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Response of Nile tilapia under biofloc system to floating or sinking feed and feeding rates: Water quality, plankton community, growth, intestinal enzymes, serum biochemical and antioxidant status

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ABSTRACT

The current study examined the combined effects of feeding rates and feeding types on the water quality, growth performance, digestive enzymes, blood parameters, and liver antioxidant enzymes of Nile tilapia (*Oreochromis niloticus*) fingerlings reared under a biofloc system. A 3 × 2 factorial experimental design was used with three feeding rates (2%, 3%, and 4%) and two feeding types (sinking or floating feed), comprising six treatments with three replicates. Fingerlings with an initial body weight of 4.50 ± 0.25 g were stocked in eighteen circular plastic tanks (0.5 m^3) at a stocking density of 35 fish per tank fed three times a day at 10:00, 12:00 and 15:00. Starch was added to all treatments as an organic carbon source at a C/N ratio of 10:1. The phytoplankton community was determined to consist of twenty-two species, including individuals from the classes Cyanobacteria, Chlorophyceae, and Bacillariophyta. The most common phytoplankton classes were Chlorophyceae, followed by Cyanobacteria, and fish-fed floating feed at a feeding rate of 2% of total biomass yielded the greatest number of phytoplankton communities. Eight zooplankton species belonging to rotifers and protozoa were identified during this experiment. The highest values of final body weight, weight gain, and specific growth rate were recorded for a fish-fed floating diet with the highest feeding rate (4% of biomass). The highest hepatosomatic index (HIS) was detected in a fish-fed floating diet at a rate of 4% of total biomass. Though fish-fed sinking feed with a 4% biomass feeding rate presented the highest spleen index (SI). The highest significant ($P < 0.05$) level of amylase was found in fish-fed sinking feed at a 4% feeding rate. While lipase activity was higher ($P < 0.05$) in the group fed floating feed at a feeding rate of 3% of total biomass. Feeding rates, feed types, and their interactions had no appreciable effect ($P > 0.05$) on hemoglobin (Hb), hematocrit (Hct), and red blood cells (RBCs). Feeding rate, feed type, and their interactions had no significant impact ($P > 0.05$) on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), or albumin, but feeding rates or feed types had an effect ($P < 0.05$) on total protein and globulin ($P < 0.05$). The highest levels of growth hormone (GH) ($P < 0.05$) were found in fish-fed either sinking feed at a feeding rate of 2% or floated feed at a feeding rate of 3% of total biomass. The fish group fed floating or sinking feed at a feeding rate of 2%, floating feed at 3%, and sinking feed at 4% recorded the highest high-density lipoprotein (HDL-C) values with an insignificant difference. Considering the liver's antioxidant enzymes, the highest catalase (CAT) and glutathione (GSH) levels were shown in fish that received either sinking or floating feed at feeding rates of 2% and 3%, respectively. Whereas, the lowest melanodialdehyde (MDA) was found in fish-fed sinking feed at a feeding rate of 3% of total biomass. According to the study's findings, the biofloc system significantly improved both the water quality and the efficiency of Nile tilapia. In addition, feeding tilapia 4% floating feed resulted in maximum feed consumption with minimal waste, improved nutritional efficiency and feed conversion efficiency, lowered production costs, and lessened water pollution.

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1. Introduction

A sine qua non for sustainable aquaculture, particularly in intensive production systems, is the ability to supply an adequate amount of feed. Feeding should be optimized so that the yield gap is as small as possible and that marginal feed costs are equivalent to marginal profits (Mengistu et al., 2020). Considering that between 60% and 70% of the expenditures of rearing fish are devoted to fish feeding (Anderson et al., 1997; Kannadhason et al., 2009; Limbu and Jumanne, 2014). Overfeeding causes a fish's stomach and intestines to become overloaded, which reduces nutritional digestion and absorption, harms water quality parameters, and raises production costs (Cho et al., 2003; Khan and Abidi, 2010; Tian et al., 2015). Additionally, a shortage of feed supplies hinders animal growth and immune system effectiveness, increasing their susceptibility to diseases and mortality (Bu et al., 2017). In commercial fish farming, the optimum exogenous feed supply for fish species in various production systems, strains, and life stages must be determined (Gelineau et al., 1998; Wang et al., 2009; Ng and Romano, 2013; Domínguez-May et al., 2020). Therefore, thorough planning and execution of production management strategies and technologies are necessary to improve the economic and environmental impacts of aquaculture systems.

Interest in numerous aspects of filter-feeding tilapia production using the Biofloc technology (BFT) system has increased recently to ensure the sustainability of their production (Kabir et al., 2020; de Moraes et al., 2020; Hisano et al., 2021). Biofloc contains a variety of helpful microorganisms, including bacteria, protozoa, rotifers, nematodes, ciliates, and copepods (Azim and little, 2008; Emerenciano et al., 2012; Rajkumar et al., 2016). The ideal environment for heterotrophic bacteria biomass to develop optimally and be able to digest nitrogenous waste from cultured spices and keeping acceptable water quality is an external carbon source and increased oxygenation (Avnimelech, 2009; Emerenciano et al., 2017; El-Sayed, 2021). By using microbial biomass as a protein source in biofloc, filter feeder species like tilapia and shrimp can reduce feed requirements and production costs (Wasielesky et al., 2006; Crab, 2010; Xu and Pan, 2014). However, recent research has revealed contradictory results on the ability of microbial flocs to maintain appropriate growth of Nile tilapia when the artificial feed supply was limited (Liu et al., 2018; Silva et al., 2020).

As a result, it's important to figure out the feeding rates of each production system to improve the link between fish intake of exogenous and endogenous food. In the nursery phase of the BFT system, feeding rates for Nile tilapia range from 2% to 3% (Kishawy et al., 2020; Saseendran et al., 2021) to 8–10% (Gallardo-Collí et al., 2020; Sgnaulin et al., 2020). When compared to growing conditions without biofloc, prior research has revealed that biofloc may contribute from 15% to 50% of the total daily feed supply (Avnimelech, 2009). Thus, to maximize the effectiveness of additional microbial feed by culture species as well as to lessen reliance on artificial feed, a restricted feed supply under a biofloc system is a smart alternative (Pérez-Fuentes et al., 2018). In this regard, earlier research on tilapia (Pérez-Fuentes et al., 2018; da Silva et al., 2020; Oliveira et al., 2021) and shrimp (Luna-González et al., 2017; Weldon et al., 2021) evaluated the effect of different feeding frequencies or different feeding ratios under biofloc.

Moreover, the type of feed (sinking or floating) has an impact on the growth and success of fish farming (Hossain et al., 2018). High-growth and high-profit Nile tilapia can be raised by farmers with high-quality food. The tilapia monosex farming industry's growth and success depend on the availability of high-quality sinking and floating feeds (Craig and Helfrich, 2009). When opposed to the sinking feed, the floating feed has advantages since it has superior physical properties such as improved water stability, digestibility, water protection, zero water pollution, and no raw material waste (Almaraaj, 2011). On the other hand, the extrusion process used to produce the floating diets requires high temperatures and pressure in addition to other costs, it is typically more expensive than sinking feed. While floating feeds are more expensive to make, sinking feeds are less (Adewumi and Olaleye, 2011). According to earlier research that investigated the effect of feed types on fish performance, tilapia-fed floating feed performed better than those fed sinking feed (Creswell, 2005; Hossain et al., 2018; Abdelhamid et al., 2019).

As far as the authors are aware, there have been no studies done on the effects of feeding types (sinking or floating) on fish raised in a biofloc system or their combined impact on the performance of fish reared in biofloc system with different feeding rates. Therefore, the goal of this study was to ascertain the effects of various feeding rates and types when combined on Nile tilapia (*Oreochromis niloticus*) fingerlings raised in a biofloc system and assess the function of microbial floc as an endogenous supplemental feed source based on water quality, growth, nutritional efficiency, digestive enzymes, hematological, biochemical blood indices, and liver antioxidant enzymes.

2. Material and methods

2.1. Experimental design and Diets

The experiment was conducted inside a greenhouse held in the Faculty of Agriculture, Benha University, Egypt. A factorial experiment (3×2) was carried out to study the effects of three feeding rates R1 (2%), R2 (3%), and R3 (4%), and two types of feed: floating (F) or sinking (S) and their interaction under the biofloc system on water quality, plankton community, growth, feed utilization parameters, digestive enzymes, hematological and biochemical indices, lipid profiles, and antioxidant enzymes of tilapia for 70 days. The floating and sinking feed were purchased from Aller Aqua Feed, Cairo, Egypt. Two types of diet were isonirtignous (30% crude protein) and isocaloric (19.1 MJ kg^{-1} diet). The experiment was performed under a biofloc system (zero water exchange).

2.2. Fish and raring technique

Nile tilapia, *Oreochromis niloticus* monosex were obtained from the farm of the Faculty of Agriculture, Benha University, Egypt. Fish were acclimated (15 days) to the environmental conditions and fed with a commercial feed (30% crude protein), three times daily (10:00, 12:00, and 15.00 h). After the acclimation period, 630 Nile tilapia fingerlings (average initial weight of 4.50 ± 0.25 g) were randomly distributed in eighteen circular plastic tanks (0.5 m^3) at a stocking density of 35 fish per tank. Each tank received constant aeration from two air stones. Before starting the experiment, biofloc preparation was performed to stimulate the heterotrophic bacteria in tilapia tanks, where the C:N ratio was 10:1 and adjusted according to Soaudy et al. (2021), by using starch as a source of carbon. The starch was added daily to all tanks in a split-order manner at intermediate feeding times. The experiment began when the floc volume reached 4 mL L^{-1}, which remained constant in all tanks after two weeks. Fish were fed their respective feed type (floating or sinking feed, 2 mm diameter) three times daily (10:00, 12:00, and 15.00 h), at different feeding rates (2%, 3%, and 4% of the total biomass). Fish were weighed every 15 days to adjust the amount of respective feed during the experiment.

2.3. Water quality assessment

The measures of the water quality were analyzed to remain within the permitted limit for Nile tilapia. Throughout the experiment trial, a mercury thermometer suspended at a depth of 15 cm was used to measure the temperature (°C), and a Jenway 970 Dissolved Oxygen Meter was used to measure the amount of dissolved oxygen (DO, in mg/L) (Keison Company, UK). The pH was recorded by using a pH meter (Orion pH meter, Abilene, Texas, USA) twice a day (at 08:00 a.m. and 04:00 p.m.). Water samples (50 mL) were collected once a week from each tank. One part of each water sample was analyzed

spectrophotometrically for total ammonia (NH_4, mg L^{-1}), nitrate (NO_3, mg L^{-1}), and nitrite (NO_2, mg L^{-1}) which were monitored once a week according to APHA (2005). Also, weekly biofloc volume (FV, mL L^{-1}) measurements were made with the aid of Imhoff cones after 30 min of settling (Avnimelech and Kochba, 2009). The titrimetric method was used to measure the alkalinity (Eaton et al., 2005).

2.4. Plankton community assessment in biofloc

2.4.1. Assessment and identification of phytoplankton

The phytoplankton counts were determined by using an inverted ZEISS microscope with 100X magnification (APHA, 2017). The number of cells per mg (mgL^{-1}) was used to display the phytoplankton data results. Identification of phytoplankton was carried out according to Bellinger and Sigee (2015) and Munshi et al. (2010).

2.4.2. Assessment and identification of the zooplankton population

Zooplankton samples were obtained using a zooplankton net (55 m, 25 cm diameter, and 80 cm length) from various biofloc treatments. 5 liters of water were filtered using a zooplankton net after the water had been well stirred. Following filtration, the formaldehyde solution (4–7%) was used to fix the samples right away. After fixing, two millimeters of Rose Bengal stain (0.5%) were added. The filtrated samples were examined under an optical research microscope using a Rafter cell with magnification varying from 100X to 400X. Based on the following equation, zooplankton were estimated after examining all of the identified species in each sample (APHA, 2005): No of organisms/litter = N * D/S*C, Whereas N = Number of organisms for the calculated species, D = Volume of sample after filtration, and S = Number of subsamples. C = Total volume of the collected water sample.

2.5. Biofloc's collection and chemical analysis

A 10-μm mesh nylon bag was used to collect concentrated biofloc samples from each group (Xu et al., 2013). The samples were dried in a hot air oven at 105 °C until constant weight and then stored in a refrigerator (−20 °C) until proximate composition analysis. The chemical analysis of biofloc samples were estimated according to the AOAC (2012).

2.6. Growth, feed efficiency, and biometric indices

The number of fish in each tank was counted and recorded before the feeding trial began and after it ended. In the footnote of Table 3 are all the formulae used to calculate the growth parameters, feed utilization efficiency, and a few biometric indices.

2.7. Determination of intestinal digestive enzymes

Samples of fish intestines (n = 4 per treatment) were promptly homogenized in 10 volumes (w/v) of ice-cold physiological saline solution, and the supernatant was then kept for endogenous enzyme activity measurement (Furné et al., 2008). Lipase activity was determined as described by Zamani et al. (2009), and the titration method was detailed by using olive oil gum. Amylase activity was estimated according to Bernfeld (1951) at 540 nm, and starch was used as the substrate.

2.8. Hemato- biochemical parameters

Blood was obtained from a fish's caudal vein (five fish from each treatment) and divided into two halves using clean syringes at the termination of the experiment. The first half was gathered using the anticoagulant ethylenediaminetetraacetate (EDTA) at a concentration of 10% to determine hematocrit (Htc), hemoglobin (Hb), the total count of white blood cells (WBCs), and red blood cells (RBCs) as described in the standard procedures of Rawling et al. (2009). The remaining part of blood sample was then centrifuged for 10 min at 3000 rpm after clotting overnight at 4 °C. A serum that had not been hemolysis was collected and kept at 20 °C until needed. The levels of alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were measured using Reitman and Frankel (1957). Standard kits (Modern laboratory kits) were used to determine the serum lipid profile, which included triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Total serum protein and albumin were assessed according to Henry (1964) and Wotton and Freeman (1982), respectively. By subtracting total serum albumin from total serum protein, the total serum globulin was calculated (Coles, 1974). According to Lugo et al. (2008), an ELISA with 96-well MaxiSorp plates was used to assess the growth hormone in the serum (Nalge Nunc International, Roskilde, Denmark). The absorbance was determined at 492 nm in a spectrophotometer (Titertek Multiskan Plus).

2.9. Measurements of hepatic antioxidant activities

Three fish's livers from each replicate were weighed, rinsed, and ground in glass homogenizer tubes with ice-cold saline (0.1 g of liver was added to 0.9 mL of saline, pH 7.0), and centrifuged at 3000 g for 10 min. The collected supernatant was used for the activity of superoxide dismutase (SOD) measurement according to the method of Peskin and Winterbourn (2000). The modified method of Beers and Sizer (1952) was used for the catalase (CAT) activity assay. Melanodialdehyde (MDA) activity was measured according to Dogru et al. (2008). Glutathione (GSH) was measured according to the method of Beutler et al. (1963).

2.10. Data analysis

All the data were analyzed by ANOVA using the SAS ANOVA procedure (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 1993). A one-way analysis of variance (One-way ANOVA) was used to determine whether there was significant variation among the treatments. When overall differences were found, differences between means were tested by Duncan's (1955) new multiple range test. A two-way ANOVA was used for analyzing the individual effects of different feeding rates and two types of floating or sinking feed. All differences were considered significant at $P < 0.05$, and the results are presented as means with standard errors of the mean. The data were arc-sin-transformed before analysis (Zar, 1984); though, data are presented untransformed to facilitate the comparisons.

3. Results

3.1. Water quality

Table (1) represented the findings of the water quality monitoring. The levels of total ammonia (NH_4, mg L^{-1}), and nitrate (NO_3, mg L^{-1}), as well as total suspended solid (TSS) (Fig. 1), and alkalinity weren't significantly ($P < 0.05$) affected by feeding rate, feed type, and their interaction. Increasing the feeding rate from 2% to 4% gradually raised TSS (from 370 to 792 mgL^{-1}, respectively) and alkalinity (from 320 to 350 mgL^{-1}, respectively) ($P > 0.05$) regardless of the feed type, but they decreased ($P > 0.05$) in response to the feed type (sinking or floating). Fish that were fed floating feed at a rate of 4% had the greatest TSS, while those that were fed sinking feed at a rate of 2% had the lowest. Generally, over the experimental period, the average water temperature varied between 27 and 28C, and the pH varied between 8.00 and 8.22. The dissolved oxygen ranged from 5.2 to 5.9 mg^{-1}, respectively. With an insignificant difference among all treatments ($P > 0.05$), the interaction between feeding rates and feed types considerably enhanced the floc volume (Fig. 1). The maximum volume of floc is found in fish-fed sinking feed at a 2% feeding rate of total biomass.

Table 1
Water quality parameters of tilapia reared in biofloc system with different feeding rate or feeding type and their interaction.

Treatment	Feeding rate	Feed type	Floc Volume (mlL^{-1})		NH_4 (mgL^{-1})	NO_2 (mgL^{-1})	NO_3 (mgL^{-1})	pH	TSS (mgL^{-1})	Alkalinity (mgL^{-1})
Individual treatment means[†]										
T1 (R1F)	R1 (2%)	Floated (F)	3.25	0.15		0.01	0.91	8.16	500	335
T2 (R1S)	R1 (2%)	Sinking (S)	6.00	0.23		0.01	0.81	8.19	240	310
T3 (R2F)	R2 (3%)	Floated (F)	3.50	0.19		0.01	0.73	8.00	620	340
T4 (R2S)	R2 (3%)	Sinking (S)	4.50	0.17		0.01	0.62	8.22	245	340
T5 (R3F)	R3 (4%)	Floated (F)	4.50	0.21		0.02	0.93	8.15	980	340
T6 (R3S)	R3 (4%)	Sinking(S)	5.00	0.25		0.01	0.75	8.14	605	350
Pooled SE			0.51	0.02		0.01	0.14	0.04	187.43	11.35
Means of the main effect[‡]										
	R1 2%		4.63	0.19		0.01	0.86	8.18	370.0	320.50
	R2 3%		4.00	0.18		0.01	0.67	8.11	432.5	340.00
	R3 4%		4.75	0.23		0.01	0.84	8.15	792.5	345.00
		Floated	3.75	0.18		0.01	0.86	8.11	700.0	338.33
		sinking	5.17	0.22		0.01	0.73	8.18	363.3	333.33
ANOVA (P-value)										
Feeding rate			0.5689	0.3775		0.4312	0.5902	0.5647	0.3126	0.4063
Feed type			0.0595	0.2403		0.3632	0.4463	0.1629	0.1805	0.7184
Feeding rate × feed type			0.3360	0.4265		0.4312	0.9749	0.2198	0.9693	0.5697

[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡] Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.

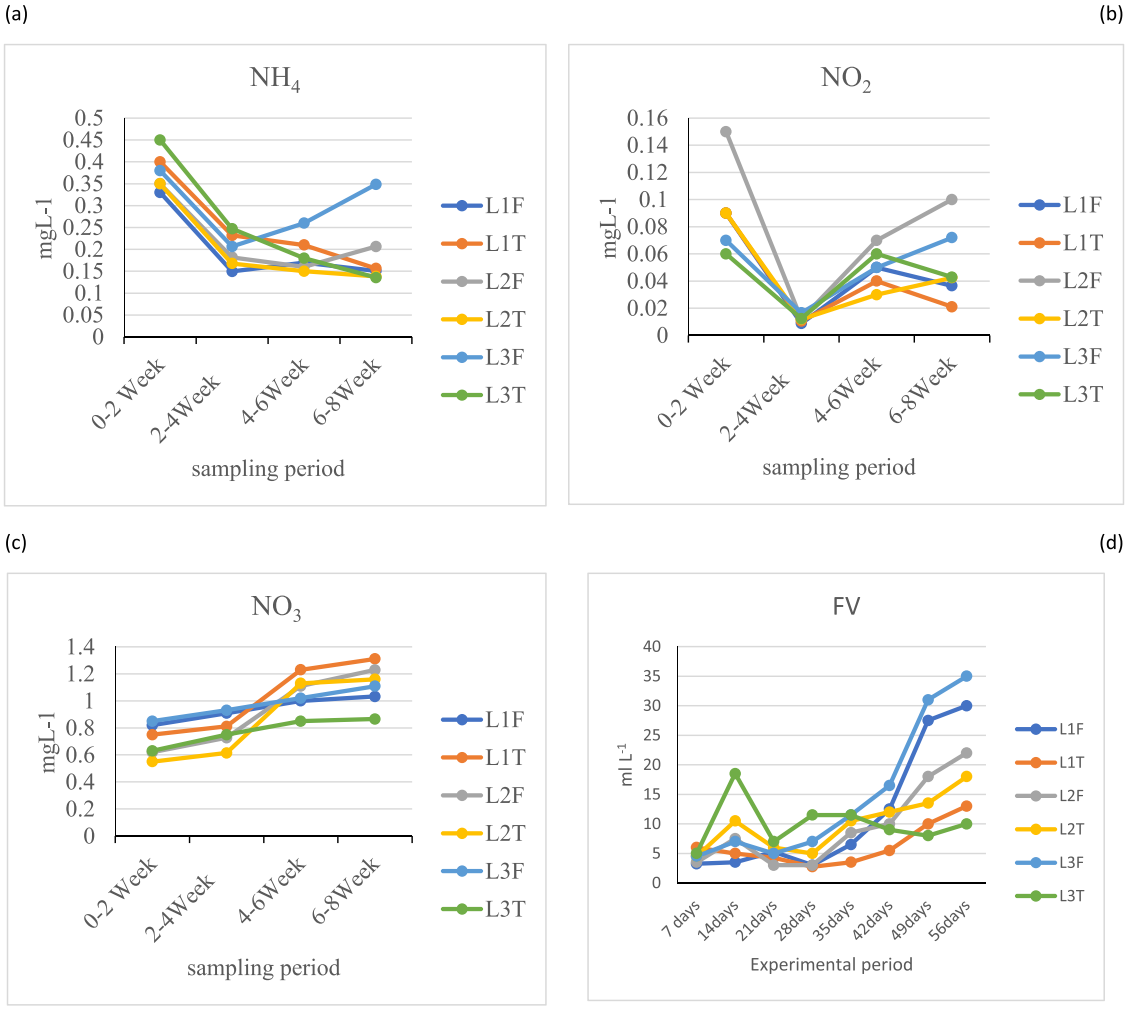

Fig. 1. Water quality parameters of biofloc system with different feeding rate or feeding type and their interaction; a) total ammonia (NH_4, mg/L), nitrate (NO_3, mg/L), and nitrite (No_2, mg/L), floc volume (FV, mL^{-1}).

3.2. Plankton communities in biofloc

Twenty-two species, including members of the classes Cyanobacteria, Chlorophyceae, and Bacillariophyta, were identified as belonging to the phytoplankton communities based on the present data (Table 2). The most prevalent classes of phytoplankton were Chlorophyceae followed by Cyanobacteria, while fish-fed floating feed at a feeding rate of 2% of total biomass recorded the greatest number of phytoplankton community. *Neglectella solitaria* was the primary dominant species in the phytoplankton community, which included 22 species.

Eight zooplankton species were identified during this experiment (Table 3). It indicates that the feeding rates, type of feed, and interactions between them had an influence ($P < 0.05$) on the zooplankton community. The increase in feeding rates combined with an increase in numerical total zooplankton communities, as the highest count was recorded for fish-fed sinking feed at a 4% feeding rate, while groups feed 3% sinking feed (T4R2) were noted for the lowest count. In the zooplankton community, rotifer species dominated. Rotifer included five species: *Polyarthera vulgaris*, *Anuraeopsis fissa*, *Brachionus calciflours*, *Brachionus quadridentata*, and *Philodena* sp. *Anuraeopsis fissa* was the dominant species within the rotifer group. The highest rotifer counts among the other treatments were observed in the fish-fed sinking feed at a 4% feeding rate. Also, protozoa were represented by three species: *Trichocerca* sp., *Vorticella campanula*, and *Lecane closterocerca*. *Trichocerca* sp. was the most prevalent protozoan species in fish-fed floating feed at a 3% feeding rate, but it was found in lesser densities in all other treatments.

3.3. Biofloc's chemical analysis

Regarding the proximate analysis (Table 4) of biofloc, crude lipid and ash are significantly ($P < 0.05$) affected by feeding rates. The highest values of crude lipid and ash were recorded in biofloc collected from tanks treated with low feeding rates of 2% and 3%, respectively. Feed types significantly ($P < 0.05$) affected the biofloc's crude protein and ash content. Biofloc gathered from tanks treated with the floating feed was found to have the highest ($P < 0.05$) protein and ash contents. So, the interaction between feeding rates and feeding types had a substantial impact ($P < 0.05$) on crude lipid but not on crude protein or ash ($P > 0.05$). The biofloc obtained from tanks treated with 2% sinking feed had the highest ($P < 0.05$) observed lipid content.

3.4. Growth performance

The effect of feeding rates, feeding types (sinking or floating), and their interaction on the growth performance and feed utilization for experimental groups are presented in Table 5. In tilapia, variable feeding rates, feed types, and their interactions had a significant ($P < 0.05$) influence on the growth performance and the feeding parameters. Regardless of the effect of feed types, fish-fed 4% of total biomass recorded higher FBW, WG, and SGR. While, the floating feed showed higher FBW, WG, and SGR. The highest values of FBW, WG and SGR were recorded for the fish-fed floating diet with the highest (4% of biomass) feeding rate. Decreased the feeding rates from 4% to 2% of body biomass significant improved feed conversion ratio (FCR), protein efficiency ratio (PER), and apparent protein utilization (APU), irrespective to the effects of the type of feed. The opposite trend was detected in feed intake (FI). No significant ($P > 0.05$) differences were

Table 2
Phytoplankton community (mgL^{-1}) of tilapia reared in biofloc system with different feeding rate or feeding type and their interaction.

Phytoplankton species	Treatment (T)					
	T1 (R1F)	T2 (R1S)	T3 (R2F)	T4 (R2S)	T5 (R3F)	T6 (R3S)
	Feeding rate					
	R1 (2%)		R2 (3%)		R3 (4%)	
	Feed type					
	Floated (F)	Sinking (S)	Floated (F)	Sinking (S)	Floated (F)	Sinking (S)
Cyanobacteria						
Chroococcus limniticus	8.203	15.605	7.400	7.196	6.668	0.746
Planktolyngbya limnetica	0.000	0.056	0.169	0.071	0.043	0.168
Microcystis flosaquae	24.769	5.510	20.883	1.613	11.121	0.646
Limnothrix planctonica	0.001	0.000	0.020	0.086	0.000	0.000
Oscillatoria tenuis	0.003	0.022	0.043	0.000	0.000	0.000
Oscillatoria sp	0.001	0.000	0.000	0.000	0.074	0.079
Spirulina sp	0.001	0.000	0.000	0.000	0.007	0.000
Subtotal	**32.977**	**21.194**	**28.514**	**8.966**	**17.914**	**1.639**
Chlorophyceae						
Chlorella vulgaris	0.014	0.000	0.000	0.000	0.001	0.000
Neglectella solitaria	36.046	0.000	0.000	0.000	0.021	0.000
Stauridium tetras	0.454	0.025	0.147	0.032	0.069	0.000
Tetradesmus obliquus	0.001	0.000	0.001	0.000	0.001	
Desmodesmus armatus	0.029	0.001	0.000	0.002	0.000	0.000
Desmodesmus denticulatus	0.042	0.003	0.000	0.000	0.000	0.000
Tetradesmus dimorphus	0.016	0.000	0.004	0.000	0.000	0.000
Scenedesmus ecornis	0.024	0.000	0.004	0.000	0.000	0.000
Scenedesmus quadricauda	0.363	0.000	0.012	0.008	0.000	0.000
Desmodesmus spinosus	0.017	0.002	0.002	0.000	0.000	0.000
Subtotal	**37.005**	**0.031**	**0.169**	**0.042**	**0.092**	**0.000**
Bacillariophyta						
Fragilaria sp	0.033	0.001	0.000	0.000	0.000	0.000
Navicula cryptocephala	0.045	0.000	0.000	0.000	0.000	0.000
Nitzschia amphibia	0.149	0.003	0.000	0.000	0.000	0.000
Nitzschia palea	0.242	0.002	0.000	0.000	0.000	0.000
Nitzschia sp	0.383	0.000	0.002	0.000	0.000	0.000
Subtotal	**0.852**	**0.006**	**0.002**	**0.000**	**0.000**	**0.000**
Total phytoplankton biomass	**70.836**	**21.231**	**28.685**	**9.008**	**18.014**	**1.639**

Table 3
Mean zooplankton density (organism mL^{-1}) of tilapia reared in biofloc system with different feeding rate or feeding type and their interaction.

Treatment (T)	Feeding rate	Feed type	Protozoa group	Rotifer group							Total zooplankton
			Vorticella campanula	*Lecane closterocerca*	*Trichocerca sp.*	*Polyarthera vulgaris*	*Anuraeopsis fissa*	*Brachionus calciflours*	*Brachionus quadridentata*	*Philodena sp.*	
Individual treatment means[†]											
T1 (R1F)	R1 (2%)	Floated (F)	505	3925^{a}	1105^{ab}	1025^{c}	110^{c}	315^{c}	315^{c}	625^{c}	7705^{cd}
T2 (R1S)	R1 (2%)	Sinking (S)	550	677^{d}	1915^{ab}	1663^{b}	343^{bc}	0	0	2227^{b}	9807^{bc}
T3 (R2F)	R2 (3%)	Floated (F)	1600	1000^{d}	4700^{a}	1300^{bc}	600^{b}	0	0	2600^{b}	11800^{b}
T4 (R2S)	R2 (3%)	Sinking (S)	0	1515^{c}	110^{b}	313^{d}	208^{bc}	0	0	815^{c}	5105^{d}
T5 (R3F)	R3 (4%)	Floated (F)	1510	2115^{b}	1025^{ab}	2895^{a}	107^{c}	618^{b}	618^{b}	410^{c}	9005^{bc}
T6 (R3S)	R3 (4%)	Sinking (S)	420	1895^{bc}	0	3112^{a}	24025^{a}	2418^{a}	2418^{a}	7825^{a}	39605^{a}
Pooled SE			410.82	126.41	1053.28	125.24	101.31	8.59	8.59	146.39	750
ANOVA (P-value)											
Feeding rate			< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001
Feed type			< .0001	< .0001	< .0001	0.8300	< .0001	< .0001	< .0001	< .0001	< .0001
Feeding rate × feed type			< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001	< .0001

[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡] Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.

Table 4
Proximate analysis of biofloc collected from tanks treated with different feeding rate or feeding type and their interaction.

Treatment (T)	Feeding rate	Feed type	Crude protein	Crude lipid	Crude ash
Individual treatment means[†]					
T1 (R1F)	R1 (2%)	Floated (F)	19.76	5.54^{b}	56.33
T2 (R1S)	R1 (2%)	Sinking (S)	18.61	7.22^{a}	51.13
T3 (R2F)	R2 (3%)	Floated (F)	20.00	5.80^{b}	59.12
T4 (R2S)	R2 (3%)	Sinking (S)	15.65	3.30^{c}	60.79
T5 (R3F)	R3 (4%)	Floated (F)	18.90	4.36^{c}	52.14
T6 (R3S)	R3 (4%)	Sinking (S)	18.59	5.65^{b}	52.93
Pooled SE			0.75	0.29	3.67
Means of the main effect[‡]					
R1 (2%)			18.58	6.45^{a}	51.31^{b}
R2 (3%)			18.08	5.01^{b}	60.38^{a}
R3 (4%)			18.74	4.84^{b}	52.53^{b}
Floated (F)			19.72^{a}	5.43	56.14^{a}
Sinking (S)			17.22^{b}	5.44	53.34^{b}
ANOVA (P-value)					
Feeding rate			0.7343	0.0065	< 0.0001
Feed type			0.0115	0.9640	0.0071
Feeding rate × feed type			0.0959	0.0008	0.0010

[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡] Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.

detected in the FCR, PER, and APU of fish-fed floating or sinking feed. The best FCR, PER and APU were detected in fish-fed floating feed at a rate of 2% of total biomass.

3.5. Biometric parameters

The effect of feeding rates, types of feed and their interaction had no significantly ($P > 0.05$) effect on the biometric parameters; relative intestine length (RIL) and spleen index (SI) (Table 5). The highest RIL was observed in fish-fed either floating feed at the rate of 2% of total biomass. Also, feeding rates or types had no significant ($P > 0.05$) effect on the hepatosomatic index (HIS), but their interaction did ($P < 0.05$). The highest HIS was detected in fish-fed floating diet at a rate of 4% of total biomass. In addition, fish-fed sinking feed with the 4% of biomass feeding rate presented the highest SI.

3.6. Digestive endogenous enzymes

The tilapia fish's endogenous enzymes, lipase and amylase varied significantly ($P < 0.05$) depending on feeding rates, types, and their interactions (Table 6). The highest lipase and amylase levels were found in the fish-fed feeding rate at 4% of total biomass, irrespective to feed type. Regardless of the feeding rates, fish-fed floating feed had the greatest levels of lipase, while fish-fed sinking feed had the highest levels of amylase. Fish-fed sinking feed at a 4% feeding rate had the highest significant ($P < 0.05$) level of amylase. While lipase levels were greater ($P < 0.05$) in the group fed floating feed at a feeding rate of 3% of total biomass.

3.7. Hematological and biochemical indices

Table 7 displays the effect of feeding rates, feed types, and their interactions on the hematological parameters of Nile tilapia reared in a biofloc system. Feeding rates, feed types, and their interactions had no appreciable effect ($P > 0.05$) on hemoglobin (Hb), hematocrit (Hct), and red blood cells (RBCs). Regardless of feed types, the fish group that received 4% of total biomass had the highest ($P < 0.05$) values of hematocrit (Hct), and white blood cells (WBC). The hematological parameters of fish-fed either floating or sinking feed were not significantly ($P > 0.05$) affected. With regard to Hb, Htc, RBCs, and WBCs, group fed floating feed at 4% of total biomass had the greatest values.

The results of biochemical parameters are shown in Table 8. Feeding rates, feed types, and their interactions had no significant impact ($P > 0.05$) on serum ALT, AST, or albumin, but feeding rates or feed types have an effect ($P < 0.05$) on total protein and globulin ($P < 0.05$). With an increase in feeding rates from 2% to 4%, ALT and AST were marginally elevated, regardless of the feed types. Also, total protein, albumin, and globulin levels in floating feed were high, whereas ALT

Table 5
Growth performance, feed utilization and biometric indices of tilapia fed different feeding rate or feed type and their interaction under biofloc system.

Treatment (T)	Feeding rate	Feed type	Growth performance				Feed utilization				Morphometric index		
			IBW[1] (g fish^{-1})	FBW[2] (g fish^{-1})	WG[3] (g fish^{-1})	SGR[4] (% day^{-1})	FI g fish^{-1}	FCR	PER	APU%	RIL%	HSI%	SI%
Individual treatment means[†]													
T1 (R1F)	R1 (2%)	Floated (F)	4.76	13.00^{d}	8.25^{d}	1.25^{c}	7.10^{c}	0.86^{e}	3.88^{a}	96.92^{a}	3.40^{a}	1.75^{ab}	0.51^{b}
T2 (R1S)	R1 (2%)	Sinking (S)	4.81	11.94^{d}	7.13^{d}	0.70^{d}	6.36^{c}	0.90^{d}	3.73^{b}	93.08^{b}	3.21^{a}	2.02^{ab}	0.41^{c}
T3 (R2F)	R2 (3%)	Floated (F)	4.86	15.60^{c}	10.75^{c}	1.62^{b}	11.09^{b}	1.04^{c}	3.25^{c}	81.16^{c}	2.12^{b}	1.20^{b}	0.43^{c}
T4 (R2S)	R2 (3%)	Sinking (S)	4.78	14.91^{c}	10.13^{c}	1.35^{c}	9.92^{c}	0.99^{c}	3.40^{c}	85.00^{d}	3.02^{a}	2.11^{ab}	0.60^{a}
T5 (R3F)	R3 (4%)	Floated (F)	4.88	19.73^{a}	14.85^{a}	1.97^{a}	17.03^{a}	1.16^{b}	2.91^{d}	72.66^{e}	2.50^{b}	2.54^{a}	0.62^{a}
T6 (R3S)	R3 (4%)	Sinking (S)	4.78	16.96^{b}	12.18^{b}	1.59^{b}	16.09^{a}	1.35^{a}	2.55^{e}	63.75^{f}	1.95^{c}	1.90^{ab}	0.66^{a}
Pooled SE		0.04	0.10	0.13	0.20	0.14	0.04	0.16	3.99	0.82	0.32	0.20	
Means of the main effect[‡]													
R1 (2%)			4.78	12.47^{c}	7.69^{c}	0.97^{b}	6.73^{c}	0.88^{b}	3.80^{a}	94.99^{a}	3.68^{a}	1.77^{c}	0.52^{b}
R2 (3%)			4.81	15.26^{b}	10.44^{b}	1.48^{a}	10.50^{b}	1.02^{ab}	3.33^{ab}	83.08^{ab}	2.47^{ab}	1.97^{b}	0.45^{c}
R3 (4%)			4.83	18.35^{a}	13.52^{a}	1.78^{a}	16.56^{a}	1.26^{a}	2.73^{b}	68.21^{b}	2.22^{b}	2.22^{a}	0.64^{a}
Floated (F)			4.83	16.11^{a}	11.28^{a}	1.61^{a}	11.74^{a}	1.02	3.34	83.58	2.61^{b}	2.04	0.48
Sinking (S)			4.79	14.60^{b}	9.81b	1.21^{b}	10.79^{b}	1.08	3.22	80.61	2.98^{a}	1.93	0.60
ANOVA (P-value)													
Feeding rate			0.9126	0.0003	0.0005	0.0196	< .0001	0.0483	0.0293	0.0294	0.0832	0.5004	0.5853
Feed type			0.6897	0.0155	0.0293	0.0480	0.1114	0.5143	0.6179	0.6164	0.4506	0.7138	0.4463
Feeding rate × Feed type			0.8083	0.0191	0.0285	0.0476	0.9377	0.6046	0.6704	0.6652	0.7953	0.0416	0.8830

Note. IBW: initial body weight; FBW: final body weight; WG: weight gain; SGR: specific growth rate; ADG: average daily gain; FI: feed intake; FCR: feed conversion ratio; HSI: hepatosomatic index; PER: protein efficiency ratio; APU: apparent protein utilization; RIL: relative intestine length; SI: spleen index.
[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡] Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.
WG = final weight (g) – initial weight (g). Specific growth rate (SGR)= LnW2 – LnW1/t, where, Ln = the natural log; W1 = initial fish weight, W2 = the final fish weight in grams and t = period in days. Feed conversion ratio (FCR) was calculated according to by the equation: FCR = Feed intake (g)/weight gain (g). Protein efficiency ratio (PER) = Weight gain (g)/protein ingested (g). Apparent protein utilization (APU %) = 100 [protein gain in fish (g)/protein intake in diet (g)]. Relative intestine length (RIL), hepatosomatic index (HSI) and spleen index (SI) were calculated using the following equations: RIL = intestine length (cm)/whole-body weight (g); HSI (%) = 100 × (liver weight [g]/whole-body weight [g]) and SI (%) = 100 × (spleen weight [g]/ whole-body weight [g]).

and AST levels in sinking feed slightly rose. The interaction between feeding rates and feed types had a statistically significant ($P < 0.05$) effect on globulin and growth hormone (GH) (Table 8). Moreover, fish-fed either sinking feed at a feeding rate 2% or floated feed at a feeding rate 3% of total biomass had the highest ($P < 0.05$) GH levels.

3.8. Lipid profile

Table (9) displays the findings of the lipid profile which includes cholesterol, triglycerides, HDL-C, and LDL-C. Cholesterol, triglycerides, and low-density lipoprotein (LDL-C) were not substantially affected ($P > 0.05$) by feeding rates, feed types, and their interactions. On the other hand, feeding rates and the interaction between feeding rates and feed types had a considerable effect ($P < 0.05$) on high-density lipoprotein (HDL-C). The highest significant ($P < 0.05$) value of HDL-C was recorded in the fish group fed a feeding rate of 4% of total biomass, irrespective of the feed types. The fish group fed floating or sinking feed at a feeding rate of 2%, floating feed at 3%, and sinking feed at 4% recorded the highest HDL-C values with an insignificant difference.

3.9. Antioxidant enzyme

Fish liver's response to antioxidant enzymes is displayed in Table 10. There was a significant effect ($P < 0.05$) on SOD and GSH in response to feeding rates, feed types, and their interaction. The fish group that received a feeding rate of 3% of total biomass had the highest ($P < 0.05$) SOD and CAT values, regardless of feed types. While the lowest GSH and MDA values were found in the fish group fed a feeding rate of 4%. Without respect to feeding rates, fish that received floating feed had the greatest levels of SOD, CAT, and GSH and the lowest levels of MDA. The highest CAT and GSH levels were shown in fish that received either sinking or floating feed at a feeding rate of 2% and 3%, respectively. Whereas, the lowest MDA was found in fish-fed sinking feed at a feeding rate 3% of total biomass.

4. Discussion

4.1. Water quality and plankton community in the biofloc system

Fish require good water quality to survive, but the high concentrations of contaminants, such as nitrogen and phosphorus, that may develop in cultured water are one of the major issues preventing the aquaculture industry from developing healthily. Floating feed performed better than sinking feed with the same composition due to more nutrients being leached from the sinking feed than the floating (Yaqoob et al., 2010). As well, various research had demonstrated that BFT is capable of efficiently converting N, P, and other contaminants in cultured water and improving water quality to lessen environmental consequences (Avnimelech, 1999). In the present trial, there was a reduction in NH_4 and NO_2 and an accumulation of NO_3 which usually

Table 6
Endogenous enzyme activity of tilapia fed different feeding rate or feeding type and their interaction under biofloc system.

Treatment (T)	Feeding rate	Feed type	Lipase (U/g tissue)	Amylase (U/g tissue)
Individual treatment means[†]				
T1 (R1F)	R1 (2%)	Floated (F)	10.50^{c}	6822^{d}
T2 (R1S)	R1 (2%)	Sinking (S)	13.67^{ab}	13507^{bc}
T3 (R2F)	R2 (3%)	Floated (F)	15.00^{a}	14585^{b}
T4 (R2S)	R2 (3%)	Sinking (S)	11.50^{bc}	11869.5^{c}
T5 (R3F)	R3 (4%)	Floated (F)	14.50^{ab}	12595^{bc}
T6 (R3S)	R3 (4%)	Sinking (S)	12.50^{abc}	19808.5^{a}
Pooled SE			0.35	170.74
Means of the main effect[‡]				
	R1 (2%)		11.50^{b}	9791.3^{c}
	R2 (3%)		13.50^{a}	13331^{b}
	R3 (4%)		13.50^{a}	16201.8^{a}
		Floated (F)	13.50^{a}	11403.2^{b}
		Sinking (S)	12.17^{b}	14812.8^{a}
ANOVA (P-value)				
Feeding rate			0.0106	< 0.0001
Feed type			0.0171	< 0.0001
Feeding rate × feed type			0.0027	< 0.0001

[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡] Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.

occurs in a biofloc system (zero water exchange) within an acceptable range for Nile tilapia (Delong et al., 2009). This reduction may be the result of the addition of a carbon source (starch), which promoted the growth of heterotrophic bacteria that increase nitrogen fixation, reduce ammonia, and oxidize it to NO_2 by nitrifying bacteria, ultimately improving water quality (Luo et al., 2013, 2014; El-Husseiny et al., 2018). These findings coincided with the previous studies that applied biofloc system for cultured carp and shrimp (Zhao et al., 2012; Emerenciano et al., 2012; Wang et al., 2015; Khanjani et al., 2017). Also, increasing the feeding rates herein from 2% to 4% gradually raised TSS (from 370 to 792 mgL^{-1}, respectively) and alkalinity (from 320 to 350 mgL^{-1}, respectively) ($P > 0.05$) regardless of the feed types, but they decreased ($P > 0.05$) in response to the feed types (sinking or floating). Fish that were fed floating feed at a rate of 4% had the greatest TSS, while those that were fed sinking feed at a rate of 2% had the lowest. Furthermore, no published data indicate the impact of feeding types, feeding rates, or a combination of both on the water quality of fish reared in a biofloc system.

Concerning phytoplankton communities in the biofloc system, twenty-two species, including members of the classes Cyanobacteria, Chlorophyceae, and Bacillariophyta, were identified as belonging to the phytoplankton community based on the present data. In line with the existing facts, Schrader et al. (2011) identified the phytoplankton communities in a biofloc technology system used for rearing channel catfish (*Ictalurus punctatus*) that contained the same genera mentioned above, while diatoms comprised co-dominant phytoplankton communities. Whereas the most prevalent classes of phytoplankton in the present data were Chlorophyceae followed by Cyanobacteria in a biofloc system for tilapia rearing. These results are consistent with those obtained in studies by Torrans (2005) and Green (2010), which found a greater chlorophyll concentration for channel catfish production in biofloc culture. The fast growth of phytoplankton herein, particularly unicellular and small colonial Chlorophyceae, is thought to act as a water oxygenator by boosting and maintaining the concentration of dissolved oxygen in aquaculture systems (Schrader et al., 2011). In contrast to the present results, in a brackish water (Ray et al., 2010) and a marine water (Vinatea et al., 2010) biofloc system, cyanobacteria and chlorophytes co-dominated phytoplankton populations. Fish-fed floating feed at a feeding rate of 2% of total biomass recorded the greatest number of phytoplankton communities.

The second most prevalent genus in the biofloc systems was cyanobacteria, which could be attributed to the high nitrogen, temperature (the water temperature was 28 ºC), and light intensity that switched the system to the dominance of cyanobacteria (Martins et al., 2016; Jankowiak et al., 2019). It is generally known that a mean of 62% TN and 70% TP of tilapia supplementary feed is released into the environment, where it is either digested by phytoplankton, denitrified by bacteria, or even sedimented in the fish pond (Wang et al., 2012; Osti et al., 2018).

Bacillariophyta (Diatoms) are marginally present when chlorophytes

Table 7
Hematological blood parameters of tilapia fed different feeding rate or feed type and their interaction under biofloc system.

Treatment (T)	Feeding rate	Feed type	Hemoglobin (g/dl)	hematocrit%	[a]RBCs ($\times 10^6$ cmm^{-1})	[b]WBC ($\times 10^3$ cmm^{-1})
T1 (R1F)	R1 (2%)	Floated (F)	7.85	24.80	2.78	87.95
T2 (R1S)	R1 (2%)	Sinking (S)	7.40	23.95	2.63	78.25
T3 (R2F)	R2 (3%)	Floated (F)	7.50	23.70	2.65	86.40
T4 (R2S)	R2 (3%)	Sinking (S)	7.50	23.25	2.67	91.85
T5 (R3F)	R3 (4%)	Floated (F)	8.35	26.80	2.95	120.25
T6 (R3S)	R3 (4%)	Sinking (S)	7.50	26.50	2.85	99.50
Pooled SE			0.33	0.69	0.15	3.46
Means of the main effect[‡]						
	R1 (2%)		7.63	24.38^{ab}	2.71	83.10^{b}
	R2 (3%)		7.50	23.48^{b}	2.66	89.13^{b}
	R3 (4%)		7.93	26.65^{a}	2.90	109.88^{a}
		Floated (F)	7.90	25.10	2.79	98.20
		Sinking (S)	7.47	24.57	2.71	89.87
ANOVA (P-value)						
Feeding rate			0.6748	0.0517	0.5117	0.0063
Feed type			0.3131	0.5311	0.6630	0.0915
Feeding rate × feed type			0.6878	0.9584	0.9214	0.1071

[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡] Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.

[a] RBCs: red blood cell

[b] WBC: white blood cell

Table 8
Biochemical blood parameters of tilapia fed different feeding rate or feeding type and their interaction under biofloc system.

Treatment (T)	Feeding type	Feed type	**ALT** (UL-1)	**AST** (UL-1)	**T. protein** (g L^{-1})	**Albumin** (g L^{-1})	**Globulin** (g L^{-1})	**GH**
Individual treatment means[†]								
T1 (R1F)	R1 (2%)	Floated (F)	31.00	61.00	3.350	1.32	2.03[a]	1.22c
T2 (R1S)	R1 (2%)	Sinking (S)	33.50	60.00	2.850	1.45	1.40[b]	2.83a
T3 (R2F)	R2 (3%)	Floated (F)	32.00	62.50	2.85	1.65	1.20[a]	2.66a
T4 (R2S)	R2 (3%)	Sinking (S)	33.50	65.00	2.40	1.20	1.21[a]	1.97b
T5 (R3F)	R3 (4%)	Floated (F)	34.50	62.500	3.10	1.14	1.96[a]	1.94b
T6 (R3S)	R3 (4%)	Sinking (S)	34.00	61.500	2.75	1.24	1.52[a]	1.92b
Pooled SE			0.80	0.07	0.08	0.09	0.03	0.33
Means of the main effect[‡]								
	R1 (2%)		60.50[b]	32.25	3.10[a]	1.39	1.72[a]	1.86
	R2 (3%)		63.75[a]	32.75	2.63[b]	1.42	1.20[b]	2.53
	R3 (4%)		62.00[ab]	34.25	2.93[a]	1.19	1.74[a]	1.93
		Floated (F)	62.00	32.50	3.10[a]	1.37	1.73[a]	2.08
		Sinking (S)	62.17	33.67	2.67[b]	1.29	1.37[b]	2.13
ANOVA (P-value)								
Feeding rate			0.0866	0.3643	0.0162	0.1664	0.0110	0.3641
Feed type			0.8636	0.3284	0.0039	0.4333	0.0150	0.8978
Feeding rate × feed type			0.2901	0.5524	0.7772	0.0782	0.0103	0.0063

[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡] Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.

Table 9
Lipid profile of tilapia fed different feeding rate or feed type and their interaction under biofloc system.

Treatment (T)	Feeding rate	Feed type	Cholesterol (mmol L^{-1})	Triglyceride (mmol L^{-1})	[a]HDL-C (mmol L^{-1})	[b]LDL-C (mmol L^{-1})
Individual treatment means[†]						
T1 (R1F)	R1 (2%)	Floated (F)	131.50	212.50	70.50[a]	26.00
T2 (R1S)	R1 (2%)	Sinking (S)	131.50	190.00	69.00[a]	19.00
T3 (R2F)	R2 (3%)	Floated (F)	132.50	219.00	60.50[a]	26.00
T4 (R2S)	R2 (3%)	Sinking (S)	109.50	260.00	38.00[b]	22.00
T5 (R3F)	R3 (4%)	Floated (F)	127	180.00	73.00[b]	20.50
T6 (R3S)	R3 (4%)	Sinking (S)	134	227.50	77.00[a]	21.50
Pooled SE			4.54	13.57	2.77	2.60
Means of the main effect[‡]						
R1 (2%)			131.50	201.25	69.75[a]	22.50
R2 (3%)			121.00	239.50	49.25[b]	24.00
R3 (4%)			130.00	203.75	75.00[a]	21.00
		Floated (F)	130.33	203.83	68.00	24.17
		Sinking (S)	125.00	225.83	61.33	20.83
ANOVA (P-value)						
Feeding rate			0.2848	0.1781	0.0027	0.7324
Feed type			0.3554	0.2194	0.0912	0.3181
Feeding rate × feed type			0.1398	0.2263	0.0419	0.5831

[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡]Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.

[a] HDL-C, High-density lipoprotein Cholesterol.

[b] LDL-C, Low-density lipoprotein Cholesterol.

and cyanobacteria co-dominate in freshwater (Schrader et al., 2011), brackish water (Ray et al., 2010), and marine (Vinatea et al., 2010) biofloc system. In the present results, diatoms biomass was 0.852 mg/L and 0.006 at tilapia fed either floated or sinking feed at a feeding rate of 2% and 0.002 mg/L at the floated of T3 (R2F) (3%), and completely disappeared in the other treatments at the experimental end. The decrease in diatoms could be related to the decrease in photosynthetic active radiation as a result of the accumulation of suspended solids, which become unfavorable to diatoms but favorable to mixotrophic cyanobacteria.

In the presence of a carbon supply, the more nitrogen waste generated, the more flocs grow, and the greater the possibility that a zooplankton community will form (Mabroke et al., 2021). In this study, regardless of the feed type (sinking or floating), an increase in feeding rates resulted in an increase in the total count of zooplankton. Further, fish-fed sinking feed at 4% feeding rate had the highest ($P < 0.05$) zooplankton counts. This result could be explained by the fact that active zooplankton growth at a high feeding rate (4%) exceeds the capacity of tilapia to consume. In the present trial, rotifer species with substantial counts were *Polyarthera vulgaris*, *Anuraeopsis fissa*, *Brachionus calciflours*, and *Brachionus quadridentata*, with *Philodena* sp. being the most prevalent species. Also, protozoa were represented by three species: *Trichocerca* sp., *Vorticella campanula*, and *Lecane closterocerca*. *Trichocerca* sp. was the most prevalent protozoan species in fish-fed floating feed at a 3% feeding rate, but it was found in lesser densities in all other treatments. The same group of organisms; Protozoa and Rotifera were detected using the biofloc system, according to Azim and Little (2008) and Mabroke et al. (2021). In the present study, T4 (R2S) had the lowest count of ciliate protozoa: *Vorticella campanula, Lecane closterocerca, and Trichocerca sp.* that are known to be elevated under the condition of activated sludge and in return consume free bacteria, and decrease turbidity (Curds, 1973; Madoni, 2011). According to Arndt (1993),

Table 10
Antioxidant enzymes activity of tilapia fed different feeding rate or feeding type and their interaction under biofloc system.

Treatment (T)	Feeding rate	Feed type	[a]SOD	[b]CAT	[c]GSH	[d]MDA
Individual treatment means[†]						
T1 (R1F)	R1 (2%)	Floated (F)	353.65[a]	104.52[e]	55.58[b]	95.84[ab]
T2 (R1S)	R1 (2%)	Sinking (S)	193.95[c]	837.79[a]	59.05[a]	126.43[a]
T3 (R2F)	R2 (3%)	Floated (F)	242.51[bc]	839.39[a]	58.88[a]	88.08[abc]
T4 (R2S)	R2 (3%)	Sinking (S)	388.36[a]	420.50[c]	42.54[c]	31.75[c]
T5 (R3F)	R3 (4%)	Floated (F)	280.47[b]	544.72[b]	41.18[d]	51.04[bc]
T6 (R3S)	R3 (4%)	Sinking (S)	112.64[d]	278.39[d]	35.41[e]	61.29[bc]
Pooled SE			18.27	8.03	0.26	14.14
Means of the main effect[‡]						
	R1 (2%)		261.54[b]	470.60[b]	57.07[a]	120.49[a]
	R2 (3%)		315.56[a]	630.10[a]	50.99[b]	60.14[b]
	R3 (4%)		196.56[c]	411.56[c]	38.29[c]	56.16[c]
		Floated (F)	292.29[a]	496.31	52.06[a]	78.47
		Sinking (S)	223.48[b]	511.86	45.51[b]	79.40
ANOVA (P-value)						
Feeding rate			< .0001	< .0001	< .0001	< .0001
Feed type			< .0001	0.0634	< .0001	0.0815
Feeding rate × feed type			0.0008	< .0001	< .0001	0.0255

[†]Treatments' means represent the average values of three aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$). Means followed by the same letter are not significantly different. [‡]Main effect means followed by the same letter are not significantly different at $P < 0.05$ by Duncan multiple range test.
[a]SOD, Superoxide dismutase; [b]CAT, Catalase; [c]GSH, Glutathione; [d]MDA, Malondialdehyde.

protozoa are also regarded as being a part of the feed web for the rotifer species, which argues for the low rotifer abundance herein in tilapia fed sinking feed at a 3% feeding rate. For Nile tilapia raised in a biofloc system, the combined effects of feeding rates and feed types on the plankton communities (zooplankton or phytoplankton) have not been researched, as far as the author is aware.

From the results obtained herein, as phytoplankton groups were formed, they were later consumed by zooplankton through which the synthesis of organic carbon was cascaded up to tilapia fish. Our study showed that inorganic nitrogen (NO_3 and NH_4) from fish excretions and feed disintegration in the biofloc system was recycled into bacterial and phytoplankton biomass to form biofloc which was grazed by zooplankton and consequently utilized by tilapia fish as an additional feed. Therefore, further in-depth research is required to comprehend the relationship between feeding rates, feed types, and the development of zooplankton and phytoplankton communities.

4.2. Proximate composition of biofloc

In the current experiment, the crude protein, crude lipid and ash content of biofloc composition bioflocs on the dry basis were 15.65–20%, 3.30–7.22% and 51.13–60.79%, respectively. According to da Silva et al. (2020) and Martins et al. (2017), feeding rates considerably altered the chemical composition of biofloc, with higher ash content of biofloc being recorded to biofloc collected from tanks treated with lower feeding rates. Additionally, the current study confirmed findings from earlier studies (Avnimelech, 2009; Crab, 2010; Emerenciano et al., 2012; López-Elías et al., 2015; Martins et al., 2019; Martins et al., 2017) by demonstrating that biofloc contains a higher ash content, a lower lipid content, and protein in all treatments. This decline in biofloc's chemical composition herein may be significantly influenced by a number of elements, including the aquatic tilapia species that have the ability to graze natural food from the biofloc system, also settling of the biofloc reduced the nutrient content of biofloc, the rearing period, feed composition, feeding rates, the nutritional profile of the feeds, the microbiota in the water source, the carbon source, and the biofloc size (Ray et al., 2010; El-Sayed, 2021). In contrast to our findings, El-kady et al. (2016) showed that biofloc had a protein content of 30.63% and a lipid content that ranged from 3.65% to 4.27% when fed to Nile tilapia at a 3% feeding rate and a 25% protein diet (*Oreochromis niloticus*). The composition of heterotrophic bacteria and other organisms in the study of El-kady et al. (2016) linked to biofilms and bioflocs may be responsible for greater protein and lipid concentration in the bioflocs of the high feeding level treatments (Fernández et al., 2008). Another factor that might be taken into account is the high concentration of zooplankton creatures (rich in protein), which may have grown due to rising levels of feeding. These species consume both bacteria and algae.

4.3. Growth indices

The proper feeding rates and the feed types (floating or sinking) utilized to minimize overfeeding, which is expensive and detrimental the water quality, are crucial factors for fish growth and nutrient efficiency in successful aquaculture (Huang et al., 2015; Dong et al., 2017; Khandan Barani et al., 2019; Mihelakakis et al., 2002). In the present data, the highest growth performance and feed utilization were observed in fish-fed floating feed with the highest feeding rate (4% of biomass). Also, variable feeding rates or feeding types significantly improved the growth performance and feed efficiency of fish (Table 4). This improvement indicated that the highest of feeding fish at a rate of 4% of biomass leads to the greatest utilization of ingested nutrients for growth (Khan et al., 2004; Luo et al., 2015). The results of the current study are consistent with earlier studies on *Labeo rohita* and *Heteropneustes fossilis* by Ahmed (2007) and Khan and Abidi (2010) as well as those on pigfish by Oberg et al. (2014), which found that ration levels frequently affect the growth performances, feed intake, and conversion efficiencies of cultured fish. Contrasting with the present results, Khandan Barani et al. (2019) revealed that juvenile snow trout, *Schizothorax zarudnyi* fed at a rate of 4% of body weight had poor growth and FCR, implying that these diets were mainly for maintenance purposes and the majority of the ingested nutrients are utilized to preserve life and only a tiny amount remaining for growth. Moreover, due to the different experimental conditions, it was challenging to compare the findings of this study with those of studies done on other fish. Additionally, using floating feed improved the growth performance of young olive flounder and African catfish (Kim and Shin, 2006; David et al., 2017), which was consistent with the data presented herein. Thus, the presence of pelleted floating feed above the water's surface, where fish can benefit from it, may be responsible for the improved growth indices of fish-fed floating diet. Hence, the low water stability of sinking feeds is causing excessive nutrient leaching of the food before fish consume it, which lowers feed conversion, impairs water quality, and raises production costs, consequently diminishing economic efficiency (Abdelhamid et al., 2019). In light of this, the biofloc system, which was applied

in the current study, might be effective in providing the ideal environmental conditions for promoting the growth of microorganisms as well as boosting the performance of tilapia growth and reducing the risk of eutrophication in water which are all related to viability. Additionally, the biofloc system eliminates the need for organic and/or inorganic fertilizers, which keeps costs down (Avnimelech and Kochba, 2009). The largest production expenses in aquaculture are almost typically associated with fish feed. Even if the biomass in the biofloc system increases more quickly, any increase in feed costs is immediately offset by the increased profit margins, making it still more efficient than traditional earthen pond systems (Sontakke and Haridas, 2018). Furthermore, BFT only includes the price of the carbon source, which is far less expensive, and eliminates the price of both organic and inorganic fertilizers. According to studies, BFT has a shorter culture period and more growth and survival than other methods, making it more economical (Avnimelech and Kochba, 2009). Studies by (Sontakke and Haridas, 2018) have shown that growing milkfish fingerlings in nurseries using BFT increased economic returns and guaranteed a year-round supply of fingerlings for grow-out culture operations.

Hence, the positive interaction between feeding rates and feeding types (floating or sinking) under the biofloc system on tilapia growth could be attributed to different scenarios as i) the significance of floating feed in improving digestion and feed absorption, which improves performance and nutrient utilization under the biofloc system (Avnimelech, 2007; Abdelhamid et al., 2019), ii) The presence of bacteria, macronutrients, and micronutrients in microbial biofloc is a significant source of digestive enzymes, synthesizes necessary nutrients, carotenoids, amino sugars, and vitamins, and modifies the immune system of fish. This has a probiotic effect that aids fish in better digesting and absorbing the minerals in their diet (De Schryver et al., 2008; Avnimelech, 2009; Banerjee and Ray, 2016; Mabroke et al., 2019). According to the present data, Oliveira et al. (2021) displayed better profitability and growth when Nile tilapia juveniles were reared in the BFT system and fed between 4.3% and 6.1% BW Day^{-1}. Similarly, P é rez-Fuentes (2018) found that tilapia under the biofloc system had good performance and health despite having a 20% reduction in feeding rate. The same was also seen when shrimp (*Penaeus monodon*) were fed at a 25% lower feeding rate under the biofloc system without any negative effects on their performance. Thus, the biofloc system can compensate for shrimp development through its natural food content (Panjaitan, 2010). Additionally, natural foods in the biofloc system had a favorable effect on shrimp's FCR (Xu and Pan, 2012). The results of the current study, which showed that FCR rose with feeding rates, could be explained by a decrease in floc use as a food source due to increased food availability (Zheng et al., 2008). As far as the author is aware, no study has looked into how the combination of feeding rates and feeding types (floating or sinking) affects tilapia performance in a biofloc system.

Fish's body index reveals their nutritional state, physical condition, and health condition (Mizanur et al., 2014; da Silva et al., 2020). Tilapia fed at a greater feeding rate with floated feed in the current study had the greatest value of hepatosomatic index (HSI). Our results are in line with those of da Silva et al. (2020), who found a rise in the HSI of tilapia fed at a greater feeding rate. The feeding rates have a considerable impact on HSI and are linked to liver lipid accumulation, biochemical alterations, and histological abnormalities (Huang et al., 2015). As a result, fish-fed at a slower rate mobilize their body's reserves of amino acids, lipids, and glucose more readily (Shimeno et al., 1997). By lowering the amount of feed provided, fish could utilize their body's lipid reserves as a source of energy (Dong et al., 2017).

4.4. Digestive enzymes

One of the crucial indicators for assessing the nutritional value and accessibility of aquatic animals' feed is digestive enzyme activity, which represents the body's capability for digestion and metabolism (Anand et al., 2014; Adeoye et al., 2016). In the present results, lipase and amylase activities were significantly ($P \leq 0.05$) improved by increasing feeding rates. In turn, this led to improved nutrient absorption, feed conversion rates, and growth performance. Similar to fish, shrimp (*Penaeus monodon*) also showed improved development performance when endogenous enzyme secretion was enhanced (Lara-Flores et al., 2003; Anand et al., 2013). These results herein could be attributed to the presence of microbial biofloc that contains a variety of extracellular digestive enzymes, including lipase and amylase, which may work in conjunction with endogenous digestive enzymes to break down lipids and carbohydrates in the animal's intestinal tract and may aid in the digestion and absorption of the feed which may in turn boost tilapia's feed utilization and growth performance (Xu and Pan, 2012; Long et al., 2015; Liu et al., 2018; Adineh et al., 2019). Consistently with our data, previous studies demonstrated that biofloc increased the activity of amylase in tilapia (Xu and Pan, 2012), intestinal amylase, lipase and protease in carp, in milkfish (Yu et al., 2020; Sontakke et al., 2021) and protease and amylase activity in the intestines of catfish (Zafar et al., 2021). Additionally, shrimp fed at higher feeding rates exhibited a significantly higher level of trypsin and chymotrypsin than shrimp fed at lower feeding rates (Luna-González et al., 2017). As far as we are aware, this is the first study that has investigated the combined effect of feeding rates and feeding types under the biofloc system on tilapia digestive enzymes.

4.5. Hematology blood parameters

The current results showed that none of the blood parameters of tilapia reared in a biofloc system, including Hb, Hct, RBCs, and WBCs changed significantly ($P > 0.05$) in response to the combined effect of different feeding rates and feeding types (sinking or floating), indicating healthy Nile tilapia and also the biofloc system had no detrimental effects on the tilapia's physical conditions. While, different feeding rates for tilapia reared in the biofloc system all significantly ($P \leq 0.05$) affected hematocrit, and WBCs regardless of feeding types. This increase in WBCs may be caused by the abundant natural microbes and bioactive substances (such as carotenoids, chlorophylls, phytosterols, etc.) in the suspended biofloc. These substances may have a positive impact on the physiological health of fish because their nonspecific immune responses effectively safeguard them from diseases and stressful conditions. Contrary to our results, Didlyn et al. (2015) found that GIFT strain tilapia juveniles weren't significantly affected by the feeding rates. There is no published research on the combined impact of various feeding rates and feeding types on hematological parameters in fish species maintained in a biofloc system. Thus, more research is needed to understand the combined impact of feeding rates and feeding types under the biofloc system on fish hematology.

4.6. Serum biochemical parameters

In the current data, raising the feeding ratios led to a small increase in the ALT and ALP enzymes activity regardless of the types of feed. Similar to this trend, the higher feeding rates of GIFT fry was accompanied by considerably elevated plasma ALT and AST activity that indicated to reflect liver tissue injury (Huang et al., 2015). While the liver enzymes activity; ALT and AST decreased when fish were fed diet at a low feeding rate regardless to feed types. It's possible that the presence of bioactive substances herein in the microbial biofloc herein was prevented Nile tilapia from being harmed. For that reason, it is believed that fish with higher serum protein content have a stronger innate response (Jha et al., 2007). Globulin level is frequently utilized as a measure of immunological responses and as a source of antibody formation (Bernet et al., 2001; Sahu et al., 2007). In the present results, greater levels of serum total protein and globulin were found in tilapia fed a low feeding rate (2%) or floating feed. This finding might be a clue to the tilapia's robust innate immunity because bioactive substances are present in the biofloc system.

Growth hormone (GH), which is primarily produced by the pituitary, is a crucial regulator of growth in addition to being involved in the control of osmotic pressure, nutrition, reproduction, physical activity, neuroprotection, and immunity (Vélez and Unniappan, 2021). Growth hormone herein was strongly influenced by the interaction of feeding types and feeding rates ($P < 0.05$), but not by either factor in isolation ($P > 0.05$). In order to demonstrate the beneficial effect of the interaction of feeding rates and feed types in the biofloc system on serum biochemical parameters and serum growth hormone, studies on tilapia are still lacking. Therefore, more research is required in this area.

4.7. Lipid profile

Triglycerides and cholesterol are significant markers of lipid metabolism in fish (Chen et al., 2014; Xu et al., 2017). The body's lipid composition may have increased, which may also imply slower rates of lipid catabolism, leading to higher levels of circulating triglycerides (McCue, 2010). In response to varied feeding rates, feeding types, and their interactions, cholesterol, triglycerides, and LDL-C did not differ significantly ($P > 0.05$) in the current results. Furthermore, the higher levels of CHO and TG are observed in tilapia fed higher feeding rates (3% and 4%) and sinking feed in combination which could be caused poorly lipids metabolism in fish leading to hyperlipidemia and serious liver lesions in the tilapia (Kritchevsky, 1995; Huang et al., 2015). In agreement with our findings, Huang et al. (2015) detected that tilapia fed at a greater feeding rate had higher levels of CHO and TG. To fully understand the impact of the combined effect of feeding rates and feeding types under the biofloc system on fish plasma lipid profiles, more research is required.

4.8. Antioxidant enzymes

Reactive oxidative stress (ROS), which the body produces as a result of metabolism, negatively impacted the antioxidant defense mechanisms (Lewis-McCrea and Lall, 2007). Furthermore, the presence of ROS led to lipid peroxidation, an oxidative chain reaction, and cell oxidative damage (Xu and Pan, 2013). Endogenous antioxidant enzymes, such as SOD, CAT, GSH, and MDA, have been demonstrated to be the first line of defense against oxygen toxicity and as diverse strategies intended to alleviate oxidative stress (Halliwell and Gutteridge, 1990; Lewis-McCrea and Lall, 2007; Sharawy et al., 2017; Li et al., 2019b). SOD is a natural superoxide radical scavenger with roles in protecting active cells from injury, delaying the aging process, and boosting immunity (Campa-Córdova et al., 2002). The cellular redox status is kept in balance by GSH, which is the most prevalent intracellular non-protein thiol. Free radicals are eliminated by CAT, which uses H_2O_2 as a substrate and breaks it down into H_2O and O_2 (Yin et al., 2018). Lipid peroxidation, which is an imbalance in the body's antioxidant system in animals, produces MDA as its byproduct. MDA has harmful effects on cells and can damage an organism (Storey, 1996). The current study found that fish-fed at lower feeding rates with either sinking or floating feed had increased CAT, SOD, and GSH activity. This may be explained by tilapia consuming microorganisms from microbial flocs in the biofloc system (De Schryver et al., 2008; Avnimelech, 2009). Whereas, the lowest MDA was found in fish-fed sinking feed at a feeding rate of 3% of total biomass. This finding suggests that the biofloc system may lower the lipid peroxidation level of tilapia and increase the fish's resistance to oxygen free radicals, which would improve the fish's health, resilience to environmental stress, and survival rate. Additionally, immunostimulant compounds such as peptidoglycan, beta-glucan, lipopolysaccharide in the wall of bacteria, and natural antioxidants in the biofloc, including polyunsaturated fatty acids, carotenoids, chlorophyll, phytosterol, polyphenols, polysaccharides, taurine, vitamins C and E, and certain minerals (Se and Zn) improved the effectiveness of the antioxidant defense system and could increase the immune status of tilapia fingerlings (Ju et al., 2008; Xu and Pan, 2013; Ekasari et al., 2014; Banerjee and Ray, 2016; Liu et al., 2017; Walker et al., 2020). However, information on the combined impact of feeding rates and feeding types (sinking or floating) under the biofloc system of tilapia antioxidant enzymes is not yet available.

5. Conclusion

The current study showed that floating feed at a rate of 4% of biomass improved digestive enzymes, hepatic antioxidant response, and serum biochemical response in addition to growth efficiency of Nile tilapia reared in biofloc system. Consequently, applying floating feed at a rate of 4% of biomass could ensure maximum feed consumption with minimal waste, lessen water pollution, improve nutritional efficiency, and improve feed conversion efficiency of Nile tilapia reared in a biofloc system.

CRediT authorship contribution statement

Eman Y. Mohammady: Experiment design, Collecting data, Statistical analyses, Drafting the paper, **Mohamed R. Soaudy:** Collecting data, Drafting the paper, **Marwa, M. Ali:** Collecting data, Drafting the paper, **Mohamed A. El-ashry:** Collecting data, Drafting the paper, **Mohamed S. Abd El-Karim:** Collecting data, Drafting the paper, **Sylwia Jarmołowicz:** Drafting and edition the paper, **Mohamed S. Hassaan:** Drafting and edition the paper.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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