

Eco-friendly cultivation of Keeled mullet (*Liza carinata*) in biofloc system.

**Magdy T. Khalil¹; Ragab A. R. Mohamed²; Ragaa El-Deeb¹; Ashraf Suloma³;
Basem S. Abd-alatty² and Shimaa A. henish².**

1- Zoology Dept, Fac. Sci. Ain Shams University, Egypt.

2- National Inst. of Oceanography and Fisheries (NIOF), Egypt.

3- Fish Nutrition Lab (FNL), Animal Production Dept, Faculty of Agriculture, Cairo University, Egypt.

ABSTRACT

A detailed study was carried out to evaluate the suitability of biofloc system condition for cultivation of Keeled mullet (*Liza carinata*) fish. The experimental design consisted of 2x2 factors experimental arrangement of two levels of crude protein (23 and 28% CP) and two culture systems (clear water system (CWS) and biofloc treatment (BFT) with zero water exchange): (CWS^{23%}, CWS^{28%}, BFT^{23%} and BFT^{28%}), where the superscripts refer to the levels of crude protein. Each treatment included three glass aquaria (80 X 45 X 30 cm) and stocked by 15 fry per aquarium with an average initial body weight of 0.26±0.003g. Total weight gain (TWG), average daily gain (ADG), and specific growth rate (SGR) were determined. Fish fed on 28% CP had significantly higher values of FBW, FI, FCR, EG and ER (24.41, 25.81, 1.06, 34.20 and 20.68, respectively) compared with values for fish fed on 23% CP (17.91, 1.19, 27.38 and 17.86, respectively). Fish fed on 23% CP had significantly higher PER and PPV values (3.83 and 72.91, respectively). Fish reared under CWS had significantly higher FI, FCR and ER (28.36, 1.38 and 20.22, respectively), while fish reared under BFT had significantly higher PER, EG and PPV values (4.57, 31.62 and 87.40, respectively). The highest amount of feed intake was recorded by CWS^{28%} (33.94). CWS^{28%} treatment had significantly highest FBW (25.45) value, while CWS^{23%} treatment had the lowest FBW (16.40). Fish reared under biofloc treatment recorded the highest $\sum\omega_3$ levels in their whole bodies compared to that reared under (CWS) clear water system. It could be concluded that biofloc technology is a suitable system for *Liza carinata* cultivation economically and environmentally.

Keywords: *Liza carinata*, biofloc, cultivation, water quality, growth performance.

INTRODUCTION

World aquaculture is growing very fast to participate in solving the problem of protein food shortage especially in the developing countries. This industry causes high pressure on the environment due the great waste products in water bodies (Subasinghe, 2005; Gutierrez-Wing and Malone, 2006; Matos *et al.*, 2006; De Schryver *et al.*, 2008). So, there was a need to develop a technology suitable economically and environmentally (Van Wyk *et al.*, 1999; Kuhn *et al.*, 2010). Biofloc is one of these technologies which improve water quality as it depends on adding extra carbon source in aquaculture system causing developing of bacteria which have two main principle roles; the first causing nitrogen uptake and the second is that fish can feed on it. This improve water quality and minimize water exchange and the bacteria also participate as fed for aquatic organisms rich in protein and this cause low cost of biofloc (Avnimelech, 1999; Hargreaves, 2006; Crab *et al.*, 2007, 2009, 2010a). Biofloc technology has been used in high intensive farming systems of several shrimp species, such as *Penaeus monodon*, *Litopenaeus vannamei*, and *Macrobrachium*

rosenbergii (Burford *et al.*, 2003; Hariet *et al.*, 2006; Crab *et al.*, 2010b). Dempster *et al.* (1995) and Azim *et al.* (2003) reviewed that tilapias being capable of both filter feeding and detritivory are ideal candidates for Biofloc Feeding Technology (BFT). Green (2010) and Bartholomew & Kevin (2013) demonstrated that outdoor biofloc systems can be used to produce high yields of channel catfish. Hoa *et al.* (2013) studied the possible use of biofloc as a feed for *Artemia* sp. Also *Artemia* nauplii had been reared in 1 l cones at stocking density of 2nauplii / l by Toi *et al.* (2013). Also, El-Dahhar (2007) for striped mullet used BFT under three protein levels and three metabolic rates. *Liza carinata* is one of the most popular fish farmed in Egypt. *Liza carinata* had studied from different point of view by many authors such as El-hlafawy (2004) who studied the reproductive biology of it; Gangadhara *et al.*, (1990) had studied the food and feeding habits of it. Blader (1997) and Pombo *et al.* (2005) studied the suitable environment and habitats.

To our knowledge, there is no study on *Liza carinata* cultivation in different rearing conditions as density, polyculture and its optimum protein level. So, the objective of this study is to evaluate the suitability of biofloc system for rearing *Liza carinata* under different protein levels.

MATERIALS AND METHODS

Experimental design and condition

This experiment was carried out in Fish Breeding and Production Lab at National Institute of Oceanography and Fisheries in Suez governorate. Fry of *Liza carinata* were obtained from El-hag Zaglol fish farm (Gulf of Suez) and transported alive in aerated tank to the laboratory. Fry were acclimated for two weeks to laboratory conditions, and fed on diet contain 23% crude protein (CP). The experimental design consisted of 2x2 factors arrangement of two levels of crude protein (23 and 28% CP) and two culture systems; clear water system (CWS) change and biofloc treatment (BFT) with zero water exchange, as continuous: (CWS^{23%}, CWS^{28%}, BFT^{23%} and BFT^{28%}) where the superscripts refer to the levels of crude protein. Each treatment included three glass aquaria (80 X 45 X 30 cm) and stocked by 15 fry per aquarium with average initial body weight of 0.26±0.003g. The aquaria were filled with water from Suez Canal. Aeration was continuously provided using an air blower to maintain oxygen level not less than (5-6 mg/l). Fry were left under natural light. Fish in each replicate aquarium were weighted every 15 day and the amount of daily diet readjusted accordingly. The experiment was extended for 136 days.

Biofloc system:

For biofloc treatments, starch was added at the same amount of feeding to maintain the C/N ratio at 1: 10 activate heterotrophic bacteria growth (Avnimelech, 1999). Starch was completely mixed in a beaker and spread to the tank surfaces at the afternoon time. Adding carbohydrate under natural light and aeration condition are the main suitable circumstances that cause floc growth and development (Azim and Little, 2008). Fish were fed three times per day. The starch was also added daily at 14:00 hour and the aquarium with clear water; no starch was added.

Diet formulation:

The experimental diets were formed from fish meal, yellow corn, sunflower oil, starch, vitamins and minerals. The composition (%) and chemical analysis (% dry matter bases) of experimental diets are presented in Table (1). All experimental diets were processed by blending the dry ingredients into a homogenous mixture, then sun

flower oil was added, then water till the mixture stick together well. A home mincer with small diameter was used to form the diet too small as possible. The diet was crumbled by hand and stocked at 20°C in deep freezer until use according to Bryant *et al.* (1980) procedure.

Table 1: Chemical composition percentage of experimental diets varying in protein sources

Ingredients composition	Diet 23% protein	Diet 28 % protein
Fish meal	326.9 g	423.1 g
Yellow corn	673.1 g	576.9 g
Starch	2	2
Sunflower oil	9	9
*Vitamins & minerals premix	1	1
Chemical analysis (%)		
Dry matter (DM)	9.06	5.58
Crude protein (CP)	22.86	28.43
Lipid	17.70	19.02
Ash	6.56	9.68
Crude fiber (CF)	9.06	5.58
Nitrogen free extract (NFE)	34.76	31.71
**Gross energy (GE)(kcal/100gDM)	438.88	470.28
***Protein/energy ratio (P/E) (mg CP/kcal GE)	52.09	60.45

*Vitamins and minerals mixture each 3Kg of mixture content: 12m.IUvit.A, 22mlU vit.D3, 10g vit.E, 2g vit.K, 1g vit.B2, 1.5g vit.B6, 10mg. vit.B12, 30g.niacin, 1000mg. Folic acid, 50mg.Biotin, 10g banathonic acid, 50g Zinc, 30g.Iron, 60g. Manganese, 4g Copper, 100mg. Coblat, 100mg Selenium, 1000mg iodine.

** GE (Gross energy) (kcal / 100 g DM) = CP x 5.64 + EE x 9.44 + NFE x 4.11 calculated according to Macdonald *et al.* (1973).

*** Protein/energy (P/E) ratio = crude protein x 10000 / Digestible energy according to Garling and Wilson (1976).

Water quality:

During the experimental period water temperature, salinity, dissolved oxygen (DO) and pH were measured. Water samples were collected weekly for total ammonia nitrogen (TAN), nitrite nitrogen (NO₂) and nitrate nitrogen (NO₃) following the standard methods for the examination of water and wastewater (APHA.1998). Biofloc volume (FV) was determined on site using Imhoff cones weekly, registering the volume taken in by the flocs in 1000 ml of the tank water after 30 min sedimentation (Avnimelech and Kochba, 2009)

Chemical analysis:

At the beginning and the end of each experiment a random sample of fish and feed were taken. Fish was dissected to take a piece of white muscle in closed containers and stored in the deep freezer for chemical analysis to determine the proximate composition analysis of fish and diets including dry matter (DM), for crude protein by the Kjeldahl method using a Kjeltach auto-analyzer (Model 1030, Tecator, Hoganas, Sweden) (Bligh and Dyer., 1959). Etherextracts (EE) and ash contents were also determined. The chemical composition of the whole fish body and diet was determined according to the procedure of AOAC (1995).

Measurement of Growth performance:

Fish samples were taken every 15 day to determine total body weight (g) and total body length (cm). Feed quantity was always readjusted according to the increase in the body weight of the fish.

Total weight gain (TWG) (g), average daily body weight gain (ADG), specific growth rate (SGR), feed conversion ratio protein (FCR) and energy retention (ER) were determined according to Castell and Tiews (1980) as follows: $TWG = [FBW - IBW]$, $ADG = TWG / \text{time}$, $SGR \% = 100 \times (\ln FBW - \ln IBW) / (t)$ Where: Ln: Natural log, and t is the duration period, $(SR \%) = (\text{No. of fish at the end} / \text{No. of fish at start}) \times 100$, $FCR = FI / WG$, $PER = WG / PI$, $PPV = 100 (PG / PI)$, $ER = 100 \times EG / EI$ and $Kc = W / L^3 \times 100$

Fatty acid Analysis

For the analysis of fatty acid methyl esters, 0.1g of lipid weighted into 5ml tube with screw cap, adding 2ml hexane for dissolving, then 0.2ml 2N methanolic KOH was added. The tube was vortexed 30 second and waited for a while, up to the upper layer clarified. Clarified hexane solution was put into vials and analyzed in duplicate by gas chromatography method (GC) (Pearson *et al.*, 1981).

Gas Chromatography Condition

In order to determine fatty acid composition, the method of Bligh and Dyer (1959) was used. A homogenized fresh sample (25g) was extracted using chloroform/methanol/water mixture (5V/10V/5V). Fats extracts were converted into fatty acid methyl esters (FAME) using acetyl chloride and then analyzed by gas-liquid chromatography HP (Hewlett Packard) 6890 GC J. Chrom. Science 16,538-542(1978). A fused silica capillary column HP-5 (5% diphenyl, 95% dimethyl polysiloxane), 30m, 0.32mm ID, 0.25 μm film thickness was employed and the temperature program was as follows: initiated for 2 min 150°C then increased to 200°C for 10 min and to 250 °C for 5.0 min; held for 9 min at 250 °C. The carrier gas was nitrogen at 10 psig and detection was performed with a flame ionization detector at 250 °C. A programmed temperature vaporizer injector was employed in the split mode (50:1) and was heated at 220°C. Peaks corresponding to FAME were identified by comparing their retention times with those of standard mixtures (FAME Mix, Supelco, Inc.). Peak areas were automatically integrated. Content of each fatty acid was expressed as percentage weight of total fatty acids (% wt).

Statistics:

Data of the experiment were analyzed by two-way analysis of variance ANOVA. Significant differences were considered at $P < 0.05$. When significant differences were found, Duncan's multiple range tests was used to identify differences among experimental groups. All statistical analyses were performed using Duncan multiple range test at ($P < 0.05$) level (SPSS, 1997). Floc volume (FV) was performed according to t-test

RESULTS AND DISCUSSION

Water quality:

The results of water quality and biofloc levels are shown in Table (2). In the present study, bioflocs were promoted in the BFT tanks through addition of starch as a carbon source. FV levels increased gradually, and were kept within acceptable ranges. The formation and development of bioflocs in BFT water was linked with the direct assimilation of dissolved nitrogenous matters from diets and fish excretions by heterophilic bacteria (Avnimelech, 1999; Ebeling *et al.*, 2006). It should be noted that no water was discharged into the BFT tanks during the all experimental period. The measured water quality in all experimental groups remained within acceptable ranges.

Table 2: Water quality values of aquaria used in rearing *Liza carinata* under different aquaculture systems and protein levels

Parameters	Experimental groups			
	CWS		BFT	
	23% CP	28% CP	23% CP	28% CP
FV	—	—	14.22±7.46	15.59±8.1
Salinity (g/L)	37.83±2.3	37.81±2.3	37.76±2.2	37.3±2.3
Temperature (°C)	27.7±1.5	27.6±1.4	27.2±1.5	27.2±1.5
DO(mg/L)	5.16±0.75	5.11±0.89	5.58±0.43	5.72±0.45
pH	8.83 ±0.57	8.86 ±0.59	8.89 ±0.48	8.93 ±0.65
NH ₃ (mg/L)	1.07±1.16	1.05 ±1.2	0.55±1.02	0.61±1.03
Nitrite (mg/L)	0.024±0.21	0.022±0.23	0.052±0.17	0.077±0.14
Nitrate (mg/L)	0.86±0.69	0.92±0.65	1.1±0.62	1.4±0.63

The values in the same column with different superscript letters indicate statically significant difference.

Growth performance:

Crude protein levels had significantly effect on the FBW of fish. Fish fed on 28% CP was significantly having higher values of TWG, ADG, and SGR (24.15, 0.189 and 3.32, respectively) than Fish fed on 23% CP (17.65, 0.138 and 3.09, respectively). On the other hand, SR was insignificantly different between all treatments. The interaction between BFT and CWS was significantly has an effect on FBW as it was noticed that fish reared under CWS^{28%} conditions had significantly higher FBW (25.45) compared to fish reared under BFT^{28%}, BFT^{23%} and CWS^{23%} (23.39, 19.42 and 16.40, respectively). The same trend was recorded for TWG and ADG, while SGR was not affected with aquaculture system change and significantly affected by change in protein level (Table 3). This result disagrees with Xu *et al.* (2012) who recorded that the final weight, weight gain and SGR of *L. vannamei* in the biofloc treatments fed on diets with 30% and 35% CP were significantly higher than those obtained in the control fed on the diet with 25% CP and there were no significant difference among the BFT^{25%}, BFT^{30%} and BFT^{35%}.

Table 3: Growth performance and survival rate of *Liza carinata* reared under different aquaculture systems and protein levels

Parameter		IBW	FBW	TWG	ADG	SGR	SR
aquaculture system	CWS	0.26±0.02	20.92±5.02 ^a	20.66±5.02 ^a	0.161±0.039 ^a	3.23±0.016 ^a	99.17±0.98 ^a
	BFT	0.26±0.02	21.40±2.31 ^a	21.13±2.30 ^a	0.165±0.018 ^a	3.24±0.061 ^a	99.59±0.54 ^a
Protein levels	23%	0.258±0.02	17.91±1.79 ^b	17.65±1.79 ^b	0.138±0.013 ^b	3.09±0.042 ^a	99.67±0.57 ^a
	28%	0.268±0.02	24.41±1.44 ^a	24.15±1.43 ^a	0.189±0.011 ^a	3.32 ±0.071 ^b	99.33±0.57 ^a
interaction	CWS ^{23%}	0.257±0.002	16.40±1.08 ^c	16.14±1.08 ^c	0.126±0.006 ^c	3.02±0.036 ^c	99.68±0.78 ^a
	CWS ^{28%}	0.27±0.002	25.45±0.69 ^a	25.18±0.68 ^a	0.197±0.006 ^a	3.34±0.012 ^a	99.33±0.81 ^a
	BFT ^{23%}	0.26±0.002	19.42±0.19 ^b	19.16±0.19 ^b	0.15±0.0001 ^b	3.14±0.006 ^a	99.33±0.81 ^a
	BFT ^{28%}	0.267±0.002	23.39±0.1.21 ^a	23.11±1.22 ^a	0.18±0.01 ^a	3.27±0.042 ^a	99.00±1.00 ^a

The values in the same column with different superscript letters indicate statically significant difference.

Waselesky *et al.* (2006) demonstrated that *L. vannamei* juveniles grown in a BFT had higher growth rate than that grown in CWS. Likewise, Arnold *et al.* (2009) found that using biofloc in a high intensity tank system with zero exchange water could significantly enhance the growth of *Penaeus monodon* juveniles. In the same context, Megahed (2010) reported that *Penaeus semisulcatus* fed on dietary protein 16.25% with biofloc could even show better growth rate than shrimp fed on 42.95% CP without biofloc. Decamp *et al.* (2002) found that the growth performance of *L.*

vannamei reared in unfiltered pond water and fed either on 25%CP or 35% CP showed no significant differences. Also, *Hari et al.* (2004) found no significant differences between the specific growth rates of *P. monodon* fed on 25% CP and 40% CP in extensive shrimp culture system and biofloc system. *Ballester et al.* (2010) also demonstrated that the dietary protein content of *Farfante penaeus paulensis* can be reduced up to 10% (from 45% to 35%) without affecting shrimp growth performance under biofloc system. Reduction of dietary protein levels without affecting shrimp growth has been reported by several authors and microbial proteins have been provided as an important source of protein available for shrimp in these systems (*Decamp, et al.*, 2002; *Hari et al.*, 2004; *Ballester et al.*, 2010). *Avnimelech & Co-workers* (1994) and *Azim & Little* (2008) did not find any difference in tilapia growth when protein level in feed has been rose from 25% to 35%.

Survival rate:

High survival rates of the *Liza carinata* in the biofloc treatments were similar to that in the control group. This proves that the culture conditions in BFT aquaria were suitable for *Liza carinata* culture. This is in agreement with *Azim and Little* (2008), who stated that tilapia survival was 100% in all treatment and control aquariums. Also, *Krummenauer et al.* (2011) found that survival rate ranged from 75.0 to 92.0% at stocking densities ranging from 150 to 450shrimp/m²; however *Suresh and Lin* (1992) and *Rostika* (2014) recorded 93.56% and 94% survival in *Tilapia* and *L. vannamei* biofloc culture system respectively. In line with them, *Faizullah et al.* (2015) recorded that survival rate of 91.8 was recorded in the rearing of the goldfish *C. auratus* young in biofloc condition and 88.8% survival rate was recorded in the control.

Feed and nutrient utilization:

Feed intake and utilization results are presented in Table (4). Fish fed on 28% CP had significantly higher values for FI, FCR, EG and ER (25.81, 1.06, 34.20 and 20.68 respectively) compared with fish fed on 23% CP (20.58, 1.19, 27.38 and 17.86, respectively). Fish fed on 23% CP had significantly higher values of PER and PPV (3.83 and 72.91, respectively).

Table 4: Feed and nutrient utilization values of *Liza carinata* reared under different aquaculture systems and protein levels.

Parameter		FI	FCR	PER	PPV	EG	ER
aquaculture system	CWS	28.36±6.12 ^a	1.38±0.072 ^a	2.85 ±0.067 ^b	52.43± 1.7 ^b	29.96±5.3 ^b	20.22±1.48 ^a
	BFT	18.34±0.039 ^b	0.86 ±0.11 ^b	4.57 ±0.22 ^a	87.40 ±0.17 ^a	31.62 ±2.8 ^a	18.32±4.2 ^b
Protein levels	23%	20.58±2.4 ^b	1.17 ±0.26 ^b	3.83±0.39 ^a	72.91±1.7 ^a	27.38 ±4.9 ^b	17.86 ±2.7 ^b
	28%	25.81±8.9 ^a	1.06 ±0.32 ^a	3.60±0.075 ^b	66.92±1.7 ^b	34.20 ±2.5 ^a	20.68 ±3.1 ^a
interaction	CWS ^{23%}	22.77±0.083 ^b	1.37 ±0.091 ^a	3.10±0.94 ^b	58.07 ±2.47 ^b	25.39 ±1.20 ^c	21.13 ±1.0 ^a
	CWS ^{28%}	33.94±0.085 ^a	1.35 ±0.035 ^a	2.61±0.18 ^c	46.80 ±2.47 ^c	34.53 ±2.5 ^a	19.30±1.41 ^b
	BFT ^{23%}	18.39±0.068 ^c	0.96 ±0.009 ^b	4.56±0.29 ^a	87.75±2.47 ^a	29.37 ±0.31 ^b	14.58±0.15 ^c
	BFT ^{28%}	17.69±0.046 ^c	0.77 ±0.0341 ^c	4.60±0.21 ^a	87.04±2.47 ^a	33.88±2.11 ^a	22.06±1.38 ^a

The values in the same column with different superscript letters indicate statically significant difference.

Fish reared under CWS condition had significantly higher values of FI, FCR and ER (28.36, 1.38 and 20.22, respectively), while fish reared under BFT had significantly higher values for PER, EG and PPV (4.57, 31.62 and 87.40, respectively).

Two-way ANOVA showed a significant impact due to the interaction between aquaculture system and protein levels on feed intake. The highest amount of feed intake was recorded by fish reared under CWS^{28%} conditions (33.94) followed by CWS^{23%}, BFT^{23%} and BFT^{28%} (22.7, 18.39 and 17.69, respectively).

The highest PER and PPV values were recorded in fish reared under BFT compared to fish reared under CWS. The same results reported by Xu *et al.* (2012) on shrimp, and they indicated that this may be due to proteinases enzymes activities in feed digestion and biofloc that was used as food protein source. Avnimelech (2006) recorded that the increase of heterotrophic biofloc and microorganisms could break down and recycle shrimp residual feeds and wastes. Hari *et al.* (2004) also recorded that PER of the shrimp reared under BFT was higher than shrimp reared under CWS and that agrees with our results. Burford *et al.* (2003) assumed that starch addition in BFT bonds activates growth of bacterial floc and algae which in turn act as secondary protein source for fish causing improve of growth parameters.

Conditional factor:

Results recorded in Table (5) showed fish fed on 28% CP had higher FBL than fish fed on 23% CP (12.8 and 11.25, respectively). Fish reared under BFT system had higher FBL than CWS system (12.55 and 11.50, respectively). The highest K_C value was recorded by fish reared under CWS^{23%} (1.50) followed by fish reared under CWS²⁸, BFT^{23%} and BFT^{28%} conditions (1.24, 1.07 and 1.09, respectively). Fish fed on 23% CP had condition factor (K_C) significantly higher than fish fed on 28% CP (1.29 and 1.17, respectively). Fish reared under CWS condition had significantly higher K_C than that of BFT (1.37 and 1.08, respectively).

Table 5: Initial, final body length and conditional factor (K_C) values of *Liza carinata* reared under different aquaculture systems and protein levels.

Parameter		IBL	FBL	K_C
aquaculture system	CWS	2.5±0.0001	11.50±1.31	1.37±0.16 ^b
	BFT	2.5±0.0001	12.55±0.38	1.08±0.037 ^a
Protein levels	23%	2.5±0.0001	11.25±1.42	1.29±0.23 ^a
	28%	2.5±0.0001	12.8±0.27	1.17±0.097 ^b
Interaction	CWS ^{23%}	2.5±0.0001	10.30±0.0001	1.50±0.10 ^a
	CWS ^{28%}	2.5±0.0001	12.7±0.0001	1.24±0.03 ^b
	BFT ^{23%}	2.5±0.0001	12.2±0.0001	1.07±0.01 ^c
	BFT ^{28%}	2.5±0.0001	12.9±0.0001	1.09±0.037 ^c

The values in the same column with different superscript letters indicate statically significant difference.

These results disagreed with Wassef *et al.* (2001) who showed that mullet (*Mugil cephalus*) fingerlings were fed on four diets containing 10, 15, 20 and 25% algal meal-based diets. A fifth test diet containing 40% dietary yeast enriched with vitamin E was further investigated for 15 weeks. They found that condition factor (K_C value) was not significantly affected by various dietary treatments. Also, Kheir *et al.* (1998) attributed this fluctuation in K_C value to the increase of food consumption as a result of increased metabolism, minimized oxygen consumption and an increase in growth hormone.

Body chemical composition:

The whole body chemical composition is recorded in (Table 6). In the present study, fish reared under BFT system had the highest dry matter (DM) and ash content (27.81 and 15.42, respectively) compared to fish reared under CWS system (26.67 and 14.43, respectively). The highest DM content was recorded by fish reared under BFT^{28%} conditions (28.04), followed by BFT^{22%}, CWS^{22%} and CWS^{28%} (27.57, 27.44 and 25.89, respectively). Protein levels revealed significant effects on CP. Fish reared under CWS had the highest CP and EE content (68.83 and 17.21, respectively) when compared to fish reared under BFT (68.65 and 16.05, respectively). The highest EE content was recorded by fish reared under CWS^{23%} (19.79) followed by fish reared

under BFT^{23%}, BFT^{28%} and fish reared under CWS^{28%} (17.05, 15.43 and 14.65, respectively), where no significant difference were found between fish reared under CWS and fish reared under BFT on the whole body dry matter (DM) and crude protein (CP). This agreed with Azim and Little (2008) who revealed that no significant difference between clear water system (CWS) and Biofloc technology (BFT) system were recognized.

Table 6: Chemical composition values of muscle of *Liza carinata* reared under different aquaculture systems and protein levels.

System		DM	CP	EE	ASH
aquaculture system	CWS	26.67±1.55 ^b	68.83±0.63 ^a	17.21 ±2.8 ^a	14.43 ±1.89 ^b
	BFT	27.81±0.58 ^a	68.65±1.42 ^a	16.05 ±1.19 ^b	15.42 ±3.22 ^a
Protein levels	23%	27.51±1.13 ^a	69.13±0.62 ^a	18.42 ±2.63 ^a	12.68 ±2.06 ^b
	28%	26.96±1.28 ^b	68.34 ±0.71 ^b	14.85 ±1.39 ^b	17.18 ±3.06 ^a
Interaction	CWS ^{23%}	27.44±1.53 ^b	68.41 ±0.37 ^b	19.79 ±0.31 ^a	12.81 ±0.75 ^c
	CWS ^{28%}	25.89±1.35 ^c	69.24±0.58 ^a	14.65 ±0.59 ^c	16.07 ±0.58 ^b
	BFT ^{23%}	27.57 ±0.33 ^b	69.85±0.58 ^a	17.05 ±0.39 ^b	12.55 ±0.98 ^c
	BFT ^{28%}	28.04 ±0.76 ^a	67.44 ±0.64 ^c	15.43 ±0.58 ^c	18.29 ±0.50 ^a

The values in the same column with different superscript letters indicate statically significant difference.

CWS: Clear water system.

BFT: Biofloc food technology.

In the same context, no significant difference between change water system (CWS), and BFT system in shrimp dry matter and crude protein content, but biofloc showed superiority for ether extract and ash content, while a significant in biofloc composition in terms of protein and ash content between different protein levels (Xu and Pan, 2012).

On the other hand, the difference of DM, CP, and ASH between CWS and BFT and protein levels were significant in the present study. The same trend was observed by Xu *et al.* (2012) who noticed significantly decrease in biofloc protein content as dietary protein level decreased, while no change in lipid and ash content were reported between treatments. Azim *et al.* (2008) reported that there were significant differences in protein and fat composition of biofloc with the highest protein in the diet, but the opposite was recorded for ash and fiber composition.

Only slight differences were found in the carbohydrate, fat and ash compositions among shrimps grown in the control and biofloc tanks, representing that the quality of flesh was not affected by any of the culture conditions used in the study of Karakoltsidis *et al.* (1995), but proximate compositions in shrimps' flesh were affected by several factors such as season, species, growth stage and feed.

Fatty acids:

The highest $\sum\omega_3$ values were recorded in fish reared under BFT^{28%} condition, followed by BFT^{23%}, CWS^{28%} and CWS^{23%} (12.38, 9.80, 5.96 and 4.99, respectively) (Table 7), and the highest $\sum\omega_6$ values were recorded in fish reared under BFT^{28%}, followed by BFT^{23%}, CWS^{28%} and CWS^{23%} (3.84, 3.56, 2.64, and 2.49, respectively) (Table 7), which give an advantage as producing functional food, forming simple and eco-friendly system. The $\sum\omega_3$ values were lower than that of Özogul *et al.* (2009) who found it ranged from 12.66% for annular sea bream to 36.54% for European hake, whereas $\sum\omega_6$ value was 1.24% for Oceanic puffer and 12.7% for Flathead mullet. These values were different than that of our study as this difference in values may be attributed to the variation in species and areas of fish under study.

In the present study, the highest average $\sum\omega_3/\sum\omega_6$ ratio was recorded in fish reared under BFT^{28%}, followed by BFT^{23%}, CWS^{28%} and CWS^{23%} (3.22, 2.75, 2.26 and 2.00, respectively) (Table 7). The $\sum\omega_3/\sum\omega_6$ ratio increased within the same treatment by increasing protein level and it was higher in BFT than CWS. These values were lower than 4.0, so they have useful effect on human health and higher than that recorded by HMSO UK(1994), who found the highest ratio was recorded in striped sea bream (1.63), followed by the wide-eyed flounder (1.45), the sand sole (1.34), while the lowest values were recorded in golden grey mullet and African tread fishes (0.51).

Table 7: Fatty acid composition in *Liza carinata* muscles affected by different treatments.

Nature	Fatty acid	CWS		Biofloc	
		23%CP	28%CP	23%CP	28% CP
SFA	C6:0		0.183	1.719	
	C8:0	0.031	0.093		0.070
	C10:0	0.012	0.053		0.026
	C11:0				0.132
	C12:0	0.256	0.198	0.066	0.223
	C13:0	1.058	0.545	0.18	0.162
	C14:0	4.854	0.274	5.65	12.07
	C15:0	5.41	0.089	4.959	9.12
	C16:0	38.55	31.06	39.66	16.01
	C17:0		11.59	7.82	
	C18:0	13.91	25.46	9.15	16.1
C20:0	5.31	1.64	1.05		
C21:0					
MUFA	C14:1	0.478		0.051	0.323
	C15:1	0.868		0.17	0.903
	C16:1	6.58	7.789	6.064	
	C17:1	6.478	7.789		15.23
	C18:1n1&9	8.719	10.2	10.30	11.69
PUFA	C18:3				
	C18: 2n 6(LA)	1.856	1.247	0.547	2.045
	C20: 4n 6(ARA)	1.993	4.088	4.973	6.637
	C20: 5n3 (EPA)	2.188	1.394	1.017	1.804
	C22: 6n3 (DHA)	1.45	1.873	4.831	5.749
	C22:2		1.411		1.91
ω_3	4.993	5.961	9.804	12.386	
ω_6	2.494	2.641	3.564	3.849	
ω_3/ω_6	2.000	2.26	2.75	3.22	
\sum SFA	69.39	71.19	70.25	53.91	
\sum MUSFA	23.12	17.99	16.58	28.15	
\sum PUSFA	7.49	10.01	13.37	18.15	
PUSFA/SFA	0.108	0.141	0.190	0.337	

The recommended minimum value of $\sum\omega_3/\sum\omega_6$ ratio is 0.45 (HMSOUK, 1994) and this agrees with UK department and also the maximum recommended value in the diet should be 4.0; this value is very useful for health as it promote cardiovascular disease, atherosclerosis and many chronic diseases reported by HMSO UK (1994); Simpoulos. (2009); Polak-Juszczak and Komar-Szymczak (2009).

CONCLUSION

In conclusion, using biofloc system in aquaculture decreases the negative impact of fish farms on the environment and also improves the economic efficiency. Biofloc system is suitable for *Liza carinata* culture, and the obtained fish is healthy as the $\sum\omega_3/\sum\omega_6$ ratio within the recommended UK values.

REFERENCES

- American Public Health Association. Water Environment Federation (APHA) (1998). Standard Methods for the Examination of Waters and Wastewaters.
- AOAC, (1995). Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.
- Arnold, S. J.; Coman, F. E.; Jackson, C. J. and Groves, S. A. (2009). High-intensity, zero water exchange production of juvenile tiger shrimp, *Penaeus monodon*. An evaluation of artificial substrates and stocking density. *Aquaculture*, 293: 42-18.
- Avnimelech, Y. (2006). Bio-filters: the need for a new comprehensive approach. *Aquaculture*, 34 (3): 172-178.
- Avnimelech, Y. and Kochba, M. (2009). Evaluation of nitrogen uptake and excretion by tilapia in bio floe tanks, using N15 tracing. *Aquaculture*, 287:163-168.
- Avnimelech, Y.(1999). Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176: 227–235.
- Avnimelech, Y.; Kochva, M. and Diab, S. (1994). Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. *Israeli Journal of Aquaculture-Bamidgeh*, 46(3): 119-131.
- Azim, M.E. and Little D.C. (2008). The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*) *Aquaculture*, 283: 29-35.
- Azim, M. E.; Little, D. C. and Bron, J. E. (2008). Microbial protein production in activated suspension tanks manipulating C: N ratio in feed and the implications for fish culture. *Bio resource Technology*, 99: 3590-3599.
- Azim-, M. E.; Verdegem, M. C. J.; Mantingh, L; van Dam, A. A. and Beveridge, M. C. M. (2003). Ingestion and utilization of periphyton grown on artificial substrates by Nile tilapia (*Oreochromis niloticus*) *Aquacult. Res.*, 34:85-92.
- Ballester, E. L. C.; Abreu, P. C.; Cavalli, R. O.; Emerenciano, M.; Abreu, L. and Wasielesky, W. (2010). Effect of practical diets with different protein levels on the performance of *Farfante penaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. *Aquaculture Nutrition*, 16: 163-172.
- Bartholomew, G. and Kevin K. S. (2013). Effect of initial biomass on Channel Catfish yield and water quality in a biofloc technology Production System. *World Aquaculture Society Meetings*. Saturday, February 23.
- Blaber, S. J. M. (1997). *Fish and fisheries of tropical estuaries*. Chapman and Hall, London.
- Bligh, E. G., and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology*, 37(8): 911-917.
- Bryant, P.; Jauncey, K. and Attack, T. (1980). *Backyard fish farming*. Prism press, 170 pp.
- Burford, M.A.; Thompson, P.J.; McIntosh, R.P.; Bauman, R.H. and Pearson, D.C. (2003). Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. *Aquaculture*, 219:393-11. Cambridge, NY, USA, 388pp.
- Castell, J. D. and Tiewes, K. (1980): Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Research, Hamburg, FRG, 21-23 March 1979. IFAC Tech. Pap. (3) 24.
- Crab, R.; Avnimelech, Y.; Defoirdt, T.; Bossier, P. and Verstraete, W. (2007). Nitrogen removal techniques in aquaculture for a sustainable production. *Aquaculture* 270: 1–14.

- Crab, R.; Chielens, B.; Wille, M.; Bossier, P. and Verstraete, W. (2010a). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. *Aquaculture Research*, 41: 559–567.
- Crab, R.; Chielens, B.; Wille, M.; Bossier, P. and Verstraete, W. (2010b). The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* post larvae. *Aquaculture Research*, 41: 559–567.
- Crab, R.; Kochva, M.; Verstraete, W. and Avnimelech, Y. (2009). Bio-flocs technology application in over-wintering of tilapia. *Aquaculture Engineering*, 40: 105–112.
- De Schryver, P.; Crab, R.; Defoirdt, T.; Boon, N. and Verstraete, W. (2008). The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*, 277:125-137.
- Decamp, O.; Conuest, L.; Forster, I. and Tacon, A. G.J. (2002). The nutrition and feeding of marine shrimp within zero-water exchange aquaculture production system: role of Eukaryotic microorganisms. In: Lee, C.S., O'Bryen, P. (Eds.), *Microbial Approaches to Aquatic Nutrition within Environmentally Sound Aquaculture Production systems*. World Aquaculture Society, Baton Rouge, USA, pp. 79-86.
- Dempster, P.; Baird, D. J. and Beveridge, M.C.M. (1995). Can fish survive by filter-feeding on micro crop articles? Energy balance in tilapia grazing on algal suspension. *Journal of Fish Biology*, 47: 7-17.
- Ebeling, J. M.; Timmons, M. B. and Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photo autotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*, 257: 346-358.
- El-Dahhar, A. A. (2007). Review Article on Protein and Energy Requirements of Tilapia and Mullet. *Journal of the Arabian Aquaculture Society*. 2(1): (28).
- EL-Halfawy, M. M. (2004). Reproductive biology of the *Mugil seheli* (Family mugilidae) reared in fish farm lake, Suez Canal. *Egypt. J. Aquat. Res.* 30(B): 234-240.
- Faizullah, M.; Rajagopalsamy, C. B. T.; Ahilan, B. and Francis, T. (2015). Impact of Biofloc Technology on the Growth of Goldfish Young Ones. *Indian J. Sci. Technology*, 8(12).
- Gangadhara, G.; Shanbhogue, S. and Gowda, H. (1990). Studies on the food and feeding habits of the grey mullet, *Valamugil seheli* (Forsk.) from Mangalore (India) waters. *Mysore J. Agric. Sci.*, 23 (3): 406- 410.
- Green, B.W. (2010). Effect of channel catfish stocking rate on yield and water quality
- Gutierrez-Wing, M.T.; Malone, R. F. (2006). Biological filters in aquaculture: trends and research directions for freshwater and marine applications. *Aquac. Eng.*, 34 (3): 163-171.
- Hargreaves, J.A. (2006). Photosynthetic suspended-growth systems in aquaculture. *Aquaculture Engineering*, 34: 344–363.
- Hari, B.; Kurup, B.M.; Varghese, J.T.; Schrama, J.W. and Verdegem, M.C.J. (2006). The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. *Aquaculture*, 252 (2-4): 248-263.
- Hari, B.; Madhusoodana, K.; Varghese, J.T.; Schrama, J.W. and Verdegem, M. C. J. (2004). Effects of carbohydrate addition on production in extensive shrimp culture systems. *Aquaculture*, 241: 179-194.
- HMSO UK. (1994). Nutritional aspects of cardiovascular disease. Report on health and social subjects no.46. London: HMSO.
- Ho, N.V.; Dieu, D. K.; Anh, N. T. and Van, N. T. (2013). Use of bioflocs grown at different salinities as a feed for *Artemia* in laboratory conditions. *World Aquaculture Society Meetings*. Saturday, February 23, in an intensive, mixed suspended-growth production system. *N. Am. J. Aquacult.*, 72: 97–106.

- Kheir, M. T.; Mechail, M. M. and Abo-Hegab, S. (1998). Some biochemical changes in *Oreochromis niloticus* reared at different saline concentration. Egypt Journal. Zool, 30:107-129.
- Krummenauer, D.; Peixoto, S.; Cavalli, R. O.; Poersch, L. H. and Wasielesky, W. (2011). Super intensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in southern Brazil at different stocking densities. Journal of the World Aquaculture Society, 42:726–733.
- Kuhn, D. D.; Lawrence, A. L.; Boardman, G. D.; Patnaik, S.; Marsh, L. and Flick, J. G. J. (2010). Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 303: 28–33
- Matos, J.; Costa, S.; Rodrigues, A.; Pereira, R. and Pinto, I. S. (2006). Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. Aquaculture, 252(1): 31-42.
- Megahed, M.E. (2010). The effect of microbial biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus Semisulcatus*) fed with different crude protein levels. Journal of the Arabian Aquaculture Society, 5: 119-142.
- Özogul, Y.; Özogul, F. H.; Çiçek, E.; Polat, A. and Kuley, E. (2009). Fat content and fatty acid compositions of 34 marine water fish species from the Mediterranean Sea. International journal of food sciences and nutrition, 60(6): 464-475.
- Pearson, D.; Harold, E.; Ronald, S.K. and Ronald, S. (1981). Pearson's Chemical Analysis of Foods Book, Eighth Edition.
- Polak-Juszczak, L. and Komar-Szymczak, K. (2009). Fatty acid profiles and fat contents of commercially important fish from Vistula Lagoon. Polish J. Food and Nutrition Sci., 59(3): 225-229.
- Pombo, L.; Elliott, M. and Rebelo, J. E. (2005). Environmental influences on fish assemblage distribution of an estuarine coastal lagoon, Ria de Aveiro (Portugal). Sci. Mar., 69: 143-159.
- Rostika, R. (2014). The reduction feed on shrimp vaname (*Litopenaeus vannamei*) replaced by the addition biofloc in Ciamis District. Res J Biotech, 9(2):56–9.
- SPSS (1997). Statistical package for the social sciences, Versions16, SPSS in Ch, Chi-USA.
- Subasinghe, R. P. (2005). Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. Preventive Veterinary Medicine, 67(2): 117-124.
- Suresh, A.V. and Lin, C. K. (1992). Effect of stocking density on water quality and production of red tilapia in a recirculated water system. Aquaculture Engineering, 11: 1–22.
- Toi, H.T.; Ahyani, N.; Sorgeloos, P.; Bossier, P. and Van, G. (2013). Manipulation of C/N ratio to stimulate the growth of bacteria as food for filter feeders in a laboratory culture system: a demonstration on *Artemia*. World Aquaculture Society Meetings. Saturday, February 23.
- Van Wyk, P.; Davis-Hodgkins, M.; Laramore, C.R.; Main, K.; Mountain, J. and Scarpa, J. (1999). Farming Marine Shrimp in Recirculating Freshwater Production Systems: A Practical Manual. FDACS Contract 4520. Florida Department of Agriculture Consumer Services, Tallahassee, Florida, US.
- Wasielesky, W.; Atwood, H.; Stokes, A. and Browdy, C.L. (2006). Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture, 258: 396-403.

- Wassef, E. A.; El Masry, M. H. and Mikhail, F. R. (2001). Growth enhancement and muscle structure of striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets. *Aquaculture Research*, 32 (Suppl. 1): 315-322.
- Xu, W. J.; Pan, L. Q.; Zhao, D. H.; and Huang, J. (2012). Preliminary investigation into the contribution of bioflocs on protein nutrition of *Litopenaeus vannamei* fed with different dietary protein levels in zero-water exchange culture tanks. *Aquaculture*, 350: 147-153.
- Xu, W. J. and Pan, L.Q. (2012). Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture*, 356-357:147-152.
- Zaki, M. I. and El Gharabawy, M. M. (1991). Reproductive biology of *Mugil capito* in Egypt. *Assiut Veterinary Medical Journal*.

ARABIC SUMMARY

استزراع سمكه السهلبيه باستخدام نظام البيوفلوك الصديق للبيئه

مجدي توفيق خليل^١، رجب محمد^٢، رجاء الديب^١، اشرف سلومه^٣، باسم عبدالعاطي^٢، شيماء حنيش^٢

١- قسم علم الحيوان، كلية العلوم، جامعه عين شمس، مصر.

٢- المعهد القومي لعلم البحار والمصايد، مصر.

٣- معمل تغذيه الاسماك، قسم انتاج الحيوان، كلية الزراعة جامعه القاهرة، مصر.

تم دراسته وتقييم امكانيه استخدام نظام البيوفلوك لاستزراع سمكه السهلبيه. اجريت هذه التجربه بمعمل تربيته و انتاج الاسماك بالمعهد القومي لعلم البحار والمصايد فرع السويس بمحافظة السويس. صممت هذه التجربه لتتكون من ٢X٢ عاملين عباره عن مستويين من البروتين (٢٣% و ٢٨% بروتين خام) و نظامين للاستزراع هما (نظام المياه النقيه حيث يتم تغيير المياه ونظام البيوفلوك حيث لا يتم تغيير المياه): (CWS^{23%}, CWS^{28%}, BFT^{23%} and BFT^{28%}) حيث تشير الارقام العلويه لنسبه البروتين. اجريت كل معامله في ثلاث احواض زجاجيه ابعادها (٨٠ X ٤٥ X ٣٠ سم) بمعدل ١٥ سمكه للحوض ووزن ابتدائي ٠.٢٦±٠.٠٠٣ جم. تم تقدير الوزن المكتسب النهائي، معدل الزيادة اليوميه في الوزن، ومعدل النمو النسبي للاسماك. لقد اظهرت النتائج ان الاسماك التي غذيت على عليقه تحتوي على ٢٨% بروتين خام اعطت اعلى وزن نهائي للجسم، كما سجلت النتائج انها استهلكت اكثر كميته من الغذاء، واعلى معدل في التحول الغذائي، والطاقيه المكتسبه، والطاقيه المحتجزه بقيم (٢٤.٤١، ٢٥.٨١، ١.٠٦، ٣٤.٢٠، ٢٠.٦٨، ١٧.٩١، ٢٧.٣٨، ١٧.٨٦، على التوالي) بالمقارنه بالاسماك التي غذيت على عليقه تحتوي على ٢٣% بروتين وكانت قيمتها (١٧.٩١، ١٧.٩١، ١.١٩، ٢٧.٣٨، ١٧.٨٦، على التوالي). كما اوضحت النتائج ان الاسماك التي غذيت على ٢٣% بروتين سجلت اعلى كفاءه تحول انتاجيه للبروتين، وكانت قيمتها (٣.٨٣ و ٧٢.٩١، على التوالي). ووضحت النتائج ان الاسماك التي كانت تحت تأثير نظام المياه النقيه اظهرت اعلى معدل تحول للغذاء وكميه طاقه محتجزه كما انها استهلكت اكثر كميته من الغذاء المأكول وكانت قيمتها (٢٨.٣٦، ١.٣٨ و ٢٠.٢٢، على التوالي) بينما اظهرت النتائج ان الاسماك تحت تأثير نظام البيوفلوك سجلت اعلى طاقه مختزنه، انتاجيه للبروتين وكفاءه بروتين، وكانت قيمتها (٤.٥٧، ٣١.٦٢، ٨٧.٤٠، على التوالي). وقد اوضحت النتائج ايضا ان الاسماك تحت تأثير التداخل بين نسبه البروتين المتناول ونظام الاستزراع استهلكت اعلى كميته غذاء في الاسماك تحت تأثير نظام المياه النقيه وتتناول بروتين ٢٨% وكانت قيمتها (٣٣.٩٤). كما سجل ايضا اعلى وزن جسم نهائي (١٦.٤٠). ولقد سجل اعلى (٣) في جسم الاسماك تحت نظام البيوفلوك بالمقارنه بالاسماك تحت تأثير نظام المياه النقيه. ومن هذا نستنتج ان نظام البيوفلوك مناسب لاستزراع سمكه السهلبيه اقتصادياً وبيئياً.