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Feed restriction and compensatory growth of the Nile tilapia, *Oreochromis niloticus* under Biofloc system condition

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ABSTRACT

For compensatory growth of Nile tilapia, a 182-day feeding trial was conducted to evaluate the effects of bioflocs nutritional content. The three cyclical regimes of feed deprivation and re-feeding were R0:F7 (feed restriction 0 days, feeding 7 days), R2:F5 (feed restriction 2 days, feeding 5 days), and R4:F3 (feed restriction 4 days, feeding 3 days). There were significant differences in floc volume, total alkalinity, ammonia, nitrite, and nitrate, between treatments, according to the results of the experiment. Surprisingly, we found that the number of bacteria in the digestive tract of tilapia was significantly higher than that found in water samples. From 60×10^4 to 220×10^7 CFU g^{-1} of aerobic plate count (APC) were found in the gut. The bioflocs in the R0:F7, R2:F5 and R4:F3 groups had average crude protein contents of 33.42± 1.32%, 30.99± 0.98%, and 29.65± 1.06%, respectively. Twenty-six phytoplankton species were found in the fish gut, including genera from Cyanobacteria, Bacillariophyta, Chlorophyta, and Euglenozoa phyla. At the beginning and end of the experiment, 12 and 10 species of zooplankton were found in the biofloc system, respectively. The growth performance in terms of final body weight, weight gain, and specific growth rate) of the Nile tilapia in treatment R2:F5 was significantly higher ($P<0.05$) than that obtained in the treatment R4:F3 and slightly increased ($P>0.05$) in treatment R0:F7. Lipid content tended to decrease significantly ($P<0.05$) in feed deprivation treatment more than in continuous feed treatment. However, total serum protein, cholesterol, triglycerides, and immunoglobulin showed significant differences ($P<0.05$) between regimens, with the highest value in R0:F7 and the lowest value observed in both R2:F5 and R4:F3 experimental feeding restrictions. Overall, these findings suggest that growth compensation induced by cycles of feed deprivation and re-feeding treatment R2:F5 does confer a huge advantage in enhancing the growth performance and immunoglobulin biofloc system.

INTRODUCTION

Aquaculture's growth is constrained by a lack of available land and water **(Widanarni *et al*., 2012)**. In intensive aquaculture, the quality of the environment is harmed by a heavy reliance on artificial feed **(Cordova *et al*., 2009)**. As a result, optimizing feed

formulations and cultural practices is necessary if aquaculture is sustainable in both the environment and economics. Biofloc technology (BFT) is widely used because of its ability to purify culture water, improve feeding efficiency, enhance immunity, and reduce disease incidence **(Poli *et al*., 2019)**. As a sustainable farming technology, BFT can reuse nutrient waste, reduce pollution, and provide a high-quality protein source **(Crab *et al*., 2009)**. The success of this technology is heavily reliant on the type of culture being used to implement it. Cultured species should be capable of efficiently grazing on and digesting bacterial flocculates and incorporating the microbial protein **(Burford *et al*., 2004)**. It has been shown that biofloc can be a source of potential probiotics, especially *Lactobacillus sp*., and provide valuable protein inputs **(Das and Mandal, 2018)**. Microalgae are thought to be one of the most promising aquaculture feedstocks in the future **(Ben Halima, 2017)**. Algae play an important role in removing ammonia (**Ahmed *et al*., 2019)**, and are used as natural feed additives for Nile tilapia (**Flefil *et al*., 2021**). Bacteria, microalgae, yeast, rotifers, ciliates, protozoa, nematodes, and copepods all contribute to the formation of bioflocs **(Collazos and CJ, 2015)**. Aquaculture relies on these microorganisms because of their nutritional value and ecological significance, as they form the foundation of all aquatic food webs **(Muller-Feuga, 2000)**. Some species of zooplankton, which belong to rotifers, cladocerans, and copepods, contain high protein levels. Feed costs can be reduced by feeding fish and shrimp aggregates as a natural live food source that produces additional protein sources and reduces the feed conversion ratio **(Emerenciano *et al*. 2017 and Khanjani and Sharifinia 2020)**. Tilapia *Oreochromis niloticus* is the most studied species in these systems; this is mainly due to their eating habits and tolerance to a high concentration of suspended solids, as demonstrated in several studies **(López-Elías *et al*., 2015)**. Due to improved retention efficiency of ingested protein, flexible feeding management reduced labor and waste, and thus, a reduction in the costs of production and environmental impact, compensatory growth can be achieved in aquaculture for various fish species **(Yengkokpam *et al*., 2014)**.

This study evaluates growth performance and feeds utilization for Nile tilapia after being subjected to regular periodic fasting periods to force the tilapia to rely on the biological nutritional content of the biofloc system.

MATERIALS AND METHODS

Experimental design:

A completely randomized design with a single factor was used in three treatment groups. Fish were subjected to three different feeding protocols (the ratio of feed restriction (R) and feeding (F) sequence for each treatment) was denoted as follows:

R0:F7 (feed restriction 0 days, feeding 7 days), R2:F5 (feed restriction 2 days, feeding 5 days), R4:F3 (feed restriction 4 days, feeding 3 days). Wheat bran as a carbon source **(Wei *et al*., 2016)** was added daily after feeding to their respective treatments to maintain

a high C: N ratio (10:1) **(Avnimelech, 2009 and Panigrahi *et al.*, 2019)**. Feed ingredients and proximate composition of carbohydrates sources are showen in Table (1).

Table (1): Formulation (%) and proximate composition (% dry matter basis) of the basal experimental treatment

Ingredients	g/100g
Fishmeal local	10
Soybean meal	20
Corn gluten meal	4
Yellow corn	24
Wheat bran w	20
Rice polish	15
Soybean oil + cottonseed. Oil (1:1)	5
Lysine	0.5
Methionine	0.5
Premix	0.5
NaCl	0.5
Dry Matter %	89.8
Crude protein %	25.05
GE MJ/kg*	12.71
Crude Fat %	8.63
Crude fibre %	6.106
Ash %	6.104
NFE %	54.11

*Calculated using gross caloric values of 23.62, 39.52, and 17.15 kJ/g for protein, fat, and carbohydrate, respectively, according to **Brett and Groves (1979).**

Experimental setup:

Six outdoor experimental rectangular cement ponds with a water volume of 40 m^3 (4m width × 10m length × 1.0m depth) were used for this study. During the test, uninterrupted 24h aeration was supplied using an air stone connected to an air pump. Each pond received constant aeration through three air diffusers made of rubber, a diameter of 25cm, located at the bottom of the pond and connected to a 3 HP blower type aerator (Sweetwater; Aquatic Ecosystems). During the experiment, no water change was made, but evaporation water lost was compensated with freshwater.

Experimental Fish and feeding:

Sexually inverted male fingerlings of Nile tilapia, *O. niloticus* were purchased from a commercial hatchery in Kafr El Sheikh Governorate, Egypt. Fish weight about 4.54 ± 0.64 SD (Initial average weight), were used for the study, maintained at a density of 40 fish/m^3. After arrival, all fish were acclimatized to experimental tanks; fish were stocked

into a fiberglass tank (water volume 2000L). Fish were hand-fed with a commercial diet during the acclimation period (two weeks). After they acclimated, the fish were deprived of feed for 24 h and then pooled, batch weighed, and randomly distributed into 6 experimental ponds in groups of fish each. At the beginning of the trial, the fish were fed a basal experimental diet (Table 1). Daily feed rations were split into two equal quantities, and fish in each of the tanks were fed at 9:00 and 15:00 h. The amount of diet was calculated during the experiment period based on the change in fish weight every 14 days.

Physico-chemical parameters of the water:

Dissolved oxygen and temperature were monitored using a dissolved oxygen meter (Professional Plus, USA), pH was measured in the water column of the tanks by pH meter (HI 8314 model). Settle able solids (measured in milliliters per liter) were monitored every 10-day interval using 1 Imhoff cone of 1,000 ml and considering a settlement period of 25 min. The settled volume of solids was noted from the Imhoff cones reading **(Avnimelech and Kochba, 2009)**. Chemical variables (NH_3, NO_2, NO_3, and Total Alkalinity) were estimated according to the procedures laid down in **APHA (2017)**

Phytoplankton identification and enumeration

The drop method was applied to count and identify phytoplankton species **(APHA, 2017)**, and triplicate samples (2µl) were taken and examined under an inverted microscope ZEISS IM 4738, with a magnification power of 40x. The results of phytoplankton density were presented as the number of cells per liter (unit/l). Phytoplankton identification was performed according to **Bellinger and Sigee, 2015**.

Zooplankton collection and analysis

Zooplankton samples were collected from biofloc systems using a zooplankton net (55µm, 25cm diameter, and 80cm length). Three liters of water were collected and filtered by the zooplankton net from each treatment. After filtration, each sample was fixed immediately using formaldehyde solution (4-7%) and was stained afterwards using Rose Bengal stain **(Goswami, 2004)**.

The organisms were identified and counted on the counting tray with a magnifying lens of magnifying power ranging from 100X to 400X. The samples were examined, counted, classified, identified, and described in the laboratory according to the description and keys constructed by **Dang et al. (2015)** and **APHA (2017)**.

Bacteriological sampling and analysis

Sampling was done for microbial investigations of Biofloc water and tilapia gut for each Biofloc treatment pond; all samples were processed and plated within 2h of collection.

1- Water sampling

Water samples were collected directly from each treatment (after good agitation) in sterilized glass bottles.

2- Tilapia intestine

Two representative fish from each pond were used for bacteriological examination. 1g of the digestive canal was taken aseptically from each fish, weighed, and homogenized in a mortar. Then, the homogenate was transferred to a tube containing 10ml of sterile (121°C, 15min) 0.85% (w/v) NaCl prepared in deionized water. Examine aerobic plate count (APC) by **Al-Harbi and Uddin (2005)**; total diazotrophs, and Spore-forming diazotrophs count by **(Hegazi et al., 1998)**; total spore-forming bacteria **(APHA, 2005)** and Lactic acid bacteria count by **Harrigan and McCance (1990)**.

Proximate composition

Diets, fish muscle, and biofloc samples were analyzed for dry matter (DM), ash content, and crude protein (N x 6.25) by the Kjeldahl method using a Kjeltech auto-analyzer according to **AOAC (2012)** and **Liu et al. (2016)**. Crude fat was measured according to **Bligh and Dyer (1959)**.

The gross energy content of the diets was calculated using kJ/g^{-1} DM values of 23.0, 38.1, and 17.2 for protein, lipid, and carbohydrate, respectively **(Tacon, 1990)**.

Blood collection and hematological parameters

After 182 days of tilapia rearing, twenty fish from each pond (treatment) were anaesthetized using clove oil (40µl/L, Merck).

Blood was collected from the caudal vein below the lateral line using a 1.0ml hypodermal syringe and 24 gauge needles. Hematological parameters of the Nile tilapia, *O. niloticus* determine by **Reitman and Frankel, 1957 and Tietz, 1986**.

Data calculation and growth Indices:

The mean final body weight (FBW) of each experimental treatment was determined by dividing the total fish weight in each pen by the number of fish. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and compensation coefficient (CC) were calculated using the following equations:

WG = Final body weight (g) - Initial body weight (g); SGR = (ln FBW - ln IBW)/t ×100; where: FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic; t = time in days; FCR = Feed intake (g)/weight gain (g). Protein efficiency ratio (PER)=weight gain (g)/protein intake (g) ; Protein productive value (PPV)= (protein gain (g)/protein intake (g)) ×100

Compensation coefficient (CC) = ΔT x ΔC-1

where: ΔT was the average weight gain (g) in the treatment group tanks divided by the number of feeding days.

ΔC was the average weight gain (g) in the control group tanks divided by the number of feeding days; thus, CC>1.0 would indicate compensation, according to **Adaklı and Taşbozan, 2015**.

Statistical Analysis

All data were presented as mean ± standard deviation. Statistical analyses were conducted using SPSS 19.0 (SPSS, Chicago, IL, United States) and checked for normality and

homogeneity of variance before analysis. The growth performance, proximate composition of muscle, and blood chemical analysis levels were subjected to a one-way analysis of variance (ANOVA). Descriptive statistics for water quality variables were used to show maximum and minimum values for each treatment. The effects of treatment on weekly and diurnal water quality, biofloc-related, and periphyton relay parameters were analyzed using Repeated measure ANOVA. A probability value (P) of less than 0.05 indicated significant differences. The differences among treatment means were resolved using Duncan's test for unplanned multiple comparisons (**Duncan, 1955**). P values <0.05 were considered statistically significant.

RESULTS

The water quality parameters monitored during the experiment period did not show a significant difference in temperature, dissolved oxygen, and pH between control and treatments, but significant differences were in floc volume, total alkalinity, total ammonia, nitrite, and nitrate (Table 2); and R0:F7 treatment showed the highest concentration of all parameters.

The present study shows bacterial loads in the water and tilapia gut are mentioned in (Tables 3 and 4). Each sample was investigated twice (duplicate agar plates), and the mean of Aerobic heterotrophic bacteria (APC) in water samples were ranged from 55 $\times 10^4$ to 78 $\times 10^4$ and 60 $\times 10^4$ to 15 $\times 10^7$ CFU ml^{-1} at the beginning and end of the experiment, respectively. The Aerobic Plate Count bacteria inside the tilapia gut recorded higher than bacterial counts in water samples, where APC in the gut ranged from 60×10^4 to 220 $\times 10^7$ CFU g^{-1}. Though these results reflect that the highest values of APC were recorded at R0:F7 treatment, and the lowest values were recorded at R4:F3 treatment.

Table (2): Physicochemical parameters of different experimental treatments.

Variable	R0:F7	R2:F5	R4:F3
Temperature (°C)	30.23±0.03	30.23±0.02	30.27±0.13
Dissolved Oxygen (mg L^{-1})	5.19±0.04	5.28±0.06	5.32±0.08
pH	8.18±0.05	8.14±0.03	8.20±0.05
Floc Volume (ml L^{-1})	62.33 ± 1.52^{a}	58.00 ± 0.85^{ab}	56.40 ± 0.54^{b}
Total Alkalinity (mg L^{-1})	143.86±2.11	119.76±0.66	122.75±2.19
Total Ammonia, NH_3 (mg L^{-1})	0.25 ± 0.01^{a}	0.23 ± 0.00^{b}	0.21 ± 0.00^{c}
Nitrite, NO_2 (mg L^{-1})	0.22 ± 0.01^{a}	0.20 ± 0.01^{ab}	0.18 ± 0.01^{b}
Nitrate, NO_3 (mg L^{-1})	0.87 ± 0.08^{a}	0.65 ± 0.05^{b}	0.55 ± 0.06^{b}

Each value represents mean SD± (n = 6). Values in the same rows with different superscript letters are significantly different (P <0.05).

Table (3): Means variation ± Standard deviation of the bacterial flora (CFU ml^{-1}) of different experimental pond water

Treatment	First				End			
	APC	TD	SFD	LAB	APC	TD	SFD	LAB
R0:F7	$78 \times 10^4 \pm 1.4 \times 10^4$	$21 \times 10^2 \pm 2.8 \times 10^2$	$15 \times 10^2 \pm 7.07 \times 10^2$	420 ± 9.6	$150 \times 10^6 \pm 59.4 \times 10^6$	$420 \times 10^2 \pm 113.1 \times 10^2$	$250 \times 10^2 \pm 18.4 \times 10^2$	$11 \times 10^2 \pm 4.2 \times 10^2$
R2:F5	$62 \times 10^4 \pm 4.2 \times 10^4$	$18 \times 10^2 \pm 6.4 \times 10^2$	$13 \times 10^2 \pm 1.4 \times 10^2$	425 ±7.07	$45 \times 10^6 \pm 26.9 \times 10^6$	$310 \times 10^2 \pm 91.9 \times 10^2$	$250 \times 10^2 \pm 24.04 \times 10^2$	$98 \times 10^2 \pm 9.9 \times 10^2$
R4:F3	$55 \times 10^4 \pm 7.9 \times 10^4$	$19 \times 10^2 \pm 4.7 \times 10^2$	$13 \times 10^2 \pm 1.4 \times 10^2$	408 ± 2.8	$0.6 \times 10^6 \pm 0.7 \times 10^6$	$20 \times 10^2 \pm 15.6 \times 10^2$	$15 \times 10^2 \pm 5.7 \times 10^2$	$4.2 \times 10^2 \pm 4.8 \times 10^2$

APC = Aerobic plate count, TD =Total Diazotroph, SFD = Spore forming Diazotroph, LAB = lactic acid bacteria, CFU ml^{-1} = colony forming unit per one millimeter.

Table (4): Means variation ± Standard deviation of the bacterial flora (CFU g^{-1}) in the different experimental tilapia gut

Treatment	APC	TD	SFD	LAB
R0:F7	$22000 \times 10^5 \pm 4242.6 \times 10^5$	$110 \times 10^3 \pm 15.6 \times 10^3$	$90 \times 10^3 \pm 8.5 \times 10^3$	$87 \times 10^4 \pm 21.2 \times 10^4$
R2:F5	$21 \times 10^5 \pm 12.7 \times 10^5$	$142 \times 10^3 \pm 9.9 \times 10^3$	$112 \times 10^3 \pm 14.1 \times 10^3$	$80 \times 10^4 \pm 9.9 \times 10^4$
R4:F3	$6 \times 10^5 \pm 2.8 \times 10^5$	$9 \times 10^3 \pm 1.4 \times 10^3$	$7 \times 10^3 \pm 4.2 \times 10^3$	$2.4 \times 10^4 \pm 1.7 \times 10^4$

APC = Aerobic plate count, CFU g^{-1} = colony forming unit per one gram, TD =Total Diazotroph, SFD = Spore forming Diazotroph, LAB = lactic acid bacteria.

The chemical composition of the biofloc in this experiment is shown in Table (5). The average crude protein contents of the biofloc systems in the R0: F7, R2:F5 and R4: F3 groups were 33.42± 1.32%, 30.99 ± 0.98%, and 29.65 ± 1.06%, respectively.

Table (5): Proximate composition of bioflocs (% on a dry-matter basis)

Proximate composition Treatments	Crude protein	Lipids	Ash	Total carbohydrate
R0:F7	33.42±1.32	1.18±0.41	28.31±0.26	37.10±2.00
R2:F5	30.99±0.98	1.02±0.36	31.68±1.28	36.33±1.88
R4:F3	29.65±1.06	1.28±0.47	30.65±1.49	38.44±2.18

Microscopic examination of phytoplankton in this study showed that 53 and 32 species were recorded in the biofloc system at the beginning and end of the experiment, respectively. Phytoplankton belonged to 5 & 4 major phyla, respectively (Table 6), in which they recorded Chlorophyta belonged to (24 & 11 sp.); cyanobacteria (14 and 12 S.); Bacillariophyta (9 & 8 sp.); Euglenozoa (3 & 1 sp.) and Charophyta (1 & 0 sp.) at the beginning and end of the experiment, respectively.

Table (6): Phytoplankton phyla density (No. of units ×10^4/l) in Biofloc treatments

Phytoplankton groups	First			End		
	R0:F7	R2:F5	R4:F3	R0:F7	R2:F5	R4:F3
Chlorophyta	810	755	475	160	295	150
Bacillariophyta	170	1395	260	100	280	85
Cyanobacteria	1155	815	1160	745	1440	790
Euglinophyta	0	20	10	0	10	0
Charophyta	55	160	75	0	0	0
Total	2190	3145	1980	1005	2025	1025

The results of the microscopic examination of the three treatments in the experiment showed a diversity of phytoplankton densities, where treatment (R2:F5) recorded the highest density of phytoplankton (3145 & 2025× 10^4 unit/l) at the beginning and end of the experiment, respectively. Diatoms prevailed at the beginning of the experiment by 44.4%, while cyanobacteria predominated at the end of the experiment by 71.1% (Table 7).

Table (7): Phytoplankton phyla density (No. of units ×10^4/l) in Gut content

Phytoplankton groups	Treatments		
	R0:F7	R2:F5	R4:F3
Chlorophyta	45	122	98
Bacillariophyta	8	72	29
Cyanobacteria	63	382	128
Euglinophyta	0	5	0
Total	116	581	255

The results revealed that the phytoplankton community at the end of the experiment in the fish gut (Table 7 and Figures 1, 2 & 3) revealed that the phytoplankton community was represented by 26 species in gut content, including genera of the Cyanobacteria, Bacillariophyta, Chlorophyta, and Euglenozoa phyla.

Samples were taken from cultivation ponds to examine Zooplankton twice: the first after the biofloc system was started (about one month), and the second after the tilapia had grown and before the end of the experiment (182 days). The microscopic examination of Zooplankton in the studied BFT system showed that 12 & 10 species were recorded in the biofloc system at the first and end of the experiment, respectively; Zooplankton belonged to 3 major phyla (Table 8 and Figure 4). Rotifera (11 & 6 sp.); Protozoa (1 & 2 sp.) and Arthropoda (0 & 2 sp.), respectively.

The results of the microscopic examination of the three treatments in the experiment showed a diversity of zooplankton densities, where treatment (R0:F7) recorded the highest density of zooplankton (11500 & 6500 org/l) at the beginning and end of the experiment, respectively.

The growth performance (in terms of final body weight, weight gain, and specific growth rate) of the Nile tilapia in treatment R2:F5 was significantly higher (P< 0.05) than that obtained in the treatment R4:F3 and slightly increased (P>0.05) in treatment R0:F7 (Table 9). Though, in the present study, the fish deprived and refeed cyclically had higher significantly (P >0.05) improved feed conversion efficiency (FCE) than that of the control fish and showed a high PER, PPV value in R4:F3 treatment. Additionally, the tilapia in all treatments also showed a high survival rate (%) (Table 9). At the end of the trial, CC values of groups R2:F5 and R4:F3 were 1.51 and 1.88, respectively (Table 9).

Table (8): Zooplankton groups density in Biofloc treatments and Gut content (No. of org/l)

Zooplankton groups	Treatments								
	R0:F7			R2:F5			R4:F3		
	First	End	Gut	First	End	Gut	First	End	Gut
Rotifera	10500	5000	100	7000	1000	0	7000	2500	150
Protozoa	1000	500	100	1000	500	0	0	500	50
Arthropoda	0	1000	300	0	4500	150	0	3000	350
Total Zooplankton	11500	6500	500	8000	6000	150	7000	6000	550

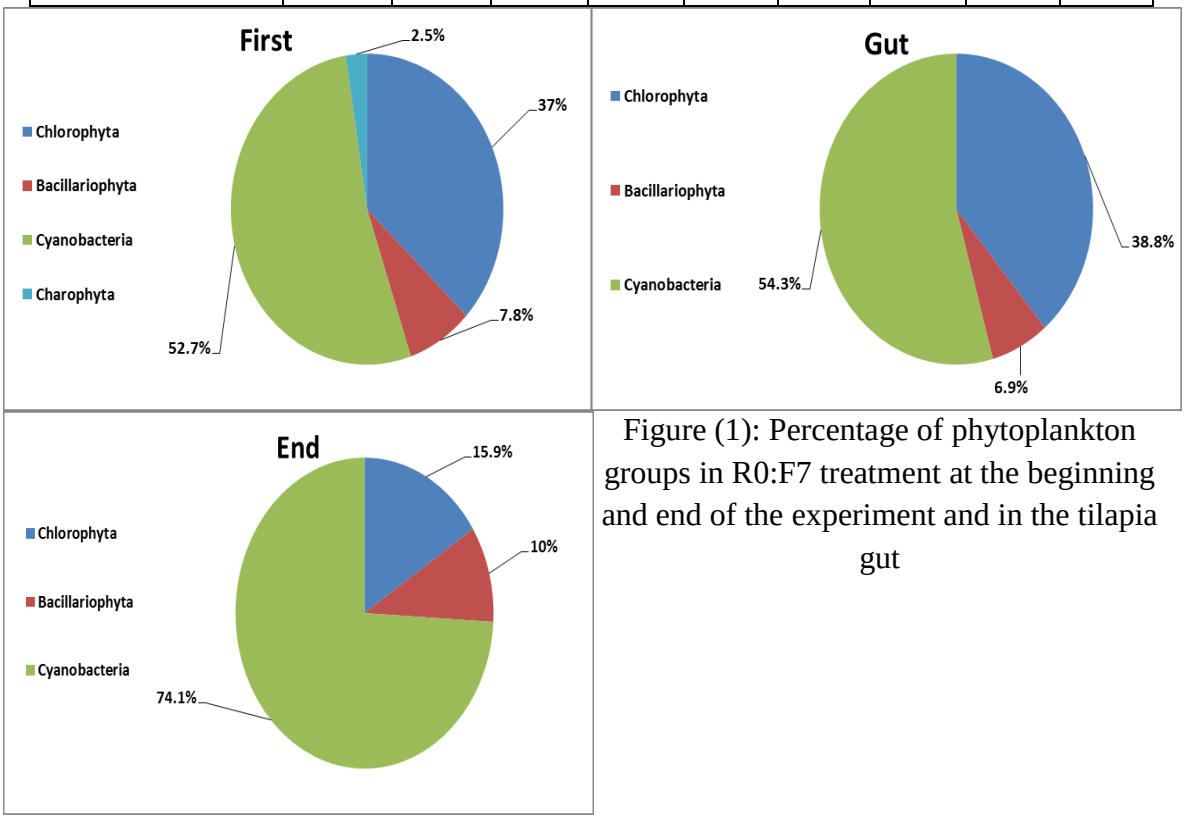

Figure (1): Percentage of phytoplankton groups in R0:F7 treatment at the beginning and end of the experiment and in the tilapia gut

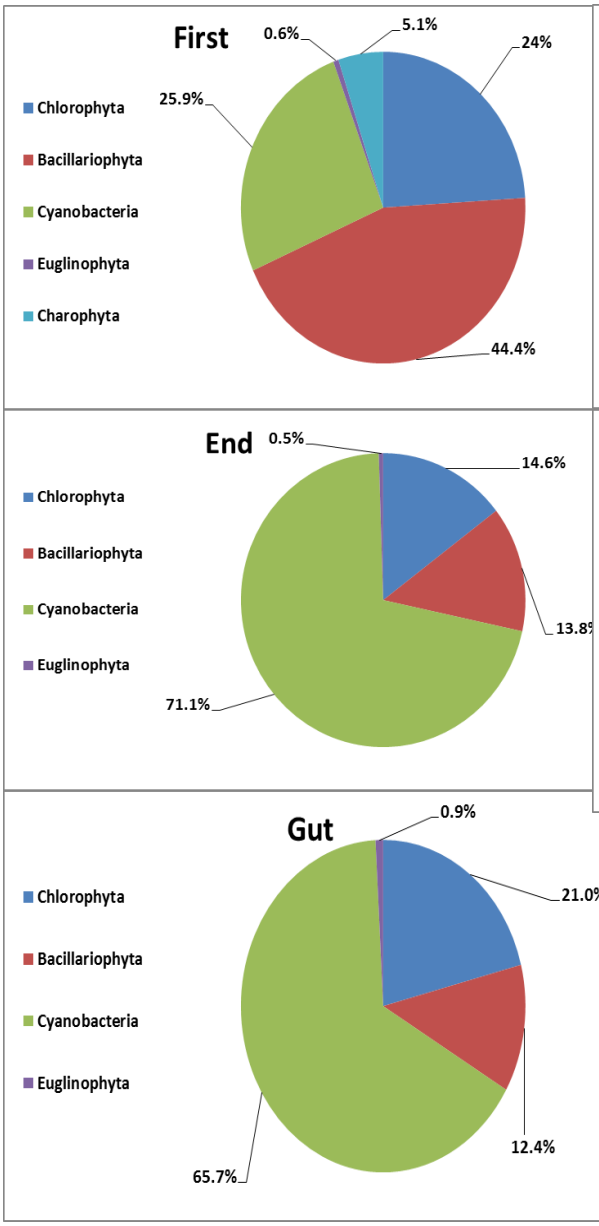

Figure (2): Percentage of phytoplankton groups in R2:F5 treatment at the beginning and end of the experiment and in the tilapia gut

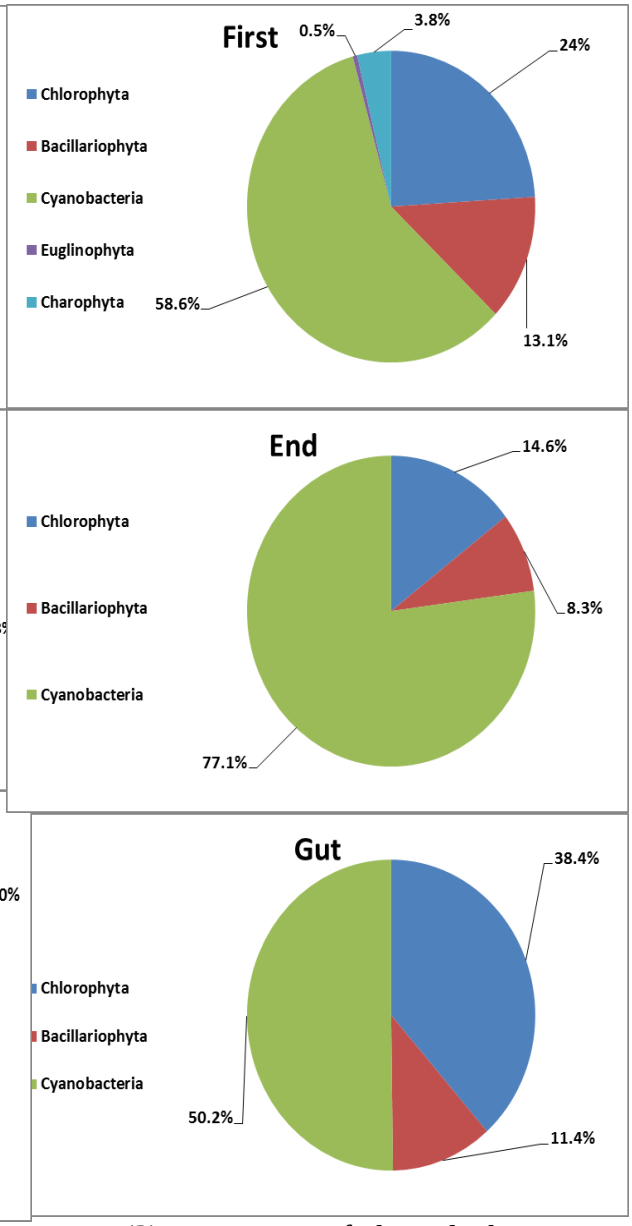

Figure (3): Percentage of phytoplankton groups in R4:F3 treatment at the beginning and end of the experiment and in the tilapia gut

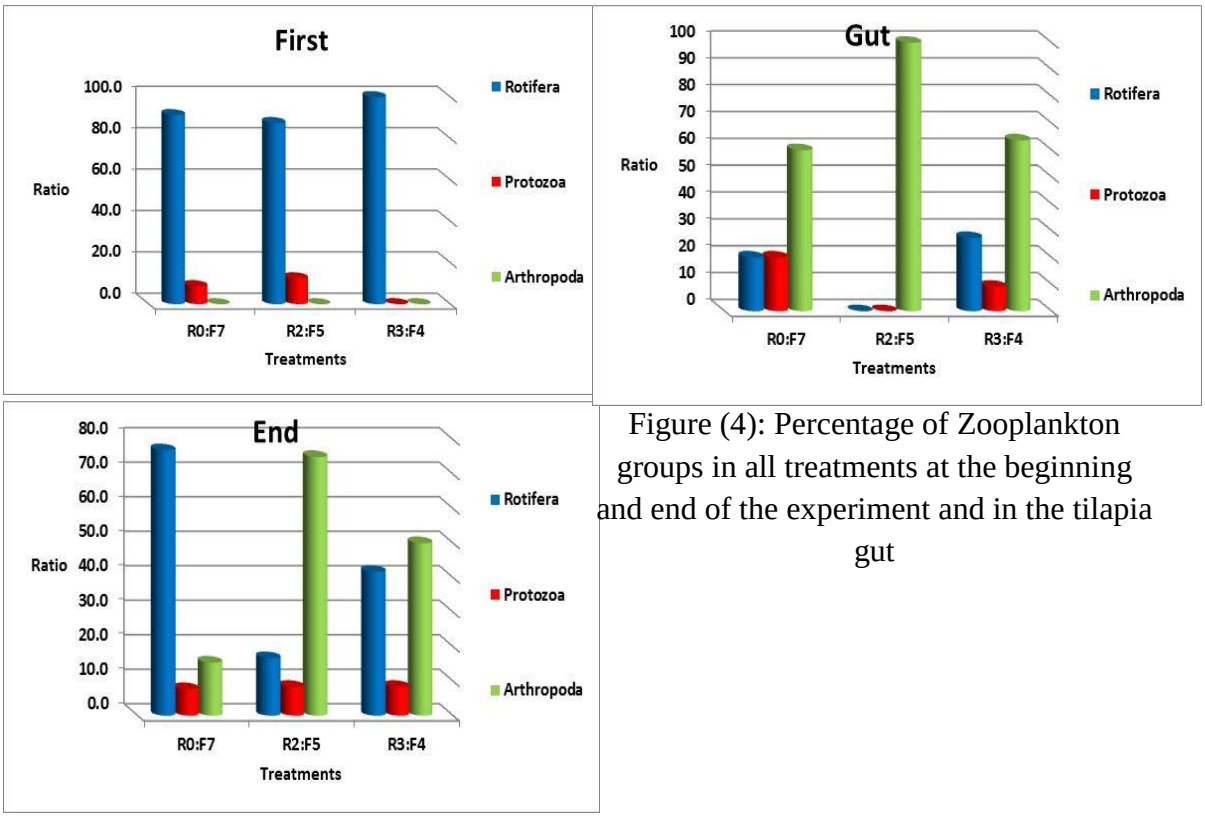

Figure (4): Percentage of Zooplankton groups in all treatments at the beginning and end of the experiment and in the tilapia gut

Table (9): Growth performance and feed efficiency of the Nile tilapia *Oreochromis niloticus* during the culture with different experimental biofloc technology (mean ± *SD*)

Parameters	Treatments		
	R0:F7	**R2:F5**	**R4:F3**
Initial body weight (g)	4.46 ± 0.75[a]	4.23 ± 0.62[a]	4.95 ± 0.55[a]
Final body weight (g)	218.58 ± 6.49[a]	223.98 ± 5.20[a]	169.19 ±6.46[b]
Gain (g)	214.12 ± 5.37[a]	219.55 ± 4.89[a]	164.43 ±5.91[b]
SGR (%/day)	2.13± 0.04[a]	2.18 ± 0.07[a]	1.94 ±0.04[b]
Feeding days	182	130	78
Feed intake (g)	444.22 ±11.62[a]	338.69 ±10.21[b]	149.62±7.51[b]
FCR	2.08±0.13[a]	1.54 ± 0.09[b]	1.10±0.02[b]
PER	1.91±0.36[c]	2.60±0.12[b]	3.92±0.14[a]
PPV	28.09±3.16[c]	38.09±6.17[b]	57.59±8.37[a]
Survival (%)	94.26 ± 5.44[a]	95.18 ± 4.36[a]	94.78 ± 5.22[a]
Compensation coefficient (CC)	–	1.44 ± 0.04	1.79 ± 0.05

Data are mean±SD. Different superscripted letters within the same row mean a significant difference ($p<0.05$)

In the present study, muscle protein and lipid content were affected significantly ($p< 0.05$) in the cyclical fasted treatments (Table 10). However, ash content was not affected ($p<0.05$) in Long-fasting treatment (R4:F3). Although differences in lipid content between continued feed (R0:F7) and the Long-fasting treatment (R4:F3) were detected, lipid content tended to decrease significantly ($p<0.05$) in feed deprivation treatment (R4:F3) (Table 10).

Table (10): Muscle Proximate composition of Nile tilapia *Oreochromis niloticus* during the culture with different experimental biofloc technology (mean ± *SD*)

Treatments	Proximate composition (%)				
	Moisture	Crude Protein	Ether Extract	Ash	Gross Energy
Initial	80.92±0.13	61.91±0.94	17.81±1.14	20.24±0.84	217.08±5.03
R0:F7	69.92±0.52^{a}	56.60±0.43^{b}	22.40±0.57^{a}	20.95±0.39^{a}	222.62±3-32^{a}
R2:F5	70.01±1.02^{a}	57.08±0.57^{ab}	22.34±0.75^{a}	20.57±0.42^{a}	223.52±7.52^{a}
R4:F3	67.19±0.88^{a}	58.14±0.72^{a}	20.97±0.68^{b}	20.81±0.35^{a}	220.62±4.49^{a}

Each value represents mean SD± (n = 6).Values in the same columns with different superscript letters are significantly different (P <0.05).

The different regime was used to detect the effect of biofloc rearing and feed restriction on hematological parameters (Table 11). It was observed that hemoglobin content, hematocrit value, RBC, and WBC, showed a significant difference ($P < 0.05$) between regime systems, with the lowest value observed in R0:F7 from both the experimental feeding restrictions R2:F5 and R4:F3. However, total serum protein, total serum cholesterol, total serum triglycerides, and immunoglobulin exhibited (Table 11) significant ($P < 0.05$) differences among the regime systems, with the lowest value noticed in both experimental feed restrictions R2:F5 and R4:F3; however, the highest in R0: F7.

Table (11): Haematological parameters of the Nile tilapia *Oreochromis niloticus* during the culture with different experimental biofloc technology (mean ± *SD*)

Haematological parameters	unit	R0:F7	R2:F5	R4:F3
Haemoglobin	g/dl	10.75±0.15$_{c}$	12.7±0.10$_{b}$	13.95±0.05$_{a}$
Haematocrit	%	21.00±0.70$_{b}$	26.00±1.00$_{a}$	26.35±0.45$_{a}$
Red cell count	millions/ ul	1.65±0.05$_{b}$	1.90±0.00$_{a}$	2.05±0.05$_{a}$
White cell count	x10˛3/ul	52.50±1.00$_{b}$	64.15±0.85$_{a}$	64.50±3.50$_{a}$
Serum total cholesterol	mg/dl	96.5±1.50$_{a}$	54.00±3.00$_{b}$	54.00±3.00$_{b}$
Serum triglycerides	mg/dl	129.50±29.50$_{a}$	50.50±2.50$_{b}$	50.00±1.00$_{b}$
total protein	g/dl	5.05±0.85$_{a}$	1.40±0.10$_{b}$	1.00±0.60$_{b}$
immunoglobulin (igm)	mg/dl	123.50±3.50$_{a}$	55.80±4.50$_{b}$	53.80±3.90$_{b}$

DISCUSSION

In the present study, the physicochemical parameters of the water were within the recommended range for the normal growth of Nile tilapia (Table 2) **(DeLong *et al*., 2009)**. Regardless of salinity, it is recommended that total alkalinity be greater than 75 mg L^{-1} to provide buffering capacity **(Roy *et al*., 2010)**. According to **Becerril-Cortés *et al*., 2018**, the water quality variable showed tolerable levels for cultivating of species in Biofloc. Treatment R2:F5 had slightly lower pH levels than the other biofloc treatments (Table 2), which may be due to the higher respiration activity of bacteria and other microorganisms in biofloc medium, increasing CO_2 levels as interpreted by **Wasielesky *et al*., 2006**. This pattern may indicate that tilapia consumed a significant portion of the macrocosm pond's biofloc, or that ecological succession in the food chain has altered the composition and abundance of the microbiota **(Moriarty, 1997 and Aboseif *et al*., 2022)**. The nitrite and nitrate concentration in treatment R0:F7 was higher than in other treatments (Table 2). According to **Chen *et al*., 2018**, which found that increases in NO_3-N concentrations in the treatments could be attributed to feeding and microbial respiration inputs, this finding supports their hypothesis. Therefore, evaluating the relationship between the microbes in the biofloc system and the animal intestinal tract microbiota is important to study further the effects of bioflocs in aquaculture **(Miao *et al*., 2017)**. Both chemoautotrophic nitrifying bacteria and heterotrophic ammonia assimilating bacteria exist in the Biofloc System **(Emerenciano *et al*., 2017)**. Heterotrophic bacteria remove TAN from water and incorporate it into cellular protein, which can be consumed by the cultured species **(Lezama-Cervantes and Paniagua-Michel, 2010)**. Heterotrophic bacteria's ability to remove noxious gases may be influenced by the type and quantity of organic carbon sources used to keep the C/N ratio within biofloc systems proper **(Ahmed *et al*., 2019)**. The current study enumerated the numbers of lactic acid bacteria (LAB) in the water sample and Tilapia gut which recorded the lowest density in long fasting treatment (R4:F3) compared to other treatments (Tables 3 and 4). *Lactobacillus* can stimulate the immune response to *Oreochromis niloticus* and crustaceans due to its natural production of antimicrobial compounds, enzymatic contribution, competition for surface adhesion sites, enhancement of antioxidant and immune parameters, improvement of intestinal morphology and microbiota, and ease of storage and processing. **(Sherif *et al*., 2022).** For the fish, bioflocs are a valuable source of additional nutrients and vitamins, including protein and lipids (**Ju *et al*., 2008 and Emerenciano *et al*., 2012**). This natural productivity is normally present in the form of bacteria, microalgae, protozoa, nematodes, copepods, and rotifers (**Ray *et al*., 2010**). These microorganisms are a rich source of lipids (**Maicá *et al*., 2012**), vitamins, and essential amino acids (**Ju *et al*., 2008**).

Plankton is an important food source for fish, and it can grow rapidly in freshwater, brackish water, and seawater (**Pamukas *et al*., 2020**). Before, research by **Kadim *et al*. (2018)** and **Das *et al*. (2018**) found that, in addition to its role in the food chain, plankton could be a bio-indicator of the state of the ecosystem. The results of the microscopic examination of all treatments in the experiment showed a diversity of phytoplankton densities (Table 6). Phytoplankton abundance

was limited in all biofloc treatments due to light limitation and carbon source application, as **Moss *et al*. (2001)** reported. On the other hand, in the biofloc system, 12 zooplankton species were found in the first experiment, and 10 species were found at the end of the experiment, indicating that in the studied BFT system, zooplankton belonged to three major phyla (Table 8), including Rotifera (11 & 6 sp.), Protozoa (1 & 2 sp.) and Arthropoda (0 & 2 sp.) respectively. **Ahmad *et al*. (2019)** found in biofloc tanks, that zooplankton was the dominant species, with only a small number of phytoplankton visible under the microscope. According to **Rajkumar *et al*. (2016)**, nematodes, ciliates, and copepods were present in low abundance in biofloc tanks at the beginning of the study and became less as a result of fish grazing. The results showed that treatment (R2:F5) had the highest density of phytoplankton (581×10^4unit/l) in the fish gut (Figures 1, 2 & 3). Treatments R4:F3 and R0:F7 had the highest zooplankton density (550 and 500 org /l, respectively) when looking at the species composition in the fish gut (Table 8). Arthropoda recorded the highest percentages (60-100%) predominated from the total zooplankton density, followed by Rotifera (0.0-27.27%) and Protozoa (0.0-20.0%). Tilapia (*Oreochromis niloticus*) feeds on a variety of organisms, including phytoplankton, zooplankton, insects, and aquatic plants, according to **Arfiati *et al*., 2019**. They also discovered that fish preferred Chlorophyceae but avoided Bacillariophyceae and Cyanophytes, while copepods and cladocera preferred rotifers. In the guts of *O. niloticus* of total lengths greater than 35cm, zooplankton was found in higher proportions than previously reported. Other plankton species, such as Rotifera, Protozoa, and Arthropoda, were abundant in the water ponds shown (Tables 7 and 8). Tilapia fish have changed their feeding habits, according to **Attayde and Menezes (2008)**.

After a period of feed limitation, individuals who are given an abundance of food experience compensatory or "catch-up" growth, a physiological process that causes their growth to accelerate extremely quickly (**Jiwyam, 2010**). Based on how much biomass the starved fish accumulates, compensatory reactions might vary greatly from excess, full, partial, or no compensation (**Ali *et al*., 2003**). Without compromising fish welfare, fish producers could benefit from this phenomenon, lower production costs and boost earnings (**Paz *et al*., 2018**). In cyclical feeding, growth compensation is more successful when fasting is brief and severe. This is true for specific species of animals (**Peres *et al*., 2011**).

There is evidence to suggest that tilapia are better adapted physiologically to cycles of feed restriction and re-feeding than other species; in this study, tilapia R2:F5 had a slightly increased resistance ($P>0.05$), while R0:F7 had an increased resistance because they were cultivated in a biofloc system, which has previously been shown to increase the resistance of organisms even in stressful situations (**Wasielesky *et al*., 2013**). A 24-hour supply of bioflocs is provided to the tilapia via biofloc technology in tilapia farming (**Emerenciano *et al*., 2017**). According to **Browdy *et al*., 2001**, the natural productivity of aquaculture systems may be an effective way to reduce the amount of feed required in these systems. Treatment R4:F3 caused less tilapia growth than R0:F7, which is likely attributable partly to the nutritional stress the tilapia was under, as the energy received from the diet was, used less for growth (**Sadoul and Vijayan, 2016**).

Interestingly enough, **Sarker *et al*. (2019)** showed that biofloc alone was insufficient for a test fish's equivalent development when fed in addition to food.

Treatments R4:F3 and R2:F5 had a better feed conversion ratio along with compensatory growth than treatment R0:F7 and showed a high PER, PPV value in R4:F3 tretment (Table 9). **Sakyia *et al*., 2020** showed that short term starvation had significant effects on growth performance and feed utilaization, haematological and biochemical parameters, and immunological parameters in Nile tilapia and recovered positively after re-feeding. According to **Freetly *et al*. (1995)**, the compensatory growth response is a physiological mechanism that raises the proportion of energy directed toward growth in organisms. Increasing feed utilization efficiency and reducing energy losses through feces and ecdysis is how this process occurs (**Wei *et al*., 2008**).

A high survival (%) was in all treatments (Table 9), suggesting that the feed restriction did not have a significant impact on the fish's physiological and nutritional status, as the fish were in good health and exhibited minimal stress symptoms at the conclusion of the study. **Crab *et al*., 2009** agreed that the metabolic response of fish subjected to periods of feed restriction and feeding could be represented by a model consisting of four events: stress, transition, adaptation and recovery.

During the study's interim periods, both the R2:F5 and R4:F3 starvation groups displayed compensatory tendencies with compensation coefficients higher than 1 (CC>1) (Table 9). It has also been found that starvation regimes increase the CC values of whitefish, *Coregonus lavaretus* (L) (**Känkänen and Pirhonen, 2009**). Short starvation periods and frequent cycles affected the compensation coefficient in this study, as in previous studies. During the short-term and multi-cycled feedings, compensation was found. In the same way, the extent of compensatory growth in fish is related to the length of time they have been deprived of food (**Wieser *et al*., 1992**). Deprivation-refeeding cycles of 3 weeks and 3 weeks resulted in full growth compensation in rainbow trout, but only partial compensation when the deprivation-re-feeding cycles were reduced to 1 to 2 weeks (**Quinton and Blake, 1990**). Short fast (R2:F5), where weight gain was slightly higher than continuing feed but significantly higher ($p < 0.05$) than long fast (Table 9), was observed (R4:F3). Fasted fish outgrew continuously fed fish throughout the entire study period, according to the findings of **Argüello-Guevara *et al*. (2020)**. **Liu *et al*., 2018** proposed that after re-feeding, fish experience a state of craving and increased appetite, which leads to an increase in their dietary intake rate levels (hyperphagia).

Protein and lipid deposition was significantly affected ($p<0.05$) by cyclical fasting and re-feeding (Table 10). Due to the use of nutrients (crude protein and total lipids) as an energy source for necessary physiologic processes, some researchers believe that feed restriction affects the fish's biochemical composition (**Cho, 2005**). It was hypothesized that muscle fat would be more slowly mobilized to meet metabolic energy needs during a reduction in feed intake than glycogen. However, according to the findings, there was no discernible difference in muscle composition between the experimental groups R0:F7 and R2:F5 (Table 10). **Avnimelech, 2009**, found that fish used the biofloc nutrients as a source of energy to meet their metabolic needs, preventing muscle protein catabolism and the depletion of lipid reserves in the fish muscle.

Indicators of fish health, such as haematological parameters, are reflected in fish (**Harikrishnan *et al.*, 2011**). A different regime was used to study the effect of biofloc rearing and feeding restriction on haematological parameters (Table 11). As **Ahmed *et al.* (2019)** found, biofloc rearing positively impacted in the *L. rohita* fingerlings' RBC, WBC, and Hb parameters indicating a positive impact on their physiological conditions. The blood Hb content of Nile tilapia reared in biofloc did not improve (**Azim and Little, 2008**). Hb and Hct, RBC, and WBC studies in Table (11) yielded results in contrast to these findings. Fasted treatments (R2:F5 and R4:F3) had a significant ($p < 0.05$) effect on total serum protein, total serum cholesterol, total serum triglycerides, and immunoglobulin levels compared to those that continued to feed (R0:F7). A negative correlation between total serum protein and total cholesterol and triglycerides in starvation conditions by **Lin and colleagues (2012)**.

Furthermore, increased immunoglobulin in the control treatment (Table 11) may be because the micro-ecological balance of aquatic microorganisms in the BFT system can effectively control ammonia, nitrogen and nitrite, reduce the probability of disease caused by conditional pathogenic microorganisms and provide protection for the health of cultured animals **(Hu *et al.*, 2017)**. Though fish continue feeding in R0:F7 (Table 11), enhanced immunity and improved ability to resist environmental stress through the promotion of growth of fish and induction of changes in microbial flora in their digestive tracts **(Zhou *et al.*, 2010)**. However, conditions such as long-term starvation **(Lin *et al.*, 2012)** were reported to induce immune fatigue in shrimp. The same authors showed that all immune parameters of 7day-starved shrimp could return to their baseline values after 5 days of re-feeding.

CONCLUSION

Based on the results of the current study, the best group in terms of partial compensation growth, feed utilization and economic treatment were the R2:F5 (feed restriction 2 days, feeding 5 days). The feeding model applied in this group is thought to be useful for the aquaculture industry. Allowing the fish to benefit from the structural structure of the biofloc colonies which has an economic return on production. our experiment was a long-term 182-day trial period which supported the data.

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