Effect of Protein Levels on Growth Performance, Feed Utilization and Economic Evaluation of Fingerlings Nile Tilapia Fingerlings under Biofloc System

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Abstract: The current experiment was designed to test the effect of crude protein content (19, 25 and 30% C.P.) on growth performance of monosex Nile tilapia (*Oreochromis niloticus*) fingerlings on clear and biofloc system. Three dietary protein levels (19, 25 and 30% CP) were used under biofloc system (no water exchange) in comparison with clear system three replicates for each treatment. The experiment was lasted for 84 days. The daily ration was 5% of the total stocked biomass divided into two equal amounts and offered two times a day (8.00 and 12.00). Fish in each treatment was weighed every 15 days and the amount of the daily allowance feed was readjusted accordingly. The C: N ratio was maintained at 15: 1 by daily addition of rice bean as an organic source of carbon. For biofloc treatments, rice bran was activated heterotrophic bacteria growth. Rice bran was dissolved in water at dry feed to molasses ratio of 1:1 on a daily basis in order to develop biofloc and nourish heterotrophic bacteria. The highest final body weight (FBW), weight gain (WG) and specific growth rate (SGR) values were recorded for fish fed 30% (DP). Biofloc system (BS) showed superiority over clear system (CS) for FBW, WG and SGR values. It could be concluded that 30% crude protein was the best in terms of growth performance, feed utilization and economic evaluation for monosex Nile tilapia fingerlings under this experimental conditions.

Keywords: Protein levels - monosex - Nile tilapia - Biofloc system - Growth

INTRODUCTION

Dietary protein is the most expensive ingredient in fish feed which is required to sustain normal growth of aquatic animals. In aquaculture feed accounts 40-60% of the production costs, with protein sources a significant proportion of this cost (Fotedar, 2004).

The use of rich protein diets above optimum levels, increases operating costs required to raise fish. Optimum protein levels in biofloc systems should be evaluated in terms of growth and feed performance as well as water quality parameters in culture units. Optimum levels of dietary protein not only reduce production costs, but also ameliorate water quality and fish growth conditions. Protein is main major dietary nutrient affecting performance of fish (Lovell, 1989). It provides the essential and non-essential amino acids which is necessary for muscle formation and enzymatic function and in part provides energy for maintenance (Yang *et al*., 2002). It is important to minimize the amount of protein used for energy, because protein is usually the most expensive constituents in the diet. Excess protein in fish diet may be wasteful and cause diets to be unnecessarily expensive (Ahmad, 2000). The protein requirement of Nile tilapia was estimated to be from 25% to 45% of diet (Swick, 2001). Monosex (males) Nile tilapia have been widely cultured in Egypt. Males are used for monosex culture because male tilapia grows faster than females (Bahnasawy, 2009).

It has been described that biofloc intake enhances the growth performance of aquatic organisms (Luo *et al*., 2014). However, there is little information on the recommended crude protein levels when using biofloc technology (BFT). For this reason, there is wide variation in the crude protein (CP) concentrations of Nile tilapia feeds used in studies with BFT. In the literature, crude protein in Nile tilapia juvenile represent 30%, with a range between 20-40% of the diet (Mansour and Esteban, 2017). However, there is little information on the recommended crude protein levels when using BFT. For this reason the present study aimed to investigate the effect of protein levels on growth performance, feed utilization and economical evaluation of fingerlings monosex Nile tilapia under clear and biofloc system.

MATERIALS AND METHODS

The present study was carried out at the experimental fish laboratory of Suez National Institute of Oceanography and Fisheries (NIOF), experiments were designed to illustrate effect of biofloc and protein levels on growth response, feed utilization and body composition of mono-sex tilapia fingerlings.

Experimental fish and Culture techniques

Nile tilapia, *O. niloticus* fingerlings with an initial body weight of 8.00±0.05 g were obtained from the National Institute of Oceanography and Fisheries (NIOF). Prior to the start of the experiment, fish was acclimatized to laboratory conditions for two weeks. The fingerlings were stocked into 18 cylindrical fiberglass tanks (with water capacity of 500 Leach) at a rate of 50 fish tank^{-1}, representing six experimental treatments in triplicate. The tanks were supplied with well water source. Aeration was continuously provided using an air blower.

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In the tanks representing the control treatments (clear system); water was exchanged biweekly, while for experimental BFT tanks, no water exchange was done (zero water exchange) except for the added water to compensate the evaporation.

The dietary protein levels (19, 25 and 30% CP) under clear and biofloc system. The experiment lasted for 84 days; the fish was fed different experimental diets (19, 25 and 30%) under clear and biofloc systems. The daily ration of food was 5% of the total stocked biomass divided into two equal amounts and offered two times a day (8.00 and 12.00). Fish in each replicate aquarium was weighed every 15 days and the amount of the daily allowance feed was readjusted accordingly.

Experimental design:

Feeding system	**% Crude protein**		
	19%	**25%**	**30%**
Clear system(CS)	T1	T3	T5
Biofloc system (BS)	T2	T4	T6

The C: N ratio was maintained at 15: 1 by daily addition of rice bean as an organic source of carbon. For biofloc treatments, rice bran was activated heterotrophic bacteria growth. For biofloc tanks, only a proper amount of fresh water was supplied to compensate water losses due to evaporation without any water exchange, whereas the control tanks were renewed weekly. At the beginning of the experiment, all tanks were filled with fresh water. No organic carbon was added to the tanks of the clear water groups, in which a daily water exchange rate of 20% was applied. For their part, each of the tanks containing the fish exposed to the BFT treatments was inoculated with 100 ml of concentrated biofloc from old biofloc tanks on the first day of the experiment. Furthermore, the BFT tanks were supplied daily with rice brane as a carbon source, two hours after feeding to maintain the C: N ratio of 15:1.

Biofloc tanks preparation (stock / fermentation):

C : N ratio calculations are presented as followed. For examples, the carbon content of the feed will be considered 50% (based on dry matter). For the carbon source, rice bran was chosen and its content in such case is also 50%. In a practical way, dry matter of the feed will be 90%. Fish assimilation will be considered 25%. It was found that fish or shrimp in a pond (Avnimelech and Lacher, 1979; Boyd, 1990; Muthuwani and Lin, 1995) assimilate only about 25% of the nitrogen added in the feed (Avnimelech, 1999).

Calculation C in the feed:

1000 g feed × 0.9 (90% dry matter) × 0.7 (30% of fish assimilation or 70% of waste that remains in water)/2 (carbon content of the feed is ~50% based on dry matter) = 315 g of C.

Calculation N in the water

1000 g of feed × 0.9 (90% dry matter) × 0.7 (30% of fish assimilation or 70% of waste that remains in water) × 0.3 (30% crude protein content of feed) ×0.16 (constant) = 30.24 g of N.

Adjusting the C : N ratio (15:1)

If I want a C: N ratio of 15:1, 30.24 g of N in feed × 15 = I need 453.6 g of C. But I already have 315 g of C (calculated in feed). So 453.6 g–315 g of C = I really need 138.6 g of C.

If the rice bran has 50% of carbon content (based on dry matter), 1 kg of rice bran represents 500 g of carbon. So 138.6 g of carbon requirement will represent 277.2 g of rice bran.

The ingredients used in preparing the fermentation were 1000 g (30% crude protein content of feed), 15 g baker's yeast and carbohydrate sources 277.2 g of rice bran. No carbohydrate source was added to the control group. All the ingredients were dissolved with 25 L of fish water, and aerated for 20 days until the aroma was odorless and flocs were formatted. After 20 days the floc was distributed to each aquarium as much as 2 liter and allowed to stand with continuous aeration. The fingerlings were stocked after seeding the tanks with flocs (Adipu *et al.,* 2019). Carbon sources were completely mixed with water sample of the experimental tanks prior spreading into the whole water body.

Because the growth rate of heterotrophic bacteria is so much greater than that of nitrifying bacteria, ammonia control through immobilization by heterotrophic bacteria occurs rapidly, usually within hours or days if a sufficient quantity of simple organic carbon (*e.g.*, sugar or starch) is added (Hargreaves, 2013).

Aeration was continuously provided using an air blower. More oxygen will be needed to support the respiratory demands of a greater bacterial load, and additional energy is needed to keep solids in suspension. High rates of water respiration (oxygen consumption) reduce response time in the event of system failure. Tanks were always covered with black plastic sheets. Water in the tanks never exchanged during the experimental period except for compensate for evaporated.

Biofloc meal precipitation

Imhoff cone was used daily to monitor the developing of biofloc. Imhoff or settling cones are a simple way to index suspended solids concentration. The cones have marked graduations on the outside that can be used to measure the volume of solids that settle from 1 liter of system water. The interval of time should be standardized and convenient, usually 10 to 20 minutes. Solids also can be measured with a turbidity meter. Maintaining a settleable solids concentration of 25 to 50 mL/L will provide good functionality in biofloc systems for tilapia (Hargreaves, 2013).

Experimental diets

All feed-grade ingredients including soybean meal, yellow corn, Rice bran, corn oil and starch were already purchased from local markets.

Experimental diets were processed by blending the dry ingredients into a homogenous mixture, and then the mixture feed was passed through mill. The experimental diet, were then solar dried and stored.

Dietary gross energy (GE) contents were calculated according to gross caloric values of using the values of 5.6, 9.5 and 4.1 cal g-1 for crude protein, crude fat, and total carbohydrate, respectively, according to (NRC, 1993). Table (1) showed the proximate composition of ingredients used in the Experimental diet.

Growth parameters

Growth parameters were calculated using the following equations:

WG = Final body weight (g) - Initial body weight (g)

SGR% = (ln FBW - ln IBW) /t ×100; where: FBW is final body weight (g); IBW is initial body weight (g); ln = natural logarithmic; t = time in days

FCR = diet intake (g) /weight gain (g)

PER = weight gain (g) /protein intake (g)

Assessment of water quality parameters

Water quality parameters, such as temperature, pH, ammonia, Nitrite and Nitrate were monitored to follow the effect of biofloc system in comparison with clear system30 cm water depth. Water temperature and dissolved oxygen was measured using a portable oxygen meter (HI9164 DO meter). pH was detected by sunflower paper test. Ammonia nitrogen (TAN), nitrite-nitrogen and nitrate-nitrogen using analytical kits (Lovebird®, Multidirect, co 210070 England.

Table (1): The proximate composition of ingredients used in the Experimental diet

Chemical composition / Ingredients	Dry mater	Protein %	Lipid %	Ash %	CF %	NFE %
Soya bean meal	90.00	44.00	1.50	5.9	6.30	42.3
Yellow corn	90.85	7.70	3.80	0.86	2,30	85.34
Rice bran	86.2	12.8	13.7	15.7	29.0	28.8

Chemical analysis

At the beginning and the end of the each trial, a random pooled sample of fish was collected and sacrificed for determination of initial whole-body proximate composition. Fish sample were oven-dried, ground, and stored at -20ºC for subsequent analysis. The chemical composition of fish and diet samples were determined according to the procedures of AOAC (2005). Dry matter was determined after drying the samples in an oven (105ºC) for 24 h. Ash by incineration at 550ºC for 12 h. Crude protein was determined by micro-Kjeldhal method, % N × 6.25 (using Kjeltechautoanalyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Soxhlet extraction with diethyl ether (40-60ºC). Table (2) presents the formulation and chemical composition of the experimental diets.

Table (2): Formulation and chemical composition of the basal diet

Ingredients	Protein Levels %		
	19	25	30
Soya bean meal	25	43	57
Yellow corn	20	20	15
Rice bran	45	27	18
Soya oil	6	6	6
Vit. and Min. premix[1]	3	3	3
CMC (carboxy methyl cellulose)	1	1	1
Proximate Analysis			
Dry matter (%)	88	88.97	87.8
Protein (%)	19.34	25	30.08
Lipid %	12.35	11.65	10.49
Total carbohydrate (%)	46.35	45.20	44.71
Ash %	3.60	4.87	5.69
Gross energy (Kcal /100g)	416.477	437.11	452.84

1. Each Kg vitamin & mineral mixture premix contained Vitamin D3, 0.8 million IU; A, 4.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg, Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg,
2. Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11Kcal/g), according to (NRC, 1993).

Statistical analysis

All variables measured at harvest (fish growth performance and feed utilization) and various components in the nitrogen budgets were analyzed by two-way ANOVA to determine the effect of dietary protein level, culture technique and their interaction (EXPI). All the ANOVA were performed using the SAS v9.0.0 (2004) program. The ANOVA was followed by Duncan test (1955) at P < 0.05 level of significant. The data were statistically analyzed by 2x3 factorial designs according to the following model:

Y**ijk**= μ + C**i** + P**j** + CP**ij** + **eijk**

Where:

μis the overall mean,

Ciis the fixed effect of culture technique (i = 1 and 2),

Pjis the fixed effect of protein levels (j = 1, 2 and 3),

CPijis the interaction between effect of culture technique and protein levels and

eijkis random error.

RESULTS AND DISCUSSION

Water quality Parameters

Table (3) present water quality criteria of monosex tilapia as affected by protein levels which reared in tanks under clear and biofloc system. As apparent difference in water quality parameters were found among treatments; the data for each experiment for the whole experimental period were pooled. Water quality was found to be within the acceptable range for tilapia growth (Crab *et al.*, 2009). Temperature and pH concentration did not differ significantly among treatments, while the increase in protein levels resulted in lower dissolved oxygen and an increase in TAN, NH_2 and NH_3 with biofloc (Table 3). Generally, the average water temperature during the experimental period ranged between 25.54 and 26.60°C, while pH ranged from 7.00 to 8.00, respectively. Dissolved oxygen 6.52±0.4 and 7.48 mg^{-1}, NO_2 0.11± 0.01 to 0.25±0.01 NO3 10.33±0.10 to 13.83±0.10 mg^{-1} measured during the study period were all within the optimum range for rearing *O. niloticus* (Marcos *et al.*, 2018).

Table (3): Effect of protein levels on average* physico-chemical parameters of water quality (Mean ± SE) of monosex of Nile tilapia (*O. niloticus*) fingerlings under clear and biofloc system throughout the experimental period (84 days)

Items	**Protein levels %**					
	19		**25**		**30**	
	C.S	**B.S**	**C.S**	**B.S**	**C.S**	**B.S**
DO	7.48±1.10	6.52±1.10	7.44±1.10	6.52±1.10	7.39±1.10	6.63±1.10
Temp. °C	25.54±1.20	25.85±1.01	26.66±1.0	26.68±1.10	26.40±1.10	26.50±1.10
pH	7.00±1.10	8.00±0.50	7.00±0.50	8.00±0.50	7.00±0.50	8.00±0.50
TAN mg^{-1}	$0.43±0.10^d$	$0.35±0.10^e$	$0.56±0.10^b$	$0.41±0.10^d$	$0.60±0.10^a$	$0.45±0.10^c$
NO_2 mg^{-1}	$0.33±0.10^a$	$0.11±0.10^f$	$0.22±0.10^c$	$0.13±0.10^e$	$0.25±0.10^b$	$0.15±0.10^d$
NO_3mg^{-1}	$11.3±1.10^b$	$10.8±1.10^c$	$11.8±0.10^b$	$10.33±0.10^c$	$13.83±0.10^a$	$11.6±0.10^b$

* Means in the same row having the same superscript letter are not significantly different (P<0.05)

Under biofloc (no water exchange), addition of carbohydrate kept water quality (TAN and NO_2) within the normal ranges as (CS) where, water in (CS) was biweekly exchanged. There are three principal pathways to remove hazardous N species in aquaculture: (1) photoautotrophic removal by algae, (2) immobilization by heterotrophic bacteria as protein acetous microbial biomass and (3) chemo-autotrophic oxidation to nitrate by nitrifying bacteria (Ebeling *et al.,* 2006). When the C/N ratio of feed is adjusted to about 20 by adding rice bran, the water quality could be controlled by the bioflocs growing in the tanks. At this C/N ratio, the inorganic nitrogen was converted into organic nitrogen, due to assimilation by the dense floc cultures (heterotrophic bacteria) (Crab *et al.*, 2009). This corresponds with the theoretical predictions made by Avnimelech (1999). Carbohydrate addition to the water column under extensive shrimp culture conditions reduced the concentration of both TAN and NO_2. Meanwhile nitrogen discharge was reduced also making extensive shrimp farming more ecologically sustainable and economically viable, by high rates of nitrification, indicated by constant nitrate accumulation coupled with ammonia immobilization into bacteria (Azim *et al.,* 2007). Similar results were suggested by Crab *et al.* (2009) for tilapia fish. Additionally, Kuhn *et al.* (2009) reported that no differences were observed between any water quality parameters, by confirming system uniformity. Water quality levels were within safe levels for normal fish health, growth, and survival under biofloc system.

Temperature is suitable for all chemical, physical and biological processes in ponds water as cited by Boyd (1979). The results indicate that there are significant differences (p<0.05) in water quality, TAN, DO, and pH due to different culture system types (CS and BFT) without any significant effect on water temperature. Total ammonia nitrogen (TAN) values used to calculate unionized ammonia NH_3 changed numerically within the normal range (0.35-0.15 mg^{-1})

for both (CS) and (BFT). In the same context normal nitrite (NO_2) concentration (0.42-0.45 mg^{-1}) was observed for both CS and BFT during the experimental period. The weekly data for NO_2 showed numerically decrease for BFT tanks compared to CS. The result of nitrate (NO_3) was noticed at the normal range (5-9 mg^{-1}) for both BFT system and CS system during the experimental period. These values were beneficial for fish culture (Boyd, 1979 and 1990). Under biofloc system BFT (zero exchange water), addition of rice bean as a carbohydrate source kept water quality (TAN and No_2) within the normal ranges as change water system (CS) where ,water in (CS) were daily changed.

Effect of protein levels on growth performance of monosex Nile Tilapia fingerlings under clear and biofloc system

Growth performances of Nile tilapia fed three dietary protein (DP) levels 19, 25% and 30% under clear (CS) or biofloc system (BS) are presented in Table (4). The two-factorial analysis of variance indicates overall significant effects of dietary protein levels and management conditions (CS, BS) effects of dietary protein levels and management conditions (CS, BS) ($p<0.05$). The highest final body weight (FBW), weight gain (WG) and specific growth rate (SGR) values recorded for fish fed 30% (DP). Biofloc system (BS) showed superiority over clear system (CS) for FBW, WG and SGR values. Regards interaction results, fish fed 30% DP under biofoc system noticed for the highest FBW, WG and SGR, while the lowest results noticed for 19% CP under clear system (CS). Generally, growth parameters improved under biofloc system. It could assume that rice bran addition to biofloc tanks activate growth of bacterial floc and algae, which in turn act as secondary protein source for fish under those treatments. These results are in agreement with the finding of Burford *et al.* (2003) who suggested that adding starch helps to develop and control of dense heterotrophic microbial bioflocs in the water column. Carbohydrate addition leads to elevate the C/N ratio, which helps to convert inorganic nitrogen into organic nitrogen as dense floc. That cause doubling of protein utilization (Avnimelech *et al*., 2008). Azim *et al.* (2007) concluded that growth performance for Nile tilapia in biofloc system improved and it contributed 43% of growth compared with system without BFT. Dan and Little (2000) found that, SGR of Nile tilapia was significantly increased as diet protein level increased from 25-45% (with increment of 5%). Also, Ogunji and Wirth (2002) with the same fish species found that, SGR increased with increasing dietary protein level from 7.3 to 44.24%.

These results revealed that, increasing dietary protein level for tilapia *O. niloticus* significantly ($P<0.05$) increased body weight (El-Saidy and Gaber, 2005). The optimum protein requirement for tilapia has been determined by several investigators and the results are not consistent. For instance, estimates of 30% (Wang *et al*. 1985), 32% (Shiau *et al.,* 1987), 29-38% (Cruz and Laudencia, 1976), 30-35% (Mazid *et al*., 1979), 36% (Davis and Stickney, 1979) and 40% (Jauncey, 1982) have been reported. In the present study, the growth performance had a significant increase ($P<0.05$) with increasing dietary protein levels.

Table (4): Effect of protein levels on growth performance, feed utilization and survival rate of monosex Nile tilapia (*O. niloticus*) fingerlings under biofloc and clear water throughout the experimental period (84 days)

Items	19%		25%		30%	
	T1 (C.S)	T2 (B.S)	T3 (C.S)	T4 (B.S)	T5 (C.S)	T (B.S)
Initial weight g	8.00 ± 0.10	8.00 ± 0.10	8.00 ± 0.10	8.00 ± 0.10	8.00 ± 0.10	8.00 ± 0.10
Final weight g	25.79±0.20	28.11±0.20^{e}	31.75±0.20^{d}	34.47±0.20^{c}	40.25±0.20^{b}	43.97±0.20^{a}
WG(g)	17.79±0.10^{f}	20.11±0.10^{e}	23.75±0.10^{d}	26.47±0.10^{c}	32.25±0.10b	35.47±0.10^{a}
WG %	222.37±1.20^{f}	251.37±1.20^{e}	296.87±1.20^{d}	330.87±1.20^{c}	403. 13±1.20^{b}	443.37±1.2^{a}
SGR	1.39±0.10^{f}	1.49±0.10^{e}	1.64±0.10^{d}	1.73±0.10^{c}	1.92±0.10^{b}	2.02±0.10^{a}
Survival rate%	78.00±1.00^{e}	80.00±1.00^{e}	85.00±1.00^{d}	86.00±1.00^{c}	92.00±1.00^{b}	94.00±1.00^{a}

* Means in the same row having the same superscript letter are not significantly different ($P<0.05$)

In many fish including tilapia, it has been reported that the protein requirement of fish decreased with increasing size and age of fish Wilson (1989). Considerable variation has been reported in the optimum dietary protein requirement for maximum growth. This variation could be the result of different experimental conditions, which include species, size and age of fish, stocking density, protein quality and environmental conditions, particularly temperature, all of which influence the dietary protein requirement in tilapia (Jauncey and Ross, 1982) and in other fish species (Wilson, 1989).

In the same context, Megahed (2010) reported that *Penaeus semisulcatus* fed dietary protein 16.25% with bioflocs could even show better growth rate than shrimp fed 42.95% CP without bioflocs. The development and regeneration of the bioflocs in the culture tanks can recycle residual feeds and associated wastes, resulting in the recycling and reutilization of feed nutrients and eventually improving overall feed assimilation, especially under zero exchange water (Avnimelech, 2006, 2007). Similar results were suggested by Zhao *et al.* (2012) who reported that the bioflocs technology significantly increased the individual shrimp weight at harvest. Similar results were obtained by Azim and Little (2008) and Mishra *et al.* (2008). Contrastively, some other studies suggest that biofloc led to the level of production well below commercially viable levels (Little *et al.,* 2008). Same phenomena was confirmed for shrimp as Xu *et al.* (2012) reported that shrimp in both dietary protein level 30% and 35% under (BFT) gained better growth performance (in terms of final weight, weight gain and specific growth rate) than that in the control (under clear system) ($P<0.05$). Wasielesky *et al.* (2006) demonstrated that, shrimp (*L. vannamei*) juveniles grown in a bioflocs-based system had higher growth rates compared to juveniles grown in a clear water system. Generally the group of monosex Nile tilapia fingerlings on biofloc system had as significantly highest ($P<0.05$) weight gain SGR, FE and PER than the group of fish on clear water system. These results are in agreement with Azim and Little (2008) and Xu *et al.* (2012).

The interaction between protein levels and biofloc system was significant ($P<0.05$). In agreement with Azim and Little (2008) and Xu *et al*. (2012). There were a significant ($P<0.05$) differences in survival rate between experiment group (Table 4). The lower survival rate (78%) in the control of T1 (19% CP) and the highest survival rate was found (96%) in T6 biofloc 30% crude protein. It is possible that survival is related to minimum CP requirements, as optimum CP levels found for final weight and daily weight gain. Biofloc has been proving itself to be an option to control mortality in aquaculture (Ekasari *et al.*, 2015; Perez-Fuentes *et al.,* 2016).

Feed utilization.

Feed utilization of different treatment is presented in Table (5). Regards of dietary protein, tilapia fed 30% CP showed the highest values for Feed intake (FI), the best feed conversation ratio (FCR). While the highest protein efficiency ratio (PER) noticed for tilapia fed (T2) 20% protein. Regards the interaction, tilapia fed 30% CP under biofloc system recorded the highest feed intake, the best FCR values. With increasing dietary protein under biofloc system a decrease in PER values were noticed. Despite that increasing dietary protein causes significant improvement in tilapia weight gain, PER results suggested that protein intake did not efficiently utilized for both 25 and 30% diets under clear and biofloc system.

Table (5): Effect of protein levels on growth performance, feed utilization and survival rat of mono Nile tilapia (*O. niloticus*) fingerlings under clear and biofloc system throughout the experimental period (84 days)

Items	19%		25%		30%	
	C.S	B.S	C.S	B.S	C.S	B.S
Feed intake	42.7±1.10^{d}	37.74±1.10^{e}	47.92±1.10^{c}	42.92±1.10^{d}	55.45±1.10^{a}	50.45±1.10^{b}
FCR	2.40±0.10^{a}	1.87±0.10^{c}	2.01±0.10^{b}	1.62±0.10^{e}	1.71±0.10^{d}	1.42±0.10^{f}
FE	0.41±0.10^{f}	0.53±0.10^{d}	0.49±0.10^{e}	0.61±0.10^{b}	0.58±0.10^{c}	0.70±0.10^{a}
PER	2.19±0.20^{d}	2.80±0.20^{a}	1.98±0.20^{e}	2.46±0.20^{b}	1.93±0.20^{f}	2.34±0.20^{c}

* Data are presented as means ± standard error (SE)
** Means followed by different letters in each row are significantly ($P<0.05$) different

Otherwise, biofloc system improved tilapia feed utilization compared with clear system. Avnimelech *et al*. (1995) estimated that feed utilization is higher under biofloc system. For Tilapia better values for feed conversion ratio, protein efficiency ratio and protein productive value were observed in all the bioflocs treatments compared to those treatments without biofloc (Avnimelech, 2007). Same trend was noticed for tilapia feed utilization under biofloc system by Azim and Little (2008). These observations were suggested also for shrimps by Zhao *et al*. (2012) who reported that the biofloc treatment for shrimp resulted in 12.0% higher protein efficiency ratio, and 7.22% lower feed conversion rate comparing with treatments without biofloc. Also, Hari *et al*. (2006) reported that shrimp in treatments supplemented with dietary protein 25% and carbohydrate recorded lower FCR and highest PER ($P \leq 0.05$) than treatment without carbohydrate supplementation. Similar result was suggested by Xu *et al*. (2012) who showed that the feed conversion ratio of the shrimp in dietary protein level 35% under (BFT) was significantly lower than that in the control (treatment without biofloc) ($P<0.05$). Additionally, Hari *et al.* (2004) demonstrated that carbohydrate addition in extensive shrimp ponds improved the nitrogen retention efficiency and had a positive effect on production. Under biofloc system addition of carbohydrate enhances the total heterotrophic bacteria in the pond, which in turn result further 35 reductions in inorganic nitrogen (Wahab *et al.,* 2003). Low toxic inorganic nitrogen levels and utilization of microbial cells are demonstrated to be an effective potential food source for tilapia and shrimp (Burford *et al*., 2003, 2004; Avnimelech, 2007). Furthermore, lower ammonia nitrogen in the sediment positively influenced the food intake and health of the shrimps (Avnimelech and Ritvo, 2003). Another reason for the improvement of feed utilization under biofoc system is that the increased activities of digestive proteinases indicated enhanced digestive capabilities of the feed (Xu *et al*., 2012). As a massive number of live microorganisms existed in the bioflocs, they could transit through the stomach into the intestine and interfere with resident intestinal micro flora balance which plays an important role in the production or secretion of digestive enzymes (Matos *et al*., 2006; Xu *et al*., 2012). In contrast with our result, the FCR was poor (3.51 and 3.44) for tilapia fed on dietary protein 35% and 24% under biofloc system respectively (Azim and Little, 2008). The interaction between protein levels and biofloc system was significant ($P<0.05$). These are in agreement with Avnimelech (2007) and Azim and Little (2008).

Economic Evaluation

Table (6) Present economical evaluation of protein levels of mono and mixed sex of Nile tilapia (*O. niloticus*) fingerlings under biofloc and clear water system throughout the experimental period (84 days). It is well known that feeding cost in fish production is about 50% and more of the total production costs as declared by. Under the present experimental condition, all other costs are constant; therefore, the feeding cost to produce one kilogram of fresh body weight could be used as a measure to compare between the tested diets. It is expected that, future of aquaculture developments will be in the form of semi-intensive or intensive culture systems which and these require appreciable inputs of fertilizers and/or artificial feeds.

Calculations of economic efficiency of the tested diets based on the cost of feed, cost of one Kg gain in weight and its ratio with the highest group T6 are shown in Table (5) because the other costs were equal for all studied treatments.

The results of the current study demonstrated that the biofloc system is more economical in aquaculture in terms of water consumption needed to produce fish under limited water availability. When fingerlings of Nile tilapia were reared in the biofloc tanks, the amount of daily feed inputs can be reduced without affecting production costs, indicating that biofloc could contribute to the nutrition and physiological health of Nile tilapia. Feed costs for producing one kilogram of Nile tilapia were better for the 30% treatment (13.49 L.E./kg). Feed costs required to produce one kilogram of fish were higher in 30% crude protein (T5 and T6) treatment. The high feed cost primarily resulted from the high cost of higher protein content as well as the inferior PER ratio obtained when protein input was reduced.

Table (6): Economical evaluation of protein levels of monosex of Nile tilapia (*O. niloticus*) fingerlings under biofloc and clear water system throughout the experimental period (84 days)

Protein level	**19%**		**25%**		**30%**	
Treatments	**T1 (C.S)**	**T2 (B.S)**	**T3 (C.S)**	**T4 (B.S)**	**T5 (C.S)**	**T6 (B.S)**
Feed intake	42.70	38.70	47.92	42.92	55.45	50.45
Feed cost/kg L.E	6.50	6.50	8.00	8.00	9.50	9.50
Feed cost L.E[1]	277.55	251.55	383.36	343.36	526.77	479.27
Relative to feed cost[2] %	0.53	0.47	0.73	0.65	100	0.91
FCR[3]	2.40	1.92	2.01	1.62	1.71	1.42
Feed cost/kg fresh fish L.E[4]	15.60	12.48	16.08	12.96	16.24	13.49
Relative feed cost/kg[5]	0.96	0.76	0.99	0.80	100	0.83

1. Feed cost X Feed intake 2. Relative % of feed cost/ kg gain 3. Feed Conversion ratio
4. Feed cost /Kg gain 5. Relative % of feed cost/kg fresh fish

CONCLUSION

It could be concluded that 30% crude protein level was the best under biofloc system under in terms of growth performance, feed utilization and economical evaluation under this experimental conditions.

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تأثير مستويات البروتين الغذائي على أداء النمو والاستفادة الغذائية والتقييم الاقتصادي لإصبعيات أسماك البلطي النيلي (*Oreochromis niloticus*) وحيد الجنس تحت نظام البيوفلوك

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صممت هذه الدراسة تأثير مستوى البروتين الخام (١٩، ٢٥، ٣٠%) على أداء نمو والاستفادة الغذائية والتقييم الاقتصادي لإصبعيات أسماك البلطي النيلي وحيد الجنس تحت نظام البيوفلوك والمياه النقية. كان متوسط وزن الإصبعيات ٨.٠٠ جم/سمكة (كل معامله ٣ مكررات) واستمرت التجربة لمدة ٨٤ يومًا. كان معدل التغذية اليومية ٥٪ من إجمالي وزن الأسماك المخزنة مقسمة على مرتين متساويين في اليوم الساعة (٨.٠٠ و ١٢.٠٠). وكان يتم وزن الأسماك في كل حوض مكرر كل ١٥ يومًا وتم تعديل كمية العلف اليومي وفقًا لذلك. تم الحفاظ على نسبة C: N عند ١٥: ١ عن طريق إضافة رجيع الكون يوميًا كمصدر عضوي للكربون. بالنسبة للمعاملة بالبيوفلوك، كان رجيع الكون ينشط نمو البكتيريا غير ذاتية التغذية. حيث تم إذابة رجيع الكون في الماء مع المولاس بنسبة ١:١ يوميا من أجل تطوير الكتلة الحيوية وتغذية البكتيريا غير ذاتية التغذية. معدل نمو البلطي النيلي وحيد الجنس لوزن ٧٨-٩٤ جم/سمكة. كان أعلى قيمة لوزن الجسم النهائي (FBW)، والزيادة في الوزن (WG) وقيم معدل النمو النوعي (SGR) المسجلة للأسماك التي تتغذى على عليقة تحتوى على ٣٠٪ بروتين خام تحت نظام البيوفلوك BS. ويستنتج من هذه الدراسة أن ٣٠٪ من البروتين الخام في نظام البيوفلوك كان الأفضل من حيث أداء النمو الاستفادة الغذائية والتقييم الاقتصادي لإصبعيات سمك البلطي النيلي وحيد الجنس تحت هذه الظروف التجريبية.