemicals, USA) were used for the following determinations: serum total proteins (g dL^{-1}) using the Biuret reagent method (Henry 1964); albumin content (ALB) by Dumas et al. (1972)'s method; cholesterol (CHL) (mg dL^{-1}) and glucose-6-phosphatedehydogenase (G6PD) (mg dL^{-1}) by the enzymatic colorimetric technique of Thomas (1992) and Trinder (1969), respectively; triglycerides (TRG) (mg dL^{-1}) using Friedewald et al. (1972)'s method; and activity of alanine aminotransferase enzyme (ALT) according to Reitman and Frankel (1975). All analyses were determined spectrophotometrically (Jasco-V530, USA). Finally, immunoglobulin M (IgM) and immunoglobulin G (IgG) were determined using ELISA Kit (Catalog No. CSB-E12045Fh, 96 k test, Cusabio Biotech Co., USA).

Taxonomic and biochemical composition of periphyton

At end of the experiment, periphyton samples were collected from the substrate surfaces by carefully scraping a surface area of 4 × 4 cm of the bamboo poles, to determine the composition and abundance of the attached microorganisms. Samples were sieved by a 20-μm plankton net, autoclaved to ensure that the water was free from any living organisms and fixed in a 4% neutral formalin solution. They were then identified to the phylum level by light microscopy. Periphyton abundance was calculated using Sedgwick-rafter cell, according to the following formula: $N = 100$ P x C/S, where N is the single cell or multicellular counts of periphyton per cm^2, P is total number of periphyton units counted in 10 fields of Sedgwick-rafter cell, C is volume of final concentrate sample (mL), and S is the total area of scraped surface (cm^2). The periphyton samples were further analyzed, in triplicates, to determine their proximate biochemical composition, using the standard AOAC (2005) methods. Gross energy contents were calculated based on 23.64, 39.54, and 17.57 KJ g^{-1} for protein, lipid, and carbohydrate, respectively.

Fish performance

Fish growth and feed utilization were calculated as follows: weight gain (WG) = FBW-IBW (g fish^{-1}); specific growth rate (SGR, % day^{-1}) = 100{ln FBW – ln IBW} / t, where *IBW* and *FBW* are initial and final body weight (g fish^{-1}), t is time of feeding trial (days). Feed conversion ratio (FCR) = dry feed consumed (g)/fish live weight gain (g); protein efficiency ratio (PER) = fish weight gain (g)/protein intake (g); protein productive value (PPV) = 100 [protein gain (g)/protein fed (g)]; energy utilization (%) = 100 [energy gain (MJ kg^{-1})/energy intake (MJ kg^{-1})]; Fulton's condition factor K = 100 W L^{-3}, where W = total fish weight (g), L = total fish length (cm); and survival (S, %) = 100 final fish number/initial fish number.

Statistical analyses

A 2 × 3 factorial design, with three replications, in a randomized complete block way was applied with two fish stocking densities (SD) and three periphyton substrate units (PS). Statistical analysis was conducted by two-way ANOVA, F-test, and least significant difference (LSD) procedures using the SAS software package. Results were presented as means ± standard error (SE) and coefficient of variation (CV, %). The data in percentages were arc-sin transformed prior to analysis. F-test and ANOVA were performed at $P = 0.05$.

Results

Growth performance

The present results revealed that growth performance and feed utilization of Nile tilapia were significantly ($P < 0.05$) affected by SD and the number PS (Table 2). The highest growth rates were obtained in fish reared at 70 fish m^{-3} in the presence of 2 periphyton units (T2 group), followed by T1 (70 fish + 1PS), whereas the lowest performance was obtained at the highest SD and PS (90 fish m^{-3} + 3PS). Similarly, the best feed utilization indices (FCR, PER, PPV, EU) were recorded in T2 fish group, followed by T1. Fish survival ranged from 76 to 87% ($P < 0.05$), but showed irregular pattern of variation among treatments. Similar irregular pattern was also observed in the condition factor (K) values of fish.

Fish biochemical composition

The whole body composition of Nile tilapia for all treatments is given in Table 3. Protein, lipids, ash, and moisture contents were all significantly affected by SD and PS, with the highest protein and lowest lipid contents being recorded in T2-fish (70 fish + 2PS, surface area of 1.4 m^2) followed by T4-fish (90 fish + 1PS). Meanwhile, T3- and T6-fish had the lowest protein content and the highest moisture and ash contents for T6-fish. Accordingly, fish of both T2 and T4 recorded the best body biochemical composition among all dietary treatments.

Health status

Major blood parameters

Blood profile, including hemoglobin content (Hb), hematocrit (Ht), red blood cells/erythrocytes (RBC), and white blood cells/leukocytes (WBC) counts of Nile tilapia, at the end of the trial, is given in Table 4. Fish of T2, T3, and T4 showed the highest Hb, Ht, RBC, and WBC counts ($P < 0.05$). At 90 fish m^{-3}, increasing PS units significantly decreased these blood parameters ($P < 0.05$). These results revealed that fish reared at 70 m^{-3} in the presence of 2PS, or at 90 m^{-3} with only 1PS achieved the best hematological records among all dietary treatments.

Physiological and immunity biomarkers

The present results indicated that the values of serum total proteins, albumin, immunoglobulin M (IgM), and immunoglobulin G (IgG) were highest in fish of T1 and T2 (70 fish + 1PS or 2PS) among all treatments ($P < 0.05$), while the lowest values were recorded in fish of T6 (90 fish + 3PS) (Table 4). At 90 fish m^{-3}, albumin level was significantly lower than that at 70 fish m^{-3} ($P < 0.05$). Serum cholesterol (CHL) levels were elevated in fish raised at 3PS m^{-3} at both stocking densities (T4, T6) compared to other fish groups. Therefore, serum CHL was affected by number of PS, but not by SD. Similar trend of variation was observed for triglycerides (TRG). Additionally, higher number of PS (2 and 3 units) led to higher values of alanine transaminase (ALT) concentrations than those in fish grown with only 1PS (T1 and T4). In brief, SD affected albumin content and PS impacted CHL, TRG, and ALT levels in fish serum, but total proteins and glucose-6-phosphate dehydrogenase (G6PD) were not significantly affected by SD or PS ($P > 0.05$).

Periphyton biochemical composition and communities

Biochemical composition of periphyton

The biochemical composition of periphyton revealed significant variation among dietary treatments ($P < 0.05$). Group T2 (70 fish m^{-3} + 2PS) showed the highest protein, ash, and fiber contents, whereas the lowest values were recorded in T6 (90 fish m^{-3} + 3PS). On the other hand, lipids and energy contents were the highest in T1 (70 fish m^{-3} + 1PS) (Table 5). In the mean time, nitrogen-free extract (NFE) ranged from 53.29 to

Table 2 Effect of stocking density and number of periphyton substrates on growth, feed utilization efficiency of Nile tilapia, *O. niloticus*, reared at different stocking densities and number of periphyton substrate in cages

Stocking density (fish m^{-3})	Periphyton substrate (no. m^{-3})[a]	IBW (g)	FBW (g)	WG (g)	SGR (% day^{-1})	FCR	PER	PPV	EU (%)	S (%)	K
70	1 (T1)	1.00	46.84b	45.84b	3.20a	1.93c	2.44b	35.74b	18.34a	87.14a	1.68b
	2 (T2)	1.05	56.30a	55.24a	3.31a	1.61d	2.92a	44.03a	18.68a	83.33b	1.72a
	3 (T3)	1.08	40.37b	39.29c	3.01b	2.23b	2.11c	36.89b	14.56c	79.26c	1.49c
90	1 (T4)	1.18	42.13b	40.95b	2.96b	2.33b	2.02c	34.23b	13.04c	81.85cb	1.69b
	2 (T5)	1.20	47.14b	45.94b	3.05b	1.94c	2.43c	41.98a	16.99b	75.93c	1.69b
	3 (T6)	1.21	38.92c	37.71b	2.89b	2.57a	1.84a	32.98b	11.45d	86.67a	1.71b
Mean		1.12	45.28	44.16	3.07	2.10	2.29	37.64	15.51	82.36	1.66
Pooled SE		0.10	1.80	1.80	0.04	0.07	0.08	2.23	0.60	1.76	0.03
C.V. (%)		4.23	9.59	9.84	3.23	5.69	5.80	15.01	5.78	4.96	2.46
LSD ($P < 0.05$)		0.08	7.88	7.87	0.17	0.21	0.23	10.24	1.62	7.41	0.05

Means in the same column bearing different lowercase letters are significantly different ($P < 0.05$)

IBW initial body weight, *FBW* final body weight, *WG* weight gain, *SGR* specific growth rate, *FCR* feed conversion ratio, *PER* protein efficiency ratio, *PPV* protein productive value, *EU* energy utilization, *S* survival, *K* condition factor, *C.V. (%)* coefficient of variation, *LSD* least significant difference

[a] Surface area: 1PS = 0.7 m^2 ; 2PS = 1.4 m^2 ; 3PS = 2.0 m^2

58.84%, with the highest values being recorded in T4 and T6 and the lowest value was found in T2 group.

Periphyton communities

Figure 1 shows the relative abundance of different aquatic species developed on PS units within the Nile tilapia cages throughout the study period for T2 and T5 (which boosted growth and have similar surface area of 1.4 m^2). The periphyton communities on bamboo substrates comprised members of three main groups of phytoplankton (Chlorophyta, Cyanobacteria, and Charophyta) and three main groups of zooplankton communities (Copepods, small Crustacean and Annelida). Other groups with minor abundance (one to < 10^3 individual mL^{-1}) were also present. The most abundant species were Chlorophyta (52 and 75%), followed by Cyanobacteria (17 and 23%) and Charophyta (4 and 21%), while the contribution of the rest of periphytic communities (annelida, small crustaceans, and copepods) was only 1 and 2% for T2 and T5 respectively.

Table 3 Body composition (on dry weight basis) of Nile tilapia, *O. niloticus*, reared at different stocking densities and number of periphyton substrate in cages

Stocking density (fish m^{-3})	Periphyton substrate (no. m^{-3})[a]	Protein	Lipids	Ash	Moisture
Initial body composition		67.50 ± 0.20	7.20 ± 0.05	24.27 ± 0.31	76.01 ± 0.15
Final body composition					
70	1 (T1)	69.33b	10.46c	20.20a	74.20bc
	2 (T2)	72.40a	6.94d	19.08a	74.12b
	3 (T3)	67.69c	11.92b	20.40a	75.66bc
90	1 (T4)	71.79a	9.00c	17.42b	74.80bc
	2 (T5)	68.45b	10.79c	18.14b	76.40a
	3 (T6)	67.59c	13.41a	20.66a	78.03d
Mean		70.26	10.42	19.31	75.54
Pooled SE		0.82	0.19	0.49	0.61
C.V. (%)		0.90	5.28	4.56	1.98
LSD ($P < 0.05$)		1.14	0.99	1.58	0.87

Means in the same column bearing different lowercase letters are significantly different ($P < 0.05$)

C.V. (%) coefficient of variation, *LSD* least significant difference ($P < 0.05$)

[a] Surface area: 1PS = 0.7 m^2 ; 2PS = 1.4 m^2 ; 3PS = 2.0 m^2

Table 4 Hematological and immunological parameters of Nile tilapia, *O. niloticus*, reared at different stocking densities and number of periphyton substrate in cages

Treatment		Blood parameters				Serum immunity indicators				Serum physiological parameters			
Stocking density (fish m^{-3})	Periphyton substrate (no. m^{-3}),[a]	Hb (g dL^{-1})	RBC (10^6 μL^{-1})	Ht (%)	WBC (10^3 μL^{-1})	Total protein (mg dL^{-1})	Albumin (mg dL^{-1})	IgM (mg dL^{-1})	IgG (mg dL^{-1})	ALT (U L^{-1})	CHL (mg dL^{-1})	TRG (mg dL^{-1})	G6PD (Ug Hb^{-1})
70	1 (T1)	7.30b	1.10b	17.20c	63.10b	3.10	2.00a	3.00a	0.69a	75.0c	81.05c	72.50b	10.75
	2 (T2)	7.65a	1.64a	25.65a	100.7a	3.35	2.05a	2.50a	0.62ab	80.0b	85.51c	76.00b	10.40
	3 (T3)	7.40b	1.50a	22.10b	99.30a	2.50	2.00a	1.80b	0.57b	89.0a	100.00b	81.00ab	10.35
90	1 (T4)	7.65a	1.60a	25.20a	99.95a	2.70	1.90b	1.80b	0.57b	67.5d	81.53c	79.00b	9.10
	2 (T5)	7.35b	1.06b	18.80c	65.60b	2.35	1.90b	1.70b	0.56b	88.5a	95.50b	83.00ab	9.60
	3 (T6)	7.00c	0.86c	16.50c	40.60c	2.41	1.85b	1.25c	0.51c	102.0e	127.52a	88.00a	8.60
Mean		7.39	1.30	19.24	78.20	2.74	1.95	1.90	1.95	83.6	95.17	79.91	9.83
Pooled SE		0.12	0.04	0.06	2.04	0.11	0.05	0.10	0.05	2.89	2.05	2.04	0.21
C.V. (%)		2.86	9.26	0.84	6.12	0.97	7.55	11.16	7.55	8.45	6.03	7.99	9.05
LSD ($P < 0.05$)		0.38	0.21	0.26	4.23	0.78	0.26	0.38	0.05	2.81	4.66	7.01	1.57

Means in the same column bearing different lowercase letters are significantly different ($P < 0.05$)

Hb hemoglobin, *RBC* red blood cells (erythrocytes) count, *Ht* hematocrit, *WBC* white blood cells (leukocytes) count, *IgM* immunoglobulin M, *IgG* immunoglobulin G, *ALT* alanine transaminase, *CHL* cholesterol, *TRG* triglycerides, *G6PD* glucose-6-phosphate dehydrogenase, *C.V. (%)* coefficient of variation, *LSD* least significant difference

[a] Surface area: 1PS = 0.7 m^2; 2PS = 1.4 m^2; 3PS = 2.0 m^2

Discussion

The production of tilapia in periphyton-based system has been considered by several authors (Huchette and Beveridge 2003; Garg et al. 2007; Sakr et al. 2015; Garg and Bhatnagar 2016; Garcia et al. 2017, and reviewed by El-Sayed 2020). These studies indicated that these systems can be highly profitable if proper species and sizes, stoking density, periphyton substrate material, and surface area and supplemental feeding are adopted. However, the combined effects of SD and the surface area of PS on the growth performance, general health status, and immune response of farmed fish are not well understood. Growing fish within cages may cause stressful conditions to them, particularly under high stocking densities. Fish undergo a series of biochemical and physiological changes, in response to stress, in an attempt to cope with the stressful conditions (Conceiçao et al. 2012; Tort 2011). In the mean time, periphyton substrates can provide an additional natural food source for growing fish. Besides, periphyton microorganisms contain many natural bioactive compounds which may improve fish resistance to stressful conditions such as crowding.

In the current study, juvenile Nile tilapia, reared in cages mainly on a natural periphyton-based system grew well and acquired a good basal health status (Table 4). This means that fish have benefited from both supplemental feed and natural food of periphyton assemblage. Furthermore, inserting 2 units of PS (1.4 m^2) within the 1-m^3 cage had led to significant improvement in fish growth rates and feed utilization efficiency, especially at a SD of 70 fish m^{-3}, compared to other treatments (Table 2). This may be attributed to reduced mechanical stress due to the presence of only 2PS at the appropriate fish density. The present results also suggest that the added supplementary feed (20% CP), in the presence of appropriate periphyton assemblage, may serve as an energy source and spare protein for growth, leading to better PER and PPV. Similar findings were reported by Yi et al. (1996), where better food utilization efficiency was attained at the lower SD in Nile tilapia cultured in cage-in pond stocked at 60 or 70 fish m^{-3}. The present findings are also in agreement with the results obtained by Garcia et al. (2017), Garg and Bhatnagar (2016), and Garg et al. (2007) where the growth of Nile tilapia was significantly enhanced in cages or ponds supplied with PS. Similarly, Sakr et al. (2015) found that the provision of PS to encaged-Nile tilapia enhanced growth performance and led to a reduction in exogenous dietary protein level to only 15%.

On the other hand, increasing fish density to 90 fish m^{-3} and PS to 3 units (T6) resulted in depressed fish growth rates and feed efficiency. This may have been due to fish crowding and increasing the surface area of PS, which may have limited the free water body within the rearing units and probably partially hampered fish movement. In addition, the presence of heavy periphyton living microorganisms within the farming

Table 5 Biochemical composition (% dry weight) of the periphyton developed on the substrates within the cages of Nile tilapia, *O. niloticus*. Means in the same column bearing different lowercase letters are significantly different ($P < 0.05$)

Treatment		(% DM)							
Stocking density (fish m^{-3})	Periphyton substrate no. m^{-3})[a]	Dry matter (DM)	Ash	Ash-free dry matter (AFDM)	Protein	Lipids	Fiber	Nitrogen free extract (NFE)	Energy (MJ kg^{-1})
70	1 (T1)	12.40a	11.61b	0.79a	25.80d	4.98a	1.69a	55.92c	10.10a
	2 (T2)	12.77a	12.68a	0.09b	28.00a	4.49b	1.54b	53.29d	10.02a
	3 (T3)	15.94b	12.69a	3.25c	26.30c	4.21c	1.56b	55.24c	9.92b
90	1 (T4)	15.01b	11.71b	3.30c	24.20e	4.69b	1.68a	57.72b	9.99b
	2 (T5)	17.81c	11.79b	6.02d	26.40b	4.50b	1.56b	55.75c	10.05a
	3 (T6)	17.41c	11.40b	6.00d	23.80f	4.41c	1.54b	58.84a	9.98b
Mean		15.22	11.98	3.24	25.75	4.54	1.59	56.12	10.51
Pooled SE		1.78	0.54	1.61	0.08	0.92	0.02	0.79	0.25
C.V. (%)		0.15	1.38	0.77	0.40	2.44	1.25	0.56	1.12
LSD ($P < 0.05$)		0.42	0.39	0.64	0.19	0.19	0.03	0.65	0.25

C.V. (%) coefficient of variation, *LSD* least significant difference

[a] Surface area: 1PS = 0.7 m^2 ; 2PS = 1.4 m^2 ; 3PS = 2.0 m^2

cages in T6 may have increased the organic matter, leading to increased biochemical oxygen demand and reduced carrying capacity of the cages. Similar results were reported by Garcia et al. (2016), who found that the growth of Nile tilapia reared in cages in the presence of PS (bamboo poles) was improved but the carrying capacity of the cages was reduced at the highest fish density (110 fish m^{-3}). Keshavanath et al. (2004) also reported that increasing number of bamboo poles above the optimal did not increase the yield of tilapia hybrids.

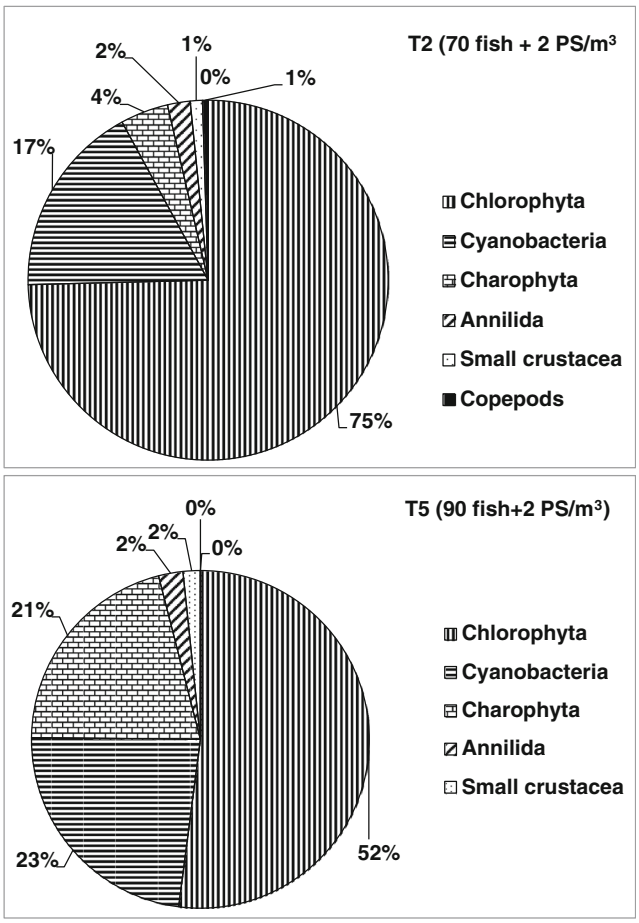

Fig. 1 Relative abundance of phytoplankton and zooplankton groups developed on periphyton substrates (PS) within the cages of Nile tilapia for treatments T2 and T5

The present study revealed also that at appropriate fish density and periphyton substrates, body protein was significantly increased. In the meantime, periphyton biochemical composition may vary depending on the substrate used, water productivity, and environmental conditions. Protein, lipids, and ash contents of periphyton in our research are within the previously reported levels (Gangadhara and Keshavanath 2008).

The accumulative mortality in the present study was relatively high (13–24%), presumably due to the small initial stocking size (only 1 g $fish^{-1}$). At this early life stage, the fish is generally more susceptible to stressful conditions, leading to higher mortality rates, than at larger sizes, as has been reported by Bolivar et al. (2004). These authors recorded relatively higher mortality rates of 43%, 25%, and 19.5% of pond-reared Nile tilapia at stoking sizes of 0.2, 1.72, and 6 g $fish^{-1}$, respectively. Similarly, Haque et al. (2015) recorded lower survival of fingerling Nile tilapia reared in periphyton-based system, whereas higher survival rates were found when larger sizes were used (Asaduzzaman et al. 2009).

Fish hematology is a very important tool in evaluating the health status and welfare of fish (Hrubec et al. 2000). For example, hemoglobin is directly related to the oxygen-binding capacity of blood (De Souza et al. 2007). In the current study, Nile tilapia reared at 70 fish m^{-3} in the presence of 2PS showed the highest values of major blood parameters among all treatments, indicating that appropriate SD and PS

would improve fish health status. Similar results have been obtained by Kopp et al. (2010) who recorded a decrease in Hb, Ht, and RBC values in silver carp reared at high density. In addition, the number of WBC together with some other biochemical parameters such as serum proteins, albumin (and globulin) (IgM and IgG) play a crucial role in fish innate immune response, especially during stressful conditions (e.g., dietary imbalance, high SD, infections, and environmental stressors) (Roberts 1978). Therefore, these parameters are used as indicators of health, stress, humoral defense system, and welfare of aquatic organisms. Thus, the high values of these parameters in the present study at lower stocking density (70 fish m^{-3}) and appropriate periphyton concentration (2PS m^{-3}) are likely to be linked with the improvement of the non-specific immune response of Nile tilapia.

Cholesterol (CHL) is one of the structural components of cell membrane as well as the outer layer of plasma lipoproteins and is the precursor of all steroid hormones (Yang and Chen 2003). Triglycerides (TRG) play an important role in providing cellular energy and can be used as an indicator of nutritional status of fish. In the present study, the relatively higher values of serum CHL and TRG concomitant with increased alanine aminotransferase (ALT) activity in fish grew at higher density (90 fish m^{-3}) at each PS unit, which suggested an increased utilization of blood lipids. High stocking densities may have also activated the mobilization of TRG to cope with the increased energy demands. Therefore, TRGs seem to be another energy substrate which is utilized during stress (Wu et al. 2018). Similar trends in CHL and TRG variations were observed in GIFT Nile tilapia reared at high stocking density (Wu et al. 2018).

In addition, the amino acid-metabolizing enzyme ALT is necessary for transamination of amino acids, thereby allowing interplay between carbohydrate and protein metabolism during the fluctuation in energy demands (Verma et al. 1981). It is also an indicator of stress (Shirdel et al. 2016). The elevated ALT values recorded in fish reared in the highly crowded cages with 3PS in the present research may have been an indication of a slightly stressful condition. These findings are in agreement with those reported by Abdel-Tawwab (2012) and Wu et al. (2018), where the increased values of ALT with increasing SD of Nile tilapia indicate the consistent stress effect on liver functions of the fish. Moreover, the increase in ALT and AST activities may reflect the use of excess hydrocarbons from amino acids to meet the increased energy demands (Wu et al. 2018).

The biomass of periphyton communities attached to periphyton substrates varied significantly among all dietary treatments in the present study. This might have been due to the significant variation in fish densities caused by the wide range in mortality rates (13–24%), which may have affected the grazing pressure. This wide variation has also been reported in similar systems used for growing tilapia and freshwater prawn *Macrobrachium rosenbergii* (Haque et al. 2015).

In the present study, periphyton biomass (DM and AFDM) were lower in T2, which exhibited the highest performance, compared to other treatments. This finding indicates that Nile tilapia prefer periphyton as food, at appropriate fish stocking density and substrate surface area. This is in agreement with the trophic plasticity of Nile tilapia, which are known to feed on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, and detritus aggregates (See El-Sayed 2020, for review). Similar results were reported by other authors (Huchette et al. 2000; Azim et al. 2003; Asaduzzaman et al. 2009). The DM in T3–T6 (at high fish density and substrate units), which showed lower performance, was much higher, suggesting that grazing pressure in these treatments was low. The variation in ash content of periphyton in the present study (11.4–12.69%) was less pronounced than that reported by Azim et al. (2001) and Uddin (2007), who reported much higher variations in ash content of the periphytic mats (16–42%). These variations may have been due to the type and density of substrate on which the periphyton was grown, and also on fish size and stocking density.

Periphyton populations collected from the substrates in the two treatments which showed the best performance in the present study (T2 and T5, respectively), belonged mainly to Chlorophyta (75 and 52%), Cyanobacteria (17 and 23%), and Charophyta (4 and 21%). Other periphytic groups were much less abundant, ranging from 0.5 to 5%. This may probably have been due to heavy grazing by Nile tilapia on phytoplankton for their growth. In support, it has been reported that constant grazing of the algal components in the periphytic biofilms maintains periphyton growth and productivity (Swamikannu and Hoagland 1989; Huchette et al. 2000; Asaduzzaman et al. 2009). Similar trends of periphytic biomass and abundance in periphyton-based tilapia ponds were observed by other authors (Asaduzzaman et al. 2009; Haque et al. 2015).

In conclusion, the present study revealed a significant combined effect of fish density and the surface area of periphyton substrates on the growth performance, immune response, and some physiological biomarkers of juvenile Nile tilapia fed a low-protein diet in periphyton-based system. The optimum fish density and periphyton area were 70 fish and 1.4 m^2 per m^3, respectively, whereas further increase in either variable has resulted in retarded growth performance and immune response.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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