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ORIGINAL ARTICLE

Biofloc system improves protein utilization efficiency and growth performance of Nile tilapia, *Oreochromis niloticus* fry: Experimental evidence

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Abstract

This study evaluated the effect of biofloc technology (BFT) on protein utilization and growth performance of *Oreochromis niloticus* fry under green house for 14 weeks under a 3 × 2 factorial design involving three crude protein (CP) levels (22, 27 and 35%) and two different carbon sources. Molasses and glucose were independently used as carbon sources in the BFT tanks with aeration using air stones. Mono-sex fish fry of mean weight 0.07 ± 0.01 g and total length 13.1 ± 0.01 mm were stocked at density of 1 fish per litre. The fishes were fed on the three commercial diets that were randomly assigned in triplicates, with the control treatment being 35% CP. Feeding was done twice daily at 5% body weight, while sludge was siphoned weekly. Calculations of specific growth rate (SGR), protein efficiency ratio (PER), food conversion ratio (FCR), survival and measurement of water quality parameters were also performed. Protein levels and carbon sources had significant effects ($p < 0.05$) on dissolved oxygen (DO) and NH_3 protein levels and carbon sources had significant interaction ($p < 0.05$) on pH. There was a significantly higher FCR in the control treatment (0.89) than in glucose (0.56–0.57) and molasses (0.59–0.63) bioflocs; furthermore, it was significantly different between the carbon sources. The PER was significantly higher in the control (8.42) than in glucose (5.03–7.99) and molasses (4.81–7.23) bioflocs. No significant interactions ($p > 0.05$) of protein levels and carbon sources were recorded on PER. However, it was significantly affected ($p < 0.05$) by protein levels and carbon sources. No significant effects ($p > 0.05$) of dietary protein level, carbon source, or their interaction were observed on SGR and condition factor. The SGR was significantly lower in the control (2.91) than glucose (3.52–3.59) and molasses (3.49-3.56) bioflocs. The condition factor was significantly lower in the control (0.81) than glucose (1.72–1.83) and molasses (1.82–1.84) bioflocs. Survival rates were significantly higher in glucose (>97%) and molasses (>94%) than the control with a lower value of 74.7%. The biofloc increased protein utilization efficiency, which improved FCR and enhanced fish growth rate even

with a lower dietary protein level. Further studies should evaluate the applicability of BFT in outdoor cultures.

KEYWORDS
Biofloc Technology, body weight, protein levels

1 | INTRODUCTION

Aquaculture production is increasing annually and contributing toward food security. However, the cost of quality fish feed is a major obstacle to aquaculture growth and development especially in developing countries. Fish feeds account for more than 60% of total aquaculture production cost (Ogello et al., 2017). The most expensive component of fish diet is crude protein (CP), which accounts for approximately 60% of total feed cost and plays a major role in the growth performance of aquatic organisms (Yassir et al., 2010; Hamidoghli et al., 2018). CP requirement for best growth performance in tilapia species depends on the quality of the protein source, energy content of diets, physiological state of fish, fish size, production status and environmental conditions (Yassir et al., 2010). Understanding these requirements will help to maximize the utilization of feeds, reduce production costs and decrease the nutrient load in effluents discharged to the environment (Mohsen et al., 2010). Only up to a maximum of 30% of nitrogen and phosphorus can be recovered from formulated feeds using conventional aquaculture systems (Cavalcante et al., 2017). Avnimelech (2006) reported that only 25% of CP in the feed is converted into harvestable products, while the rest is lost to the system in the form of organic nitrogen, feed remnants and faecal matter.

Apart from this wastage of expensive feed, the waste generated compromises water quality of culture systems and at higher loads these wastes may be detrimental to cultured fish (Crab et al., 2009). While it is possible to exchange water frequently under conventional culture methods, this requires more water to be used, hence, being more expensive for farmers in developing countries. Achieving sustainability in future aquaculture requires production of high-quality products at affordable costs using available natural resources (Avnimelech, 2009; Crab et al., 2012). This approach has led to technological innovations that are friendly to the environment. Recently, for instance, the use of biofloc technology (BFT) has been termed as a rather efficient approach to tackle such issues (Khanjani & Sharifinia, 2020). With bioflocs culture system, there is a reduction in the production of nitrogenous wastes (Emerenciano et al., 2017), a lower use of natural resources (Avnimelech, 2012; García-Ríos et al., 2019) and increased stocking densities can be employed (Lima et al., 2018).

BFT involves altering the carbon nitrogen ratio (C:N) balance of culturing water to be above 15:1 (Avnimelech, 2009) by addition of a suitable carbon source, for instance, starch, glucose, sugar, cellulose, wheat, acetate, or glycerol (De schryver et al., 2008; Avnimelech, 2009). This makes possible that toxic wastes in the aquatic system (ammonia and nitrite) to be assimilated by microorganisms, the majority being heterotrophic bacteria, hence, maintaining the quality of water (Crab, 2010; Avnimelech, 2012). Furthermore, it encourages removal of nitrogen, bacterial growth and formation of microbial proteins (Avnimelech, 1999; Crab, 2010). Nitrogenous wastes fluctuate in a biofloc culture system (Avnimelech, 1999; Emerenciano et al., 2012) and are maintained at species tolerable levels. Using a biofloc system results in the recycling of nitrogen compounds leading to a lower or lack of water exchange, hence, generating a lower environmental impact (Avnimelech, 2012). Lorenzo et al. (2016) observed ammonia and nitrite to be removed from a C:N ratio adjustment culture tanks. Bioflocs are formed when microorganisms aggregate and are utilized in situ by shrimp and fish as additional nutrients (Crab, 2010), which results in a reduction of fish feed cost. Bioflocs constitute organic particles, faces, food debris, chemoautotrophic and heterotrophic bacteria (Khanjani & Sharifinia, 2020), which are nutritious and can successfully be used by some filter feeder aquatic organisms, ultimately resulting in improved growth performances (Hu et al., 2015).

Nile tilapia, *Oreochromis niloticus* is one of the most cultured freshwater species globally and is also an important source of animal protein, especially in developing countries (Ogello et al., 2014), presenting a white firm texture meat which lacks bones in the muscle (Wang & Lu, 2016). *Oreochromis niloticus* is highly preferred for aquaculture (Fitzsimmons, 2005), because it readily accepts formulated diets and breeds in a wide range of environmental conditions (Siddik et al., 2014a), grows fast (El-Sayed, 2006), is well adapted to high stocking densities (Avnimelech, 2007). Tilapia species are suitably adapted to feed on bioflocs, therefore, protein content in formulated diets should be optimized to account for (Green et al., 2019). It is, therefore, cost effective to feed aquatic organisms with a relatively lower protein feed in a biofloc culture system (Xu & Pan, 2013). Biofloc use in aquaculture has been determined to be in a range of 10–20% of feed gain (De Schryver et al., 2008). The use of bioflocs as protein supplement in fish feed has been studied by several researchers (Khatoon et al., 2016; Khanjani et al., 2020a, b). Management of feeds through sustainable technologies, such as biofloc systems, is crucially important to enhance the growth performance of a given culture species (Ogello et al., 2014).

Previous research has demonstrated optimal growth performances using a combination of biofloc systems and conventional feeding (Xu & Pan, 2012). Hence, the main objective of this study was to determine the effect of a biofloc system and the interaction between protein levels and carbon sources in improving protein utilization efficiency and growth performance of *O. niloticus* cultured under experimental greenhouse conditions.

2 | MATERIALS AND METHODS

2.1 | Ethics approval statement

The experiment was performed according to the Kenya Marine and Fisheries Research Institute (**KMFRI**) established in 1979 by the Science and Technology **Act**, Cap 250 of the laws of Kenya, which has since been repealed by the Science, Technology and Innovation **Act** No. 28 of 2013 which has recognized **KMFRI** as a National Research Institution under section 56, fourth schedule. The research was also granted research licenses permit (License No: NACOSTI/P/19/490) by National Commission for Science, Technology and Innovation (NACOSTI) regulations, 2014

2.2 | Experimental site, design and conditions

This research was conducted for 14 weeks at the KMFRI. Twenty-one glass aquariums, each containing 50 L of water, were used for the experiment. The experiment involved two biofloc treatments under 3 × 2 factorial design with three levels of CP (22, 27 and 35% CP), two carbon sources (glucose and molasses) and an additional treatment of 35% CP for the control which was a non-biofloc system with three replicates. The carbon to nitrogen ratio (C/N) was calculated biweekly according to Avnimelech (2009), and maintained at 20:1. The quantity of carbon sources was calculated as follows: Quantity of carbon = (Feed quantity × percentage nitrogen in excretion × percentage nitrogen in feed)/0.05. The C:N ratio was maintained by adding prepared solution of molasses/glucose in a bowel, and applying the solution to each biofloc aquarium daily before feeding the fish (Avnimelech, 1999; Samocha et al., 2007). Male fry of *O. niloticus* (initial mean weight 0.07 ± 0.03 g and mean length 13.00 ± 0.24 mm) were stocked at one fish per litre (Karunaarachchi et al., 2018). The fishes were fed twice a day at 5% body weight (Karunaarachchi et al., 2018) at 0900 h and 1630 h on different commercial fish fry diets containing 22, 27 and 35% protein levels (Manufactured by KMFRI Sangoro, Kenya). The control experiment was fed on 35% CP, which is the standard diet for fry in most *O. niloticus* hatcheries. Nutrient, chemical analysis and feed formulation of the experimental diets are presented in Table 1. The treatments were labelled M-22, M-27 and M-35 for molasses treatments, and G-22, G-27 and G-35 for glucose treatments. Each biofloc aquaria was inoculated with 5 L of green pond water to introduce live food microorganisms (Ogello et al., 2020). The experiment was conducted under a greenhouse, where the water temperature was maintained at 28.1 ± 0.2°C. Supplementary aeration was supplied to all the aquaria via air stones connected to a centralized 10 HP air pump.

Measurements of selected water quality parameters, that is, temperature (°C), dissolved oxygen (DO) mg/L, total dissolved solids (TDS) mg/L, conductivity (μsiemens) and pH were recorded daily using a multi-parameter meter (Procomm11, ITEM: 605404, L/N: 17L100001, YSI). Every 2 weeks, water samples were collected before fish sampling, for nutrient analysis using a mass spectrophotometer (Genesis 10s vis) (Azim & Little, 2008; Liu et al., 2014).

TABLE 1 Nutrient and chemical composition for the different experimental feeds (dry matter)

Nutrients	Percentage constituents		
Crude protein level	**35%**	**27%**	**22%**
Fish meal	22.0	12.0	12.0
Soya bean meal	46.0	36.0	19.0
Maize bran	6.6	12.6	17.6
Wheat pollard	5.0	10.0	14.0
Sunflower meal	3.0	3.0	3.0
Cassava	17.0	26.0	34.0
Mycoban	0.2	0.2	0.2
Vitamin pre-mix	0.1	0.1	0.1
Mineral pre-mix	0.1	0.1	0.1
Total	100.0	100.0	100.0
Chemical analysis (%)			
Moisture	9.41 ± 0.20	9.39 ± 0.17	9.07 ± 0.12
Crude protein	35.2 ± 0.09	27.7 ± 0.12	22.38 ± 0.19
Crude fat	2.50 ± 0.04	2.40 ± 0.03	1.31 ± 0.08
Ash	7.20 ± 0.13	8.41 ± 0.10	7.74 ± 0.07
Fibre	4.62 ± 0.17	5.33 ± 0.22	5.38 ± 0.05

Values are mean ± SD.

2.3 | Samplings of fish, microorganisms and bioflocs for proximate analysis

A sample of 30 fishes per tank was sampled and measured for body weight and total length.

A Leica microscope with an ocular magnification of 10*/20 and objective lens magnification of 4*/0.01 was used to observe water samples from the aquariums in triplicates by the use of lugol solution for microbial live food densities. Total quantity of feed consumed in the aquaria was recorded for the whole experimental period. Biofloc particles were carefully collected from the aquariums using fine net (50 μm), dried to constant weight at 105°C in an oven, then preserved at −20°C for proximate analysis of crude protein, lipids, crude fibre and ash (AOAC, 2019).

2.4 | Fish growth parameters

Fish growth parameters were calculated as follows:

1. Specific growth rate (SGR) %/day = (In *Wt*-In*W0*)/*t* × 100
2. Protein efficiency ratio (PER) = (*Wt*–*W0*)/protein consumed (dry weight)
3. Feed conversion ratio (FCR) = Quantity of feed consumed (g)/(*Wt*–*W0*), where *W0* and *Wt* are the initial and final fish weight (g), respectively and *t* is the culture time in days.
4. Survival rate = (final fish count/initial fish count)*100%

TABLE 2 Water quality parameters for *Oreochromis niloticus* fry in the experimental treatments

Treatments							
Parameters	G-22	G-27	G-35	M-22	M-27	M-35	Control
Temperature	27.9 ± 0.1^{a}	28.1 ± 0.1^{a}	28.1 ± 0.1^{a}	27.9 ± 0.1^{a}	27.9 ± 0.1^{a}	28.1 ± 0.2^{a}	28.2 ± 0.1^{a}
DO	5.10 ± 0.02^{b}	4.99 ± 0.01^{b}	4.96 ± 0.06^{b}	4.81 ± 0.07^{c}	4.80 ± 0.06^{c}	4.74 ± 0.09^{c}	5.22 ± 0.11^{a}
Conductivity	182.9 ± 2.1^{b}	191.5 ± 2.2^{b}	185.5 ± 1.8^{b}	199.3 ± 2.7^{a}	199.7 ± 2.4^{a}	197.6 ± 2.6^{a}	167.2 ± 3.3^{c}
TDS	112.43 ± 1.08^{b}	114.67 ± 1.53^{b}	112.92 ± 2.01^{b}	123.12 ± 1.82^{a}	122.72 ± 2.10^{a}	122.25 ± 2.44^{a}	102.41 ± 3.62^{c}
pH	6.9 ± 0.1^{b}	6.9 ± 0.1^{b}	6.9 ± 0.1^{b}	6.8 ± 0.1^{b}	6.9 ± 0.1^{b}	6.8 ± 0.1^{b}	7.1 ± 0.1^{a}
NH_3	0.21 ± 0.03^{b}	0.19 ± 0.04^{b}	0.22 ± 0.07^{b}	0.24 ± 0.05^{b}	0.25 ± 0.04^{b}	0.23 ± 0.05^{b}	0.33 ± 0.05^{a}
NO_2^-	0.03 ± 0.01^{b}	0.04 ± 0.01^{b}	0.04 ± 0.02^{b}	0.04 ± 0.02^{b}	0.04 ± 0.04^{b}	0.04 ± 0.05^{b}	0.07 ± 0.01^{a}
PO_3^-	1.78 ± 0.12^{b}	1.79 ± 0.24^{b}	2.04 ± 0.09^{b}	2.09 ± 0.02^{a}	2.11 ± 0.11^{a}	2.13 ± 0.15^{a}	1.79 ± 0.21^{b}

Values are mean ± SE as analysed by two-way ANOVA and one-way ANOVA. Different superscripts indicate significant difference at $p < 0.05$, where a > b > c.

5. Net biomass (kg/m^3) = Total biomass at harvest−Total biomass at stocking
6. Condition factor (K) = (W*100)/L^3, where L = Measured total length of fish (cm) and W = Measured weight of fish (g) (Blackwell et al., 2000).

2.5 | Statistical analysis

Data were tested for normality and homogeneity using the Shapiro-Wilk and Levene's test, respectively. Statistical analyses for the effects of protein levels, carbon sources and their interactions on the physicochemical water quality parameters, growth parameters, proximate analysis of bioflocs and abundance of microorganisms were done in R software (R-Core Team, 2019) using a two-way ANOVA. The one-way ANOVA was then employed to test for each biofloc treatment with the control. Tukey's Honestly Significance (HSD) was used to locate specific differences. All descriptive statistics performed on the data were expressed as mean ± standard error (SE) and the observed differences were considered statistically significant at a predetermined significance level of $p < 0.05$.

3 | RESULTS

3.1 | Physicochemical parameters

Average water quality parameters are presented in Table 2. Among the physicochemical water quality parameters protein levels and carbon sources had significant interaction ($p < 0.05$) on pH. There was no significant difference ($p > 0.05$) in temperature between the biofloc and the control units and among the protein levels ($p > 0.05$). Throughout the study, TDS concentration was observed to vary, increasing from week one and decreasing when sludge was drained, reaching the highest value of 138.125 mg/L in glucose formed bioflocs, 149.93 mg/L in molasses formed bioflocs and 94.01 mg/L in the control treatments. TDS was significantly different ($p < 0.05$) between the biofloc and the control experiments. TDS also exhibited a significant difference ($p < 0.05$) between molasses and the control treatments. DO, pH, NH_3 and NO_2 were significantly higher in the control than biofloc units. Water conductivity and phosphorus were higher in the biofloc units than in the control. The protein levels and carbon sources had significant effects ($p < 0.05$) on DO and NO_2.

3.2 | Fish growth performance

There was a significant difference ($p < 0.05$) in average mean weight gain between the biofloc units and the control with values of 2.07 g in glucose biofloc, 1.99 g in molasses biofloc and 1.73 g in the control treatments. SGR was significantly different (one-way ANOVA, $p < 0.05$) between biofloc units and the control, with SGR of fish reared in glucose and molasses units being superior than those reared in control (Figure 1A). However, no significant effects ($p > 0.05$) of protein level, carbon source, or their interaction was observed on SGR. The average FCR was significantly different ($p < 0.05$) between the biofloc units and the control with values of 0.56, 0.64 and to 0.89 in glucose, molasses and the control, respectively (Figure 1B). FCR was also significantly different between the carbon sources. The PER was affected by protein level ($p < 0.05$) and carbon source ($p < 0.05$), but no interaction effects were observed ($p > 0.05$). There was significantly higher PER ($p < 0.05$) in the control than the bioflocs (Figure 2C). Furthermore, it was significantly different ($p < 0.05$) not only between the biofloc and control but also among the protein level treatments and between the bioflocs. The fish condition average values were 1.85, 1.83 and 0.78 in glucose, molasses biofloc units and the control, respectively (Figure 2D) with significant difference ($p < 0.05$) between the biofloc units and the control. No significant effects ($p > 0.05$) of protein level, carbon source, or their interaction were detected on condition factor. Survival rates in the biofloc units were significantly different ($p < 0.05$) than the control, being more than 92% compared to 74.7% in the control (Figure 3) while survival rates in glucose bioflocs showed significantly

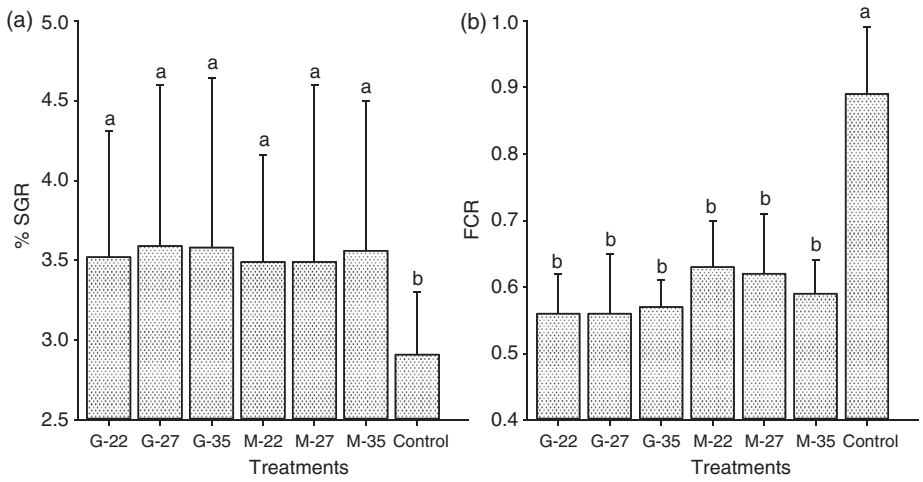

FIGURE 1 Specific growth rate (SGR) and feed conversion ratio (FCR) for the experimental treatments. (mean data ± SE, error bars indicate SE)

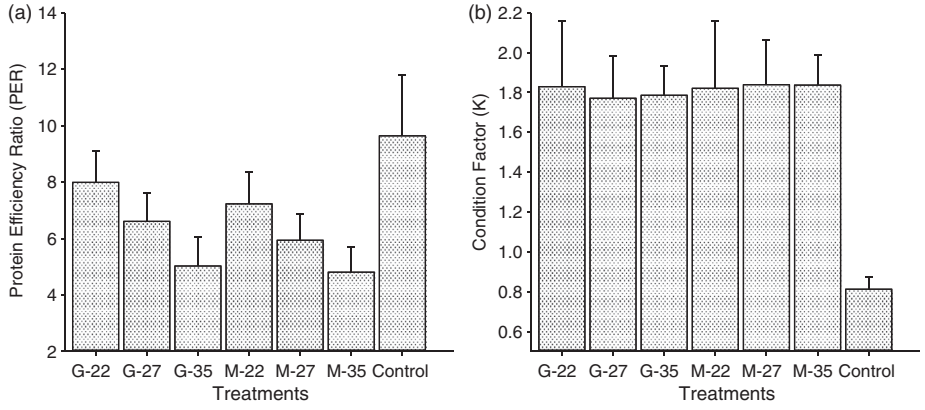

FIGURE 2 Protein efficiency ratio (PER) and condition factor for the experimental treatments (mean data ± SE, error bars indicate SE)

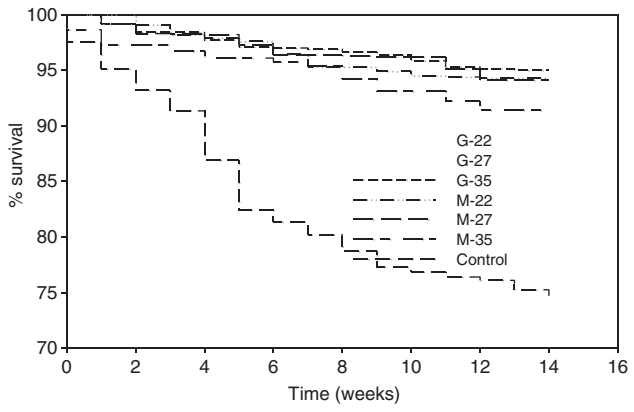

FIGURE 3 Percentage survivals for the experimental treatments (mean data ± SE, error bars indicate SE)

higher ($p < 0.05$) values than that in molasses bioflocs. Highest survival in glucose bioflocs was observed in G-22 and lowest in G-27, while molasses treatment M-35 (94.6%) showed the highest survival, with M-22 and M-27 having similar values (94.2%). Survival did not show significant differences ($p > 0.05$) among protein levels in biofloc treatments. The growth curves for different biofloc treatments in comparison to the control showed growth homogeneity indicated by weight increase irrespective of the protein level used for glucose and molasses bioflocs (Figures 4 and 5). The final fish biomass in glucose and molasses bioflocs was significantly higher ($p < 0.05$) than the control.

3.3 | Proximate analysis of bioflocs

The mean comparison of proximate analysis tests of the bioflocs is represented in Figure 6. CP was significantly different ($p < 0.05$) in glucose and molasses bioflocs with average values of 34 and 31%, respectively. Glucose bioflocs had higher lipid and ash content compared to molasses bioflocs without significant difference ($p > 0.05$). There was a significant difference ($p < 0.05$) in the crude fibre between the biofloc units. The CP and crude fibre contents of bioflocs were significantly affected by carbon source ($p < 0.05$) and not protein level ($p > 0.05$), or their interaction effects ($p > 0.05$).

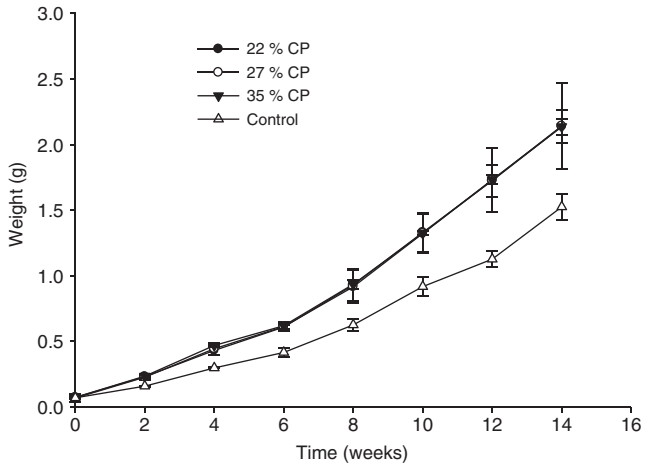

FIGURE 4 Growth curves of *Oreochromis niloticus* fry in glucose biofloc with the control treatments (mean data ± SE, error bars indicate SE)

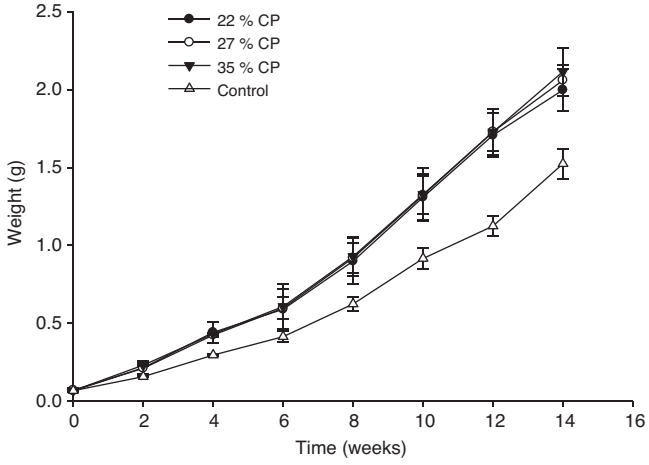

FIGURE 5 Growth curves of *Oreochromis niloticus* fry in molasses biofloc with the control (mean data ± SE, error bars indicate SE)

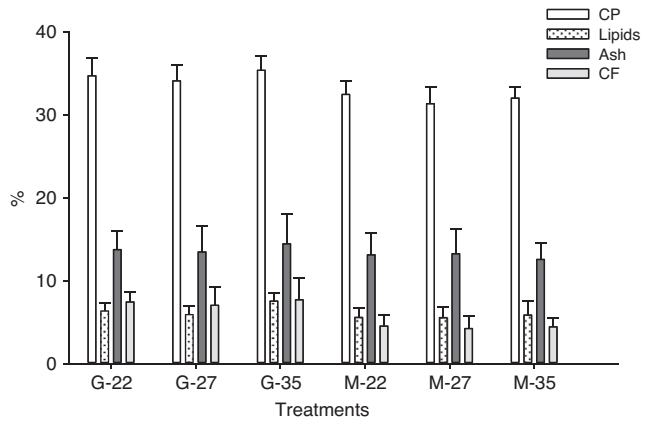

FIGURE 6 Proximate analysis of the bioflocs in the experimental treatments (mean data ± SE, error bars indicate SE)

3.4 | Abundance of microorganisms

The microorganisms in the water of bioflocs included–amoeboids, ciliates, flagellates and rotifer. The mean abundance of the microorganisms was significantly different ($p < 0.05$) between the biofloc and the control units (Table 3). The abundance of amoeboids, ciliates, flagellates and rotifers was affected by carbon source ($p < 0.05$) but not protein level ($p > 0.05$), or their interaction ($p > 0.05$).

4 | DISCUSSION

4.1 | Water quality

Physicochemical water quality is a limiting factor and of importance in considering the health of aquaculture species (Khanjani et al., 2021). The average water temperature for the biofloc units and the control experiment was within the range of 20–35°C recorded by El-Sayed (2006) for tilapia normal growth. pH ranges observed in biofloc units were near to optimum level. Due to low variation of pH in biofloc treatments, it is a stable culture systems when compared to the control because pH affects a number of physical and chemical water quality parameters (Boyd et al., 2011).

The reduction of DO in biofloc units may be due to the accumulation of organic matter, high respiration rates and metabolism activities by bacteria which are features of a Biofloc culture system (Khanjani et al., 2020a, b), as compared to the control. The higher DO in the control may be due to exchange of water carried out daily and low microbial density (Mirzakhani et al., 2019; Khanjani et al., 2021). Conductivity and TDS were observed to increase gradually in all treatments but were consistent with the results of other studies (Avnimelech, 2012; Lima et al., 2018). This was probably due to an increase in organic matter concentration following C:N adjustment (Crab et al., 2012). It was evident that adding glucose and molasses daily to culture units resulted in a better heterotrophic system compared to the control, leading to intense microbial production and more TDS (Khanjani et al., 2021).

Ammonia levels calculated were less than 0.5 mg/L and were higher in the control than in the biofloc units despite water exchange ratio. Furthermore, ammonia increased with protein level in the diet treatments for the biofloc units due to the nitrogen in the feed being supplied. Ammonia was observed to be directly proportional to nitrogen in the feed (Hari et al., 2006). The majority of the commercially farmed fish, including tilapia fry, tolerate toxic ammonia levels below 1.5 mg/L (Neori et al., 2004). This level is above what was observed in this study. High ammonia concentrations in the first 2 weeks of the study may be attributed to low numbers of heterotrophic bacteria in the biofloc units which can transform nitrites to nitrates (Azim & Little, 2008; Avnimelech, 2012). Heterotrophic bacteria have been found to assimilate ammonia more rapidly than nitrifying bacteria, as they grow tenfold faster as compared to nitrifying bacteria (Crab et al., 2012; Cavalcante et al., 2017).

The mean nitrite concentration was suitable for tilapia culture in all treatments with the control having the highest concentration,

TABLE 3 Mean abundance of microorganisms in *Oreochromis niloticus* fry experimental treatments (Mean ± SE)

	G-22	G-27	G-35	M-22	M-27	M-35	Control
Amoeboids	3.4 ± 1.2	3.6 ± 0.2	3.8 ± 0.1	2.2 ± 0.8	2.5 ± 0.7	2.6 ± 0.9	2.0 ± 0.3
Flagellates	18.6 ± 1.3	18.9 ± 1.2	17.5 ± 2.7	12.8 ± 1.1	14.8 ± 2.1	13.6 ± 1.2	9.3 ± 1.3
Ciliates	19.5 ± 1.7	20.3 ± 1.5	18.2 ± 2.2	13.5 ± 2.8	14.2 ± 2.8	13.1 ± 1.7	8.3 ± 1.2
Rotifers	13.7 ± 2.1	13.3 ± 1.3	14.2 ± 2.3	9.9 ± 1.2	9.5 ± 1.6	10.6 ± 1.4	1.0 ± 0.2

however, lower than the maximum optimum level (0.2 mg/L) in fish and shrimp (Taslihan et al., 2003). High concentrations of nitrite cause a reduction of survival and growth delays of aquatic organisms (Lin & Chen, 2003). The lower nitrite and ammonia levels observed in G-22 and M-22 treatments may be due to lower protein levels used in the diet treatments because ammonium nitrogen increases with corresponding increase in CP in fish feeds (Cavalli et al., 1996).

Ammonia and nitrite in the experiments were within the optimum range of Nile tilapia farming and consistent with other studies (Mirzakhani et al., 2019; Khanjani et al., 2021). The gradual increase of phosphorus could be due to the high rate of mineralization of organic matter by heterotrophic bacteria in the biofloc culture systems, resulting from the addition of more phosphorus (Lorenzo et al., 2016). Although phytoplankton could absorb phosphorus from the water, this absorption was affected by high turbidity in biofloc units which might have decreased phytoplankton populations (Xu et al., 2016).

4.2 | Growth performance

The SGR among the three crude protein treatments under biofloc showed efficient assimilation of biofloc protein into fish biomass compared to the control, resulting in growth homogeneity. Lower FCR was achieved in biofloc units than the control showing that *O. niloticus* fry had consumed bioflocs apart from commercial feeds. Researchers have documented that supplementation of commercial diets with bioflocs improves FCR and increases feed efficiency (Mirzakhani et al., 2019; Khanjani et al., 2020a; Khanjani et al., 2021). Bioflocs have been found to contain probiotic properties that improve the digestion and absorption of commercial diets resulting in better feed conversion (Aguilera-Rivera et al., 2014). FCR values from this study were better than 3.51 and 4.95 and reported by Azim and Little (2008) in systems with and without bioflocs, respectively. Nevertheless, the lower FCR and higher yield in biofloc units than in the control might have been due to improved water quality caused by increased nutrient removal and all-time microbial protein.

Different protein levels in the bioflocs promoted significant differences in PER between the carbon sources used. This is because PER considers the protein in commercial feeds and not the protein of microbes in bioflocs. Due to the presence of microbial protein, which also led to lower FCR, PER was of significant difference between the biofloc and the control treatments (Ogello et al., 2014). Between the biofloc units' values of PER were lower than the control indicating higher protein utilization efficiency. Therefore, utilization of microbial protein played a major role in reducing toxic nitrite and ammonia in the biofloc culture system, leading to low accumulation of harmful wastes in aquaria which also indicates low protein in fish faecal wastes resulting in a low concentration of TAN. Toxic nitrogenous wastes, namely ammonia and nitrite, were shown to be reduced in the biofloc units by bacterial metabolism into microbial flocculates consumed by fish, resulting to more nitrogen that is converted into harvestable biomass thereby increasing protein efficiency.

Despite the low commercial protein level, there was natural food in form of microbes available daily for the fish to consume, promoting higher utilization of protein in 22% glucose and molasses biofloc treatments in relation to 27 and 35% protein-level treatments. Similarly, Khanjani et al. (2021) observed that the presence of natural microbial protein supplemented low dietary protein resulting in higher utilization of protein in biofloc as compared to non-biofloc culture systems. Values of PER show that productivity of tilapia fry in a biofloc system, feed and nutrient utilization increased significantly while using a low crude protein feed (22%). This translates into a higher use of protein as a result of nutrient recycling by the microbial population.

Apart from heterotrophic bacteria, the recycled nutrients from organic particles promote the growth of protozoa and algae in biofloc systems (Emerenciano et al., 2012). This increases the variety of foods consumed by fish in a biofloc culture unit, resulting in higher growth performance of farmed fish species (Cavalcante et al., 2017). Bioflocs have been documented to contain chlorophylls, phytosteroids and carotenoids, which are bioactive compounds (Ju et al., 2008), as well as poly-beta-hydroxybutyrate organic compounds (De Schryver et al., 2010), all of which affect the growth performance of aquatic organisms positively. Generally, the higher growth observed in biofloc units than in the control from this study was not attributed to protein levels but the microbial community available, which relieved nitrogenous wastes and yields microbial protein. The growth performance of aquatic organisms has been reported to be improved by the presence of bioflocs in several studies (Mirzakhani et al., 2019; Khanjani et al., 2020b, 2021), growth and production of tilapia is, therefore, enhanced in a biofloc culture system in a greenhouse.

The high survival rates in biofloc units may be attributed to good culture conditions as compared to the control and corroborates the findings by Crab et al. (2009) and Mirzakhani et al. (2019) who reported up to 80–98% and 100%, respectively, while studying tilapia culture in a biofloc system. This shows that biofloc systems

offer good culture conditions for aquatic organisms. The lowest survival rate observed for the control experiment may be attributed to fluctuations of ammonia, pH and NO_2 which causes stress to aquatic organisms, thus, affecting survival and growth performance (Avnimelech, 2012). This study reported a higher condition factor in biofloc units than what had been previously reported in *O. niloticus* fry-fingerlings grown in other culture systems (Omweno et al., 2020). This shows that a biofloc system provides better culture conditions as compared to conventional fish culture systems. The three protein levels resulted to homogenous fish growth in the biofloc units indicating that by using 22% CP, fish growth was not compromised in the BFT.

The experiment involved very small tiny fry of *O. niloticus* of initial weight 0.07 ± 0.01 g, which were at lag phase, a stage of slow growth. However, the low feeding rate and frequency employed might have also contributed to the slow growth observed in this study. Nonetheless, the lowest yield results from this experiment of 3.86 ± 0.21 kg fish per metre cube was higher than 1.23 ± 0.09 kg fish per metre cube reported by Cavalcante et al. (2017) studying periphyton and biofloc integration in tanks used to culture *O. niloticus*.

4.3 | Proximate analysis of bioflocs

Bioflocs contain good nutrient, as seen from proximate analysis. CP variations for the bioflocs were from 31.3 to 35.3%, lower than what Mirzakhani et al. (2019) and García-Ríos et al. (2019) reported at 56–66% and 63.9–71%, respectively, but higher than the values reported by López-Elías et al. (2015) at 23.7–25.4%. The protein content of bioflocs from this study was in the range observed by other studies (Mirzakhani et al., 2019). The significantly higher CP in glucose formed bioflocs was a result of the glucose substrate promoting bacteria which resulted in higher natural feed for fish growth. The protein content in bioflocs formed by glucose and molasses carbon sources is consumed all the time by the fish, and it represents the recycled wasted feed (McIntosh, 2000). Bioflocs with more than 38% CP are regarded as suitable in tilapia production (Azim & Little, 2008). Lipid content was 5–7% DW and 5% DW in glucose and molasses bioflocs, respectively. Lipids supply high energy, almost twice that of carbohydrate and protein, and can substitute partially the protein in fish feed (Craig & Helfrich, 2002). Lipids constitute about 15% of the DW in fish feeds and are used to contribute essential fatty acids which significantly increase their nutritional value (Ju et al., 2008; Khatoon et al., 2016).

Bioflocs in glucose resulted to slightly higher ash (13–14%) than those in molasses (12–13%) DW. Craig and Helfrich (2002) advised a complete diet to have at most 8.5% DW of ash. Low digestibility of other ingredients can result in poor fish growth due to high ash in fish feeds. Avnimelech (2007) reported a variation of biofloc nutrition composition due to the presence of specific microbes, culture period, feeding habits of fish under culture, conditions of environment and the type of species under culture. Furthermore, the biochemical components of bioflocs have been documented to be affected by the level of feeding (Khanjani et al., 2016), carbon type (Khanjani et al., 2017), carbon/nitrogen ratio (Minabi et al., 2020) and microbial density (Ahmad et al., 2017).

4.4 | Abundance of microorganisms

Highest microbial density in bioflocs than the control showed that daily addition of glucose and molasses was effective while culturing *O. niloticus* (Emerenciano et al., 2012; Khanjani et al., 2020a, 2021). The differences in biofloc nutrient values may be due to different carbon sources used for the experiment, for instance, the significantly higher CP in glucose-formed bioflocs was possibly a result of the glucose substrate promoting more bacterial growth than the molasses. This resulted to higher natural feed for *O. niloticus* fry.

5 | CONCLUSIONS

It is evident from this study that nutritional composition of bioflocs contributed to higher fish growth and more efficient protein utilization due to the presence of heterotrophic bacteria, as biofloc units promoted a better fish performance than the control. Survival was also improved in bioflocs compared to the control. Although CP remains an expensive ingredient in fish feed, the use of a biofloc system can enhance fish growth even with lower CP feeds, as it likely enhances protein utilization efficiency in fish feeds. A future study is, therefore, necessary to assess the protein utilization efficiency in a BFT on a commercial scale based for the whole production cycle and its possible application by fish managers and farmers. Generally, it can be concluded that using a biofloc culture system with a lower protein level (22%) to culture *O. niloticus* fry, will enhance a better growth performance.

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AUTHOR CONTRIBUTIONS

Erick O. Ogello: Initiation; Methodology; Supervision; Formal analysis. Albert Getabu: Investigation; Methodology; Supervision; Validation. Reuben Omondi: Formal analysis; Investigation; Visualization

CONFLICT OF INTEREST

We certify that there is no conflict of interests among the authors. Further authors declare no conflict of interest.

AUTHOR'S ETHICAL STATEMENT

We certify that this is our original scientific research work, and it has not been submitted or published anywhere. The authors are responsible for all the content in the manuscript.

ANIMAL ETHICAL STATEMENT

We certify that the current study followed all the applicable guidelines for the care and use of fish.

DATA AVAILABILITY STATEMENT

We certify that the data used in this article was collected from the study and can only be availed through the request and permission of the third-party authors.

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