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Impacts of Intermittent Fasting on Floc Formation, Growth, Chemical Composition, and Histomorphometry of Muscles of the Nile Tilapia Cultured in the Biofloc System

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ABSTRACT

The success of the biofloc system is contingent on its management. Therefore, this study seeked to assess the effects of intermittent fasting at different feeding rates on the efficacy of the biofloc system in terms of water quality, floc composition, growth performance, muscular chemical composition, and histomorphometry changes in the Nile tilapia muscles in comparison to the traditional system. A total of six hundred Nile tilapia fingerlings (with an average initial body weight of 54.19±1.32g) were divided into five treatments: T_0 (the control) consisted of fish reared in the traditional system and fed at a rate of 4% of their body weight (BW). While, in treatments T_1 and T_2, fish received a feeding rate of 3% of their body weight, with T_1 being subjected to continuous feeding and T_2 experiencing intermittent fasting (alternating between a feeding day and a fasting day). Likewise, T_3 and T_4 fish were fed at a rate of 4% of their BW and were exposed to continuous feeding and intermittent fasting, respectively. The average quantity of floc, total bacteria in the floc, total phytoplankton, and total zooplankton increased with increasing feeding rates but decreased when subjected to intermittent fasting. Fish in the biofloc-based treatments exhibited superior growth performance and feed efficiency parameters when compared to those reared in the traditional system. Among the treatments, fish in T_3 demonstrated the highest levels of moisture, fat, and energy content while displaying the lowest levels of protein and ash contents among other treatments. Fish in treatment T_2 exhibited the lowest values of large and mean diameters and the highest values of muscular bundle intensity in comparison to the other treatments. Finally, these findings support the efficacy of intermittent fasting as a viable approach to biofloc system management, as it results in a noteworthy decrease in aquafeed expenses and overall fish production.

INTRODUCTION

Excessive exploitation of natural fisheries has led to a decline in their fish stocks. Therefore, there has become an urgent need to move toward aquaculture, which currently contributes more than 50% of the global demand for fish **(Naylor *et al.*, 2021)**. To meet this increasing demand for fish, farmers are turning to intensifying production, accompanied by increased inputs from artificial feed **(Piedrahita, 2003)**. At the same time, fish only benefit from 20-25% of the protein present in the feed, and the rest is excreted in the water in the form of ammonia and organic nitrogen in feces and unconsumed feed **(Brune *et al*., 2003)**, which is a highly toxic substance to fish. Moreover, the drainage of this water causes a significant environmental load on the surrounding water environment and enriches it with nutrients **(Widanarni *et al*., 2012)**. Among the systems used in aquaculture that have gained great popularity is the biofloc system due to its ability to promote a reduction in water use by improving the quality of culture water and reducing its environmental impact, which results in increasing the biosafety and sustainability of production **(Avnimelech, 2009; Khanjani *et al*., 2022b)**. A floc formed in a biofloc system contains various organisms, such as photoautotrophs (such as microalgae), chemoautotrophs, fungi, ciliates, protozoa, rotifers, copepods, and nematodes **(Collazos-Lasso & Arias-Castellanos, 2015; Khanjani *et al*., 2022a)**. These organisms improve water quality by eliminating inorganic nitrogen compounds through the formation of floc aggregates **(Khanjani & Sharifinia, 2020)**, which serve as a supplemental food source with properties similar to probiotics **(Khanjani *et al*., 2022a, 2023)**. The biofloc system (BFS) is primarily based on bacteria, which are the main component of the system's microorganism community. Bacteria actively utilize the organic matter dissolved in the water and convert it into other substances as food for primary organisms, which in turn become a food source for higher organisms in the food chain **(Nevejan *et al*., 2018)**.

The chemical composition of the floc produced varies according to several factors, including the type of organic organisms that make up the floc aggregates, the source of the added carbon, and the size and density of the floc **(Avnimelech, 2009; Crab, 2010)**. The formed floc has demonstrated the presence of suitable nutritional components, including protein, lipid, carbohydrate, and ash content, making it a viable option for use as aquaculture feed **(Crab, 2010)**. Utilizing floc in this manner can result in savings of approximately 10-20% of feed costs **(De Schryver *et al*., 2008)**.The chemical composition of floc contains 12–49% protein, 0.5–12.5% lipid, and 13–46% ash **(Bakhshi *et al*., 2018; Khanjani *et al*., 2022b)**. To increase the efficiency of the biofloc system, numerous researchers have followed several nutritional strategies to reduce the cost of production, including reducing feeding rates **(Magouz *et al*., 2021)**. They concluded that the cultured tilapia in BFS fed at 0.5% has favorable impacts on maintaining water quality, while boosting feed efficiency and growth performance **(Magouz *et al*., 2021)**. In addition, **Nguyen *et al*. (2021)** suggested that BFS could make up for a decreased feed protein using a protein with a lower biological value. Additionally, raising the juvenile common carp in BFS can lead to a 10% reduction in the feed protein **(Adineh *et al*., 2022)**. **Aliabad *et al*. (2022)** indicated that it is possible to decrease the 15% daily feeding rate of the Nile tilapia fry reared in a biofloc system. In this context, countless nutritional practices are used to make the feeding process more successful and reduce its cost, including feeding according to desire, restricted feeding, and intermittent feeding according to the age and stage of the growth of fish **(Lovell, 1998)** to achieve the best growth rate and utilization of food. Consequently, the current study aimed to estimate the effect of intermittent fasting (one-day feeding followed by one-day fasting) with different feeding rates on the efficiency of the biofloc system in terms of water quality, floc composition, growth performance, feed efficiency, muscular chemical composition, and histomorphometry alterations in the muscles of the Nile tilapia, *Oreochromis niloticus* compared to the traditional system.

MATERIALS AND METHODS

1. The procedures and management of the experiment

The present study was conducted at the Fish Research Unit (FRU), Faculty of Agriculture, Mansoura University, Egypt. This study employed 600 Nile tilapia fingerlings, with an average starting body weight of 54.19± 1.32g. Fish were obtained from a private farm in El-Manzala, Al Dakahlia Governorate, Egypt. Moreover, fish were adapted to the experimental conditions for 15 days; during this period, fish were fed a commercial feed containing 29.86% crude protein and 5.82% crude fat (Haid Feed Factory, Al Dakahlia Governorate, Egypt) twice daily by hand. The water in the tanks was changed twice a week, with 50% of the total volume being replaced each time. After the adaptation period, the fish were randomly assigned to five treatments, as shown in Table (1), with three replicates per treatment. Fish were cultured at a density rate of 40 fish per tank (total volume of $1m^3$ of each). Each tank was equipped with two air stones connected to an air pump to obtain a high level of ventilation compatible with the biofloc system. The light period was 12 light: 12 dark.

Table 1. Details of the experimental design

Treatment	Fish culture system	Details
T_0 (control)	Traditional	Feeding rate at 4% + water change at a rate of 50% twice/week
T_1	Biofloc	Feeding rate at 3% + continues feeding
T_2		Feeding rate at 3% + feeding a day followed by a fasting day
T_3		Feeding rate at 4% + continues feeding
T_4		Feeding rate at 4% + feeding a day followed by a fasting day

2. Preparation of the biofloc system and feeding management

To form a biofloc system, according to Avnimelech's protocol, fish in all treatments were fed a floating commercial diet which is 4% of their body weight (BW). Treatments that were cultivated using the biofloc system have a carbon source added (molasses) to maintain the ratios of nitrogen and carbon at 1:10 for 15 days before the beginning of the experiment. Through this period, the amount of molasses was calculated according to

Avnimelech (2009) and was added daily in the middle of the day. The amount of floc formation in tanks was measured every day by the Imhoff funnel.

After the formation of floc, fish weighing was conducted according to the experimental design presented in Table (1). The experiment encompassed five treatments: T_0 (the control) involved fish reared in the traditional system, with water changes occurring at 50% in each tank every two days and feeding at a rate of 4% of their BW. In treatments T_1 and T_2, fish were reared in the biofloc system and subjected to a feeding rate of 3% of their BW, with T_1 receiving continuous feeding and T_2 experiencing intermittent fasting (a feeding day followed by a fasting day). Similarly, T_3 and T_4 were fed at a rate of 4% of their BW and were exposed to continuous feeding and intermittent fasting, respectively. Fish were manually fed twice daily at 9:00 a.m. and 2:00 p.m. for 70 days.

The quantities of commercial diet and molasses were adjusted every two weeks based on the actual average BW of the fish in each tank. After one month of the experiment, the feeding rate was reduced to 3% in treatments T_0, T_3, and T_4, and 2% in treatments T_1 and T_2. This adjustment was made since the leftover feed was observed after meals. Throughout the experiment, the floc in the tanks was daily measured before feeding using the Imhoff funnel.

3. The physiochemical parameters of water

Water temperature, dissolved oxygen (DO), and pH were followed up through the experimental period two times per week. The water temperature was routinely measured by the thermometer; DO was measured by a Milwaukee MW600 PRO portable DO-meter, USA, and pH was measured by HI98129 Waterproof pH & Temperature Testers, Hungary. For total ammonia nitrogen (TAN), total dissolved solids (TDS), and total suspended solids (TSS), water samples were collected once a week to be measured according to **APHA (1992)**.

4. Floc composition

4.1. Amount of floc

During the experimental period, the floc in each tank was measured every day before feeding by the Imhoff funnel for 20-25min. Sometimes, when the floc level increased to 40mL/ L in measurement on the Imhoff funnel, water filtration was performed according to the concentration of biofloc formed in the tank.

4.2. Total count of beneficial bacteria in water

To determine the total bacterial count (TBC), water samples were collected from different tanks during the preparation of the biofloc system every two days (0, 2, 4, 6, 8, 10, and 12 days) and biweekly during the experimental period. Water samples were collected in presterilized test tubes (10mL) from all treatments and analyzed in the laboratory. Water samples were immediately used for bacteriological examinations by serial dilution (10^{10}), which was carried out using physiological saline (0.85% NaCl).

After dilution, 0.1mL of subsamples were plated, in duplicate, on general trypticase soy agar (TSA) **(APHA, 2005; Luis-Villaseñor *et al*., 2015)**. After incubation at 37°C for 24 hours, the colonies in each plate were counted. The count was then calculated by multiplying the average number of colonies per plate by the reciprocal of the dilution used and reported as a colony-forming unit per milliliter (CFU/mL) **(AOAC, 1990; APHA, 1992)**.

Many kinds of colonies were identified based on the bacterial colonies' form, size, color, and opacity. Until pure cultures were achieved, three to five examples of each colony type were streaked on extra TSA plates many times. Gram stain analysis, microscopic observations, and colony development parameters were used to classify all pure isolates.

4.3. Phytoplankton and zooplankton count

Surface water samples were collected from each treatment for phyto- and zooplankton analysis, and water samples were preserved in a 4% formalin solution. Phytoplankton samples were concentrated in a sedimentation chamber **(Utermöhl, 1958)**, while zooplankton samples were filtered through a 55μm mesh diameter, and then counted using an inverted microscope. Zooplankton numbers were expressed as the number of organisms per cubic meter.

5. Fish samples

At the end of the experiment, the fish in each tank underwent weighing to calculate their growth performance and feed efficiency parameters based on the biomass in each tank (1m^3). Subsequently, five fish per replicate (n = 15/treatment) were selected at random and subjected to anesthetic with clove oil extract (50mg/ L dissolved in alcohol and added to 10L of water). In addition, dorsal muscle samples were obtained for the histological and histometric examinations.

6. The experimental measurements

6.1. Growth performance and feed efficiency

Growth performance and feed efficiency parameters were calculated based on biomass in each tank (1 m^3) using the equations described by **Lovell (2001)** as follows:

- Weight gain biomass (WGB, kg/m^3) = FB (kg/m^3) – IB (kg/m^3)
- Average daily gain biomass (ADGB, g/m^3/day) = WGB (kg/m^3) / T_i (days)
- Relative growth rate (RGR, %) = (WGB / IB) × 100
- Specific growth rate (SGR, %/day) = [(Ln FB – Ln IB) / T_i)] × 100
- Feed conversion ratio (FCR) = FI (kg/m^3) / WGB (kg/m^3)
- Feed efficiency (%) = [WGB (kg/m^3) / FI (kg/m^3)] × 100
- Protein efficiency ratio (PER) = WGB (kg/m^3) / protein intake (kg).

Where:

FB: Final biomass (kg), IB: Initial biomass (kg), FI: Feed intake; T_i: time of the experiment (day)

6.2. Chemical composition of dorsal muscles

Muscular chemical composition samples (*n* = 6 per treatment) as crude protein, crude fat, ash, and energy content (EC) were assessed as a proportion of dry matter (DM) basis according to the guidelines of the **AOAC (2016)**.

6.3. Histological characteristics and histometric parameters of dorsal muscles

The dorsal muscle samples were collected and preserved in a 10% neutralized formalin solution. Prior to usage, the samples were rinsed with tap water and then dehydrated using varying concentrations of alcohol (70, 85, 96, and 99%). Following dehydration, xylene was used to clean the samples before they were embedded in paraffin wax. Hematoxylin and Eosin (H&E) staining was performed on the wax blocks for histological examination, in accordance with the protocol described by **Roberts (2001)**. The histometric parameters were measured using the technique described by **Radu-Rusu *et al*. (2009)**. Photomicrographs were taken at a magnification of 200× (scale bar = 50µm) using a Leica DM 500 phase-contrast microscope and an ICC50W digital camera, UK.

6.4. Microbiological analysis of muscle

The **Maturin and Peeler (2001)** technique was used for microbiological analysis. In summary, 25g of fish meat was aseptically put into a stomacher bag, and then 225mL of 0.85% saline solution was added. A stomacher was then used to homogenize the resultant sample mixture for 60 seconds. The homogenate was then logarithmically diluted in 0.85% sterile saline. The total bacteria count (TBC) was determined using a plate count agar, which was then incubated for 24 hours at 37°C. The APC was defined as log CFU per gram of fish meat.

7. Statistical analysis

To evaluate the overall effects of the treatments, we performed the one-way analysis of variance (ANOVA) on all numeric data using SAS® software version 9.1.3 for Windows **(SAS, 2006)**. Before conducting the statistical analysis, all ratios and percentages were transformed using the arcsine transformation. To compare the means of different treatments, *Tukey's post hoc* test was applied. We considered statistical significance at a probability level of $P < 0.05$.

RESULTS

1. Water quality parameters

The effect of intermittent fasting on water quality parameters is shown in Table (2). No significant variances were observed in water temperature ($P > 0.05$). Fish cultured in the biofloc system exhibited a notable reduction in DO, pH, and TAN, along with a considerable increase in TDS and TSS compared to fish cultured in the traditional system (the control treatment; $P \leq 0.05$). Additionally, intermittent fasting resulted in a marked decrease in TDS and TSS in treatments T_2 and T_4 compared to treatments T_1 and T_3, which were continuously feeding ($P \leq 0.05$).

Table 2. Effect of continuous feeding and intermittent fasting on water quality parameters in different culture systems

Parameter	Traditional system (as a control)	Biofloc system				*P*-value
		Feeding rate 3%		Feeding rate 4%		
	T_0	T_1	T_2	T_3	T_4	
Temperature (°C)	27.0±2.45	27.0±2.15	27.0±2.30	27.0±2.55	27.0±2.00	0.6708
pH	7.92±0.39^{a}	7.35±0.33^{b}	7.55±0.65^{b}	7.10±0.41^{b}	7.26±0.63^{b}	0.0521
DO (mg/L)	5.91±0.52^{a}	5.07±0.32^{c}	5.48±0.55^{b}	5.11±0.62^{c}	5.31±0.42^{b}	0.0587
TAN (mg/L)	2.356±0.24^{a}	0.116±0.05^{b}	0.092±0.04^{b}	0.114±0.09^{b}	0.097±0.06^{b}	0.0456
TDS (mg/L)	337.6±5.45^{c}	465.7±8.89^{b}	407.3±11.2^{b}	573.4±10.05^{a}	418.0±9.44^{b}	0.0578
TSS (mg/L)	209.0±7.68^{d}	573.8±3.65^{b}	418.8±9.71^{b}	655.5±10.77^{a}	478.5±6.34^{c}	0.0011

Mean in the same row having different letters are significantly different ($P \leq 0.05$). DO: Dissolved oxygen; TAN: Total ammonia nitrogen; TDS: Total dissolved solids; TSS: Total suspended solids.

2. Average amount of floc production

The data in Fig. (1) show the average amount of floc that increased with increasing the feeding rate (T_3 and T_4); however, it decreased with exposure to intermittent fasting (T_2 and T_4).

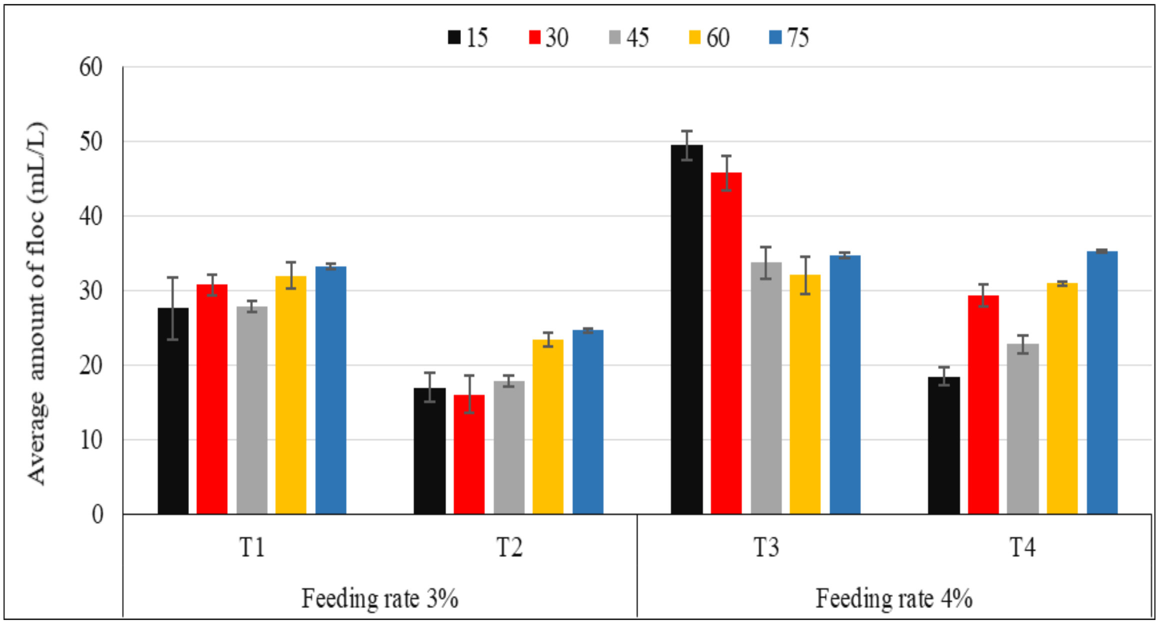

Fig. 1. Average amount of floc (mL/L) for each treatment (every two weeks) during the experimental period

3. Total count of beneficial bacteria during the floc formation period

The data shown in Fig. (2A- C) demonstrate the TBC recorded during the floc formation period (12 days). It is clear that the TBC steadily rose over time and reached its highest point on the 12th day of the floc formation period (Fig. 2A). This pattern was also observed in the biofloc system when compared to the traditional system (T_0) (Fig. 2B). Additionally, the increase in the TBC encompassed different species, such as *Bacilli*, *Short rods*, and *Cocci*, in the floc system than the traditional system (T_0) shown in Fig. (2C).

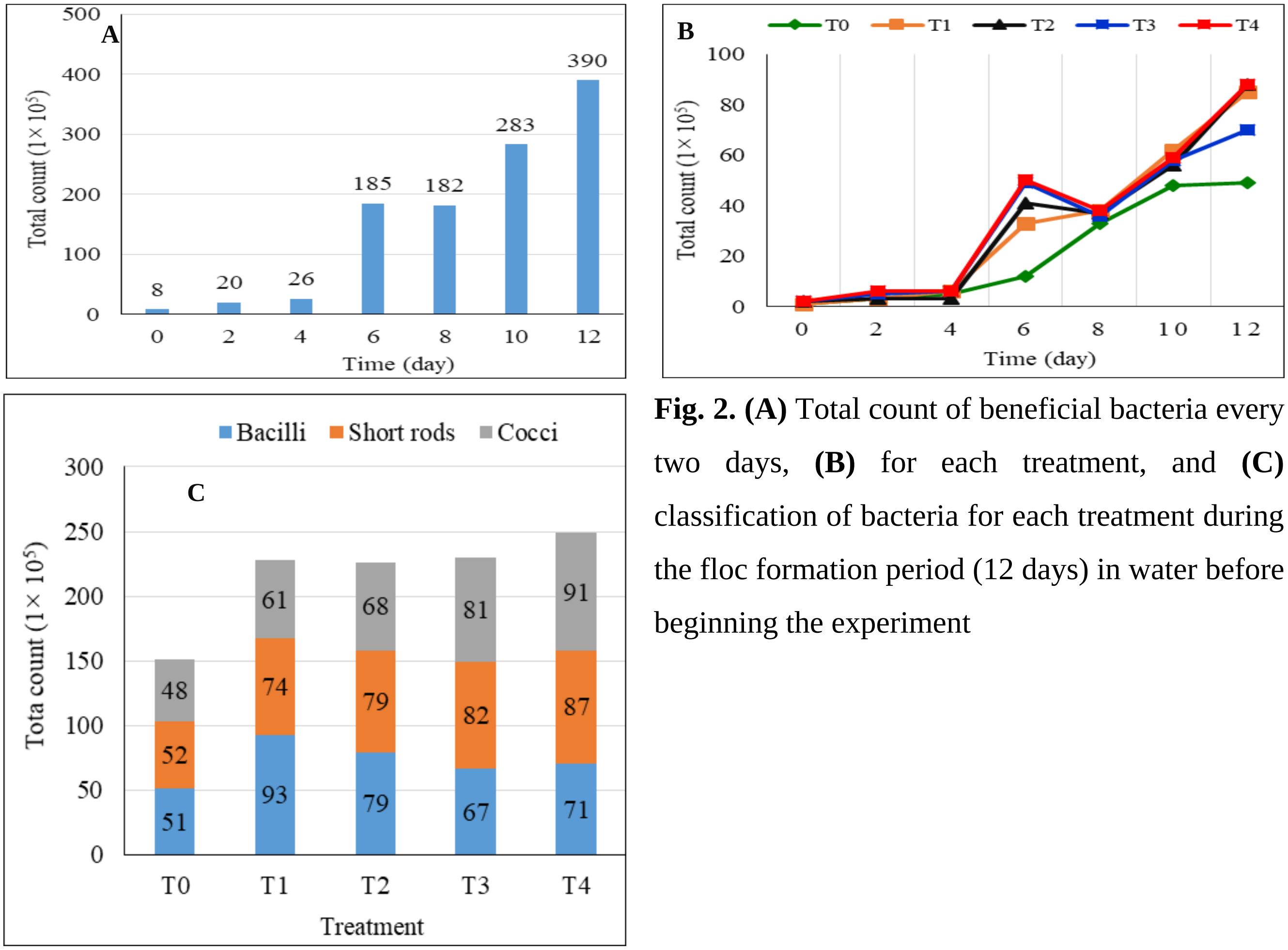

Fig. 2. (A) Total count of beneficial bacteria every two days, **(B)** for each treatment, and **(C)** classification of bacteria for each treatment during the floc formation period (12 days) in water before beginning the experiment

4. Total count of beneficial bacteria during the experimental period

The effect of intermittent fasting on the total count of beneficial bacteria and classification for each treatment in water every two weeks during the experiment period is shown in Fig. (3A- B). Fish cultured in the biofloc system showed a significant increase in the TBC, with large variations observed during the experimental period compared to the traditional system (T_0 as a control treatment). The biofloc treatments also exhibited a higher abundance of short-species bacteria, followed by *Cocci*, then *Bacilli* (Fig. 3A). Treatments using the culture biofloc system exhibited fluctuations in the quantity of bacteria present throughout the entire duration of the experiment. On the other hand, the control treatment demonstrated a noticeable decrease throughout the experiment period (Fig. 3B).

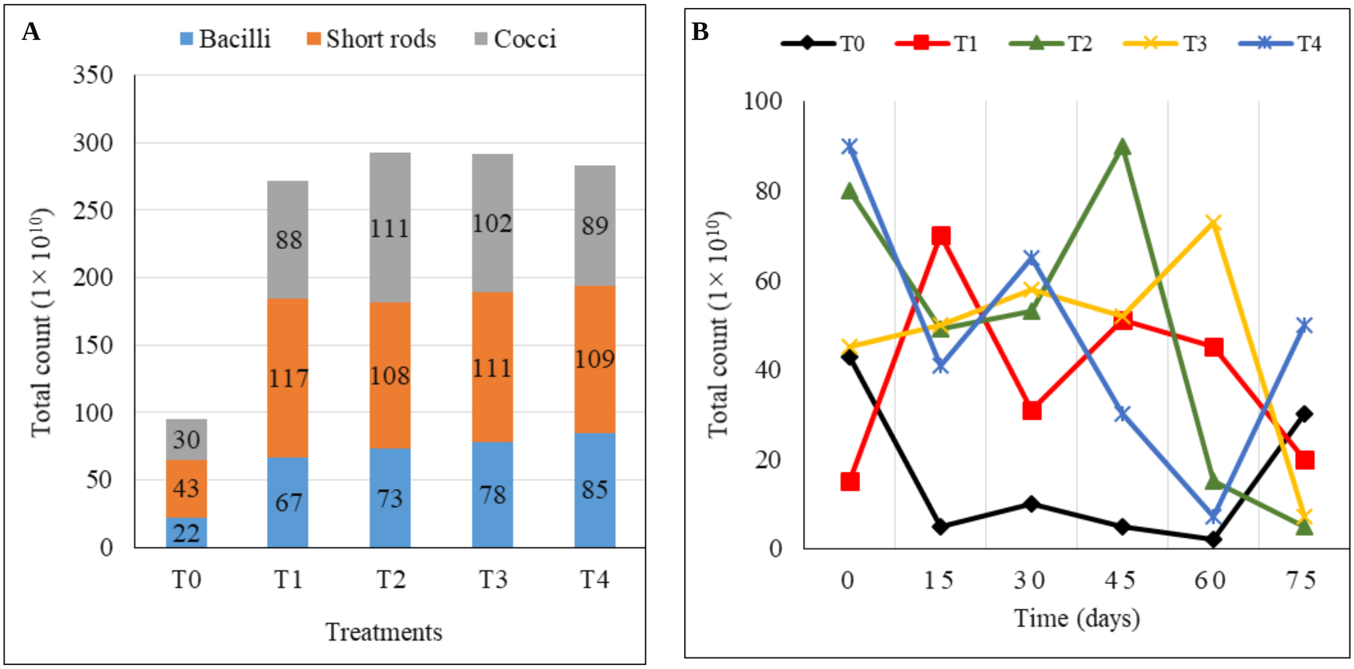

Fig. 3. (A) Total count of beneficial bacteria and its classification in each treatment and **(B)** every two weeks in water during the experimental period (70 days)

5. Total count of phytoplankton and zooplankton

The total count of both phytoplankton and zooplankton in water (Fig. 4A-B) showed an increase in the biofloc system compared to the traditional system (T_0, as a control treatment). Furthermore, the total count of phytoplankton and zooplankton in water increased with increasing feeding rate (as detected in T_3) and decreased in water of fish exposed to intermittent fasting as observed in T_2 and T_4 (Fig. 4A-B).

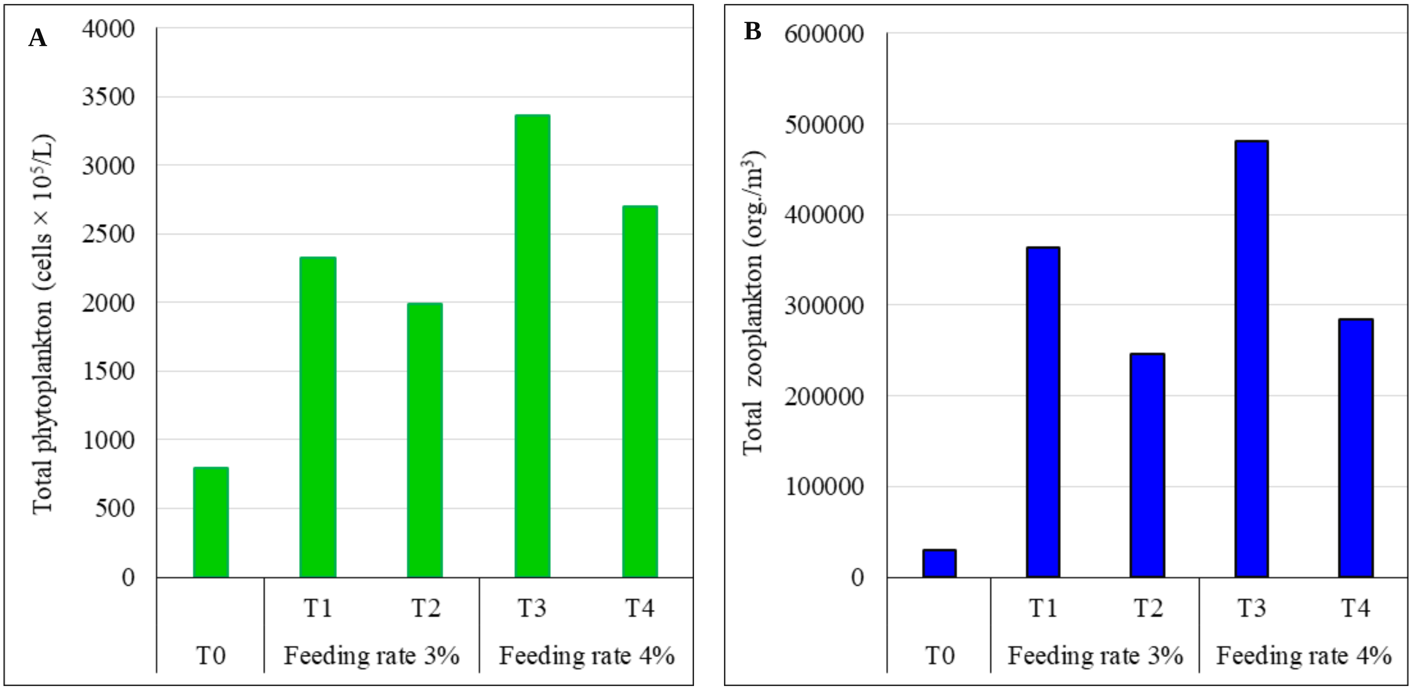

Fig. 4. (A) Effect of intermittent fasting on total count of phytoplankton and **(B)** zooplankton in water of fish reared in different culture systems

6. Growth performance and feed efficiency parameters

The effect of continuous feeding and intermittent fasting on the growth performance and feed efficiency parameters of the Nile tilapia reared in different culture systems is presented in Table (3). Fish in treatments cultured in a biofloc system exhibited higher growth performance and better feed efficiency parameters compared to those reared in a traditional system (T_0), except T_2, which displayed the lowest values for growth performance parameters. In the biofloc treatments, growth performance parameters increased with an increase in the feeding rate, as observed in T_3, which exhibited the highest values. No significant differences were recorded between T_1 (feeding at 3% with continuous feeding) and T_4 (feeding at 4% with intermittent fasting). However, T_2 (feeding rate at 3% with intermittent fasting) displayed significantly lower values for FCR and higher values for FE and PER compared to the other treatments.

Table 3. Effect of continuous feeding and intermittent fasting on growth performance and feed efficiency parameters of the Nile tilapia reared in different systems

Parameter	Traditional system (as a control)	Biofloc system				*P*-value
		Feeding rate 3%		Feeding rate 4%		
	T$_0$	T$_1$	T$_2$	T$_3$	T$_4$	
Initial biomass (kg/m^3)	2.2±1.00	2.2±1.00	2.2±1.00	2.2±1.00	2.2±1.00	<.0001
Final biomass (kg/m^3)	4.80±0.00^{c}	5.02±0.04^{b}	4.61±0.02^{c}	5.31±0.08^{a}	5.08±0.08^{b}	<.0001
WGB (kg/m^3)	2.62±0.01^{b}	2.88±0.04^{a}	2.43±0.03^{b}	3.13±0.08^{a}	2.90±0.08^{a}	<.0001
ADGB (g/m^3/day)	37.53±0.03^{c}	40.97±0.55^{b}	34.90±0.35^{c}	44.87±1.07^{a}	41.40±1.10^{b}	<.0001
RGR (%)	121.3±0.09^{bc}	132.3±1.85^{b}	112.6±1.15^{c}	145.0±3.46^{a}	133.7±3.58^{b}	<.0001
SGR (%/day)	0.97±0.01^{bc}	1.01±0.01^{b}	0.91±0.01^{c}	1.09±0.02^{a}	1.01±0.02^{b}	<.0001
Feed intake (kg/m^3)	5.67±0.03^{a}	4.30±0.00^{b}	2.03±0.03^{d}	5.97±0.15^{a}	2.97±0.03^{c}	<.0001
Feed conversion ratio	2.17±0.02^{a}	1.51±0.02^{c}	0.82±0.01^{e}	1.90±0.00^{b}	1.01±0.01^{d}	<.0001
Feed efficiency (%)	46.33±0.23^{e}	66.22±0.92^{c}	120.2±0.15^{a}	52.63±0.03^{d}	98.18±1.23^{b}	<.0001
Protein efficiency ratio (%)	1.57±0.02^{e}	2.21±0.03^{c}	4.02±0.01^{a}	1.77±0.01^{d}	3.29±0.04^{b}	<.0001

Mean in the same row having different letters are significantly different ($P \leq 0.05$). WGB: Weight gain of biomass; ADGB: Average daily gain of biomass; RGR: Relative growth rate; SGR: Specific growth rate.

7. Muscular chemical composition

The data presented in Table (4) reveal the impact of intermittent fasting on the muscular chemical composition of the Nile tilapia cultivated in various culture systems. Fish in T$_3$, which had a feeding rate of 4% with continuous feeding and was reared in a biofloc system, exhibited the highest moisture, crude fat, and EC contents while showing the lowest levels of crude protein and ash contents ($P \leq 0.05$). Conversely, treatments T$_0$ and T$_2$ had the highest crude protein content ($P \leq 0.05$) compared to other treatments. Treatments T$_1$ and T$_2$ displayed the highest levels of ash content in fish muscles compared to other treatments ($P \leq 0.05$).

Table 4. Effect of continuous feeding and intermittent fasting on the muscular chemical composition of the Nile tilapia reared in different culture systems

Parameter	Traditional system (as a control)	Biofloc system				*P*-value
		Feeding rate 3%		Feeding rate 43%		
	T$_0$	T$_1$	T$_2$	T$_3$	T$_4$	
Moisture (%)	10.19±0.18^{c}	13.69±0.02^{a}	13.28±0.34^{a}	13.73±0.02^{a}	11.87±0.05^{b}	<.0001
Crude protein (%)	82.75±0.03^{a}	81.39±0.01^{b}	82.22±0.16^{a}	81.27±0.01^{b}	81.81±0.05^{b}	<.0001
Crude fat (%)	6.57±0.05^{c}	7.40±0.07^{b}	6.74±0.12^{c}	8.64±0.01^{a}	8.10±0.03^{a}	<.0001
Ash (%)	10.67±0.08^{b}	11.21±0.06^{a}	11.04±0.04^{a}	10.09±0.01^{c}	10.09±0.04^{c}	<.0001
EC (MJ 100/g DM)	2216±2.60^{b}	2217±2.31^{b}	2210±1.15^{b}	2263±0.58^{a}	2254±1.15^{a}	<.0001

Mean in the same row having different letters are significantly different ($P \leq 0.05$). DM: dry mater; EC: Energy content (MJ 100/g DM) = [(protein × 23.64) + (fat × 39.54)].

8. Histological and histometric parameters of dorsal muscles

Data in Table (5) and Fig. (5) display the histometric parameters and histological properties of dorsal muscles of the Nile tilapia reared in different culture systems, respectively. Fish reared in T$_3$, fed at a feeding rate of 4% with continued feeding, revealed the best histometric parameters (Table 5; $P \leq 0.05$) and histological properties (Fig. 5), among other treatments. However, fish in T$_2$, which were fed at a feeding rate of 3% with intermittent fasting, showed the lowest values of large and mean diameters and the highest values of intensity of muscular bundles among other treatments ($P \leq 0.05$). Notably, there are no significant variances among treatments in the smallest/largest ratio ($P \geq 0.05$).

Table 5. Effect of continuous feeding and intermittent fasting on histometric characteristics of the Nile tilapia dorsal muscles reared in the biofloc system

Parameter	Traditional system (as a control) T$_0$	Biofloc system				*P*-value
		Feeding rate 3%		Feeding rate 4%		
		T$_1$	T$_2$	T$_3$	T$_4$	
Smallest diameter (µm)	129.3±2.29^{a}	119.7±2.42^{b}	117.2±1.52^{b}	134.3±2.24^{a}	113.5±1.82^{b}	<.0001
Largest diameter (µm)	195.0±4.35^{b}	200.6±4.06^{b}	177.9±2.79^{c}	217.0±4.66^{a}	195.3±3.78^{b}	<.0001
Mean diameter (µm)	162.2±2.63^{b}	160.2±2.29^{b}	147.6±1.55^{c}	175.7±2.52^{a}	159.4±1.98^{c}	<.0001
Smallest/largest ratio	0.71±0.02	0.65±0.03	0.69±0.01	0.68±0.03	0.67±0.03	0.5303
Intensity of muscular bundles (mm^{-2})	29.70±2.50^{b}	30.57±1.84^{b}	40.34±1.43^{a}	34.00±2.42^{ab}	36.06±2.14^{ab}	0.0027
Percentage of muscular bundles area (%)*	57.23±1.42^{c}	67.49±1.24^{b}	59.21±1.50^{c}	71.70±1.30^{a}	66.83±1.40^{b}	<.0001
Percentage of connective tissue area (%)**	42.77±1.42^{a}	32.51±1.24^{b}	40.79±1.50^{a}	28.30±1.30^{c}	33.17±1.40^{b}	<.0001

Mean in the same row having different letters are significantly different ($P \leq 0.05$).

* Percentage of muscular bundles area (PMBA) = [3.14 × (mean diameter/2)^2] × intensity of muscular bundles (per mm^2) ×100; whereas, the muscular bundles appeared as circularity shape approximately. ** Percentage of connective tissue area (PCTA, per mm^2) = (1− muscular bundles area) × 100.

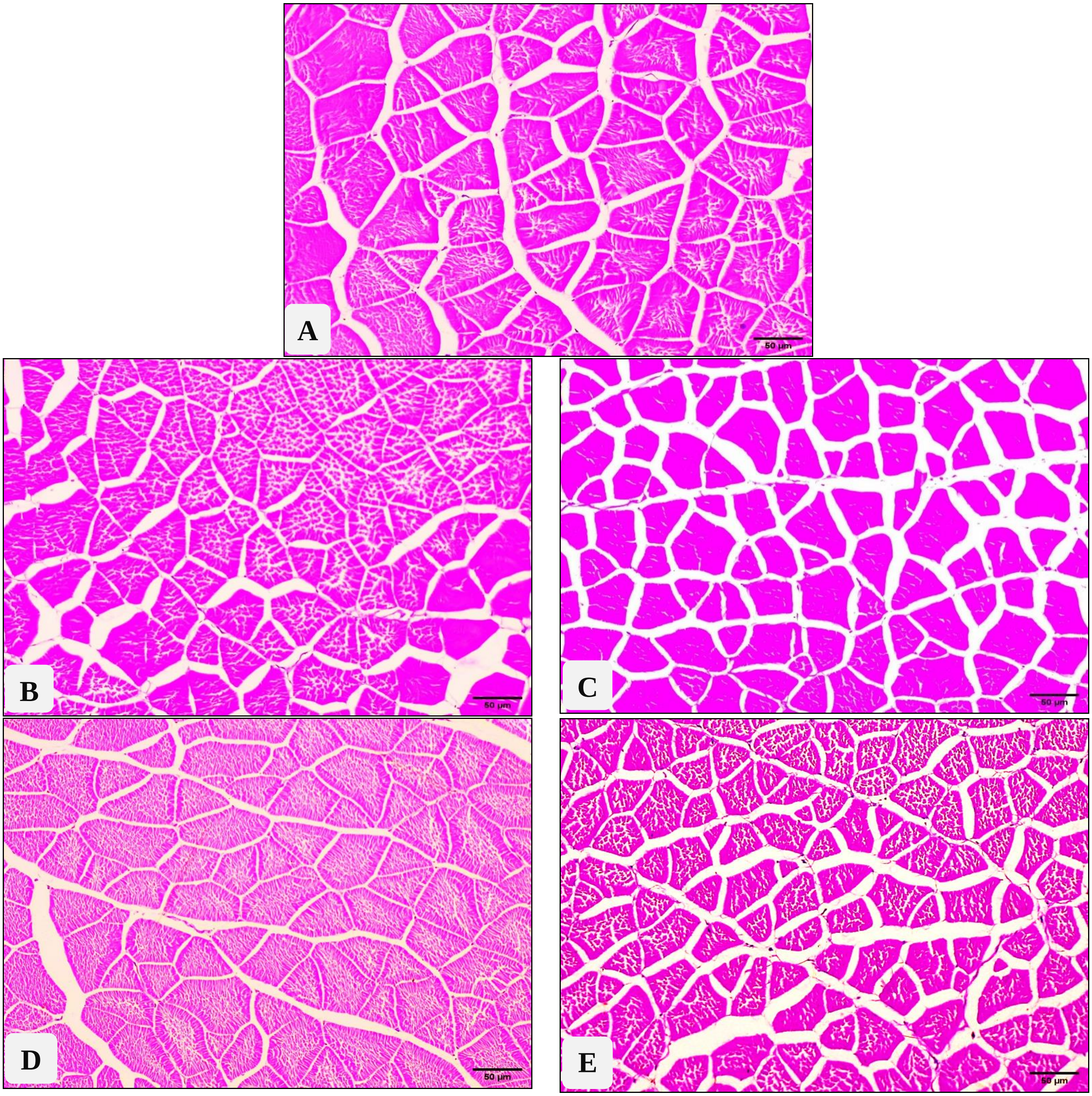

Fig. 5. Effect of continuous feeding and intermittent fasting on the histological properties of the dorsal muscles of the Nile tilapia reared in different culture systems. **(A)** Fish fed at a feeding rate of 4% and reared in the traditional aquaculture system (T_0, as a control); **(B)** Fish fed at a feeding rate of 3% with continuous feeding and reared in the biofloc system (T_1); **(C)** Fish fed at a feeding rate of 3% with an intermittent fasting and reared in the biofloc system (T_2); **(D)** Fish fed at a feeding rate of 4% with continuous feeding and reared in the biofloc system (T_3); and **(E)** Fish fed at a feeding rate of 4% with an intermittent fasting and reared in the biofloc system (T_4). Scale bar = 50µm.

9. Microbiological analysis of muscle

Throughout the storage period (1-3 months), the TBC in the muscle of the Nile tilapia increases over time (Fig. 6). The control treatment, T_0, where fish were reared in a traditional system, showed a higher increase in TBC, especially after 60 and 90 days of storage compared to other fish cultured in the biofloc system (Fig. 6).

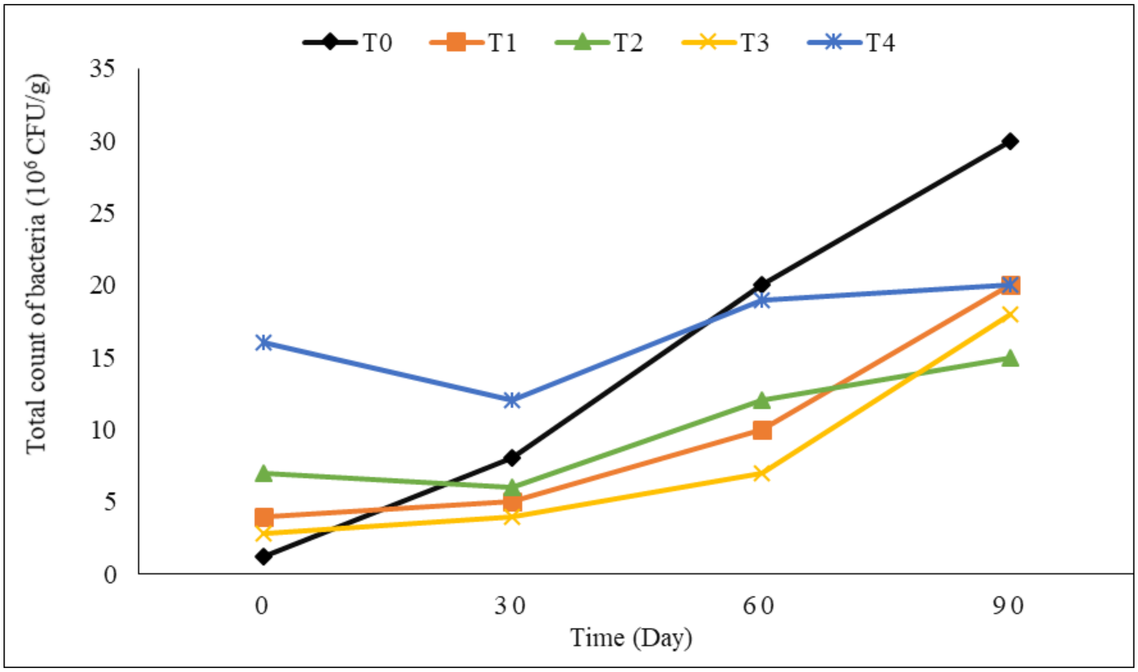

Fig. 6. Effect of intermittent fasting on total count of bacteria in the Nile tilapia muscles during storage periods

DISCUSSION

The biofloc system improves water quality parameters through its ability to convert nitrogenous wastes from a toxic form to bacterial communities (flocs), which reduces ammonia levels in water **(Avnimelech, 2009; Anjalee-Devi & Madhusoodana-Kurup, 2015; Khanjani & Sharifinia, 2020)**. This is consistent with the results obtained in the present study. Fish cultured in the biofloc system showed a significant decrease in TAN as well as in DO levels and pH compared to the conventional system. The decrease in DO is due to the increased consumption resulting from the increase in the biomass inside the biofloc system of heterotrophic bacteria, phytoplankton, and zooplankton, as well as the increase in CO_2 production in metabolic processes and uptake of nitrogenous compounds **(Khanjani *et al.*, 2017; Mirzakhani *et al.*, 2019; Sarsangi Aliabad *et al.*, 2022)**. At the same time, fish cultured in the biofloc system showed an increase in TDS and TSS due to the presence of bacterial aggregates continuously suspended in the water column to maintain the continuity of the biofloc system. Meanwhile, intermittent fasting led to a decrease in TDS and TSS in treatments (T_2 and T_4) compared to fish continuously fed (T_1 and T_3), and this may be due to the fish feeding on bacterial aggregates during the fasting period leading to a decrease in their number. Likewise, **Aliabad *et al*. (2022)** indicated that the TDS and TSS values were significantly higher in the biofloc system than those of the clear water and enhanced by elevating feeding rates. Furthermore, because there will be more substrate available for heterotrophic bacterial growth, a greater feeding rate may result in a higher quantity of settleable solids **(Pérez-Fuentes *et al*., 2016)**. This is also consistent with the current findings regarding the numbers of TBC, phytoplankton, and zooplankton forming flocs. In general, all water quality measurements were within the permissible limits for the Nile tilapia cultivation **(El-Sayed, 2006)**.

The biofloc system relies mainly on numerous microorganisms such as bacteria, algae, fungi, ciliates, rotifers, and nematodes, which form the basis of this system **(Manan *et al*., 2017)**. Analysis of the floc in the present study showed that they contain beneficial bacteria (*Bacillus*, *Short*, and *Cocci*), phytoplankton, and zooplankton. These numbers augmented with an increasing feeding rate from 3 to 4%, as well as being reduced in the treatments that were subjected to intermittent fasting (T_2 and T_4) compared to fish fed continuously (T_1 and T_3). This is due to the fish being fed flocs during the fasting period to compensate for the lack of added food. This is consistent with the amount of floc measured during the experimental period, which was observed to rise with the increase in the feeding rate and decrease with the fasting protocol. The decrease rate was greater in fish fed the experimental diet at a feeding rate of 3% than in those fed the experimental diet at a feeding rate of 4%. This agrees with the results obtained by **Magouz *et al*. (2021)**. The previous authors noted an increase in the size of the formed floc upon increasing the feeding rates, and consumption of the floc clusters by fish was not sufficient to prevent their accumulation. In addition, they concluded that there was a need to regularly remove the formed floc for an effective management and to maintain the operation of the biofloc system **(Magouz *et al*., 2021)**. Furthermore, **Helal *et al*. (2024)** found a link between biofloc concentration and zooplankton concentration, with rotifers being the dominating category **(Said & Taha, 2022)**. In addition, **Rajkumar *et al*. (2016)** found that zooplankton was dominant and the number of phytoplankton was limited.

Different fish species have shown improved growth performance when cultured in biofloc systems compared to those reared in conventional or clear water systems **(Aliabad *et al*., 2022)**. The same trend was observed in this study, that under the biofloc system and increasing the feeding rate led to an improvement in growth performance parameters, as observed in T_3, followed by T_1, which was fed at a feeding rate of 3% continuously, and T_4, which was fed at a feeding rate of 4% with the fasting protocol. Intermittent fasting led to a significant decrease in the growth performance parameters for T_2, which was fed at a feeding rate of 3% with the fasting procedure. This reduction in growth performance parameters may be attributed to the fish not receiving sufficient nutritional requirements as a result of the fasting practice. **Aliabad *et al*. (2022)** discovered that reducing the feeding rate by up to 15% resulted in a comparable growth performance to the control treatment; however, reducing the feeding rate by 30, 45, and 100% led to tilapia's growth performance decline. A biofloc system can lower tilapia fingerlings' feeding rate by up to 20% **(Pérez-Fuentes *et al.*, 2018; Amany *et al.*, 2019)**. Another study found that floc intake might fulfill 25% of tilapia's protein demand **(Avnimelech, 2009)**. This concurs with the results obtained from the current study, where fish cultured using the biofloc system showed an improvement in feed efficiency indicators. Intermittent fasting also led to an improvement in the FCR and PER due to a 50% reduction in the amounts of feed added and the fish getting what they need from the formed floc in the system. This result supported the ability of the biofloc system to provide feed, which is reflected in reducing the production cost. Additionally, they support the successful use of intermittent fasting as a promising protocol to manage the biofloc system.

In the biofloc system, **Emerenciano *et al.* (2012)** exhibited that the whole-body composition of cultured species can be influenced positively by floc produced by heterotrophic bacteria. In the present study, fish reared in the biofloc system exhibited a significant increase in moisture and fat content, along with a decrease in protein content in the muscles compared to fish reared in the conventional system. These findings align with the results obtained by **Aliabad *et al.* (2022)**, who observed an increase in moisture content among fish reared in biofloc systems. Furthermore, as the feeding rate increased, the fat and energy content also increased, whereas the ash content decreased. This is explained by the fact that fish can turn excess energy from feed that is fed to them at greater feeding rates into fish fat **(Huang *et al.*, 2015)**. In addition, **Ahmed (2007)** highlighted the significant impact of feeding rates on fish body composition, which is consistent with the findings of this study. Moreover, intermittent fasting resulted in a substantial reduction in fat content, particularly among fish fed at a feeding rate of 3% compared to other treatments. This decrease in fat content may be attributed to the utilization of visceral and intramuscular fat as an energy source **(Adebayo *et al.*, 2000)**. Similarly, as shown by **Martins *et al.* (2017)** and **da Silva *et al.* (2020)**, decreasing the feeding rate in the biofloc system may cause fish to filter more floc at lower feeding rates, which could result in a larger buildup of inorganic materials (ash).

According to **Listrat *et al.* (2016)**, the quality of fish meat is mostly determined by the three main components: muscle fibers, intramuscular connective tissue, and intramuscular fat. To the best of our knowledge, no previous studies have been conducted on the histometric parameters of fish muscles reared in a biofloc system. Several studies have reported on the histometric of muscles affected by various factors, such as stocking density (SD) **(Refaey *et al.*, 2018)** and feed additives **(Mehrim *et al.*, 2024)**. Changes in histometrics of muscles are used as indicators of flesh quality. Fish cultured under the biofloc system showed an improvement in the histometric properties of muscles compared to those reared in the conventional system. This improvement augmented with the increase of the feeding rate and reduced with the exposure of fish to intermittent fasting, which was associated with a rise in the feeding rate with a significant increase in body growth reflected in the increase in muscle tissue in fish. On the contrary, fish exposed to intermittent fasting showed a significant decrease in the histometric properties of muscle tissue, especially at a feeding rate of 3% compared to the other treatments. This decrease may be attributed to the fact that the fasting procedure led to the depletion of part of the fish's energy, as shown in the decrease in muscle fat, which reduced the rate of muscle tissue formation and reduced the histometric properties of fish muscles. Additionally, the increased numbers and size of muscle fibers are vital limits for muscle growth **(Listrat *et al*., 2016)**, which are reflected in the augmentation of body weight in channel catfish reared in the low SD. However, **Helal *et al*. (2024)** recently showed that no significant variances were detected in the histological properties of muscles of fish reared in the biofloc system or those in the control group.

CONCLUSION

Finally, based on the findings obtained in this study, the biofloc system outperforms the traditional system in all measured parameters. Additionally, it is evident that reducing the feeding rate (to 3%, as observed in T_1) or implementing an intermittent fasting protocol (T_2) while maintaining the same feeding rate (at 4% with intermittent fasting, as observed in T_4) yields positive outcomes in terms of water quality, floc formation, growth performance, feed efficiency, and flesh quality within the biofloc system compared to those reared in the traditional system. Consequently, it could be concluded that modifying feeding management in biofloc systems, especially with the use of the intermittent fasting protocol, is always necessary since it provides a significant cost reduction in feed, environmental aspects, and overall sustainability of fish production.

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