Effect of Biofloc, Feeding Rate and Dietary Protein Levels on Growth Performance and Feed Utilization of Nile Tilapia, *(Oreochromis niloticus)*, Flathead Grey Mullet, (*Mugil cephalus*) and Thin Lipped Mullet, (*liza ramada)* Fingerlings in Polyculture

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ABSTRACT: The present study was carried out to investigate the effect of biofloc technology (BFT), feeding rate (FR) and dietary protein levels (PL) on growth performance, survival (%) ,feed utilization and economical evaluation parameters of Nile tilapia *(Oreochromis niloticus)* flathead grey mullet*, (Mugil cephalus)* and thin-lipped mullet, (*Liza ramada)* fingerlings. Two different daily feeding rate ($FR^{2\%BW}$ and $FR^{3\%BW}$) and three dietary protein levels ($PL^{20\%,}$ $PL^{25\%}$ and $PL^{30\%\ CP}$) under the conditions of regular water exchange system or zero water exchange using BFT (Biofloc technology) were studied. The 12 experimental treatments were studied in duplicate and were allocated in twenty four 16 m^3 concrete ponds. Nile tilapia final body weight (FBW), total weight gain (TWG), average daily gain (ADG), specific growth rate (SGR) (%/ day) and survival (%) were significantly affected by rearing system and feeding rate. Significant difference was observed for the effect of dietary protein level on growth performance of Nile tilapia. It could be concluded that BFT system enhanced survival and growth rates of tilapia and mullet ssp. in polyculture under low feeding levels (2%) and high protein diets (30% CP) regimes.

Keywords: Biofloc, tilapia, mullet, feeding rate, dietary protein level, growth performance, feed utilization, chemical composition, water quality.

INTRODUCTION

Intensive aquaculture systems are used to efficiently produce dense biomasses of fish species. Since fish retain only 20-30% of feed nutrients (Avnimeleoh and Ritvo, 2003), the rest is excreted and typically accumulates in the water. As a result, intensive aquaculture industry faces two major problems. The first is the water quality deterioration caused by the high concentrations of metabolites and the second is the low feed utilization associated with lover water exchange rate. With almost seven billion people on earth, the demand for aquatic food carries on to increase and hence; expansion and intensification of aquaculture production are highly required. The prime goal of aquaculture expansion must be to produce more aquaculture products in sustainability (Avnimelech and Kochba , 2009; Naylor *et al*. 2000) . The second goal is to build up systems providing an equitable cost / benefit to support economic and social sustainability (Avnimelech and Kochba 2009). Biofloc technology is based upon the running of the pond using minimal water exchange; subsequent development of dense microbial population and managing the microbial population through the adjustment of the C/N ratio, so that it controls inorganic nitrogen concentration in the water. The recycling of feed and minimization of water exchange are important contribution to the economy of tilapia production. Monitoring and fast response to negative developments are essential to the success of the culture. The aim of the present work is to invetgate the effects of using biofloc technology (BFT) on growth performance; feed utilization ; chemical composition ; and water quality of Nile tilapia ,(*Oreochromis niloticus)* flathead grey mullet*, (Mugil cephalus)* and thin-lipped, (*Liza ramada)* fingerlings under two feeding rate (2 and 3 %) and three dietary protein levels 20 , 25 and 30 % in ponds.

MATERIALS AND METHODS

Experimental fish and culture techniques:

Technique and duration:

The present study was carried-out at private fish hatchery belongs to El-kady fish farms group, Mutubis province, Kafr El-Sheik Governorate, Egypt. This experiment started on August, 08, 2014 and continued for 110 days using 2X2X3 in factorial design; two daily feeding rates (2% & 3% BW), three dietary protein levels (20%, 25%, and 30% CP) under zero water exchange biofloc system (BFT) or regular water exchange system (RS). The experimental treatments were subjected to be studied as follows (Table 1)

Table (1). Experimental treatments and design

Treatments	Water exchange	Feeding rate (%)	Protein level (%)
T 1	Regular	2	20
T2	Regular	2	25
T3	Regular	2	30
T4	Regular	3	20
T5	Regular	3	25
T6	Regular	3	30
T7	Biofloc	2	20
T8	Biofloc	2	25
T9	Biofloc	2	30
T10	Biofloc	3	20
T11	Biofloc	3	25
T12	Biofloc	3	30

Concrete ponds:

Twenty four concrete ponds each measuring Approximately 16 m^3 (3×7×0.76 m) width, length and depth of respectively under green-house condition were used, ponds were filled with surface water. Drainage water from draining canal was used as a source of inoculation of microbiota, in addition to 50 gm Urea as a source of nitrogen, while the control ponds were designed under open flow system. The experimental ponds represented the twelve experimental treatments in duplicate.

Rearing techniques:

Nile Tilapia, (*Oreochromis niloticus)*, flathead grey mullet,*(Mugil cephalus)* and thin lipped mullet, (*Liza ramada)* fingerlings 4.5, 10 and 3.5 g/fish, respectively were obtained from private fish farm located in Mutubis province, Kafr El-Sheik Governorate, Egypt. Prior to the start of the experiment, experimental fish were acclimatized to the new water conditions for two weeks and fed on a formulated diet. The fingerlings were stocked in at a density of 159 (145 tilapia+ 5 flathead mullet+ 9 thin-lipped mullet) fish / pond equivalent to 10 fish / m^3. Fish were reared under natural light (12:12 h, light: dark schedule). The water volume was maintained at approximately 17 $m^{3,}$ and loss of water due to evaporation and leakage was replaced whenever necessary according to water size in BFT ponds. Water in regular system ponds was exchanged system at a rate of 16 m^3/ day equivalent to 100% daily exchange rate per pond, twice daily. Aeration was continuously provided using 5.5 Hp ring air blower (Saad Zakhary Co. for electric motors). Also, agitation was kept at biofloc ponds by continuously strong aeration.

Experimental diets formation and preparation:

The three experimental diets were formulated from fish meal, soybean meal, yellow corn, wheat bran, wheat flour, carboxy methyl cellulose (CMC), ascorbic acid, vegetable oil, vitamins and minerals mixture. Ingredients were obtained from the local market and the dry ingredients were mixed thoroughly at first and with oil thereafter. The experimental diets were pelleted, all diets were put into plastic bags after samples had been taken and stored in deep freezer until use. The composition (%) and Chemical analysis (% dry matter basis) of experimental diets are presented in Table 2.

Table (2). Formula and chemical analysis (%) of the experimental diets.

Ingredients	20% CP	25%CP	30%CP
Fish meal	20	40	50
Soy bean meal	185	225	350
Rice polishing	260.5	225.5	125.5
Wheat middling's	250	200	180
Corn gluten	50	100	110
Corn grain	200	175	150
Veg. oil	1.5	1.5	1.5
Salt	5	5	5
Di cal.	25	25	25
Vit. and Min[1]. premix	3	3	3
CMC[2]			
Total	**1000**	**1000**	**1000**
Proximate composition %			
Moisture	7.20	7.00	6.90
Dry matter	92.80	93.00	93.10
Crude protein	20.46	25.15	30.17
Ether extract	10.14	10.38	11.31
Crude fiber	7.25	6.81	6.06
Ash	7.40	8.54	8.93
NFE[3]	54.75	49.12	43.54
GE[4] Kcal/ 100 g diet)[4]	436.10	441.75	455.82

(1) Vitamins and minerals mixture : Each 1 kg contains Vit A (400000 i.u.), Vit D (100000 i.u.), Vit E (250 mg,) Vit K3 (200 mg,) Vit B1 (200 mg), Vit B2 70mg, Vit B6 (200mg), Vit B12 (1mg), Vit C 450mg, Niacin 1000mg, Methionine1000mg, Cholin chloride 10000mg, Folic acid 100mg, Biotin 2mg, Panthonic acid 220mg, Magnesium sulphate 1000mg, Copper sulphate 1000mg, Iron sulphate 3000mg, Zinc sulphate , 600mg, Cobalt sulphate 100mg, Carrier upto 1000mg.

(2) CMC: Carboxy methyl cellulose

(3) Total carbohydrate =100-(CP+EE+Ash+ CF)

(4) Gross energy (GE) was calculated as 5.64, 9.44 and 4.11 kcal/g for protein, lipid and NFE, respectively NRC, (2012).

Feeding regime:

All fish were fed the experimental diets (20, 25, and 30% CP) using daily ration of 2 or 3 % of the total stocked biomass two times daily.

Water quality and samples:

Water quality parameters were monitored during the study period to follow the changes under biofloc system compared to control treatments (regular water exchange). Temperature and pH values of the water samples were measured using graduating thermometer and portable digital pH meter Martini Instruments (Model 201/digital). Water salinity and total dissolved solid (TDS) were measured using Salinometer Y.S.I (Bekman, Model RS-10). Dissolved oxygen was measured using oxygen meter model Hanna oxy check. Organic phosphorus were measured by seal AA3 auto analyzer. Ammonia, Nitrite, and Nitrate were measured every week calorimetrically by kites according to the Animal Health Research Institute (AHRI) Biomedical Chemistry Unite.

Fish sampling:

Representative fish in each pond were weighted every 15 days to the nearest 0.00 g to adjust the feed quantity.

Carbon levels for biofloc system:

Starch was added according to the amount of feeding ration introduced to fish in order to maintain the optimal C/N ratio, (>10- 25: 1) to activate heterotrophic bacteria growth (Avnimelech, 1999). Starch had been completely dissolved in water at plastic barrel, and spread over the pond surfaces at 10 am. Adding starch as a carbohydrate source, shading ponds, and strong aeration condition are the main circumstances that cause floc growth and development (Azim and Little, 2008).

<u>Growth performances, feed utilization parameters, and Survival rate:</u>

Growth indices:

At the end of the experiment random fish samples were selected and weighted to determine mean final body weight (FBW), Total weight gain (TWG), average daily gain (ADG), specific growth rate (SGR %) and feed conversion ratio (FCR), which were calculated according to (El-Saidy and Gaber, 2004).

Feed Intake and Feed conversion ratio were also calculated according to (Azim and Little, 2008).

Survival %:

Survival % was calculated in all experimental units according to Ricker (1975) and Newman and Martin (1983) .

Survival (%) = (No. of fish at the end / No. of fish at the start) × 100

Proximate analysis:

Fish and diets analysis:

At the beginning and the end of the trial, random pooled samples of fish and diets were collected and sacrificed for determination of initial whole-body proximate or chemical composition were done according to AOAC (1995 and 2000).

Analytical methods

1) Physico-chemical parameters of water

Water dissolved oxygen, pH, , nitrite and nitrate were determined according to (APHA,1999 and Grasshoff *et al.*, 1999)

2) Biofloc volume (FV)

Biofloc volume (FV) was determined on site using Imhoff cones daily registering the volume taken in by the flocs in 1000 ml of the tank water after 30 min sedimentation (Avnimelech and Kochba, 2009).

3) Statistical analysis:

Data of the experiment were analyzed using two ways ANOVA. Significant differences ($p \leq 0.05$) among means were tested by the method of Duncan (1955).The analyses of variance (ANOVA) were made according to Snedecor and Cochran (1981).

RESULTS AND DISCUSSION

Water quality

The overall mean, standard error, and range of water temperature, dissolved oxygen and pH are displayed in Table 3. All the environmental variables during the study period were within the range considered suitable for the culture of Nile tilapia.

A temperature in water of all treatments was in optimal condition for fish culture which ranged from 26.0-27.5℃ (Table 3). Tekelioglu (1998) recommended a preferred temperature values for tilapia between 20 to 35 ℃.

No significant differences in pH were found among treatments. pH was lower in the T9 (ranged from 7.91- 8.54) compared to T1 (ranged 7.81- 8.82). The pH were lower in the T9 treatments, suggesting a reducing condition in such treatments, probably due to the activity of heterotrophic bacteria, which release CO_2 to the water column causing a pH decrease. Contrarily, in the regular water exchange system (RS) treatments, where the photosynthesis was enhanced, the phytoplankton in agriculture drain water produced CO_2 during the night, but sequesters it during the day, causing pH increases. A similar trend was observed by many authors (Tacon *et al.*, 2002 and Wasielesky *et al.*, 2006). In addition others, (Chen *et al.*, 2006; Ebeling *et al.*, 2006 and Rijn *et al.*, 2006) reported a decrease in pH during the chemoautotrophic nitrification process as a result of $CaCO_3$ consumption and the release of CO_2 and pH into the culture medium. The significant increase in pH may have been as the result of enhanced photosynthesis.

Dissolved oxygen remained within the recommended range for growth of tilapia. By aerating the DO average was kept above 5 mg/L these values within the recommended levels of DO as reported by many researchers (El-Sayed, 2006; Kutty, 1996; Tsadik and Kutty, 1987 and Bergheim, 2007). The incidents of increased DO were higher in the regular water exchange treatments T1-T6 (ranged from 5.06 to 6.7 mg/L) compared to zero-exchange water system T7-T12 (ranged from 5.0 to 5.9 mg/L). This may explained by the high consumption of dissolved oxygen by heterotrophic organisms in biofloc treatments.

The concentrations of nitrogen species measured during this study are presented in Table 3. The incidents of increased TAN and nitrite–N were higher in the regular water exchange treatments T1-T6 compared to zero-exchange water system T7-T12. T1 showed relatively higher Total Ammonia Nitrogen (TAN) (0.61 mg/L) concentrations. The difference in TAN concentrations between regular exchange water and the other BFT treatments was expected as there is increase in the heterotrophic bacteria activities in BFT treatment which process to decrease TAN by nitrification.

Within the BFT treatments nitrate–N gradually decreased in all treatments, this may be explained by the low dose of nitrogen delivered for the system (Kirchman, 1994; Middelburg and Nieuwenhuize, 2000).

The significant low TAN and NO_2 values recorded for regular water exchange treatments. This decrease probably relates to nitrogen species uptake by phytoplankton in these treatments in particular when there is limited ammonia-N available in the water (Hargreaves, 1998).

Table (3). Mean±SE of water quality criteria in ponds as affected by rearing system, feeding levels and dietary protein levels

Treatment	Temp. Cº	pH	O_2 (mg/L)	TAN (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)
T1	26.63±0.57a (26.0-27.5)	8.21±0.38a (7.81-8.82)	6.11±0.4ab (5.43-6.48)	0.58±0.4ab (0.15-0.95)	0.35±0.3a (0.20-0.95)	0.35±0.26a (0.17- 0.84)
T2	26±00c (26-26)	8.14±0.33a (7.76-8.55)	6.14±0.39ab (5.56-6.52)	0.59±0.26ab (0.17-0.83)	0.21±0.012a (0.19-0.23)	0.30±0.17a (0.18-0.54)
T3	26.2±0.31bc (26-26.6)	8.13±0.282a (7.82-8.49)	6.24±0.45a (5.56-6.7)	0.61±0.27a (0.17-0.95)	0.21±0.03a (0.16-0.25)	0.24±0.12a (0.15-0.40)
T4	26.38±0.3ab (26-26.6)	8.16±0.34a (7.73-8.52)	6.09±0.36ab (5.65-6.43)	0.49±0.31abc (0.10-0.85)	0.23±0.02a (0.20-0.27)	0.30±0.11a (0.19-0.44)
T5	26±00c (26-26)	8.11±0.34a (7.72-8.56)	6.1±0.49ab (5.43-6.43)	0.45±0.34abcd (0.1-0.89)	0.22±0.05a (0.18-0.32)	0.24±0.11a (0.15-0.45)
T6	26.25±0.274c (26-26.5)	8.12±0.32a (7.75-8.56)	6.36±0.4a (5.79-6.70)	0.31±0.23cbd (0.12-0.64)	0.22±0.02a (0.2-0.25)	0.24±0.09a (0.170-0.37)
T7	26±0c (26-26)	8.1±0.19a (7.87-8.4)	5.31±0.21bc (5.06-5.56)	0.19±0.03d (0.15-0.23)	0.35±0.39b (0.02-0.89)	0.1±0.09b (0.02-0.27)
T8	26±0.0c (26-26)	8.12±0.18a (7.94-8.41)	5.31±0.19bc (5.07-5.56)	0.22±0.05cd (0.18-0.31)	0.3±0.04b (0.02-0.85)	0.06±0.03b (0.02-0.09)
T9	26.25±0.27bc (26-26.5)	8.07±0.25a (7.91-8.54)	5.43±.32bc (5-5.93)	0.20±0.03d (0.15-0.24)	0.31±0.4b (0.02-0.85)	0.06±0.02b (0.03-0.08)
T10	26.08±0.20bc (26-26.5)	8.12±0.28a (7.9-8.66)	5.48±0.22bc (5.06-5.7)	0.2±0.02d (0.17-0.24)	0.31±0.4b (0.02-0.91)	0.06±0.03b (0.02-0.09)
T11	26.25±0.27bc (26-26.5)	8.01±0.1a (7.91-8.19)	5.46±0.3c (5.0-5.93)	0.22±0.02d (0.18-0.26)	0.28±0.36b (0.02-0.76)	0.03±0.02b (0.02-0.08)
T12	26.17±0.26bc (26-26.5)	8.09±0.24a (7.9-8.51)	5.52±0.07bc (5.43-5.61)	0.25±0.07cd (0.17-0.34)	0.31±0.39b (0.02-0.82)	0.07±0.02b (0.05-0.09)

Means in the same column having different letters are significantly ($P \leq 0.05$) different.

Growth performance

Nile tilapia

Table (4) are summarized the growth performance parameters of tilapia as affected by the experimental treatments rearing system had also effects on FBW. BFT group had significantly higher FBW (75.33 g/fish) compared to the RS group (70.54 g/fish). Feeding rate factor had effects on FBW $FR^{3\%}$ group had significantly higher FBW (75.108 g/fish) compared to the $FR^{2\%}$ group (70.77 g/fish). Dietary Protein level factor had no effects on FBW. The same trend was observed for FWG, ADG and SGR.

The interactions between rearing system, feeding level and dietary protein level had significant difference on FBW, TWG, ADG and SGR. The highest values were recorded by T12 group (77.50 g/fish, 72.90 g/fish, 0.66 g/day and 2.57 %/day, respectively), while the lowest values were recorded by T1 group (59.41 g/fish, 54.81 g/fish, 0.50 g/day and 2.33 %/day, respectively).

Flathead grey mullet

Table 5 is summarized the growth performance parameters of grey mullet as affected by the experimental treatments Rearing system factor levels had effects on FBW. BFT group had significantly higher FBW (118.83 g) compared to the RS group (103.54 g/fish). Feeding rate factor had effects on FBW. $FR^{3\%}$ group had significantly higher FBW (114.58 g) compared to the $FR^{2\%}$ group (107.79 g/fish). Dietary Protein level factor had significant effects on FBW. $PL^{30\%}$ group had significantly higher FBW (114.63 g) compared to the

$PL^{20\%}$ and $PL^{25\%}$ group (107.18 and111.57 g/fish, respectively). The same trend was observed for TWG, ADG and SGR.

The interactions between rearing system, feeding level and dietary protein level had significant difference on FBW, TWG, ADG and SGR. The highest values were recorded by T12 group (126.50 g/fish, 116.50 g/fish, 1.2 g/day and 2.31 %/day, respectively), while the lowest values were recorded by T1 group (95.73 g/fish, 85.73 g/fish, 0.85 g/day and 2.05 %/day, respectively).

Thin-lipped mullet

Table 6 is summarized the growth performance parameters of thin-lipped mullet as affected by the experimental treatments rearing system factor levels had effects on FBW. BFT group had significantly higher FBW (52.0 g/fish) compared to the RS group (44.0 g/fish). Feeding rate factor had effects on FBW. $FR^{2\%}$ group had significantly higher FBW (30.81 g/fish) compared to the $FR^{3\%}$ group (25.8 g/fish). Dietary Protein level factor had significant effects on FBW. $PL^{30\%}$ group had significantly higher FBW (51.75 g) compared to the $PL^{20\%}$ and $PL^{25\%}$ group (44.50 and 47.75 g/fish, respectively). The same trend was observed for TWG, ADG and SGR.

The interactions between rearing system, Feeding rateand dietary protein level had significant difference on FBW, TWG, ADG and SGR. The highest values were recorded by T12 group (59.0 g/fish, 55.50 g/fish, 0.5 g/day and 2.568 %/day, respectively), while the lowest values were recorded by T1 group (39.0 g/fish, 35.50 g/fish, 0.323 g/day and 2.19%/day, respectively).

Different studies have reported enhanced survival, health, and growth rates of fish and shrimps raised in ponds with high activity of algae, microbial flocs, and other natural biota (Avnimelech, 1999; Moss *et al*., 2000 and Burford *et al*., 2004). However it is not yet known exactly how microbial flocs enhance growth, but Izquierdo *et al.* (2006) suggested lipid contributions of microbial flocs are significant. Avnimelech (1999) reported that the microbial protein supplied by the stocked fish biomass was enough to supplement the protein provided by the fish feed.

In culture systems, together with microbial flocs acting as a feed also do play some important ecological roles. The deterioration of water quality due to unconsumed feed, fecal matter of cultured organisms or the presence of other organic matter in culture facilities is nullified because the floc microbes act as conditioner for water. This always control excess nitrogen. The subsequent uptake of nitrogen from the water facilitated synthesis of microbial protein. Hence biofloc based aquaculture system also offers potential to use as zero exchange recirculation aquaculture system (Avnimelech, 2007).

Many of previous studies have shown that growing shrimp (L. vannamei) in biofloc systems can improve shrimp survival and growth performance, compared to clear water (Cohen *et al.* 2005; Azim & Little 2008; Mishra *et al.* 2008). One reason for the improved performance is probably related to harvesting and consuming bioflocs by the shrimp. The second reason is therefore, it is assumed that the presumptively large quantity of bacteria associated with bioflocs may contribute to enhance the immunity as well as growth performance of shrimp when the bioflocs are consumed by shrimp (Rao *et al*., 2010).

Table (4). Effects of different experimental treatments (rearing system, feeding rates and dietary protein levels on growth performance and survival rates of Nile tilapia fingerlings.

Treatment	Rearing system	Feeding rate	Protein level	IBW (g/fish)	FBW (g/fish)	TWG (g/fish)	ADG (g/fish)	SGR	Survival %
Regular system	Regular system	-	-	4.56a ±0.02	70.54b ±1.75	65.99b ±1.75	0.60 b ±0.02	2.49 b ±0.02	98.28a ±0.2
BFT System	BFT system	-	-	4.566a ±0.014	75.33a ±0.466	70.77a ±0.459	0.64a ±0.004	2.55a ±0.005	99.66a ±0.143
$FR^{2\%}$	-	2%	-	4.575a ±0.013	70.77b ±1.779	66.19b ±1.778	0.60b ±0.016	2.49b ±0.024	98.97a ±0.30
$FR^{3\%}$	-	3%	-	4.550a ±0.015	75.11a ±0.538	70.56a ±0.527	0.64a ±0.005	2.55a ±0.005	98.97a ±0.232
$PL^{20\%CP}$	-	-	20%	4.562a ±0.018	70.57a ±2.509	66.00a ±2.515	0.60a ±0.023	2.49a ±0.035	98.88a ±0.343
$PL^{25\%CP}$	-	-	25%	4.55a ±0.019	73.59a ±1.054	69.04a ±1.051	0.63a ±0.009	2.53a ±0.013	98.88a ±0.345
$PL^{30\%CP}$	-	-	30%	4.575a ±0.016	74.66a ±1.209	70.09a ±1.198	0.64a ±0.011	2.54a ±0.013	99.14a ±0.313
T1	Regular	2%	20%	4.600a ±0.00	59.41c ±0.308	54.81d ±0.308	0.498d ±0.003	2.33d ±0.005	97.59d ±0.345
T2	Regular	2%	25%	4.550a ±0.050	69.30b ±1.300	64.75c ±1.350	0.59c ±0.012	2.48c ±0.027	97.93d ±0.69
T3	Regular	2%	30%	4.550a ±0.050	71.40ab ±4.30	66.85bc ±4.25	0.61bc ±0.038	2.50bc ±0.045	99.31d ±0
T4	Regular	3%	20%	4.550a ±0.050	73.35ab ±2.75	68.80abc ±2.70	0.63abc ±0.024	2.53abc ±0.024	98.62cd ±0
T5	Regular	3%	25%	4.550a ±0.050	74.55ab ±0.45	70.00abc ±0.40	0.64abc ±0.004	2.54ab ±0.004	98.28cd ±0.345
T6	Regular	3%	30%	4.550a ±0.050	75.25ab ±0.95	70.70abc ±0.90	0.64abc ±0.008	2.55ab ±0.001	97.93d ±0
T7	BFT	2%	20%	4.600a ±0.00	75.00ab ±1.00	70.40abc ±1.00	0.64abc ±0.009	2.54abc ±0.012	99.66ab ±0.345
T8	BFT	2%	25%	4.550a ±0.050	75.00ab ±2.00	70.45abc ±1.95	0.64abc ±0.018	2.55ab ±0.014	100 a ±0
T9	BFT	2%	30%	4.600a ±0.00	75.00ab ±1.50	69.90abc ±1.50	0.64abc ±0.014	2.53abc ±0.018	99.31abc ±0.69
T10	BFT	3%	20%	4.500a ±0.00	74.50ab ±0.50	70.00abc ±0.50	0.64abc ±0.005	2.55ab ±0.006	99.66ab ±0.345
T11	BFT	3%	25%	4.550a ±0.050	75.50a ±0.50	70.95ab ±0.45	0.65ab ±0.004	2.55ab ±0.004	99.31abc ±0
T12	BFT	3%	30%	4.600a ±0.00	77.50a ±0.50	72.90a ±0.50	0.66a ±0.005	2.57a ±0.006	100a ±0

Means in the same column having different letters are significantly ($P \leq 0.05$) different.

Table (5). Effects of different experimental treatments (rearing system, feeding rates and dietary protein levels on growth performance and survival rates of flathead grey mullet fingerlings

Treatment	Rearing system	Feeding rate	Protein level	IBW (g/fish)	FBW (g/fish)	TWG (g/fish)	ADG (g/fish)	SGR	Survival rate %
Regular system	Regular system	-	-	10.12a ±0.06	103.54b ±1.99	93.42b ±1.97	0.98b ±0.02	2.11b ±0.02	100 ±0
BFT System	BFT system	-	-	10.18a ±0.08	118.83a ±1.34	108.65a ±1.34	1.13a ±0.01	2.23a ±0.01	100 ±0
FR2%	-	2%	-	10.17a ±0.07	107.79a ±3.09	97.62a ±3.06	1.01a ±0.03	2.14a ±0.02	100 ±0
FR3%	-	3%	-	10.13a ± 0.07	114.58a ±2.17	104.45a ±2.18	1.09a ±0.02	2.20a ±0.02	100 ±0
PL20%CP	-	-	20%	10.19a ±0.09	107.18a ±3.1	97c ±3.05	1.00c ±0.04	2.14a ±0.02	100 ±0
PL25%CP	-	-	25%	10.13a ±0.08	111.75a ±3.31	101.63b ±3.31	1.06b ±0.03	2.18a ±0.03	100 ±0
PL30%CP	-	-	30%	10.14a ±0.09	114.63a ±3.74	104.49a ±3.73	1.09a ±0.03	2.2a ±0.03	100 ±0
T1	Regular	2%	20%	10.00a ±0.00	95.73e ±1.93	85.73e ±1.93	0.85e ±0.02	2.05e ±0.05	100 ±0
T2	Regular	2%	25%	10.200a ±0.2	98.00ed ±1.00	87.8e ±0.8	0.94d ±0.01	2.05e ±0.01	100 ±0
T3	Regular	2%	30%	10.00a ±0.00	100.00ed ±2.00	90.00ed ±2.00	0.95d ±0.02	2.09ed ±0.02	100 ±0
T4	Regular	3%	20%	10.20a ±0.2	103.50d ±0.5	93.30d ±0.3	0.99d ±0.01	2.11d ±0.01	100 ±0
T5	Regular	3%	25%	10.00a ±0.00	111.00c ±1.00	101.0c ±1.00	1.05c ±0.01	2.19c ±0.01	100 ±0
T6	Regular	3%	30%	10.30a ±0.3	113.00c ±1.0	102.70c ±1.3	1.07bc ±0.03	2.18c ±0.01	100 ±0
T7	BFT	2%	20%	10.25a ±0.25	115.00bc ±0.00	104.75bc ±0.25	1.09bc ±0.02	2.2c ±0.0	100 ±0
T8	BFT	2%	25%	10.30a ±0.3	119.00b ±3.00	108.7b ±2.7	1.13ab ±0.004	2.22bc ±0.03	100 ±0
T9	BFT	2%	30%	10.25a ±0.25	119.00b ±3.00	108.75b ±2.75	1.13ab ±0.0007	2.23bc ±0.03	100 ±0
T10	BFT	3%	20%	10.30a ±0.3	114.50bc ±2.5	104.20bc ±2.2	1.09bc ±0.007	2.19c ±0.022	100 ±0
T11	BFT	3%	25%	10.00a ±0.00	119.00b ±1.00	109.00b ±1.00	1.13ab ±0.01	2.25b ±0.01	100 ±0
T12	BFT	30%	30%	10.00a ±0.00	126.50a ±0.50	116.50a ±0.50	1.20a ±0.004	2.31a ±0.005	100 ±0

Means in the same column having different letters are significantly ($P \leq 0.05$) different.

Table (6). Effects of different experimental treatments (rearing system, feeding rates and dietary protein levels on growth performance and survival rates) of Liza ramada fingerlings

Treatment	Rearing system	Feeding rate	Protein level	IBW (g/fish)	FBW g/fish)	TWG (g/fish)	ADG (g/fish)	SGR	Survival %
Regular system	Regular system	-	-	3.542a ±0.023	44.00b ±1.135	40.46b ±1.135	0.368b ±0.010	2.29b ±0.024	100 ±0
BFT System	BFT system	-	-	3.51a ±0.01	52.00a ±1.308	48.491a ±1.308	0.441a ±0.012	2.45a ±0.023	100 ±0
$FR^{2\%}$	-	2%	-	3.170a ±0.063	30.81a ±3.485	27.643a ±3.513	0.251a ±0.032	2.02a ±0.095	100 ±0
$FR^{3\%}$	-	3%	-	3.143a ±0.071	25.8b ±1.401	22.654b ±1.362	0.206b ±0.012	1.902b ±0.039	100 ±0
$PL^{20\%CP}$	-	-	20%	3.537a ±0.026	44.50c ±1.822	40.962c ±1.832	0.372c ±0.017	2.296c ±0.04	100 ±0
$PL^{25\%CP}$	-	-	25%	3.500b ±0.019	47.75b ±1.943	44.25b ±1.942	0.402b ±0.018	2.370b ±0.036	100 ±0
$PL^{30\%CP}$	-	-	30%	3.537a ±0.018	51.75a ±1.75	48.21a ±1.762	0.438a ±0.016	2.436a ±0.034	100 ±0
T1	Regular	2%	20%	3.500d ±0.00	39.00j ±1.00	35.50k ±1.00	0.323k ±0.009	2.191k ±0.023	100 ±0
T2	Regular	2%	25%	3.500dv ±0.00	41.50i ±0.500	38.00i ±0.500	0.345i ±0.004	2.248i ±0.011	100 ±0
T3	Regular	2%	30%	3.600b ±0.00	47.00f ±2.00	43.40f ±2.00	0.394f ±0.018	2.335h ±0.039	100 ±0
T4	Regular	3%	20%	3.650a ±0.050	41.50i ±0.500	37.85j ±0.550	0.344j ±0.005	2.21j ±0.023	100 ±0
T5	Regular	3%	25%	3.450e ±0.050	45.50h ±0.500	42.05h ±0.550	0.382h ±0.005	2.345f ±0.023	100 ±0
T6	Regular	3%	30%	3.550c ±0.050	49.50d ±0.500	45.95d ±0.550	0.418d ±0.005	2.395d ±0.022	100 ±0
T7	BFT	2%	20%	3.500d ±0.00	46.00g ±1.00	42.50g ±1.00	0.386g ±0.009	2.341g ±0.02	100 ±0
T8	BFT	2%	25%	3.550c ±0.050	48.50e ±0.500	44.95e ±0.550	0.409e ±0.005	2.377e ±0.022	100 ±0
T9	BFT	2%	30%	3.500d ±0.00	51.50c ±0.500	48.00c ±0.500	0.436c ±0.004	2.444c ±0.01	100 ±0
T10	BFT	3%	20%	3.500d ±0.00	51.50c ±0.500	48.00c ±0.500	0.436c ±0.004	2.444c ±0.01	100 ±0
T11	BFT	3%	25%	3.500d ±0.00	55.50b ±0.500	52.00b ±0.500	0.473b ±0.004	2.512b ±0.01	100 ±0
T12	BFT	3%	30%	3.500d ±0.00	59.00a ±1.00	55.50a ±1.00	0.504a ±0.01	2.57a ±0.015	100 ±0

Means in the same column having different letters are significantly ($P \leq 0.05$) different.

Feed intake and utilization

Feed intake and utilization are tabulated in (Table 7 and 8). The rearing system factor revealed higher significant amounts on feed intake. RS fish consumed significantly higher amount of feed (84.16 g/fish) compared with fish cultured under BFT condition (75.2 g/fish). Feeding levels had significant effects on feed intake. $FR^{3\%}$ Fish consumed significantly higher amount of feed (99.42 g/fish) compared with $FR^{2\%}$ group (59.94 g/fish). Also two-way ANOVA showed a significant effect due to the interaction among rearing system, Feeding rate and dietary protein levels on feed intake.

The highest amount of feed intake was recorded by T4 group (108.40 g), which was statistically different ($P<0.05$) compared with other groups. T7 group consumed the lowest amount of feed intake (56.84 g).

Rearing system showed significant effects on mass weight gain of cultured fish and FCR ($P<0.05$). The best mass weight gain and FCR figures were obtained by fish reared in BFT system (70.7 g and 1.06), respectively compared to (65.4 g and 1.27) respectively which obtained by fish reared in regular system. The experimental feeding rate had significant effects on mass gain and FCR. $FR^{3\%}$ recorded the highest figures compared to the lowest feeding rate ($FR^{2\%}$).

The dietary protein level factor had no significant effects on mass weight gain and FCR. The interaction between rearing system feeding level and dietary protein levels showed significant difference on both mass weight gain and FCR. The range of FCR lied from 0.81 to1.6. Fish groups in T7 (raised under BFT, 2% feeding rate and at 20% crude protein diet) had achieved the best FCR (0.81) compared to other groups (Table 7).

These results might be due to the conditions of zero water exchange probably contributed to the decrease of the FCR in all the treatments because there was not any release of nutrients in effluents, which favored the formation of a nutrient cycling through the food chain. Nutrient cycling has been documented in systems without water exchange in which natural feed was promoted.

The result obtained for FCR in this study agrees with finding of Avnimelech, (2007) who reported that the feed contribution of microbial flocs in the tested ponds contributed close to 50% of fish protein requirement. The high number of protozoa and rotifers in the BFT communities' contributed to better shrimp performance in BFT treatments compared to the control as shown by Thompson *et al*. (2002). Avnimelech, (2006) showed that recovery of nitrogenous compounds from culture systems with tilapia could be increased from 25% to 50% under biofloc technology.

Table (7). Mass growth performance parameters and survival rates of Nile tilapia flathead and thin-lipped mullet as affected by experimental treatments. (rearing system, feeding rates and dietary protein levels (Mean± SE)

Treatment	Rearing system	Feeding rate %	Protein level %	IBW (g/fish)	FBW (g/fish)	TWG (g/fish)	ADG (g/fish/day)	SGR	Survival %
				Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Regular system	Regular system	-	-	4.68a ±0.01	70.07b ±1.68	65.4b ±1.68	0.59b ±0.02	2.46b ±0.02	98.43b ±0.16
BFT System	BFT system	-	-	4.68a ±0.01	75.38a ±0.49	70.7a ±0.49	0.64a ±0.00	2.53a ±0.01	99.69a ±0.09
FR[2%]	-	2%	-	4.68a ±0.01	70.50b ±1.75	65.81b ±1.75	0.6b ±0.02	2.46b ±0.02	99.06a ±0.25
FR[3%]	-	3%	-	4.67a ±0.01	74.95a ±0.62	70.28a ±0.61	0.64a ±0.01	2.52a ±0.01	99.06a ±0.21
PL[20%CP]	-	-	20%	4.68a ±0.02	70.24b ±2.44	65.56b ±2.45	0.6b ±0.2	2.458b ±0.3	98.98a ±0.31
PL[25%CP]	-	-	25%	4.67a ±0.02	73.32b ±1.14	68.65b ±1.13	0.62b ±0.01	2.5b ±0.01	98.98a ±0.29
PL[30%CP]	-	-	30%	4.69a ±0.01	74.62a ±1.13	69.93a ±1.22	0.64a ±0.01	2.51a ±0.01	99.21a ±0.26
T1	Regular	2%	20%	4.71a ±0.0	59.39d ±0.40	54.69d ±0.40	0.5d ±0	2.30e ±0.01	97.8e ±0.31
T2	Regular	2%	25%	4.67a ±0.05	68.62c ±1.18	63.95c ±1.23	0.58c ±0.01	2.44d ±0.03	98.11de ±0
T3	Regular	2%	30%	4.67a ±0.05	70.92bc ±3.74	66.25bc ±3.7	0.60bc ±0.03	2.47dc ± 0.04	99.37b ±0
T4	Regular	3%	20%	4.68a ±0.05	72.48bc ±2.55	67.81bc ±2.50	0.62bc ±0.02	2.49abc ±0.02	98.74c ±0
T5	Regular	3%	25%	4.66a ±0.05	74.04ab ±0.35	69.38ab ±0.3	0.63ab ±0.0	2.51abc ±0.01	98.43cd ±0.31
T6	Regular	3%	30%	4.67a ±0.03	74.97ab ±0.93	70.3ab ±0.89	0.64ab ±0.01	2.52abc ±0.0	98.11de± 0.0
T7	BFT	2%	20%	4.72a ±0.01	74.62ab ±0.97	69.9ab ±0.98	0.64ab ±0.01	2.51abc ±0.01	99.69ab ±0.31
T8	BFT	2%	25%	4.67a ±0.06	74.88ab ±1.9	70.21ab ±1.83	0.64ab ±0.02	2.52ab ±0.01	100a ±000
T9	BFT	2%	30%	4.72a ±0.01	74.6ab ±1.3	69.88ab ±1.31	0.64ab ±0.01	2.51abc ±0.02	99.37b ±00
T10	BFT	3%	20%	4.63a ±0.01	74.47ab ±0.35	69.83ab±0 .36	0.63ab ±0.003	2.53abc ±0.01	99.69ab ±0.31
T11	BFT	3%	25%	4.66a ±0.5	75.74ab ±0.4	71.08ab ±0.35	0.65ab ±0.003	2.53ab ±0.004	99.37b ±0
T12	BFT	3%	30%	4.71a ±0.0	77.99a ±0.53	73.29a ±0.53	0.67a ±0.004	2.55a ±0.01	100a ±00

Means in the same column having different letters are significantly (P≤0.05) different.

Table (8). Feed utilization parameters of Nile tilapia flathead and thin-lipped mullet as affected by experimental treatments (rearing system, feeding rates and dietary protein levels (Mean± SE)

Treatment	Rearing system	Feeding rate %	Protein level %	FI (g)	FCR(g)	PI	PER
				Mean± SE	Mean±SE	Mean±SE	Mean±SE
Regular system	Regular system	-	-	84.16a ±6.86	1.27a ±0.08	21.31a ±2.05	3.33b ±0.25
BFT System	BFT system	-	-	75.2b ±5.41	1.06b ±0.07	18.99b ±1.63	4.03a ±0.34
FR2%	-	2%	-	59.939b ±1.62	0.92b ±0.03	15.21b ±0.94	4.48a ±0.26
FR3%	-	3%	-	99.42a ±2.55	1.42a ±0.04	25.10a ±1.33	2.88b ±0.15
PL20%CP	-	-	20%	78.33a ±8.37	1.19a ±0.11	16.03c ±1.71	4.37a ±0.42
PL25%CP	-	-	25%	80.61a ±7.77	1.17a ±0.1	20.27b ±1.96	3.61b ±0.34
PL30%CP	-	-	30%	80.10a ±7.59	1.14a ±0.10	24.16a ±2.29	3.07c ±0.27
T1	Regular	2%	20%	57.57c ±142	1.05d ±0.03	11.78h ±0.29	4.65b ±0.15
T2	Regular	2%	25%	64.22c ±7.68	1.00d ±0.10	16.15fg ±1.93	4.01c ±0.40
T3	Regular	2%	30%	65.24c ±5.61	0.98ed±0.03	19.68ed ±1.69	3.37de ±0.10
T4	Regular	3%	20%	108.40a ±2.61	1.6a ±0.02	22.18cd ±0.53	3.06fe ±0.04
T5	Regular	3%	25%	101.82ab±8.86	1.47ab±0.12	25.61bc ±2.23	2.73f ±0.23
T6	Regular	3%	30%	107.71a ±2.82	1.53ab±0.02	32.49a ±0.85	2.16g ±0.03
T7	BFT	2%	20%	56.84c ±2.49	0.81f ±0.02	11.63h ±0.51	6.02a ±0.18
T8	BFT	2%	25%	57.92c ±0.72	0.83ef ±0.01	14.57hg ±0.18	4.82b ±0.07
T9	BFT	2%	30%	57.84c ±2.05	0.83ef ±0.04	17.45ef ±0.62	4.01c ±0.22
T10	BFT	3%	20%	90.52b ±0.32	1.3c ±0.002	18.52ef ±0.07	3.77cd ±0.01
T11	BFT	3%	25%	98.46ab ±1.63	1.39bc±0.02	24.76bc ±0.41	2.87fe ±0.03
T12	BFT	30%	30%	89.61b ±1.11	1.22c ±0.02	27.03b ±0.33	2.71f ±0.05

Means in the same column having different letters are significantly ($P \leq 0.05$) different.

Biofloc composition .

Mean values on dry matter basis of the proximate analysis from pooled samples collected during floc harvesting for the different treatments are presented in (Table 9). Proximate analysis of BFT from the current study indicates the presence of 30.63 % crude protein in the T11 BFT system, 3% feeding rate at 25% protein diet which was higher than for the other treatments (Table 9). Protein content generally was higher in T10, T11 and T12 treatments which fed at a rate of 3% (ranged from 26.250 to 30.63%) than in T10, T11 and T12 treatments which fed 2% feeding rate (ranged from 25.10 to 25.72%). The higher protein concentration in bioflocs of the high feeding level treatments may be related to the chemical composition of heterotrophic bacteria and other organisms associated to bioflocs and biofilms (Fernandes *et al.*, 2008). Also, the high Zooplankton organisms (high in protein) which maybe increased with the increasing feeding level, consume both bacteria and algae and may be considered as another reason.

There were significant differences in crude lipid among the ponds (ranged from 2.22%to 4.16%).

Lipid content generally was higher in T10, T11 and T12 treatments which fed 3% Feeding rate(ranged from 3.65 to 4.27%) than in T10, T11 and T12 treatments which fed 2% feeding rate(ranged from 2.12 to 2.51%). The higher lipid concentration in bioflocs of the high feeding level treatments may be related to the chemical composition of heterotrophic bacteria and other organisms associated to bioflocs and biofilms (Fernandes *et al.*, 2008). Also, the high Zooplankton organisms (high in lipid) which maybe increased with the increasing feeding level, consume both bacteria and algae and may be considered as another reason.

Table (9). Mean ± standard error of two replicates of biofloc composition as affected by daily feeding rates and varying dietary protein levels.

Treatments	Rearing System	Feeding rate	Dietary rotein level	Dry matter %	Cp %	Lipid %	Ash %
T7	BFT	2%	20%	11.855ab±0.555	25.10a ±1.0	2.510a±1.00	32.32±ab2.09
T8	BFT	2%	25%	10.20 a ±0.10	25.715a±3.815	2.120b±0.380	32.320a±2.090
T9	BFT	2%	30%	10.020ab±0.0100	25.715a±3.815	2.120ab±0.380	37.75ab±5.350
T10	BFT	3%	20%	11.165ab ±0.465	26.795a±0.545	4.200ab±1.200	30.395ab±3.1750
T11	BFT	3%	25%	10.600 ab ±0.900	30.630 a± 0	3.655ab±0.455	30.400ab± 5.100
T12	BFT	3%	30%	12.200b ±0.200	26.250a± 0	4.270ab±0.630	24.260b±0.690

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الملخص العربي

تأثير مساهمة البيوفلوك ومعدل التغذية ومستوي البروتين علي الاداء الانتاجي للاستزراع المتعدد لأسماك البلطي النيلي والبوري والطوبار

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صممت هذه التجربة لدراسة تأثير مساهمة البيوفلوك و معدل التغذية ومستوي البروتين علي الاداء الانتاجي للاستزراع المتعدد لأسماك البلطي النيلي والبوري والطوبار واستمرت التجربة لمدة (١١٠) وقد صممت تجربة عامليه ٢*٢*٣ لدراسة تأثير عوامل كما يلي :-

١. نظام الاستزراع (استزراع تقليدي بتغيير المياه مقابل استزراع بنظام البيوفلوك)

٢. مستوي التغذية (٢ ، ٣ % من وزن الجسم)

٣. مستوي البروتين الخام بالعليقة (٢٠ ، ٢٥ ، ٣٠ %)

لينتج ١٢ اثني عشر معاملة تجريبية تم توزيعها علي ٢٤ اربعة وعشرون حوض اسمنتي سعة الواحد ١٦م٣ بمعدل مكررتين (حوضين) لكل معاملة

ـ بعد انتهاء مدة التجربة اظهرت النتائج ما يلي :-

ان الاداء الانتاجي للبلطي النيلي و البوري والطوبار قد تأثرت معنويا بعوامل الدراسة وخاصة نظام الاستزراع ومعدل التغذية وبدرجة اقل بمستوي البروتين الخام في العليقة

ـ وتوصي الدراسة بأهمية تطبيق تكنولوجيا البيوفلوك (عدم تغيير المياه) مع مستويات التغذية المنخفضة ٢ % من وزن الجسم ومستوي البروتين الغذائي (٣٠ % بروتين خام).