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Influence of Biofloc technology on economic evaluation of culturing *Oreochromis niloticus* reared at different stocking densities and feeding rates.

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ABSTRACT

The present work aimed to investigate the effect of Biofloc technology at different densities (60 &80 fish/m^3) and feeding rates (2% & 3%) of *Oreochromis niloticus* vs control clear water groups on growth performance and protein utilization, then estimation of economic evaluation on production scale (3000 m^3) based on the output of the present work. A 20-week study was performed from June to October 2019. Triplicate four Biofloc groups vs four control once were used. All Biofloc groups had an additional carbohydrates diets composed of molasses and rice brane (1:1) Circular 250 Liter tanks with 200 Liter were used. Results of growth and feed conversion ratio in the present study were used to estimate economic evaluation of production scale (Feddan composed of 3000 m3 net volume). Generally, Biofloc improved growth performance, protein utilization and achieved more estimated net income than clear water groups net income with 9/1 provide of water consumption.

INTRODUCTION

High investment of Aquaculture's has attracted farmers and investors to the intensification systems with the application of modern technologies in order to increase profits (**Dawood *et al*., 2016**). High stocking densities combined with highly nitrogenous diets in intensive fish culture negatively affect the water quality especially the accumulation of inorganic nitrogen forms NH_3 and NO_2 (**Hargreaves & Tucker, 2004**).

Biofloc technology (BFT) is a new approach to increase aquaculture productivity in limited water and space availability. It based on assimilation of waste nutrients and converting them into microbial biomass, which grazed as a natural food source for aquatic species **(De Schryver *et al*., 2008). Kuhn *et al*. (2009)** reported that microbial floc meal in tilapia diets significantly increase the weight gain. Recently, Biofloc technique have widely been used to maximize tilapia production for its ability to support high densities cultivation, to improve water quality and simultaneously recycling feed and protein production in the same culture unit **(Rakocy *et al*., 2004; Crab *et al*., 2007** and **Azim & Little, 2008)**. Bioflocs consumption by fish could contribute about 50% of the dietary protein requirements of *O. niloticus* **(Avnimelech, 2007). Azim & Little**

(2008) reported that, the application of BFT increased total production with 45% of *O. niloticus* compared to the traditional culture (Clearwater).

So, the present work was aimed to investigate the effect of Biofloc technology on the economic evaluation of *Oreochromis niloticus* fish culture in feddan (net 3000 m^3 water) as a production scale.

MATERIALS AND METHODS

1. Fish and experimental desgin

The study was conducted in sixteen circular tanks (each tank is 250 L) filled with 200 L dechlorinated water,. Monosex fingerlings of Nile tilapia, *Oreochromis niloticus* were used. Fishes were acclimatized for six weeks at laboratory condition then healthy fishes with 30.0 ± 2.1 g weight and 9.5 ± 1.1 cm length were selected to use in the experiment. They reared for 20 weeks extended from 11 June to 30 October, 2019 at the Aquaculture Lab., Animal house, Faculty of Science, Al-Azhar University, Cairo.

The experimental fish diet was 30% protein floating pellets. The proximate composition of this basic diet was 29.8% protein, 7.5% lipid, 12.4% ash and 6.9% water content. All fishes were fed by two equal diets daily at 9:00AM and 3:00 PM, seven days a week. The feeding rate was calculated fortnightly by weighig not less than 30 % of fishes for each group. Fishes were grouped into two stocking densities and two feeding rates with or without carbohydrate addition (**Table. 1**).

Table 1. Grouping of experimental design.

Group		Stock Density	Feeding rate
Control clear water (without carbohydrate addition)	C1	12 fish (60/m^3)	Low (2%)
	C2	12 fish (60/m^3)	High (3%)
	C3	16 fish (80/m^3)	Low (2%)
	C4	16 fish (80/m^3)	High (3%)
Biofloc (with carbohydrate addition)	T1	12 fish (60/m^3)	Low (2%)
	T2	12 fish (60/m^3)	High (3%)
	T3	16 fish (80/m^3)	Low (2%)
	T4	16 fish (80/m^3)	High (3%)

Biofloc groups had a carbohydrate additional diet composed of molasses and rice bran (1:1) to achieve C:N equal to 15:1 according to **Avnimelech (1999)** with only weekly addition of evaporative water loss while control groups were reared without any additional diets and 50-70% water weekly replacement to achieve tolerable conditions.

2. Water quality

All water quality parameters (Ammonia, Nitrate, Nitrite, DO and floc volume) were maintained in a suitable limit for tilapia rearing according to **El-Sayed (2006).**

3. Experimental parameters

Total Feed intake: The total feed consumed (g/fish) is calculated for each fish.

Food conversion ratio = feed intake (g)/ total weight gain (g) (**Tacon, 1987**).

Total input/output ratio (for Biofloc groups)

Input/output ratio = (fodder intake + carbohydrate intake) g/ total weight gain (g)

4. Economic evaluation:

The total costs were calculated by the following equation:

Total costs = feed costs (LE) + fish fingerlings cost (LE) + operation cost (LE)

Operation costs include workers, electricity, transportations…etc. Electricity was estimated as 9 hp aerators for feddan working 24h a day, seven days a week according to **(Avnemelech, 2012)**. All experimental diet costs, fish fingerlings cost and operation cost were calculated according to the prices in Egyptian marketing during the study period. The economic evaluation was calculated by the following equation:

Net income (LE) = Total fish price (LE) - Total costs (LE)

5. Statistical analysis:

The Shapiro-Wilk normality test and Bartlett's homoscedasticity test were employed at 5% significance. Three-way analysis of variance (ANOVA) was applied to the growth parameters and nutritional values using **(SAS., 2003)**

RESULTS

1. Growth and feeding parameters

Data in **Table (2)** showed that final body weight ranged between 192.9 ± 20.0 g/fish at Biofloc high density high feeding rate (**T_4**) group and 156.3±16.8 g/fish at control low density low feeding rate (**C1**) group. Total weight gain fluctuated between 164.6 ± 17.2 and 129.2 ± 16.4 g/fish at Biofloc high density high feeding rate group (**T4**) and control low density low feeding rate group (**C_1**), respectively (**Table 2**).

Specific growth rate fluctuated between 1.37 ± 0.06 and 1.09 ± 0.10 g/fish at Biofloc high density high feeding rate group (**T4**) and control high density low feeding rate group (**C_3**), respectively. Survival rate showed the maximum with 100% at each of control low density low feeding rate group (**C_1**), Biofloc low density low feeding rate group (**T1**) & Biofloc high density low & high feeding rate groups (**T_3** and **T_4**). While it was minimum (87.5 ±6.25%) at control group of **C_4 (Table 2)**.

Final body weight, total weight gain and specific growth rate were statistically significant (P <0.05) between Biofloc and control groups at only high feeding rate groups. Survival rate of Biofloc groups was significant at only high feeding rate groups (**Table 2**).

Data in **Table (2)** showed that diet intake ranged between 268.1 and 159.1 g/fish at Biofloc high density high feeding rate (**T_4**) group and Biofloc high density low feeding rate (**T_3**) groups, respectively **(Fig. 1)**.

Results in **Table (2)** showed that the carbohydrate intake ranged from 112.6 to 66.8 g/fish at Biofloc high density high feeding rate (**T_4**) group and Biofloc high density low feeding rate (**T_3**) groups, respectively.

Feed conversion ratio fluctuated between 1.8 ± 0.3 and 1.2 ± 0.1 at control high density low feeding rate group (**C_3**) and Biofloc low density low feeding rate group (**T_1**) respectively, (**Table 2** and **Fig. 2).**

Feed efficiency fluctuated between 0.9 ± 0.1 and 0.5 ± 0.06 at biofloc high density low feeding rate group (**T_3**) and control high density high feeding rate group (**C4**) respectively, (**Table 2).**

Input/Output ratio for Biofloc groups fluctuated between 2.3 ± 0.3 and 1.7± 0.3 at biofloc high density high feeding rate group (**T4**) and Biofloc high density low feeding rate group (**T_3**) respectively, (**Table 2).**

Table 2. Experimental parameters of *O. niloticus* reared with different stock densities and feeding rates either at Biofloc or control groups for 140 days.

Parameter	Density	Treatment			
		Control		Biofloc	
		FR 2%	FR 3%	FR 2%	FR 3%
Final body weight (g)	Low	156.3±16.8	171.5 ± 15.3	177.7±19.7	187.9 ± 20.7*
	High	144.6±22.7	168.3 ± 17.5	160.8±21.8	192.9 ± 20.0*
Total weight gain (g/fish)	Low	129.2 ± 16.4	142.6 ± 14.6	146.9 ± 16.4	155.2 ± 17.4
	High	113.8 ± 18.8	139.5 ± 14.8	132.2 ± 20.9	164.6 ± 17.2*
Specific growth rate (SGR)	Low	1.16 ± 0.07	1.27 ± 0.06	1.24 ± 0.06*	1.25 ± 0.07
	High	1.09 ± 0.10	1.26 ± 0.04	1.23 ± 0.09*	1.37 ± 0.06*
Survival rate (%)	Low	100.0 ±0	91.7± 0	100.0±0	100.0*
	High	93.8±0	87.5 ±6.25	93.8± 0	100.0*
Diet intake (g/fish)	Low	197.0	259.5	171.9*	219.4*
	High	207.4	250.7	159.1*	268.1*
Carbohydrate intake (g/fish)	Low	0	0	72.2	92.1
	High	0	0	66.8	112.6
Food Conversion Ratio (FCR)	Low	1.7 ± 0.2	1.8 ± 0.2	1.2 ± 0.1*	1.4 ± 0.2*
	High	1.8 ± 0.3	1.9 ± 0.2	1.2 ± 0.3*	1.6 ± 0.2*
Feed efficiency	Low	0.6 ± 0.08	0.6 ± 0.08	0.9 ± 0.1*	0.7 ± 0.08*
	High	0.6 ± 0.09	0.5 ± 0.06	0.8 ± 0.13*	0.6 ± 0.06*
Input/ Output ratio	Low	--	--	1.7	2.0
	High	--	--	1.7	2.3
Biomass yield (Kg/m^3)	Low	9.4±0.3	9.4±0.2	10.7 ± 0.5*	11.3 ± 0.6*
	High	10.8± 0.5*	11.8 ±0 0.4	12.1 ± 0.6*	15.4 ±0 0.4*
Yield percent of change (%)	Low	0	0	13.8%	20.2%
	High	0	0	12.0%	30.5%

FR 2%= Low Feeding Rate, FR 3%= High Feeding Rate, Low density= 60 fish/m^3, high density= 80 fish/m^3, *= significant at p value <0.05.

Biomass yield fluctuated between 15.4 ±0 0.4 and 9.4±0.2 Kg/m^3 at Biofloc high density high feeding rate group (**T$_4$**) and control low density high feeding rate group (**C$_2$**), respectively. Yield percent of change fluctuated between 30.5 and 12.0 % at Biofloc high density high feeding rate (**T$_4$**) and Biofloc high density low feeding rate (**T$_3$**) groups, respectively (**Table 2)**.

Feed conversion ratio, Feed efficiency and biomass yield in the different groups have statistically significant differences(P <0.05) between Biofloc groups and control groups at all cases (**Table, 2)**.

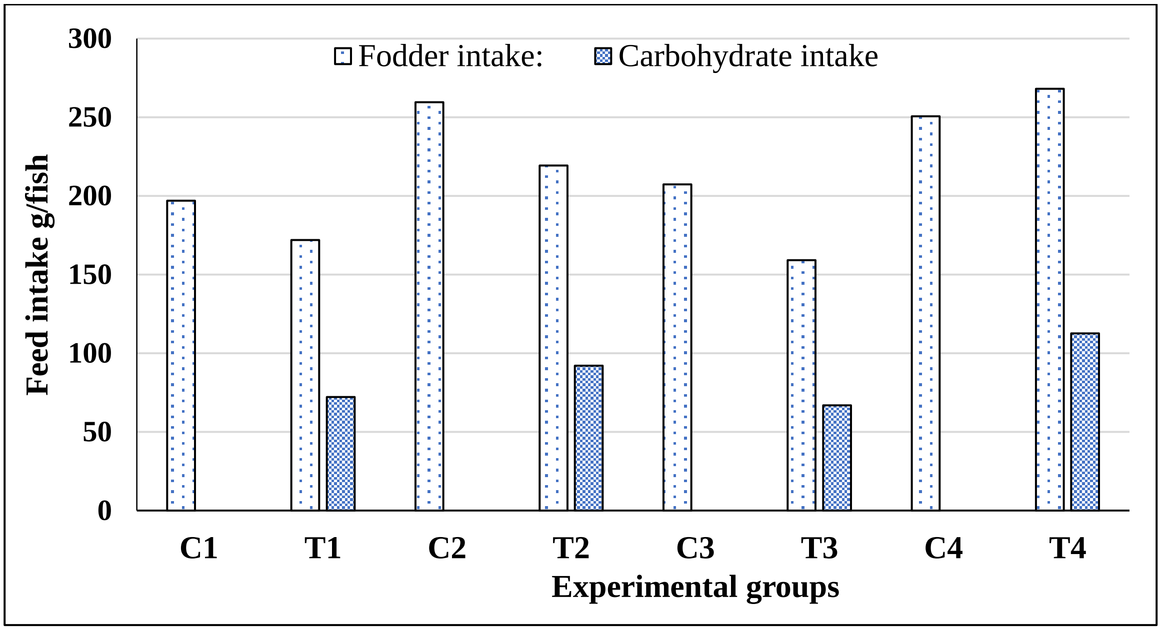

Figure 1. Feed intake by *O. niloticus* that reared at different densities and different feeding rates of Biofloc and control groups for 140 days.

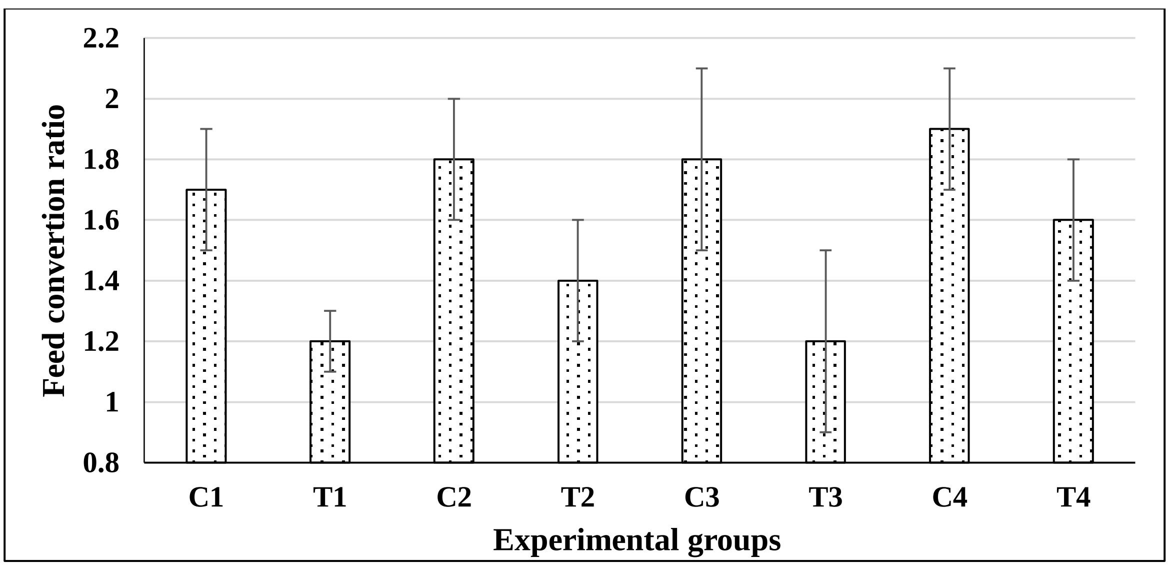

Figure 2. Feed conversion ratio of *O. niloticus* reared at different densities and different feeding rates of Biofloc and control groups for 140 days.

2. Protein parameters

Results in **Table (3)** showed that protein efficiency rate fluctuated between 2.8 ± 0.4 and 1.8 ± 0.2 for Biofloc high density low feeding rate group and control high density high feeding rate group, respectively. Protein productive values ranged between 100.4 and 62.9 for Biofloc high density low feeding rate group and control high density high feeding rate group respectively. Percent of PPV change between Biofloc vs control groups were 32.7% at low density low feeding rate (**T_1**), 19.7% at low density high feeding rate (**T_2**), 33.4% at high density low feeding rate (**T_3**) and 11.2% at high density high feeding rate (**T_4**).

3. Water consumption

Water consumption fluctuated between 1160 and 108 L/kg at control high density high feeding rate group and biofloc low density high feeding rate group (**T_2**) respectively **(Table 3** and **Fig. 3)**.

Table 3. Protein parameters and water consumption of *Oreochromis niloticus* reared at different groups for 140 days

Parameter	Density	Treatment			
		Control		Biofloc	
		2% FR	3% FR	2% FR	3% FR
Protein efficiency rate	Low	2.0 ± 0.3	1.9 ± 0.2	2.8 ± 0.3	2.3 ± 0.3
	High	1.9 ±0.3	1.8 ±0.2	2.8 ± 0.4	2.0 ± 0.2
Protein productive value	Low	67.6	64.2	100.3	83.8
	High	67.0	62.9	100.4	74.0
Participate of floc to PPV	Low	--	--	32.7%	19.7%
	High	--	--	33.4%	11.2%
Water consumption (L/Kg)	Low	1120	1166	125	131
	High	1120	1166	122	108

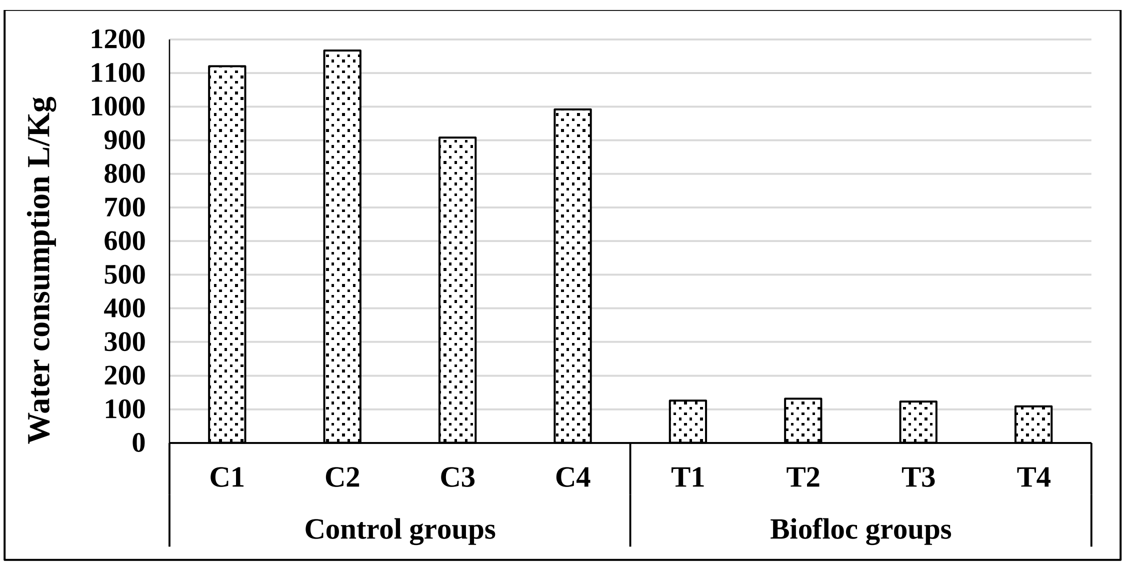

Figure 3. Water consumption of *O. niloticus* reared at different densities and different feeding rates of Biofloc and control groups for 140 days.

4. Economic evaluation:

By applying the results of growth and feed conversion ratio at the present study on production scale (Feddan composed of 3000 m^3 net water volume), we obtain the estimated results presented in **Table (4)**. Estimated number of fingerlings in feddan aquaculture as low density is 180 thousand fingerlings and in case of high density is 240 thousand fingerlings.

The maximum estimated feed intake in the present study was recorded at Biofloc high density high feeding rate (**T_4**), being 64.344 tons diet in addition of 27.024 tons carbohydrate. While, the minimum estimated feed intake was recorded at Biofloc low density low feeding rate (**T_1**), being 30.942 tons diet and 12.996 tons carbohydrate. Also, the estimated maximum total costs ($681.616*10^3$ LE) were recorded at Biofloc high density high feeding rate (**T_4**), and the minimum total costs ($346.424*10^3$ LE) were recorded at Biofloc low density low feeding rate (**T_1**).

The maximum total fish yield was recorded at Biofloc high density high feeding rate (**T_4**) being 46.3 tons. While the minimum fish yield was recorded at control low density low feeding rate (**C_1**), being 28.13 tons (**Table 4)**. Also, the maximum total fish price was recorded at Biofloc high density high feeding rate (**T_4**), being $914.346*10^3$ LE; while the minimum fish price was recorded at control low density low feeding rate (**C_1**), being $515.790*10^3$ L.E (**Table 4)**.

The highest total net income was $292.597*10^3$ LE that recorded at Biofloc high density low feeding rate (**T_3**); while the lowest total net income was recorded at control low density low feeding rate (**C_1**), being only $82.9*10^3$ LE. The highest net income ($282.3*10^3$ LE) for the high feeding rate (3%) with Biofloc treatments; while the low feeding rate (2%) without Biofloc led to the lowest net income, estimating by $127.9*10^3$ LE. The highest net income ($262.7*10^3$ LE) for the high stocking density ($240*10^3$ fish) with Biofloc treatments; while the low density ($180*10^3$ fish) without Biofloc led to the decreasing the net income to only $127.9*10^3$ LE. The results estimated that the total Biofloc treatments will give more net income, being 258.5*103 LE; but the total control experiments will give only $160.3*10^3$ LE **(Table 4** and **Figure 4)**.

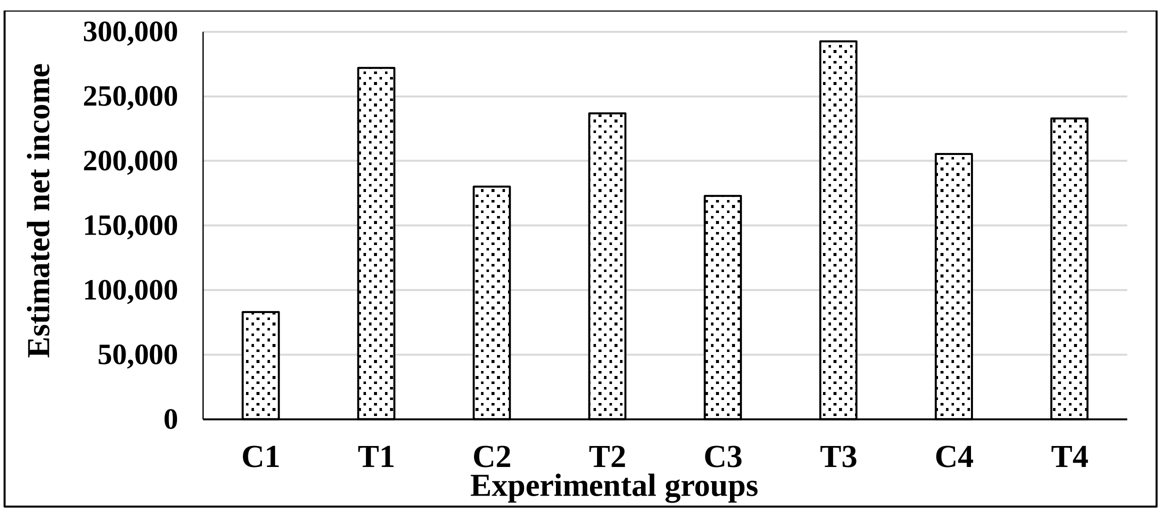

Figure 4. Estimated net income of Biofloc and control groups of the present study.

Table 4. Estimated costs and net interest of production-scale unit (Feddan with net volume of 300 m^3) depending on the present study results of fish production, density and feeding rate in addition, experimental diets at the marketable time

Assessment items		Control treatments				Biofloc treatments			
		Low density		High density		Low density		High density	
		FR 2% (C1)	FR 3% (C2)	FR 2% (C3)	FR 3% (C4)	FR 2% (T1)	FR 3% (T2)	FR 2% (T3)	FR 3% (T4)
Fingerlings (10^3 fish)		180	180	240	240	180	180	240	240
Total fingerlings cost (1000 LE)		**45**	**45**	**60**	**60**	**45**	**45**	**60**	**60**
Total diet consumed (tons)		37.33	45.13	47.28	62.28	30.94	39.49	38.18	64.34
Total diet cost (10^3 LE)		**294.9**	**356.5**	**373.5**	**492.0**	**244.43**	**311.97**	**301.62**	**508.29**
Carbohydrate consumed (ton)		0	0	0	0	12.99	16.59	16.04	27.02
Carbohydrate cost (10^3 LE)		**0**	**0**	**0**	**0**	**51.98**	**66.35**	**64.15**	**108.10**
Total feeding cost (10^3 LE)		**294.9**	**356.5**	**373.5**	**492.0**	**296.41**	**378.3**	**365.8**	**616.4**
Operating cost (10^3 LE)		**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Total costs (10^3 LE)		**432.9**	**406. 5**	**438.7**	**557.2**	**346.4**	**428.3**	**431.0**	**681.6**
Total fish wt. (g/fish)		156.3	171.5	144.6	168.3	177.7	187.9	160.8	192.9
Total fish yield (tons)		**28.13**	**30.87**	**34.70**	**40.39**	**31.99**	**33.82**	**38.59**	**46.30**
Fish grades (%)	I	25.0	58.3	12.5	43.8	66.7	83.3	43.8	87.5
	II	66.7	33.3	56.3	56.3	33.3	16.7	50.0	12.5
	III	8.3	8.3	31.3	0.0	0.0	0.0	6.3	0.0
Fish price (10^3 LE)	**I**	**140.65**	**359.94**	**86.75**	**353.82**	**426.75**	**563.44**	**338.05**	**810.25**
	II	**337.73**	**185.03**	**351.65**	**409.31**	**191.75**	**101.66**	**347.31**	**104.18**
	III	**37.36**	**41.00**	**173.78**	**0.00**	**0.00**	**0.00**	**38.90**	**0.00**
Total fish price (10^3 LE)		**515.8**	**586.5**	**611.7**	**762.4**	**618.4**	**665.2**	**723.6**	**914.4**
Net income (1000 LE)	**Total**	**82.9**	**180.0**	**173.0**	**205.2**	**272.0**	**236.8**	**292.6**	**232.7**
	Feed rate	**127.9**	**192.6**			**282.3**	**234.8**		
	Density	**131.5**		**189.1**		**254.4**		**262.7**	
	Biofloc	**160.3**				**258.5**			

Fingerlings price =250 LE/1000 fish; Diet price= 7900 LE/ton; Carbohydrate cost= 4 LE/Kg; Feeding cost = Diet cost + carbohydrate cost; Total costs = Fingerlings cost+ Feeding cost+ operation costs (workers, electricity, transport, etc...); Price list of fish grades (LE/kg): I=20LE, II=18 LEand III=16 LE.

DISCUSSION

Intensification of aquaculture became necessary to increase productivity due to agriculture aquaculture competition for land and water. High-density culture in intensive systems requires high amounts of feed to be added to the systems. This will cause water quality deterioration **(Avnimelech, 2007).** Biofloc technology (BFT) system considered as a friendly environmental strategy to establish a near to zero water exchange culture system while providing potentially consumable biomass to the cultured animal **(Bossier & Ekasari, 2017**). This operates on the principle of increasing carbon to nitrogen ratios, through the addition of an exogenous carbon source that consequently stimulates natural heterotrophic bacterial growth in the system **(Hargreaves, 2006** and **De-Schryver. *et al*., 2008).**

The present study aimed to expect the optimum level of stocking density and feeding rate of tilapia reared at Biofloc condition vs clear water to achieve maximum economic benefit. In addition, focusing on growth parameters, protein utilization and estimate economic profit of feddan production scale build upon the results of this study. In the present work, all growth parameters were significantly increased in Biofloc high density tanks (T3 and T4) this may be due to participate of floc aggregates as an additional source of feed**. Azim & Little (2008); Luo *et al*. (2014)** and **Long *et al*. (2015)** reported the same results for fish growth and feed utilization parameters. **Burford *et al*. (2003)** found that over 29% of the daily food consumed by *litopenaeus vannamei* could be biofloc. Only Feed density without regarded to feeding rate are significant in the present work this may be attributed to optimum aggregates of floc particles at high density groups. The same results were obtained by **Zaki *et al*. (2020)** who mentioned that the surface of BF and its particle size increase the surface area required for bacterial growth to increase the produced BF at high density treatments.

The present work revealed low FCR values obtained in the Biofloc treatments in comparison with control clear water groups, this may be due to the availability of nutritionally rich food. The same results were obtained by **Azim & Little (2008); Luo *et al*., (2014); Verma *et al*. (2016)** and **Zhang *et al*., (2016).** They mentioned the high nutrition values and probiotic effect of Biofloc that improve the digestion.

Feed efficiency, protein efficiency rate and protein productivity value in the different groups of Biofloc are statistically significant ($P < 0.05$) vs control groups at the present work, this due to floc aggregates that increase protein productivity by 32.7%, 19.7%, 33.4% and 11.2% for T_1, T_2, T_3 and T_4. **Azim & Little (2008)** reported that the BF system can increase the fish total production by around 45 % over the normal aquaculture systems. While **Zaki *et al.*. (2020)** explained low growth at 60 fish/m^3 as tilapia is a regional and aggressive fish competitive for food as a result of overcrowding **(Zhang *et al*., 2016).**

In the present work, survival rate of Biofloc groups were significantly differ at all cases except at T_3 this may be due to optimized environmental condition at Biofloc treatments. The same results were noticed by **Cohen *et al*. (2005), Mishra *et al*. (2008)** and **Asaduzzaman *et al*. (2009)** in fish larvae reared at Biofloc conditions. **El-Shafiey *et al*. (2018)** reported non-significant effect between glucose, sarch, molasses and cellulose as a carbon source of Nile Tilapia culturing. **De-lima *et al*. (2018)** noticed non-significant between 0, 4, 8 and 16 g/L salinity on survival of *Oreochromis niloticus*. **Da-Silva *et al.* (2018)** revealed non significant effect of crude protein percent in diets in two growth stages of Nile tilapia (*Oreochromis niloticus*) in a Biofloc system. **Fleckenstein *et al.* (2019)** reported significant survival increase of *Litopenaeus vannamei* post-larvae that reared at biofloc condition but supplemental lights negatively affect.

The present work revealed that there was statistically significant difference ($P < 0.05$) between Biofloc groups and control groups at all protein's parameters and feed efficiency. Biofloc aggregates were increase protein productivity by 32.7%, 19.7%, 33.4% and 11.2% for T_1, T_2, T_3 and T_4 , this may be due to high nutritive value of these aggregates that 24h in situ present as an additional feeding source. The same results were noticed by **Tacon *et al.* (2002)** who observed that the microbial protein, aggregated in microbial flocs serves as a rich source of amino acids and growth factors for fish leading to significant recycling of protein and higher utilization of feed.

This study revealed that the Biofloc increase net income at all cases. It increases Low density low feed treatment (T_1) with about 81.6%, low density high feed (T_2) with 65.7%, High density low feed (T_3) with 83.3% and high-density high feed (T_4) with 34.4% , this attributed to optimized water quality, 24h in situ floc aggregates with high nutritive value present as additional food source. This agreed with that obtained by **Pérez-Fuentes *et al.* (2018)** who suggested that the Biofloc could reduce the productive costs derived from feed consumption by at least 10%.

CONCLUSION

Biofloc improved growth performance and protein utilization that led to increase net income at all cases. BFT increases Low density low feed treatment (T_1) with about 81.6%, low density high feed (T_2) with 65.7%, High density low feed (T_3) with 83.3% and high-density high feed (T_4) with 34.4% . Biofloc technology provide 85% of water consumption vs control clear water groups, so it is a comprehensive promising technology suitable for aquaculture away from water bodies.

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