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RESEARCH ARTICLE

CONDITION FACTOR OF NILE TILAPIA, *OREOCHROMIS NILOTICUS* FRY UNDER THE INFLUENCE OF DIFFERENT PROTEIN LEVELS IN A BIOFLOC SYSTEM

Wilfred O. Zablon¹, Albert Getabu¹, Erick O. Ogello², Job O. Omweno¹ and Nicholas Outa²

1. Department of Fisheries and Aquatic Sciences, Kisii University, P.O. Box 408-40200, Kisii, Kenya.
2. Department of Fisheries and Natural Resources, Maseno University, P.O. Box Private Bag, Maseno, Kenya.

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Abstract

The study was conducted at Kenya Marine Fisheries Research Institute (KMFRI), Sangoro Station, Kenya to compare the growth performance of *Oreochromis niloticus* fry under different protein levels in a biofloc system using weight-length regression models and Fulton's condition factor (K). Fry of initial mean weight 0.07 ± 0.03 g and length 1.30 ± 0.24 cm were randomly stocked in 18 aquarium tanks with capacity of 50 litres at stocking density of one fish Litre⁻¹. The experiment was set in a greenhouse under controlled temperature and dissolved oxygen conditions using aerators and thermostat heaters. The biofloc used glucose and molasses as carbon sources which were assigned at 22%, 27% and 35% crude protein levels randomly to the aquaria. The fish were fed on their daily ration twice a day at 5% body weight for 14 weeks. Physicochemical parameters were recorded daily using a multiparameter meter and water samples collected before fish sampling for nutrient analysis. A sample of 30 fish per tank were used for taking body weight and total length measurements using an electronic balance and a measuring board respectively. Fry in all treatments exhibited allometric growth with b coefficient varying between 2.54 and 2.66. Average Fulton's condition factor ranged between 1.77-1.84 with no significant difference ($P > 0.05$) among the protein levels and the bioflocs. The condition factor showed both carbon sources were suitable for use in the biofloc system and hence replacement of higher protein level (35%) with lower protein level (22%) to reduce the production cost is a feasible application of the biofloc system in fish farming.

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Introduction:-

The average aquaculture growth among African countries has remained fairly constant at 5.8% annually, although in few countries, the reported aquaculture production has doubled (FAO, 2018). According to FAO (2020), the finfish production declined gradually over the past two decades inspite of inland aquaculture producing 54.3 million metric tonnes finfish but comparatively, aquaculture is doing better than other food production sectors. The global production of Tilapia which is approximately 4.5254 Million Tonnes (8.3%) supersedes production from other cultured fish species except the carps (FAO, 2020). This shows that with the available aquatic resources, aquaculture can be expanded by incorporating other culture systems. The precursor for aquaculture growth is human population

Corresponding Author:- Wilfred O. Zablon

Address:- Department of Fisheries and Aquatic Sciences, Kisii University, P.O. Box 408-40200, Kisii, Kenya.

increase which has brought about decline in wild fisheries, consequently increased prices of fish and their products (Péronet *et al.*, 2010). In aquaculture, improved technology is one of the most recent ways of managing the cultured fish to reduce the production cost (Jiang, 2010). It has been reported that the high cost of feeds accounts for 40-69% of the production cost of cultured species of which the protein is an essential component (Fotedar, 2004). To lower the operational costs, Ogello *et al.*, (2014) suggests that sustainable aquaculture should consider good culture management and feed, which can also imply replacement of fishmeal in feeds with affordable protein sources (Lim and Webster, 2006). This has led to technical innovation such as the recent biofloc technology (BFT), which aims at maximizing utilization of feeds, hence decrease the production cost, and remove effluents discharged to the recipient aquatic ecosystems (Abdel-Tawwab and Ahmad, 2009, Mohsen *et al.*, 2010). Previous studies and reports (Arnold *et al.*, 2009; Megahed, 2010; Xu & Pan, 2012) have sought to establish whether biofloc technology can be efficiently used for increased productivity and reduced environmental pollution than the conventional culture systems. However, it is also crucial to assess the condition of these cultured fish using weight-length relationships (Petrakis and Stergiou, 1995) and Fulton's condition factor and compare the fish response to different environmental feeding conditions caused by different carbon sources and protein levels (Anyanwu *et al.*, 2007).

The condition factor (LeCren, 1952), indicates the general well-being of fish and is used to explain the variation for fish weight with body length. The condition factor also represents the physiological condition of a fish for a given culture period (Ighwela *et al.*, 2011). Variation in fish condition is caused by environmental factors and feeding, sex, physico-chemical water parameters, season, feed availability and stress (Khallaf *et al.*, 2003). Feeding being a major factor affecting the fish condition, studies have been conducted to show the effect of *Tilapia* feeding habits on fish condition using weight-length relationships (Olurin and Aderibigbe, 2006). Although studies on *O. niloticus* are important given its significance in the global aquaculture, only few studies have been conducted on weight-length relationship of *O. niloticus* in a biofloc system. The present study was conducted to address this research gap and provide baseline information for future related studies.

Materials And Methods:-

Experimental site and design:

The study was conducted at Kenya Marine and Fisheries Research Institute (KMFRI), Sangoro station in Kisumu, Kenya between August and December, 2019. Sex reversed all male *O. niloticus* Fry of initial mean weight 0.07 ± 0.03 g and length 1.30 ± 0.24 cm were randomly stocked in 18 aquarium tanks with a working volume of 50L at a stocking density of one fish Litre^{-1} . The experiment was set in a greenhouse under controlled temperature and dissolved oxygen conditions with supplementary aeration provided by air stones connected to 10 HP air pump and temperature regulated by thermostat heaters. The biofloc used glucose and molasses as two carbon sources which were assigned at three protein levels of 22%, 27% and 35%. The treatments were randomly assigned to the aquaria in triplicates. The treatments were labeled as G-22, G-27 and G-35 in glucose bioflocs, M-22, M-27 and M-35 in molasses bioflocs for respective protein levels. Weekly sludge was drained and freshwater replaced to maintain water levels. The fish were fed on their daily ration twice a day at 5% body weight for 14 weeks. The percentage of carbon in molasses was calculated from density and that of glucose from the atomic weights. The carbon to nitrogen ratio (C/N) was calculated according to Avnimelech 1999, and maintained at 20:1. The quantity of carbon sources were calculated as follows: Quantity of carbon = (Feed quantity x percentage nitrogen in excretion x percentage nitrogen in feed) / 0.05 To accelerate biofloc growth, 5 litres of fertilized pond water was used as an inoculant (Correia *et al.*, 2002) and the carbon:nitrogen ratio was maintained by mixing daily the pre-weighted molasses and glucose in a bowl and applying the mixture to each aquarium before feeding the fish (Avnimelech, 1999; Samocha *et al.*, 2007).

Supplementary feeding was performed twice daily at 5% of body weight at 0900hrs and 1630hrs using a formulated trial diet. The physico-chemical water parameters were recorded daily while nutrients were sampled bi-weekly as recommended by Azim and Little (2008), Widanar *et al.*, (2012) and Liu *et al.*, (2014). Daily measurements were taken using YSI multiparameter meter (model Procomm 11) and nutrient analysis done using a mass spectrophotometer (Genesis 10s vis) while ammonia was calculated from TAN according to standard methods provided by El-Shafai *et al.*, (2004). A sample of 30 fish per tank was used for taking body weight and total length measurements using an electronic balance (readability = 0.001g) and a measuring board (readability = 0.01cm) respectively. Mortality was monitored daily and survivals calculated at the end of every two weeks. The collected data was analyzed using a two-way analysis of variance in R - Software. Regressions and Tukey's multiple comparisons was used as post hoc test to determine which particular treatment combinations differed. Fulton's condition factor (K) was determined using the equation; $K = (W \times 100) / L^3$. Where L - is the total length and W - is the body weight of fish (Blackwell *et al.*, 2000). Regression analysis was performed between log transformed weight

and length data using the log transformed form of Le Cren (1951) equation, $\text{Log } W = \log a + b \log L$, where b - is the slope and $\log a$, the intercept of weight-length regression. Descriptive results were presented as (mean \pm SE) and all significant differences determined at $P \leq 0.05$.

Results:-

Water quality parameters

The results of water quality measurements for the experimental treatments are presented in Table 1. There was no significant difference ($p > 0.05$) in temperature between the carbon sources and among the three crude protein treatments. However, the mean DO of glucose biofloc was significantly higher ($p < 0.05$) than that of molasses and exhibited significant differences ($p < 0.05$) among the crude protein levels. Similarly, the pH showed significant differences ($P < 0.05$) between the crude protein and the carbon used in all the treatments. TDS and EC showed significant differences ($p < 0.05$) between the biofloc treatments while they exhibited no significant differences ($P > 0.05$) among the crude protein treatments. There was a significant difference in the mean ammonia concentration between the carbon sources ($p < 0.05$) and among the crude protein treatments. The 22% crude protein level in glucose biofloc resulted to generation of significantly ($p < 0.05$) lowest levels of ammonia although significant differences were also observed between the treatments. Generally, glucose bioflocs resulted to lower ammonia levels than molasses bioflocs. Ammonia showed an increasing trend for the first two weeks of this study to a maximum of 0.30 mg L^{-1} recorded in M-27 treatment. However, Nitrite showed a decreasing trend in both bioflocs depending on the protein level used and its concentrations did not differ significantly ($p > 0.05$) between the carbon sources.

Table 1:- Physicochemical water quality parameters in *Oreochromis niloticus* fry presented as mean \pm SE. Means having different alphabet superscript were significantly different ($p < 0.05$) as determined by ANOVA with replication and Turkey's comparison of means. $a > b > c$

Treatments	G-22	G-27	G-35	M-22	M-27	M-35
Temp.	27.90 \pm 0.10 ^a	28.10 \pm 0.10 ^a	28.10 \pm 0.10 ^a	27.90 \pm 0.10 ^a	27.90 \pm 0.10 ^a	28.10 \pm 0.20 ^a
DO	5.10 \pm 0.02 ^a	4.99 \pm 0.01 ^a	4.96 \pm 0.06 ^a	4.81 \pm 0.07 ^b	4.80 \pm 0.06 ^b	4.74 \pm 0.09 ^b
TDS	112.4 \pm 1.08 ^b	114.6 \pm 1.53 ^b	112.9 \pm 2.01 ^b	123.1 \pm 1.82 ^a	122.7 \pm 2.10 ^a	122.2 \pm 2.44 ^a
EC	182.8 \pm 2.05 ^b	191.5 \pm 2.18 ^b	185.5 \pm 1.78 ^b	199.3 \pm 2.70 ^a	199.7 \pm 2.35 ^a	197.6 \pm 2.58 ^a
pH	6.90 \pm 0.10 ^b	6.90 \pm 0.10 ^b	6.90 \pm 0.10 ^b	6.80 \pm 0.10 ^b	6.90 \pm 0.10 ^b	6.80 \pm 0.10 ^b
NH ₃	0.21 \pm 0.03 ^b	0.19 \pm 0.04 ^c	0.22 \pm 0.07 ^b	0.24 \pm 0.05 ^a	0.25 \pm 0.04 ^a	0.23 \pm 0.05 ^a
NO ₂ ⁻	0.03 \pm 0.01 ^b	0.04 \pm 0.01 ^b	0.04 \pm 0.02 ^b	0.04 \pm 0.02 ^b	0.04 \pm 0.04 ^b	0.04 \pm 0.05 ^b

Length-weight relationship, condition factor and Survival:

There was no significant difference ($p > 0.05$) in average body weight and total length of the fry between the carbon sources at stocking (Table 2). Fish body weight resulted to significantly ($p < 0.05$) lower values in molasses compared to glucose bioflocs. The Final mean length of fish reared on glucose biofloc was not significantly different ($p > 0.05$) from the final mean total length in molasses bioflocs. The weight-length regression equations, b values, R^2 , P and F values are tabulated in Table 3 and Fig. 1, 2 and 3. The highest b coefficient was 2.66 obtained in G-35 treatment, while the lowest was 2.54 obtained from G-22 treatment. The initial and final condition factors were not significantly different for all treatments (Table 2 and Fig. 4). Survival rate were significantly higher than 96% in glucose and molasses 93% (Fig. 5).

Table 2:- Growth parameters in *Oreochromis niloticus* fry presented as mean \pm SE. Means with different alphabet superscript are significantly different ($p < 0.05$) as determined by ANOVA with replication and Turkey's comparison of means. $a > b$.

Treatments	G-22	G-27	G-35	M-22	M-27	M-35
Initial Weight	0.07 \pm 0.03 ^a	0.07 \pm 0.03 ^a	0.07 \pm 0.04 ^a	0.07 \pm 0.02 ^a	0.07 \pm 0.03 ^a	0.07 \pm 0.03 ^a
Final Weight	2.14 \pm 0.61 ^a	2.14 \pm 0.46 ^a	2.14 \pm 0.41 ^a	2.00 \pm 0.47 ^b	2.06 \pm 0.46 ^b	2.12 \pm 0.39 ^a
Weight Gain	2.07 \pm 0.61 ^a	2.08 \pm 0.46 ^a	2.07 \pm 0.42 ^a	1.93 \pm 0.47 ^b	1.99 \pm 0.46 ^b	2.05 \pm 0.38 ^a
Initial Length	1.30 \pm 0.25 ^a	1.29 \pm 0.21 ^a	1.30 \pm 0.22 ^a	1.30 \pm 0.21 ^a	1.30 \pm 0.21 ^a	1.30 \pm 0.23 ^a
Final Length	4.85 \pm 0.45 ^a	4.92 \pm 0.37 ^a	4.89 \pm 0.42 ^a	4.76 \pm 0.42 ^a	4.80 \pm 0.42 ^a	4.85 \pm 0.33 ^a
Initial K	3.33 \pm 2.00 ^a	3.08 \pm 1.91 ^a	3.13 \pm 1.66 ^a	3.31 \pm 1.56 ^a	3.32 \pm 1.57 ^a	3.13 \pm 1.53 ^a

Final K	1.83±0.33 ^a	1.77±0.22 ^a	1.79±0.15 ^a	1.82±0.34 ^a	1.84±0.23 ^a	1.84±0.15 ^a
%Survival	98.3 ±0.26 ^a	97.3 ±0.33 ^a	98.3 ±0.14 ^a	94.2 ±1.22 ^b	94.3 ±1.24 ^b	94.6 ±1.09 ^b

Glucose and molasses - 22%

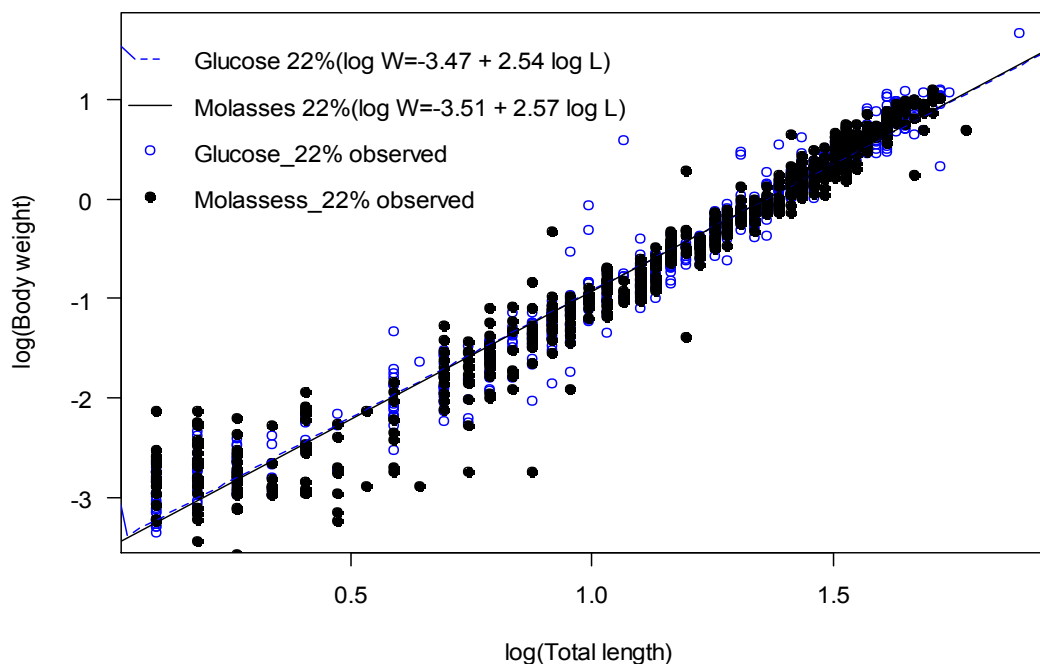


Fig.1:- Weight-length relationship of fish in glucose and molasses bioflocs compared at 22%.

Glucose vs. molasses - 27%

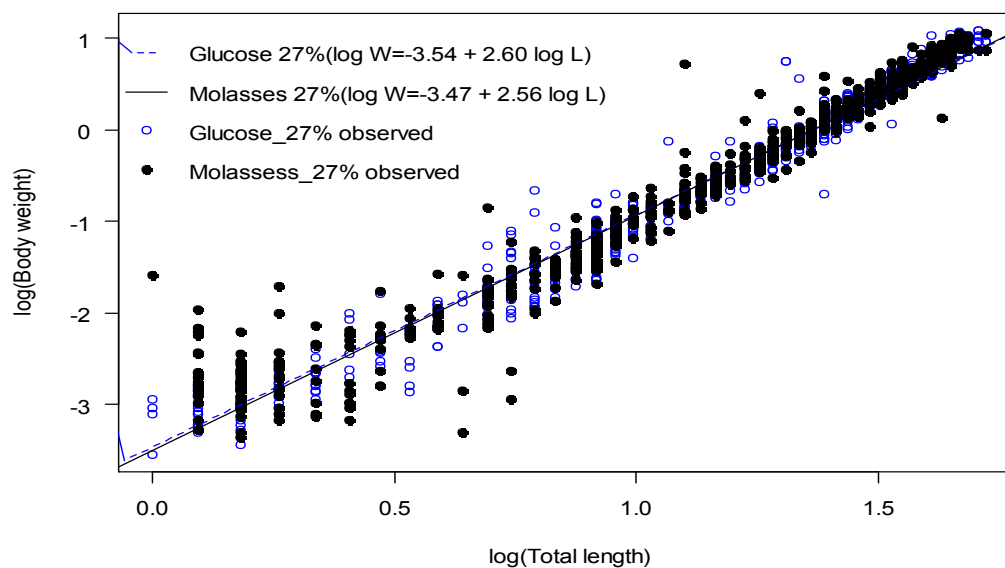


Fig.2:- Weight-length relationship of fish in glucose and molasses bioflocs compared at 27%.

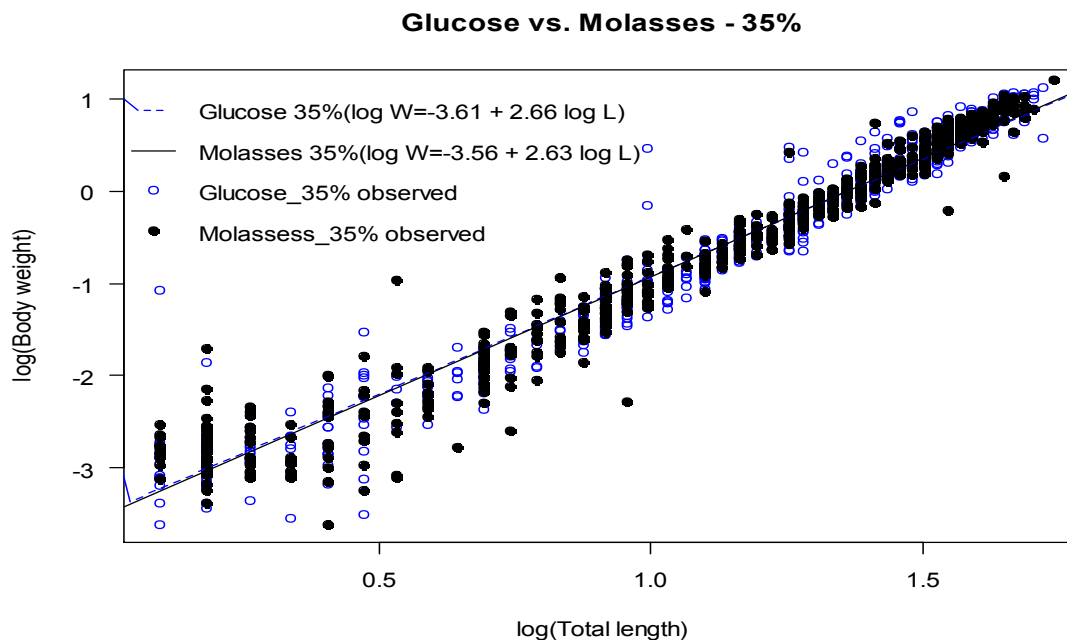


Fig. 3:-Weight-length relationship of fish in glucose and molasses bioflocs compared at 35%

Table 3:- The results of weight-length regressions obtained in the treatments.

Treatments	Log Equations	b	R ²	Signif.	F
G-22	Log W=-3.47+2.54LogL	2.54	0.967	<2.2e-16	2.10e+04
M-22	Log W=-3.51+2.57LogL	2.57	0.9572	<2.e-16	1.607e+04
G-27	Log W=-3.54+2.60LogL	2.60	0.9729	<2.2e-16	2.581e+04
M-27	Log W=-3.47+2.57LogL	2.57	0.9539	<2.2e-16	1.488e+04
G-35	Log W=-3.61+2.66LogL	2.66	0.9605	<2.2e-16	1.746e+04
M-35	Log W=-3.56+2.63LogL	2.63	0.9611	<2.2e16	1.775e+04

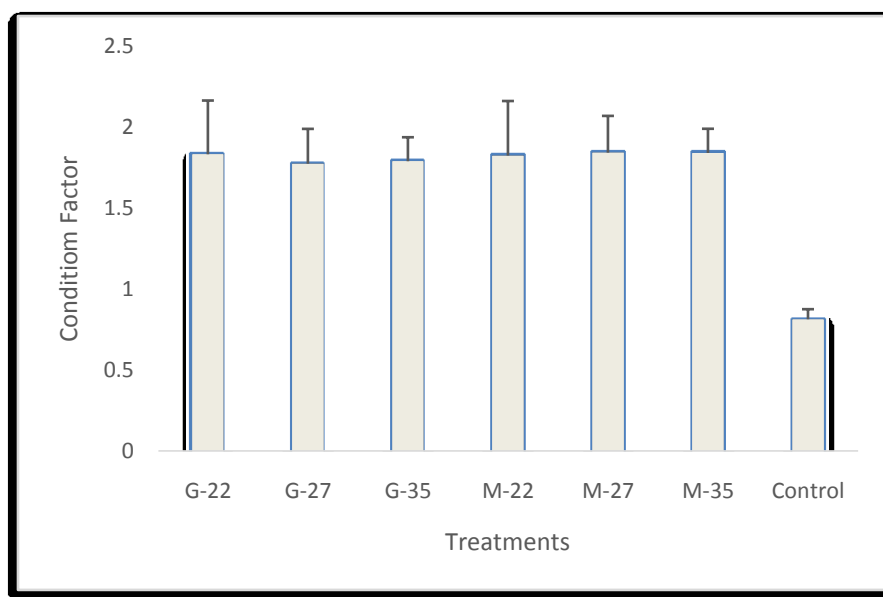


Fig.4:- Condition Factor of *Oreochromis niloticus* for the experimental treatments.

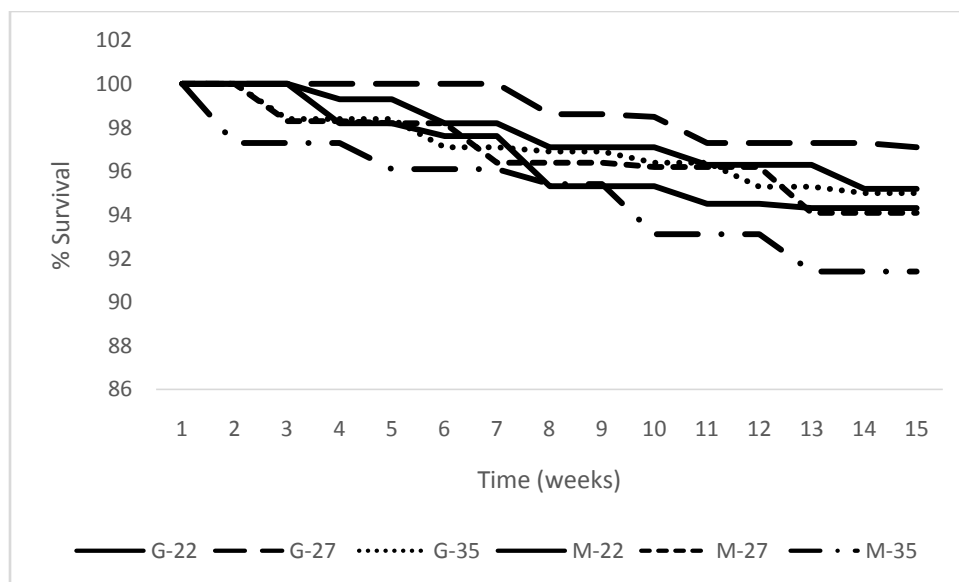


Fig.5:- Percentage survival of *Oreochromis niloticus* for the experimental treatments.

Discussions:-

The results showed that the average temperature in all treatments was within the optimum range of 20 - 35°C recommended for fish culture (El-Sayed, 2006). However, Crab *et al.*, (2009) and El-Sayed (2006) suggested that in considering this optimum for growth comparisons, it is also important to make reference to the *O. niloticus* specific optimum temperature of 25-30 °C. Although temperature in this study was controlled, it was not easy to maintain this specific optimum range in all temperature ranges due to system specific challenges and this necessitated monitoring of temperature variations in this study. Similar problems of temperature control in other studies have also been reported by Azim and Little (2008) and Luo *et al.*, (2014). Temperature is the most critical water quality variable which affects a number of biological processes in fish culture systems (Ogello *et al.*, 2014). For instance, water temperature affects the level of DO directly (Kuhn *et al.*, 2010), which in turn effects the growth of microorganisms in a biofloc system and consequently, growth and condition factor of cultured species. The pH level was also within the recommended range of 6.5 - 9.0 and the biofloc system was stable due to low pH variation exhibited (Boyd, Tucker, & Viriyatum, 2011). However, the observed low pH values of close to 6.5 could have been due to carbon-dioxide release by heterotrophic bacteria in the biofloc (Azim *et al.*, 2008).

Although the experiment recorded high DO levels at the beginning, the subsequent build-up of organic matter, resulted in increased respiration by bacteria eventually depleting oxygen (Taw, 2010 and Schweitzer *et al.*, 2013). However, the DO level did not fall below the optimum 4 mg l⁻¹ reported by Avnimelech (2011) because of supplemental aeration system provided. Higher DO level was recorded for low protein levels indicating low oxygen requirement for organic matter decomposition by heterotrophic bacteria (Asaduzzaman *et al.*, 2009a). Other factors which caused reduction in DO levels include fish respiration, temperature and competition between autotrophic and heterotrophic bacteria. The mean Ammonia levels determined in this study were below 0.5 mg L⁻¹, which is considered tolerable to most cultured fish species (Neoriet *et al.*, 2004; Avnimelech, 2012). However, the higher TAN value of 0.37 mg L⁻¹ in the molasses bioflocs was not toxic to fish because low pH levels recorded in the study lead to low TAN toxicity as a result of less toxic ammonia being favored in the equilibrium of ammonia gas and ammonium (Avnimelech, 2012). According to Nehemia *et al.*, (2012), prolonged exposure to unionized ammonia exceeding 0.2 mg L⁻¹ is highly toxic to fish at pH levels of greater than 9 and can lead to massive mortalities.

The Nitrite levels recorded were relatively low as compared to 28.1 mg L⁻¹ which Yanbo *et al.*, (2006) hypothesized to cause 50% mortality in tilapia after 96 hours of exposure. Increasing the C:N ratio to 20:1 using glucose and molasses enabled heterotrophic bacteria to colonize the biofloc resulting to bioflocs constituting of algae, protozoa and organic particles (Emerenciano *et al.*, 2012). These heterotrophic bacteria helped to maintain water quality in the biofloc system by metabolizing the carbon added to form microbial flocs, resulting to low nitrogenous wastes in the effluents (Ebeling *et al.*, 2006; Avnimelech, 2012; Emerenciano *et al.*, 2012; Hargreaves, 2013). Self-recycling of waste water which results in high fish survivals and increased growth performance makes the biofloc system a more

sustainable culture system (Mallasen and Valenti, 2006; Naqvi *et al.*, 2007; Crab *et al.*, 2007; Asaduzzaman *et al.*, 2008). Results of regression analysis showed that there were positive significant ($p < 0.05$, $R^2 > 0.5$) relationships between body weight and total length in the fitted data sets. This implies that an increase in mean weight was brought about by a corresponding length increase during the culture period. From the three linear regressions, the coefficients of determination R^2 ranged between 0.9539 to 0.9729 indicating good model fitness in which over 95% of the body weight is explained by the regression model (Omwen *et al.*, 2020). It also implies that mean body weight is highly depended on total length, although it is also depended on other factors such as the number of culture days and physicochemical parameters (Omwen *et al.*, 2020).

The values of the slope coefficient (b) showed that fish in all samples exhibited a negative allometric growth ($b < 3$), therefore becomes slender and leaner as the length increases (Pauly 1984). The b-coefficient ranged between 2.54 and 2.66, with the highest value recorded in G-35 treatment. The b value from this study lies within the range of 2.5 and 3.5 observed for most species by Froese (2006), but is on average lower than b value of 2.908 for the total length of 4.00-23.10 cm in tilapia reported by Britton and Harper (2008). Generally, fish were in good allometric condition although the b-coefficients indicated that there were many stress factors affecting the fish condition factor resulting to b-values of less than 3 (Prasad and Anvar, 2007). Similar findings have been reported by Moradinasab *et al.*, (2012), Kembanya *et al.*, (2014) and Omwen *et al.*, (2020) showing that fish at juvenile stage can exhibit allometric growth due to stress factors in the culture environment. However, in this study, the different b values may be attributed to different carbon sources and the protein levels resulting to different food items available for fish growth. According to Stewart (1998), Kleanthid *et al.*, (1999) and Hossain (2010), fish management and variation of environmental parameters may also be the cause of different b value in this study.

The Fulton's condition factor of more than 1.7 further indicated that the fish were in a healthy physiological state due to good water quality and low stress in the two biofloc systems. The condition factor was higher than what was reported for *O. niloticus* juveniles by Kembanya *et al.*, (2014) and Olurin and Aderibigbe (2006). Understanding the condition factor helps to depict the environmental aspects of the culture system as well as the level of management (Araneda *et al.*, 2008), consequently showing that the biofloc system is a better, efficient and sustainable culture system for *O. niloticus*. This can also be confirmed by high survival rates being higher than 93%. The survival results agree with Luo *et al.*, (2014) who obtained 100% survival for *O. niloticus* in a biofloc system. According to McIntosh (2000), microbial protein in the bioflocs which constitute the recycled wasted feed can be used to supplement formulated diets fed to fish. This explained why fish remained in good condition even with reduction of protein level from 35%-22% in both bioflocs evidenced from this study. Reduction of protein level in the feeds can be economical because feed account for over 50% of operational costs in aquaculture.

Conclusion and Recommendations:-

In conclusion, the findings showed that the fry of *O. niloticus* cultured in the two biofloc systems were in good condition and there was no significant influence of protein levels on the fish condition factor. Although the culture system imposed some stress factors to cultured fish, the mean weight of *O. niloticus* was highly dependent on total length and the fish in the biofloc system were in good physiological condition. A further study is recommended to determine the weight-length relationship and condition factor of other life stages in a biofloc culture system to monitor the effect of changing protein levels.

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