

CHAPTER FOUR
IV. RESULTS AND DISCUSSION

4.1. The First Experiment:

This experiment was conducted in El-Max Research Station, National Institute of Oceanography and Fisheries, Alexandria, to study the effects of C/N ratio levels on growth performance, survival rate, water quality, and economic feasibility to *Mugil cephalus* fingerlings.

4.1.1. Water quality criteria:

Differences between change water or control system (CS) and biofloc system (BFT) in water quality parameters are shown in (Table 6). Generally, the average water temperature during the experimental period ranged between (22.9-25.5^c), while pH ranged between (7.95-9.56), salinity ranged (29.8-35.5) ppt, dissolved oxygen (DO) ranged (5.9-6.6) ppm, and total dissolved solid (TDS) ranged between (27.2-35.8)g/l, and organic phosphors ranges were (0.7-1.2) ppm. However the amount of dissolved oxygen in pond, temperature and the pH are the most important physico - chemical features of fish culture (Ntengwe and Edema, 2008).

Also, total nitrogen ammonia (TNA) values which were used in calculating unionized ammonia (NH₃) Fig. (14) changed numerically within the normal range (0.5-2 mg/l) for both (CS) and (BFT). In the same context normal range of nitrite (NO₂) (0.5 mg/l) was observed for both CS and BFT during experimental period Fig. (15). Also the weekly data for NO₂ showed numerical decrease for BFT comparable to CS. A result of nitrate NO₃ was noticed for higher values than normal range (5mg/L) for both BFT system and CS system during the experimental period Fig. (16).

These results indicated that there were significant differences in water, salinity, TDS, DO, and pH due to different culture system types (CS and BFT), and no significant difference in water temperature and organic phosphors, but by referring to the output of nitrogen TNA, NO₂, and NO₃ there is a significant difference for the benefit of treatment 60% starch with CP 20% which recorded the lowest measurements. Also the water in control ponds were slightly better than biofloc bonds, which were non-transparent because of their high turbidity . Also, these values were beneficial for fish culture (Huet, 1986; Boyd, 1979 and 1990).

However, the averages of water temperature were within the range considered for fish growth. This temperature is suitable for all chemical, physical and biological processes in ponds water as cited by Boyd (1979). Same findings were observed by Bakeer *et al.*, 2008 and Boyd and Lickpoppler (1979) who demonstrated that water with pH higher 6.5 is considered the best for fish production and the pH levels were suitable for wellbeing of mullet fish.

The average dissolved oxygen concentrations in all experimental ponds were almost not less than 5.9 mg/l during the experimental period. This situation is suitable for fish cultivation and agrees with the finding of NACA (1989).

Low organic phosphors, TDS, and salinity considered to be less favorable for good mullet culture, with culture system, CS and BFT.

The proportion of unionized ammonia (NH₃) is regulated by the pH and temperature of the water. An increase of pH values increase the proportion of un-ionized ammonia (Boyd, 1990 and Wurts, 2003). In this experiment, ammonia (NH₃) was never a problem, and not exceed 0.05 mg/l.

Table (6). Difference in water quality parameters status in the experimental period between (BFT) and (CS) during the first experiment.

Items	Concrete ponds	
	Control Ponds (Change water)	Biofloc ponds (No change water)
Temperature (°C)	23.1-24.9	22.9-25.5
Salinity (mg/l)	29.8-31.5	29.9-35.5
pH	8.31-9.13	7.95-9.56
TDS(g/l)	29.3-33.8	27.2-35.8
Organic phosphors(ppm)	0.81-1.2	0.7-1.2
DO (mg/l)	5.9-6.1	6.3-6.6
Total nitrogen ammonia(TNA) ppm	0.1-0.511	0.04-0.556
Nitrogen nitrite (No ₂) ppm	0.03-0.26	0.02-0.28
Nitrogen nitrate (No ₃) ppm	2-10	2-12

Under biofloc system BFT (zero exchange water), addition of starch as a carbohydrate source kept water parameters (TNA and No₂) within the normal ranges as change water system (CS) where ,water in (CS) was daily changed. 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 for feed is adjusted to about 20:1 by adding starch, the water quality could be controlled by the bioflocs growing in the ponds. At this C/N ratio, the inorganic nitrogen was converted into organic nitrogen, due to assimilation by the dens floc cultures (heterotrophic bacteria) (Carb *et al.* 2009). This corresponds to the theoretical predictions made by Avnimelech, (1999).

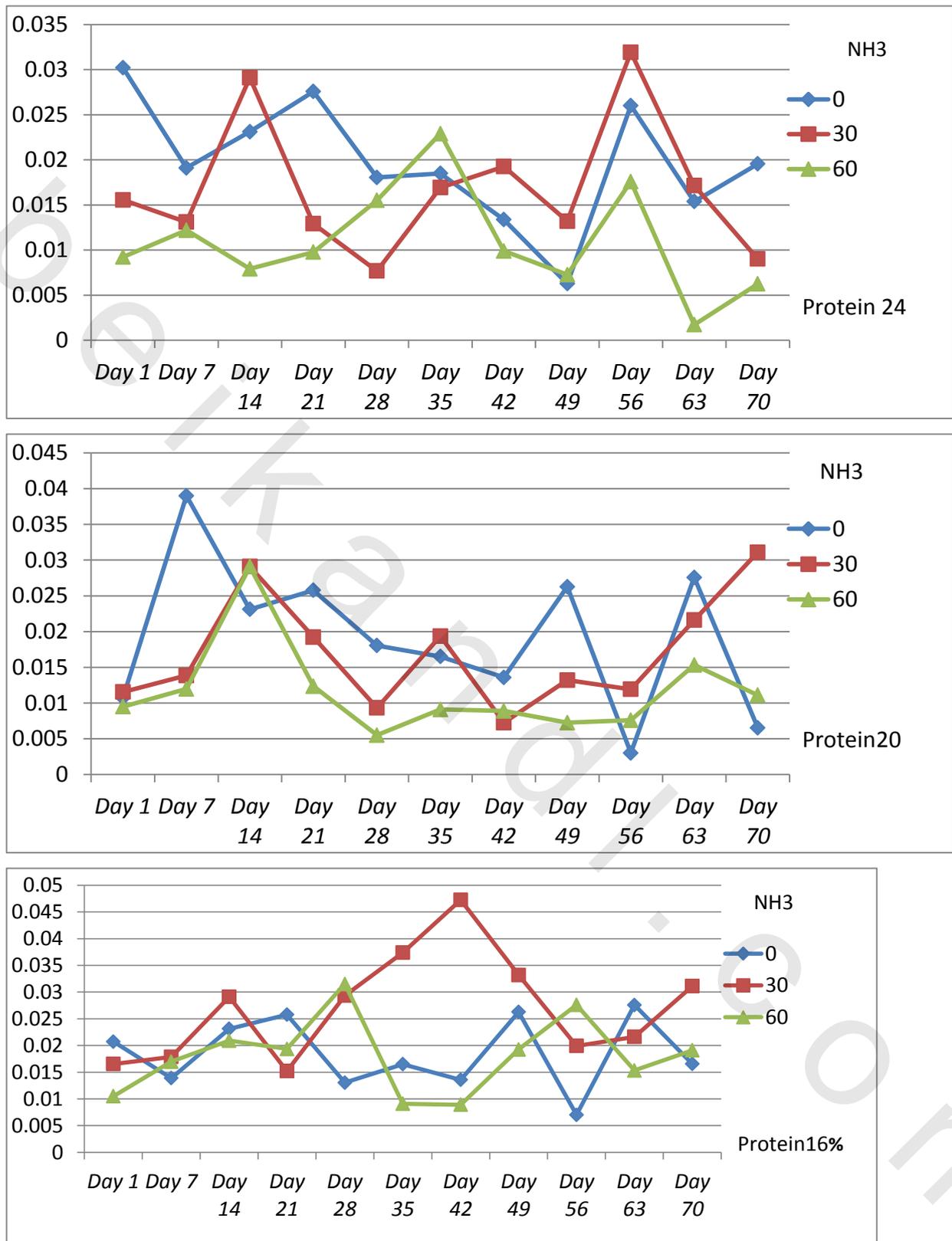


Fig. (14). Unionized ammonia (NH₃) values for control and biofloc treatments proteins (24%, 20%, and 16%).

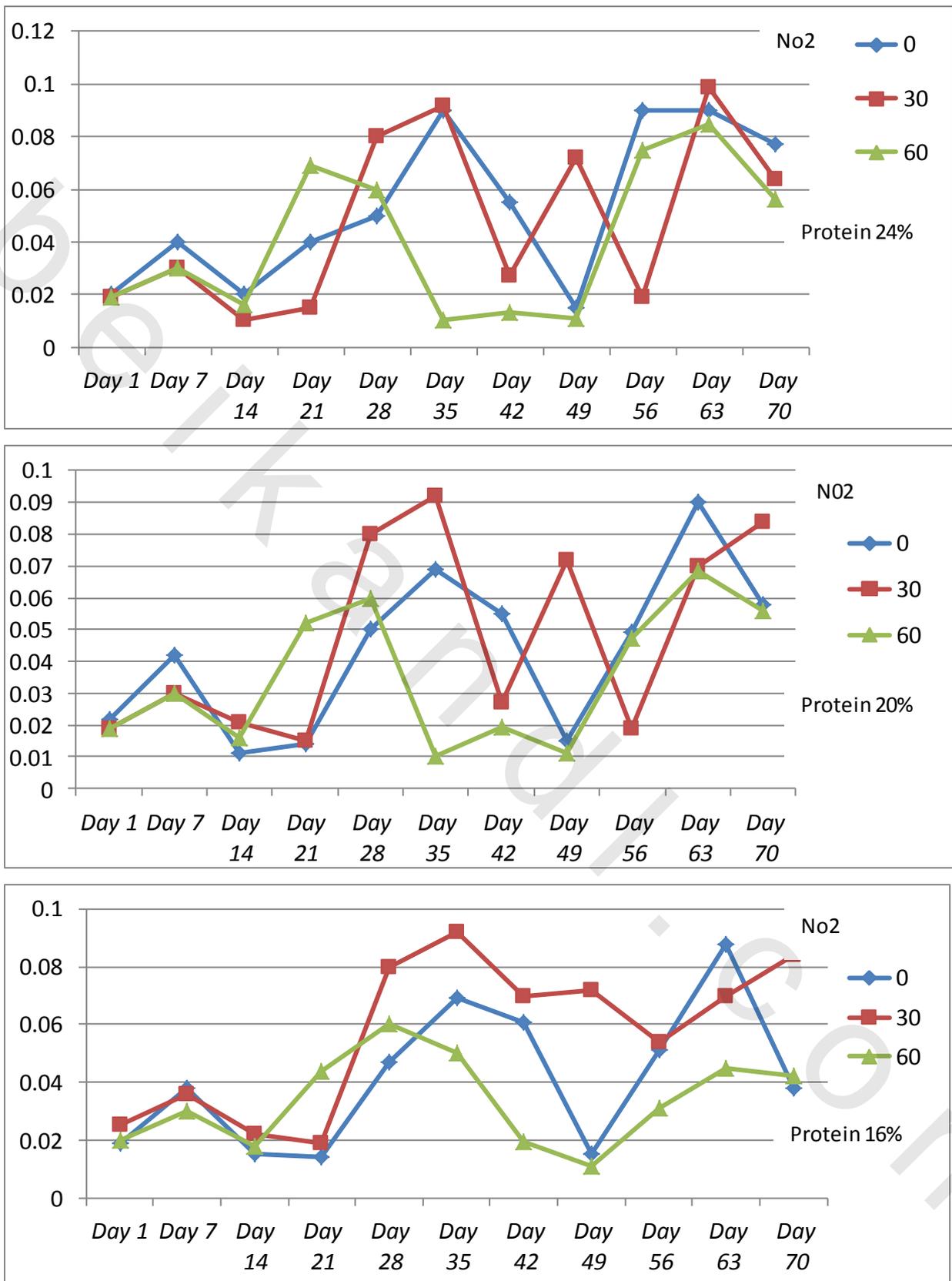


Fig. (15). Nitrite nitrogen (No₂N) values for control and biofloc treatments under proteins (24%, 20%, and 16%).

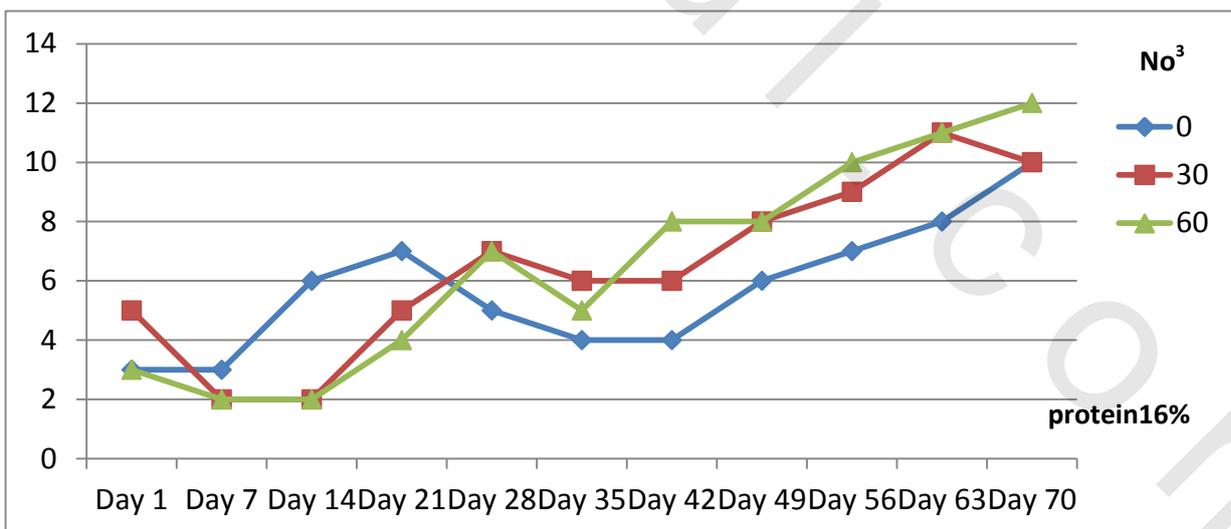
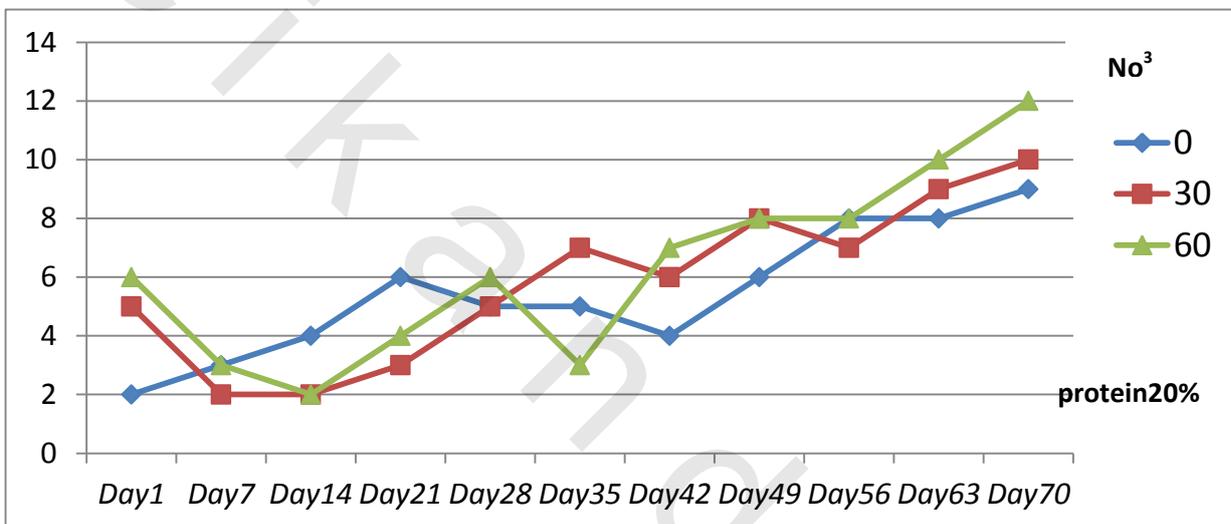
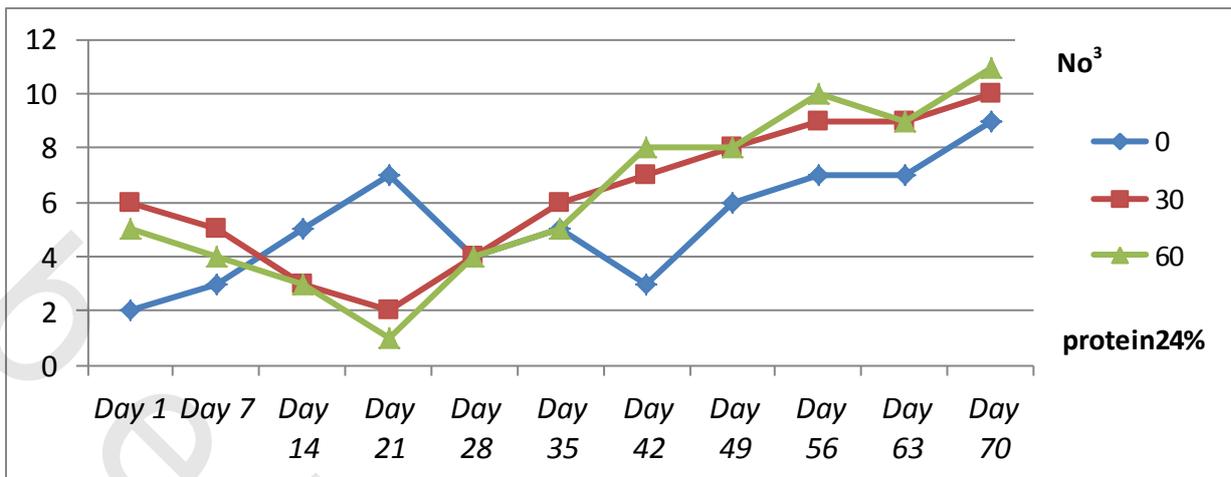


Fig. (16). Nitrate nitrogen (No_3) values for control and biofloc treatments under proteins (24%, 20%, and 16%).

Carbohydrate addition to the water column under extensive shrimp culture conditions reduced the concentration of both TNA 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 bacteria (Azim *et al.*, 2008).

Similar results were suggested by (Carb *et al.* 2009) for tilapia fish. Additionally, Kuhn *et al.* (2009) reported that no differences were observed between any water quality parameters, there by confirming system uniformity. Water quality levels were within safe levels for normal shrimp health, growth, and survival under BFT system.

Although the TAN levels remained low, they fluctuated weakly (between 0.0511 and .566 mg/l) only in the control treatment. The concentrations of TAN in the biofloc-rich water treatments remained low and were significantly lower than the levels found for the control treatment except the biofloc treatment with (16% CP protein 30% starch), It is probable that this lack of fluctuation resulted because the uptake by bacteria was higher in these treatments than in the control treatment. Ammonium can be oxidized by ammonia oxidizing bacteria to form nitrite, an intermediate product of the nitrification process (Maillard *et al.* 2005; Avnimelech 2009, while the levels of NO_2^- N in the control water and biofloc-rich water treatment with starch 30% higher than in all other treatments compared to biofloc-rich water treatment with (60% starch in the three levels of protein). These low concentrations with 60% starch can be explained as a result of uptake by autotrophic nitrifying bacteria that were introduced into the culture water with the biofloc.

According to Otoshi *et al.* (2009), bacteria-dominated systems promote more stable water quality than systems dominated by mixed algae because they do not experience the bloom-and-crash cycles typical of algal-dominated systems. This stabilization process was also observed by other researchers working with *L. vannamei* in systems with limited or no water exchange. For example, McAbee *et al.* (2003) conducted a study of intensive shrimp production in raceways with reused water and showed that this water helped to maintain low ammonia and nitrite levels throughout the trial. A similar reduction in ammonia and nitrite levels was observed in our biofloc-rich water treatments consistent with the finding of Gaona *et al.* (2011). Who used 10% of biofloc-rich water as an inoculum to accelerate the formation of the nitrifying bacteria in the culture medium with no water exchange, and they found a positive relationship between the amount of reused water and the dissolved nitrate concentrations in the culture media as expected.

4.1.2. Phytoplankton and Zooplankton count.

The changes of biofloc volume are presented in Fig. (17). Direct manual quantification of total bacteria was carried out, to determine the total bacterial counts, and the observations were as follows: All biofloc treatments except one treatment (16% CP with 30% starch) had gradually increased in floc volume through the seventy days of experiment, but the high floc volume was obtained by biofloc treatments (20%CP with 60% starch), (24%CP with 60% starch), (16%CP with 60% starch), (20%CP with 30% starch), and (24%CP with 30% starch) respectively, then the other control treatments and biofloc treatment (16% CP with 30% starch). However there were no significant differences among these treatments. These differences in floc volume among the treatments refers to the different C/N ratios, therefore increasing C/N ratio by adding starch in all treatments with (60% starch) led to increasing floc volume which reflected on fish growth positively.

The other second sub-sample (50 mL) which was removed from the original composite water sample that was collected from each pond had been analyzed, at microbiology laboratory in Faculty of Veterinary Medicine. The microscopic evaluation which had been performed to the pond water revealed presence of several species of diatoms, Chlorophyta, Rotifer, Copepod, Cyanophyta, Bacillariophyta, Euglenophyta, Protozoa, Micro green algae, presented in Table (7). Besides no undesirable species of cyanobacteria (e.g., colonial such as potential toxin producing *Microcystis spp.* or filamentous types such as *P. perornata*) were observed to be present in the pond water.

As described in this table, the highest total count of phytoplankton and zooplankton were recorded in treatments fed different dietary protein levels under BFT system compared to other treatments under change water system (CS). Meanwhile, under BFT system, the highest count of phytoplankton and zooplankton were recorded in treatment (20%CP with 60% starch), and (24%CP with 60% starch) respectively, so under BFT conditions, increasing starch levels with dietary protein led to elevation of phytoplankton and zooplankton. Eight groups of organisms were identified, (Chlorophyta) included three species, *Ankistrodesmus sp.*, *Gloeocystis sp.*, and *Scenedesmus sp.*, then (Rotifer) included four genus or species, *Brachinous plicatilis*, *paramecium sp.*, *Euchlanis sp.*, and *Trichocerca sp.*, then (Copepod) included one species, *copepotidae sp.* (Cyanophyta) included one species *Coelosphaerium sp.* (Bacillariophyta)

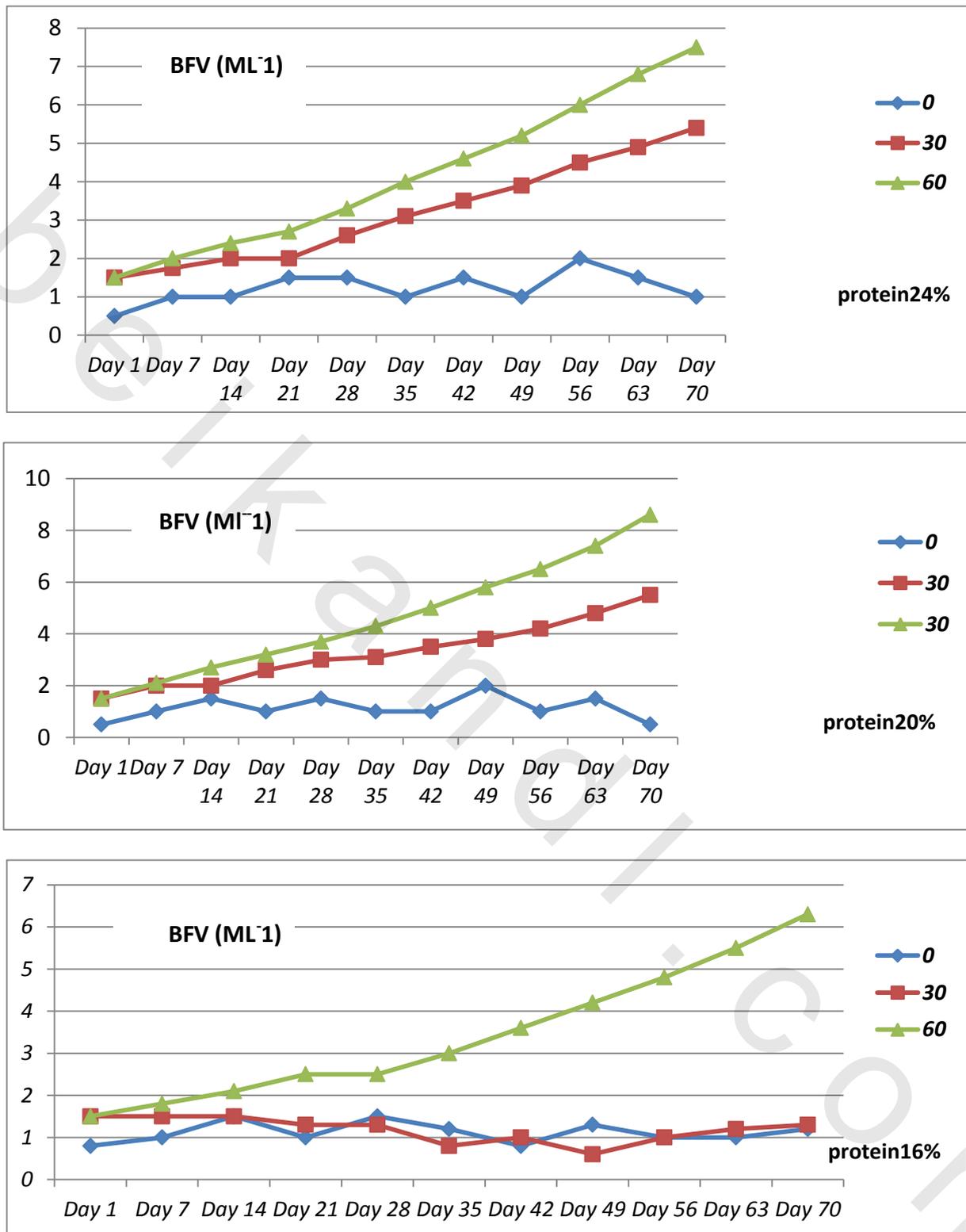


Fig. (17): Changes of biofloc volume (BFV) in the control and two bioflocs treatments with two C/N ratios (30% and, 60% starch) during the 70-days experimental period. Values are means (means \pm S.D.) of three replicate ponds per sampling time in each treatment.

Table.(7):Zooplankton and phytoplankton count and species identification in experimental treatments with dietary crude protein levels (24, 20 and 16 %) and starch ratios (0, 30 and 60 %) of daily diet.

		System conditions and count (natural unit/ml)						
		24% CP			20% CP		16% CP	
Division	Genus or species	control	30% s	60% s	30% s	60% s	30% s	60% s
Chlorophyta	<i>Ankistrodesmus sp.</i>	1000	6000	11000	7500	11300	1200	6000
	<i>Gloeocystis sp.</i>	2000	15000	21000	16000	22000	3000	16000
	<i>Scenedesmus sp.</i>	0	2000	5000	4500	13000	5000	11000
Copepoda	<i>copepodidae sp.</i>	1600	9000	13000	23000	15000	20000	13000
Rotifera	<i>Brachinous plicatilis</i>	2000	8000	84000	52000	75000	21000	51000
	<i>paramecium sp.</i>	1500	9000	49000	31000	44000	0	40000
	<i>Euchlanis sp.</i>	500	12000	36000	29000	38000	0	0
	<i>Trichocerca sp.</i>	3000	18000	90000	66000	90000	3000	22000
Cyanophyta	<i>Coelosphaerium sp.</i>	0	3100	0	0	1000	0	0
Bacillariophyta	<i>Centric diatoms</i>	0	0	0	0	3000	0	0
	<i>Pennate diatoms</i>	0	5000	35000	27000	45000	6000	29000
Euglenophyta	<i>Trachelomonas sp.</i>	4500	6000	8000	6000	4000	3200	4000
	<i>Phacus sp.</i>	0	3000	7000	9000	0	0	0
Diatomes	<i>Chaetoceros</i>	20000	30000	65000	48000	59000	12000	37000
	<i>Liptocylindrus</i>	23000	31000	99000	71000	88000	2000	50000
	<i>Melosira</i>	3000	22000	81000	63000	40000	0	3000
	<i>Rhzosolenia</i>	0	5000	24000	14000	12000	4000	0
Protozoa	<i>Arcella vulgaris</i>	2500	1000	0	2000	0	4000	0
	<i>Centropyxioculeatea</i>	3000	4000	6000	5000	5000	15000	1000
Micro Green Algae								
	<i>Tetraselmis tetrathele</i>	25000	90000	180000	166000	310000	20000	22000
	<i>Skeletonema costatum</i>	8000	63000	230000	195000	366000	49000	450000
Pooled means		Ch	Co	Ro	Baci	Eu	Di	M G A
24		10500	7866.67	104333.3	13333.333	9500	134333.333	198666.67
20		12916.667	13 200	144000	25333.333	7833.3333	146333.333	356666.67
16		7550	1533. 33	48000	12666.667	3900	50000	191333.33
0		3100	1600	7000	11000	4500	44000	33000
30		20066.667	17333.333	83000	12666.667	9066.6667	100666.667	194333.33
60		38766.667	13666.667	206333.3	36333.333	7666.6667	186000	519333.33
Total count		181900	300466.67	1004000	717333.33	1071456	168400	755000

Where: (S) starch,(Ch) *Chlorophyta*, Ro (*Rotifer*),Co (*Copepod*), Cy (*Cyanophyta*), Ba (*Bacillariophyta*), Eu (*Euglenophyta*), Pr (*Protozoa*), Di (*Diatomes*), MGA (*Micro green algae*).

Included two genus, *centric diatoms* and *Pennate diatoms*. (*Euglenophyta*) included two species, *Phacus sp.*, and *Trachelomonas sp.* (*Protozoa*) included two species *Arcella vulgaris*, and *Centropyxioculeatea*. Finally (*Diatomes* and Micro Green Algae) included seven genus or species, *Chaetoceros*, *Liptocylindrus*, *Melosira*, *Rhzosolenia*, *Tetraselmis tetrathele*, *Skeletonema costatum*. Overall, the dominance among the different phytoplankton and zooplankton groups were (green algae, diatoms, and rotifers) was variable among the biofloc ponds.

These results indicate that, addition of carbohydrate activated the growth of phytoplankton and zooplankton; the later played an important role in growth performance and feed utilization in treatments under BFT system which in agreement with the prediction of (Gao *et al.* 2012; Avnimelech 2007 and Emerenciano *et al.*, 2011).

Same observations were cited by Azim and Little (2008) who showed that under BFT system, three groups of organisms were identified: Protozoa, Rotifera and Oligochaeta. Among protozoans, three genera, namely, *Paramecium*, *Tetrahymena* and *Petalomonas* dominated. Four genera of rotifers were identified, namely, *Lecane*, *Trichocera*, *Polyarthra* and *Asplanchna*. Only *Tubifex* was found in the group Oligochaeta.

Recently, Schradera *et al.*, (2011) found that under BFT system, four groups of organisms were identified: Chlorophyta, Cyanophyta, Bacillariophyta, and Euglenophyta. The group of Chlorophyta included seventeen genera or species identified: *Ankistrodesmus sp.* *Geminella sp.* *Gloeocystis sp.* *Pediastrum sp.* *Scenedesmus sp.* *Coelastrum sp.* *Protoderma sp.* *Stigeoclonium sp.* *Actinastrum sp.* *Binuclearia sp.* *Chlorella sp.* *Closterium sp.* *Dictyosphaerium sp.* *Micractinium sp.* *Oocystis sp.* *Protococcus sp.* *Ulothrix sp.* Cyanophyta included eleven genera or species identified: *Coelosphaerium sp.* *Jaaginema subtilissimum*, *Stigonema sp.* *Anacystis cyanea*, *Anacystis sp.* *Aphanothece sp.* *Planktothrix agardhii*, *Phormidium sp.* *Raphidiopsis sp.* *Scytonema sp.* *Tolypothrix sp.* Bacillariophyta group included three genera or species identified: Centric diatoms, Pennate diatoms, *Melosira sp.* And Euglenophyta group included three genera or species identified: *Phacus sp.* *Euglena sp.* *Trachelomonas sp.*

Also Souady. (2013), confirmed the species of *Lepadella ovalis* (O.F.Muller), *Monostylaclosterocerca* (Schmarda), *Philodenasp*, Rotifer Genus including *Trichoceraca sp.* *Colurella adriatica* (Ehrenberg), *Colurella obtusa* (Gosse), *Trichocerca sp.* *Cephalodella sp.*

Euchlannis sp., *Paramecium sp.*, *Tokophyraquadripartita*(Goodrich& Jahn). Protozoa group including *Vorticella campanula* (Ehrenberg), *Centropyxisoculeatea* (Stein.), *Arcella vulgaris* (Ehrenberg), *Diffflugia corona* (Bove) *Didinium sp.* And group copepod including *Copepotidae*.

4.1.3. Mullet growth performance and survival rate.

4.1.3.1. Mullet growth performance

Growth performances of flathead grey Mullet fed three dietary protein (DP) levels 16%, 20% and 24% under change water system (CS) or biofloc system (BFT) are presented in table (8). The two factorial analysis of variance indicate overall significant effects of dietary protein levels and starch levels under management conditions (CS, BFT) ($p < 0.05$). The highest final body weight (FBW), weight gain (WG), and specific growth rate (SGR) values recorded for fish fed (20% CP with 60% starch), and (24% CP with 60% starch) under BFT system where no significant difference. BFT system showed superiority over change water system (CS) for FBW, WG, and SGR values.

Regards interaction results, fish fed (20% CP with 60% starch) under BFT system had the highest FBW, WG and SGR while the lowest results noticed for (16% CP with 30% starch) under BFT system. Also, we noticed that all treatments of different protein levels (24%, 20%, and 16% with 60% starch) under BFT system were significant increased ($p < 0.05$) than their control and 30% starch treatments. While the arrangement of the all treatments respectively came as follows. The second best treatment for FBW, WG, and SGR values was (24% CP with 60% starch), followed by (24% CP control), then (20% CP with 30% starch), (16% CP with 60% starch), (20% CP control), (24% CP with 30% starch), then (16% CP control), finally (16% CP with 30% starch).

Generally, growth parameters improved under BFT system, so it could assume that starch addition in BFT bonds 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 biofloc in the water column. Carbohydrate addition elevate the C/N ratio which helps to convert inorganic nitrogen into organic nitrogen as dense floc. That cause doubling of protein utilization and supply of essential lipids and vitamins (Avnimelech *et al.* 2009).

Azim *et al.* (2007) concluded that growth performance for Nile tilapia in biofloc system improved and increased growth 43% compared with control system. The same phenomenon was confirmed for shrimp as Xu *et al.* (2012) reported that shrimp in both dietary protein 30% and 35% under BFT system gained better growth performance (in terms of final weight, weight gain and specific growth rate) than that in the control (under change water system) ($P < 0.05$). Likewise, Arnold *et al.* (2009) found that adding carbon source (tapioca powder) to promote bioflocs 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 dietary protein 16.25% with bioflocs could even show better growth rate than shrimp fed 42.95% CP without bioflocs.

Table (8): Mean \pm standard error (SE) of initial and final body weight (BW), weight gain specific growth rate (SGR), and survival rate of flathead grey mullet (*Mugil cephalus*) fingerlings as affected with dietary crude protein levels (24, 20 and 16 %) and starch ratios (0, 30 and 60 %) of daily diet.

Protein levels %	Starch levels of dietary protein %	Initial BW (g)	Final BW (g)	Gain (g)	SGR (%/d)
<i>Flathead grey mullet (Mugil Cephalus) fingerlings</i>					
24	0	4.95 \pm 0.01 ^a	15.64 \pm 0.04 ^b	10.69 \pm 0.05 ^b	1.64 \pm 0.00 ^b
	30	4.96 \pm 0.06 ^a	13.98 \pm 0.20 ^d	9.02 \pm 0.26 ^c	1.47 \pm 0.03 ^c
	60	5.17 \pm 0.09 ^a	17.51 \pm 0.52 ^a	12.33 \pm 0.59 ^a	1.74 \pm 0.06 ^a
20	0	5.16 \pm 0.10 ^a	14.22 \pm 0.05 ^c	9.05 \pm 0.06 ^c	1.44 \pm 0.02 ^c
	30	4.98 \pm 0.00 ^a	14.25 \pm 0.14 ^c	9.27 \pm 0.13 ^c	1.5 \pm 0.01 ^c
	60	5.08 \pm 0.10 ^a	17.5 \pm 0.08 ^a	12.41 \pm 0.18 ^a	1.76 \pm 0.03 ^a
16	0	5.13 \pm 0.08 ^a	11.73 \pm 0.17 ^e	6.59 \pm 0.18 ^d	1.18 \pm 0.02 ^d
	30	5.07 \pm 0.08 ^a	10.82 \pm 0.43 ^e	5.74 \pm 0.36 ^e	1.07 \pm 0.03 ^d
	60	5.14 \pm 0.04 ^a	14.22 \pm 0.12 ^c	9.08 \pm 0.08 ^c	1.45 \pm 0.01 ^c
Pooled means					
24		5.03 \pm 0.00 ^g	15.71 \pm 0.06 ^h	10.68 \pm 0.06 ^h	1.64 \pm 0.00 ^h
20		5.07 \pm 0.00 ^g	15.32 \pm 0.06 ^h	10.24 \pm 0.06 ^h	1.57 \pm 0.00 ^h
16		5.12 \pm 0.00 ^g	12.26 \pm 0.06 ^g	7.14 \pm 0.06 ^g	1.23 \pm 0.00 ^g
	0	5.08 \pm 0.00 ^x	12.86 \pm 0.06 ^y	8.78 \pm 0.07 ^y	1.42 \pm 0.00 ^y
	30	5.00 \pm 0.00 ^x	13.02 \pm 0.06 ^y	8.01 \pm 0.06 ^y	1.35 \pm 0.00 ^y
	60	5.13 \pm 0.00 ^x	16.41 \pm 0.06 ^x	11.27 \pm 0.06 ^x	1.65 \pm 0.00 ^x

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

Moreover, bioflocs not only acts as supplemental microbial nutrition but also contribute to digestion and utilization of the feed for the cultured shrimp, consequently, enhancing growth performance and feed utilization. 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, 2008). Similar results were suggested by Zhao *et al* (2012) who reported that the bioflocs technology significantly increased the individual shrimp weight at harvest. in contrast, some other studies suggest that biofloc led to the level of production well below commercially viable levels (Little *et al.*, 2008). Timmons *et al.* (2002) reviewed that the final biomass levels of 10-28 kg fish m⁻³ achieved indoor and outdoor BFT system. These productions are even far below its counterpart traditional recirculating aquaculture systems in which standing biomass exceeding 100kg fish m⁻³ with oxygenation and 70-80kg fish m⁻³ with aeration.

Recently, Souza *et al.* (2014) stated that, Shrimp reared in the environment with molasses addition exhibited a survival rate, final weight and SGR significantly higher than those of the control. These results are in agreement with Krummenauer (2008), who demonstrated the efficiency of the BFT culture system in high-intensity shrimp culture with a production above 2.5 kg/m². Other authors (Otoshi *et al.*; 2006, and; Otoshi, *et al.*, 2007) have reported that, production values ranging from 4.5 to 10 kg/m², confirming the success of this system for shrimp production. Also, Kim *et al.* (2014) confirmed that the survival and growth rates of shrimp in biofloc group were significantly higher than those in control group in their study. Many of previous studies have shown that growing *L. vannamei* in biofloc systems can improve shrimp survival and growth performance, compared to clear water (Moss and Pruder 1995; Cohen *et al.* 2005; Azim and Little 2008; Mishra *et al.* 2008). One reason for the improved performance is probably related to harvesting and consuming bioflocs by the shrimp.

Krummenauer *et al.* (2014) concluded that the use of different fractions (25–100%) of biofloc-rich water produced results suggesting that the accelerated rate of development of the biofloc allowed the rapid establishment of the nitrifying microbial community and resulted in the rapid removal of ammonia and nitrite from the culture water. These results also suggest that the supplementation of seawater with biofloc-rich water at a level as low as 25% was effective and that the reuse of even a small amount of biofloc-rich water can provide improved nutritional conditions for shrimp growth. This approach provides a bio secure and environmentally friendly

system. Moreover, it is even more effective than closed systems without biofloc or conventional system in order to minimize the use of water.

However, there might be several reasons attributed to the poor fish growth and production. Increased turbidity due to biofloc reduces the visibility and hence artificial feed intake. Even when floc separator was used, it was not easy to maintain 500mg l^{-1} TSS. Maintaining optimum floc levels was also identified as a critical issue in managing BFT systems (Little *et al.* 2008). Another reason for the poor fish growth and production, water quality parameters were not stable, high fluctuation of pH and alkalinity, high concentrations of inorganic nitrogen, species might have chronic effects on fish health (Azim and Little, 2008).

4.1.3.2. Survival and Condition factor (K):

Survival rate was high in all treatments, where there was no mortality in any treatment except the treatment of (20% CP with 30% starch) under BFT system which recorded 98% survival; this is in agreement with Azim and Little (2008), who stated that tilapia survival was 100% in all treatment and control tanks. Also, similar survival rates for shrimp have been observed in previous studies with stocking densities higher than those used in this study by Krummenauer *et al.* (2011) who evaluated the effect of stocking density on the survival of *L. vannamei* and found that survival ranged from 75.0 to 92.0% at stocking densities ranging from 150 to 450 shrimp/ m². Otoshi *et al.* (2009) reported survival ranging from 82.3 to 91.8% in 75m² recirculation aquaculture system stocked at densities ranging from 301 to 408 shrimp/ m².

Condition factor is a measure of the suitability of the environment and food for fish growth and survival (Lagler 1956). The data presented in Table (9) and Fig. (18) shows the effect of feeding crude protein 24%, 20%, and 16% under control system and BFT system on condition factor of *M. cephalus* fingerlings. Data indicated that although condition factor of *M. cephalus* fingerlings was enhanced under BFT system compared to CS system, it was not significantly ($P < 0.05$) affected. Mean condition factor increased but not significantly with diet (20%CP with 60% starch) and it was $(1.32 \pm 0.08 \text{ g})$ followed by diet containing (24%CP with 60% starch) it was $(1.29 \pm 0.02 \text{ g})$, and the lowest value by $(0.92 \pm 0.09 \text{ g})$ was recorded in (16%CP with 30% starch).

Interaction between different dietary proteins content and starch under BFT system showed that, there were improvement in (K) values between treatments specially with starch level 60% and the three diets (24, 20, and 16%) CP. These results confirmed the result of growth and survival performance previously referenced in the present study, which demonstrated the

RESULT AND DISCUSSION

Table (9).Condition Factor values for flathead grey mullet (*Mugil cephalus*) under control treatments with different proteins (24%, 20%, and 16%).

Items		Survival %	Initial total length	Condition factor at start	Final total length	Condition Factor at the end
protein %	Starch%					
24	0	100	6.56±0.03 ^{ab}	1.7±0.02 ^a	11.10±0.2 ^a	1.14±0.06 ^{ab}
	30	100	6.53±0.08 ^{ab}	1.7±0.05 ^a	10.83±0.24 ^{ab}	1.10±0.06 ^{ab}
	60	100	6.90±0.11 ^a	1.5±0.06 ^c	11.06±0.18 ^a	1.29±0.02 ^a
20	0	100	6.80±0.15 ^a	1.6±0.08 ^{ab}	10.93±0.03 ^{ab}	1.08±0.01 ^{ab}
	30	97.60	6.56±0.03 ^{ab}	1.7±0.03 ^a	10.86±0.17 ^{ab}	1.11±0.04 ^{ab}
	60	100	6.70±0.11 ^{ab}	1.6±0.06 ^{ab}	11.00±0.25 ^a	1.32±0.08 ^a
16	0	100	6.83±0.18 ^a	1.6±0.1 ^{ab}	10.30±0.25 ^{ab}	1.07±0.06 ^{ab}
	30	100	6.66±0.12 ^{ab}	1.7±0.09 ^a	10.60±0.32 ^{ab}	0.92±0.09 ^b
	60	100	6.80±0.05 ^a	1.6±0.06 ^{ab}	10.73±0.16 ^{ab}	1.15±0.06 ^{ab}
Pooled means						
24		100	6.66±0.07 ^x	1.63±0.04 ^x	10.99±0.21 ^x	1.17±0.04 ^x
20		99.2	6.68±0.09 ^x	1.63±0.06 ^x	10.93±0.15 ^x	1.17±0.04 ^x
16		100	6.76±0.10 ^x	1.63±0.08 ^x	10.54±0.24 ^x	1.04±0.07 ^x
	0	100	6.73±0.12 ^x	1.66±0.07 ^x	10.77±0.16 ^x	1.09±0.04 ^x
	30	99.2	6.58±0.08 ^x	1.70±0.06 ^x	10.76±0.24 ^x	1.04±0.06 ^x
	60	100	6.8±0.09 ^x	1.56±0.06 ^x	10.93±0.19 ^x	1.25±0.05 ^x
LSD		0.54	0.45	0.25	0.92	0.38

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

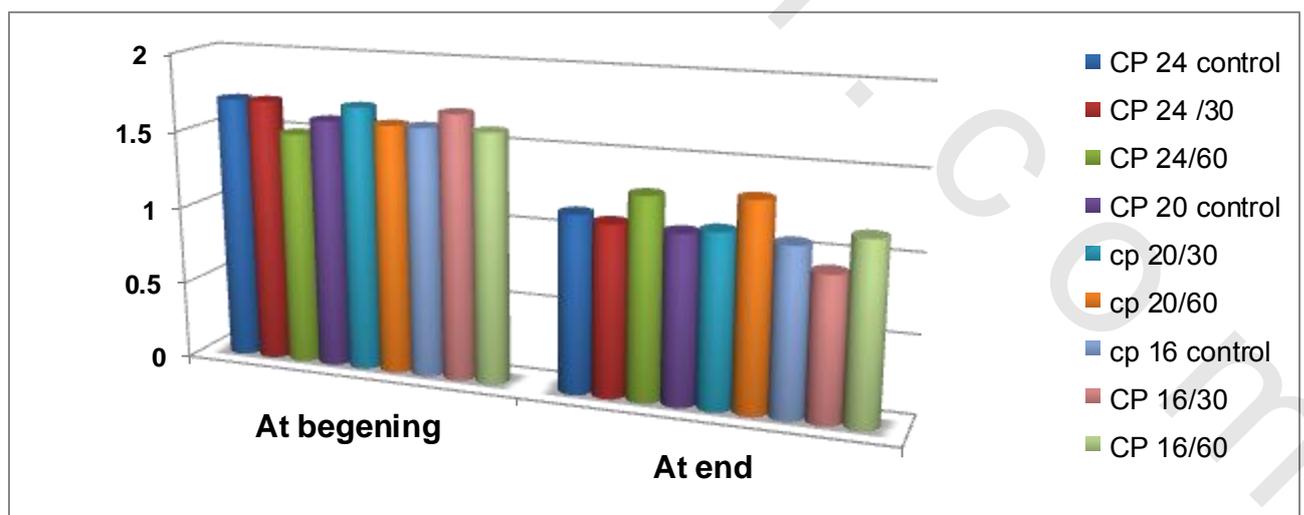


Fig. (18).Condition Factor values for flathead grey mullet (*Mugil cephalus*) under CS and BFT treatments with different proteins (24%, 20%, and 16%).

importance of bioflocs, where the increase in the value of condition factor indicates the good health of *Mugil Cephalus* fingerlings and the suitability of environment and food conditions for survival and growth of fish (Lagler, 1956; Essa *et al.*, 2010).

Also, Wassef *et al.* (2001) showed that mullet (*Mugil cephalus*) fingerlings were fed 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, value) was not significantly affected by various dietary treatments. Also, Kheir *et al.* (1998) attributed this fluctuation in K-value to THE increase of food consumption as a result of increased metabolism, minimized oxygen consumption and an increase in growth hormone.

4.1.4. Feed Utilization.

Feed utilization of different treatments is presented in Table (10). Regarding dietary protein, mullet fed (24, 20, and 16% CP with 60% starch) under biofloc conditions showed high values comparable with their control treatments. The data revealed that there were no significant differences between the two of treatments (20%CP with 60% starch and 24% CP with 60% starch) in feed intake FI and FCR (23.70 and 24.16 gm for FI, respectively) and (1.9 and 1.96 g fish/ gm feed for FCR, respectively). Also there were no significant differences between (20% CP control, 16% CP with 60% starch and 20% CP with 30% starch) in FI and FCR where their values were (22.6, 22.69, 22.38 and 2.49, 2.66, 2.52 for FI and FCR, respectively). Also, the best PER, PPV and ER were obtained by fish fed on 20%CP with 60% starch (2.18, 41.46 and 65.46 %, respectively)

Regarding the interaction, mullet fed 20% CP with 60% starch under BFT system recorded the highest feed intake FI, PER, PPV, ER, and the best FCR values. So, we can concluded that increasing dietary protein level from 20% to 24% under the same starch ratio (60 %), for flathead grey mullet, under BFT system did not significantly affect FCR, PER, PPV and ER($P < 0.05$).

Although that increasing dietary protein did not cause significant improvement in mullet weight gain, FCR, PER, PPV, and ER values, results suggested that the protein intake did not efficiently utilize for 24% diet under BFT and control system. Otherwise, biofloc system improved mullet feed utilization compared to change water system. These results agree with Avnimelech *et al.*(1994) who estimated that feed utilization is higher under biofloc . Also,

Avnimelech (2007) stated that, tilapia gave better values for feed conversion ratio, protein efficiency ratio, and protein productive value when observed in all biofloc treatments compared to those treatments without biofloc. The same trend was noticed for tilapia feed utilization under biofloc system by Azim and Little (2008).

Table (10) : Mean \pm standard error (SE) of total weight gain, offered feed and feed conversion ratio (FCR), protein energy ratio (PER), protein productive value (PPV), and energy retention of flathead grey mullet (*Mugil cephalus*) fingerlings as affected with dietary crude protein levels (24, 20 and 16 %) with three starch ratios (0, 30, and 60% of daily diet).

Protein levels	Starch levels	Feed intake	Gain	FCR	PER	PPV	ER
%	%	M \pm SD	(g)			%	%
24	0	22.84 \pm 0.16 ^c	10.69 \pm 0.05 ^b	2.13 \pm 0.00 ^b	1.94 \pm 0.00 ^b	29.15 \pm 0.26 ^d	59.09 \pm 0.12 ^a
	30	23.14 \pm 0.23 ^b	9.02 \pm 0.26 ^c	2.57 \pm 0.09 ^c	1.62 \pm 0.05 ^c	24.37 \pm 3.78 ^f	49.57 \pm 1.76 ^b
	60	24.16 \pm 0.10 ^a	12.33 \pm 0.59 ^a	1.96 \pm 0.09 ^a	2.12 \pm 0.09 ^a	34.11 \pm 6.13 ^b	64.85 \pm 2.78 ^a
20	0	22.60 \pm 0.27 ^b	9.05 \pm 0.06 ^c	2.49 \pm 0.04 ^c	1.66 \pm 0.03 ^c	30.00 \pm 1.95 ^d	50.92 \pm 0.9 ^b
	30	22.38 \pm 0.26 ^b	9.27 \pm 0.13 ^c	2.52 \pm 0.00 ^c	1.64 \pm 0.00 ^c	31.50 \pm 0.35 ^c	50.70 \pm 0.16 ^b
	60	23.70 \pm 0.20 ^a	12.41 \pm 0.18 ^a	1.90 \pm 0.04 ^a	2.18 \pm 0.04 ^a	41.46 \pm 3.23 ^a	65.46 \pm 1.47 ^a
16	0	22.45 \pm 0.06 ^b	6.59 \pm 0.18 ^d	3.4 \pm 0.09 ^e	1.22 \pm 0.03 ^d	26.31 \pm 2.31 ^e	36.65 \pm 1.07 ^c
	30	23.92 \pm 0.28 ^a	5.74 \pm 0.36 ^e	4.19 \pm 0.25 ^f	0.99 \pm 0.05 ^e	23.43 \pm 3.79 ^f	29.91 \pm 1.74 ^d
	60	22.69 \pm 0.00 ^b	9.08 \pm 0.08 ^c	2.66 \pm 0.02 ^d	1.56 \pm 0.01 ^c	27.07 \pm 0.93 ^e	48.37 \pm 0.43 ^b
Polled means							
24		23.38 \pm 0.02 ^x	10.68 \pm 0.06 ^h	2.22 \pm 0.01 ^v	1.98 \pm 0.00 ^x	29.21 \pm 0.39 ^v	57.83 \pm 0.28 ^x
20		22.89 \pm 0.02 ^x	10.24 \pm 0.06 ^h	2.30 \pm 0.01 ^v	1.83 \pm 0.03 ^x	34.32 \pm 0.51 ^x	55.86 \pm 0.3 ^x
16		22.78 \pm 0.03 ^x	7.14 \pm 0.06 ^e	3.42 \pm 0.02 ^x	1.26 \pm 0.00 ^v	25.60 \pm 0.67 ^z	38.31 \pm 0.32 ^y
	0	22.63 \pm 0.01 ^h	8.78 \pm 0.07 ^v	2.68 \pm 0.02 ^h	1.61 \pm 0.01 ^h	28.48 \pm 1.50 ^h	48.89 \pm 1.38 ^h
	30	23.48 \pm 0.02 ^e	8.01 \pm 0.06 ^v	3.09 \pm 0.03 ^e	1.42 \pm 0.01 ^h	26.43 \pm 2.67 ⁱ	43.39 \pm 0.4 ^z
	60	23.51 \pm 0.01 ^e	11.27 \pm 0.06 ^x	2.18 \pm 0.01 ^h	1.95 \pm 0.01 ^e	34.21 \pm 3.43 ^e	59.72 \pm 0.35 ^e

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

These observations were also suggested for shrimp by Zhao *et al.* (2012), who reported that biofloc treatment for shrimp resulted in 12% higher protein efficiency ratio, and 7.22% lower feed conversion ratio rate compared to 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 ($p < 0.05$) than treatment without

carbohydrate supplementation. Similar results were suggested by Xu *et al.* (2012) who showed that feed conversion ratio for 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 ponds, which in turn result further reduction 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 and 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 biofloc 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 (Harris 1993, Moss *et al.*, 2000, and Xu *et al.* 2012)

4.1.4. Chemical composition for mullet and biofloc.

Mullet whole body chemical composition is presented in Table (11). Regarding dietary protein, the highest dry matter recorded for the treatment (24% CP, control), and the treatment (24%CP with 30% starch) under BFT system (32.70, 31.73%) respectively, the highest crude protein recorded for the treatments (24%CP with 60% starch), and (20% with 60% starch) under BFT system (65.85, 65.16%) respectively, the highest ether extract contents recorded for the treatments (24%CP with 30% starch), and (20%CP with 30% starch) under BFT system (21.33, 20.94%) respectively. While, the highest chemical composition values was noticed for BFT system except dry matter. Interaction results showed that highest crude protein content was recorded for fish fed dietary protein (24% with 60% starch) under BFT system, while the highest ether extract content was noticed for fish fed dietary protein (24% with 30% starch) under BFT system. Also fish fed dietary protein (16% with 60% starch) presented the highest ash value (16.2%).

Table (11) : Mean ± standard error (SE) of chemical composition of flathead grey mullet (*Mugil cephalus*) fingerlings as affected with dietary crude protein levels (24, 20 and 16 %) with three starch ratios (0, 30, and 60% of daily diet).

Protein levels	Starch Levels%	Dry Matter %	Protein %	Fat %	Ash %
24	0	32.70±0.02 ^a	64.51±0.18 ^b	20.87±0.09 ^b	14.13±0.17 ^{bc}
	30	31.73±0.07 ^b	64.26±0.11 ^{bc}	21.33±0.13 ^a	14.38±0.17 ^{bc}
	60	29.80±0.55 ^c	65.85±0.28 ^a	20.69±0.29 ^{bc}	13.32±0.17 ^d
20	0	30.24±0.36 ^{bc}	64.66±0.41 ^b	20.90±0.10 ^b	14.23±0.42 ^{bc}
	30	30.49±0.33 ^{bc}	64.34±0.10 ^{bc}	20.94±0.29 ^b	14.19±0.13 ^{bc}
	60	29.29±0.60 ^c	65.16±0.18 ^{ab}	20.88±0.37 ^b	13.93±0.19 ^c
16	0	30.70±0.29 ^{bc}	63.52±0.17 ^d	20.61±0.02 ^{bc}	15.19±0.14 ^b
	30	29.71±0.46 ^c	63.29±0.33 ^d	20.3±0.07 ^c	15.6±0.09 ^{ab}
	60	29.39±0.41 ^c	64.37±0.24 ^{bc}	19.45±0.00 ^d	16.2±0.14 ^a
Pooled means					
24		31.41±0.05 ^x	64.87±0.03 ^x	20.96±0.01 ^x	13.97±0.02 ^y
20		30.00±0.03 ^y	64.12±0.03 ^y	20.90±0.01 ^x	13.85±0.02 ^y
16		29.03±0.03 ^z	64.02±0.03 ^y	20.62±0.01 ^y	15.22±0.01 ^x
	0	31.21±0.04 ^g	64.03±0.02 ^h	20.79±0.00 ^g	14.48±0.02 ^g
	30	30.64±0.03 ^h	63.96±0.02 ^z	20.85±0.02 ^g	14.72±0.02 ^g
	60	29.49±0.03 ^z	65.62±0.01 ^g	20.84±0.01 ^g	13.48±0.03 ^h

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

Biofloc compositions are summarized in Table (12). General increase in biofloc nutrient composition occurred with elevation of starch levels except ether extract. Previous results suggest that biofloc conditions stimulate accumulation of both fat and ash in tilapia carcass especially with the elevation of dietary protein level. Biofloc chemical analysis with mullet confirmed this hypothesis for lipid under BFT system (24% CP, with 30% starch). But not for ash where, the highest accumulation of ash was in the fish under BFT system (16% CP, with 60% starch).

In contrast, (Azim and Little, 2008) revealed that no significant difference between change water system (CS), and BFT system were recognized. In the same context, no significant difference between change water system (CS), and BFT system in shrimp dry matter and crude protein content, but biofloc showed superiority for ether extract and ash content (Xu and Pan, 2012). It was hypothesized that under BFT system shrimp (*L. vannamei*) have better nutrient assimilation compared to those fed only on formulated feed because of the greater amount of essential amino acids, fatty acids (PUFA and HUFA), and other nutritional elements supplied by

the bioflocs (Izquierdo *et al.* 2006; Ju *et al.* 2008 and Tacon *et al.* 2002). This findings support our results as increase in biofloc protein and ether extract content recorded with the elevation of dietary protein.

Table (12): Mean ± standard error (SE) of chemical composition of biofloc.

Protein Levels %	Starch Levels%	Protein %	Fat %	Ash %
24	30	32.06±0.23 ^c	8.19±0.17 ^a	8.53±0.17 ^{bc}
	60	37.18±0.37 ^a	7.53±0.17 ^b	7.79±0.45 ^c
20	30	33.23±0.14 ^{bc}	7.15±0.45 ^c	7.58±0.39 ^c
	60	37.43±0.14 ^a	7.22±0.45 ^{bc}	8.71±0.39 ^b
16	30	30.67±0.34 ^d	6.86±0.08 ^d	6.59±0.22 ^d
	60	35.31±0.12 ^b	7.16±0.17 ^c	9.96±0.18 ^a
Pooled means				
24		34.62±0.00 ^v	7.86±0.17 ^x	8.07±0.32 ^z
20		35.33±0.04 ^x	7.18±0.45 ^v	8.14±0.39 ^v
16		32.98±0.03 ^z	6.92±0.12 ^z	8.27±0.15 ^x
	30	31.98±0.22 ^h	7.4±0.20 ^e	7.56±0.22 ^h
	60	36.36±0.00 ^e	7.30±0.26 ^h	8.82±0.01 ^e

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

Also, Tacon *et al.* (2002) reported that the increased whole body ash content of the shrimp might be explained by continuous availability of abundant minerals and trace elements from the bioflocs as indicated by ash content. Our results agree with this phenomenon where, mullet under biofloc system recorded the highest ash contents. Meanwhile, chemical analysis of biofloc samples showed elevation in protein and fat contents with increasing dietary protein and starch levels, but ash decreased with increasing dietary protein. Other results were suggested by (Azim *et al.* 2008), where they observed significant in biofloc composition in terms of protein and ash content between different protein levels. The opposite 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 diet, but the opposite recorded for ash and fiber composition.

4.1.5. Economic efficiency and evaluation:

Calculation of economic efficiency of the tested diet based on the cost of feed, cost of one kg weight gain is shown in table (13). As described in this table feed cost per kg gain (LE) which fed 24% CP, 20% CP, and 16% CP with control system and/or BFT system with two

levels of starch 30%, and 60% from daily diet. In table (9), shown that the diet of 20% CP with 60% starch was the lowest one in the relative feed cost/kg compared to the other diets, so the rearing of flathead grey mullet fingerlings with crude protein level 20% and starch level 60% from daily diet seemed to be economic at (*Mugil Cephalus*) fingerlings.

It is well known that feeding cost in fish production is about 50% and more of the total production costs as declared by (Eid and Magouz, 1995 and El-Sayed, 1998). 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 the future of aquaculture developments will be in the form of semi-intensive or intensive culture systems which require appreciable inputs of fertilizers and/ or artificial feeds. In these systems, feed accounts between 30-60% of the production cost thus feed therefore, has a major role in the economics of such enterprises. When stocking density exceed the level that can be supported by natural food, supplementary feeds, however, assume that natural food makes a significant contribution to the nutrient requirements of culture species, and is not formulated to provide well-balanced nutrient profiles. They are based on that ever low cost feed nutrients are available. As the level of intensity of aquaculture increases (Fish maintained under intensive culture systems), complete formulated diets are designed to supply all the nutrient levels required (Fouda, 1990; Tacon, 1992; Tahoun, 2002).

Table (13): Cost of feed required for producing one Kg gain of *M cephalus* fingerlings as reared under control and/or BFT conditions.

Treatments		Feed cost per kg	FCR	Cost/kg fresh fish	Relative Feed cost/kg
24% CP	control	3.98	2.13	5.88	114.8
	30% starch	4.13	2.57	6.74	131.6
	60% starch	4.29	1.96	5.80	113.2
20%CP	control	3.56	2.94	5.66	110.5
	30% starch	3.70	2.52	5.73	111.9
	60% starch	3.85	1.9	5.12	100
16%CP	control	3.14	3.4	6.01	117.3
	30% starch	3.30	4.19	7.10	138.6
	60% starch	3.45	2.66	5.64	110.1

$$\text{Relative feed cost/kg fresh fish} = \frac{\text{Values of feed cost/kg fresh fish}}{\text{The minimum value of the same parameter}}$$

Therefore, it could be concluded that, the optimal level of crude protein 20% and level of starch 60% of performance of economic analysis.

4.2. The Second Experiment:

This experiment was conducted to study the effect of biofloc system at different salinities, with crude protein (20% and 24%) on water quality, growth performance, survival rate, and economic feasibility of *Mugil cephalus*. in El-Max Research Station, National Institute of Oceanography and Fisheries, Alexandria.

4.2.1. Water quality criteria:

In this experiment all treatments were bioflocs, two levels of crude protein (20%, and 24% with 60% starch) under different salinities (fresh, brackish, and underground marine water). Differences among the treatments in water quality parameters are shown in (Table 14). Generally, the average water temperature during the experimental period ranged between (17.5-25.3^o), while pH ranged (8.31-9.0), salinity ranged (0.4- 41.5) ppt, dissolved oxygen ranged (5.5-6.6) ppm, and total dissolved solid (TDS) ranged (0.445-35.8)g/l, and organic phosphors ranged (0.06-1.1) ppm.

Also, the recorded values of total nitrogen ammonia (TNA) which were used in calculating unionized ammonia (NH₃) Fig. (19) changed numerically within the normal range (0.5-2 mg/l) under different salinities. In the same context, normal range of nitrite (NO₂) (< 0.5 mg/l) was observed for all treatments during experimental period Fig. (20). A result of nitrate NO₃ was noticed for higher values than normal range (5mg/l) in all treatments during the experimental period Fig. (21).

Table (14). Difference in water quality parameters status in the second experiment between different salinities (marine, brackish, and fresh) water under 20% and 24% CP with 60% starch.

Items	Concrete ponds		
	Marine water ponds under BFT system with 20,24% CP and 60% starch	Brackish water ponds under BFT system with 20,24% CP and 60% starch	Fresh water ponds under BFT system with 20,24% CP and 60% starch
Temperature (°C)	18-25.3 ^c	17.9-24.8 ^c	17.5-24.8 ^c
Salinity (mg/l)	31.9-41.5	14-17	0.4-0.642
pH	8.40-8.77	8.31-8.70	8.79-9.09
TDS(g/l)	33.21-41.89	16.59-19.76	0.445-0.695
Organic phosphors(ppm)	0.7-1.1	0.57-1.2	0.07-0.44
DO (mg/l)	5.5-6.2	5.7-6.3	5.9-6.6
Total nitrogen ammonia (TNA) ppm	0.1-0.4	0.12-0.38	0.07-0.38
Nitrogen nitrite (No2) ppm	0.00-0.22	0.00-0.18	0.00-0.16
Nitrogen nitrate (No3) ppm	2-9	2-9	1-10

These results indicated that there were significant differences in water parameter, total dissolved solid TDS, dissolved oxygen DO, pH, and organic phosphors due to different salinities, and no significant difference in water temperature, by referring to the output of nitrogen TNA, NO₂, and NO₃ there is no significant difference due to salinity under BFT conditions, but the treatments (24 and 20%) CP with 60% starch in fresh water recorded the lowest measurements.

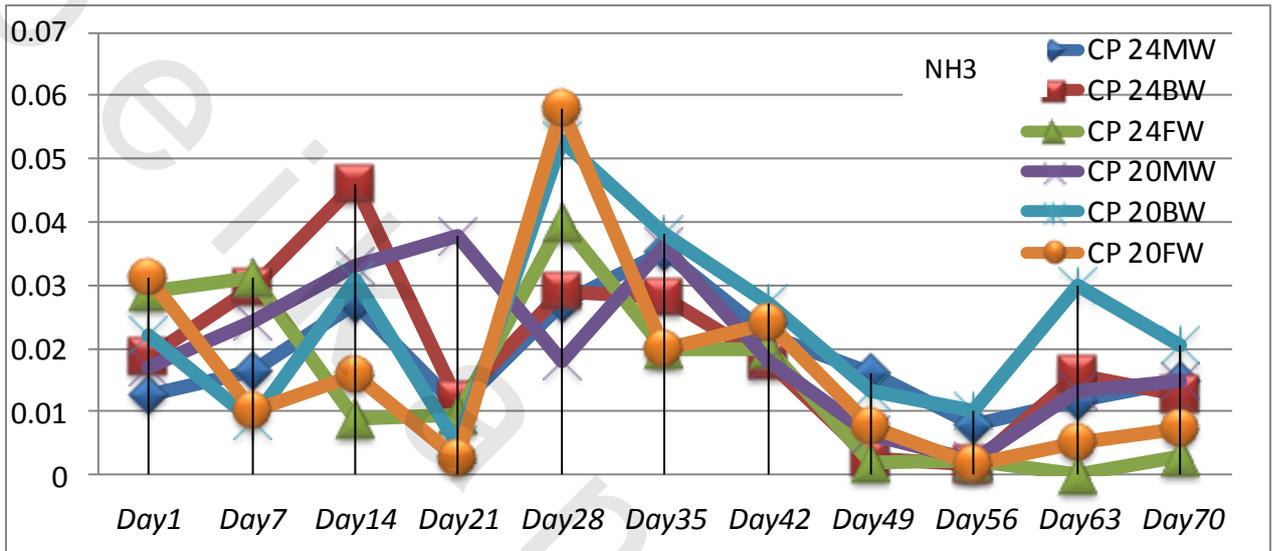


Fig. (19).Unionized ammonia (NH₃) values for biofloc treatments under different Salinities with (24%, 20% CP, and 60%starch). Where (MW): marine water, (B W): brackish water, (F W): fresh water. (CP): crude protein.

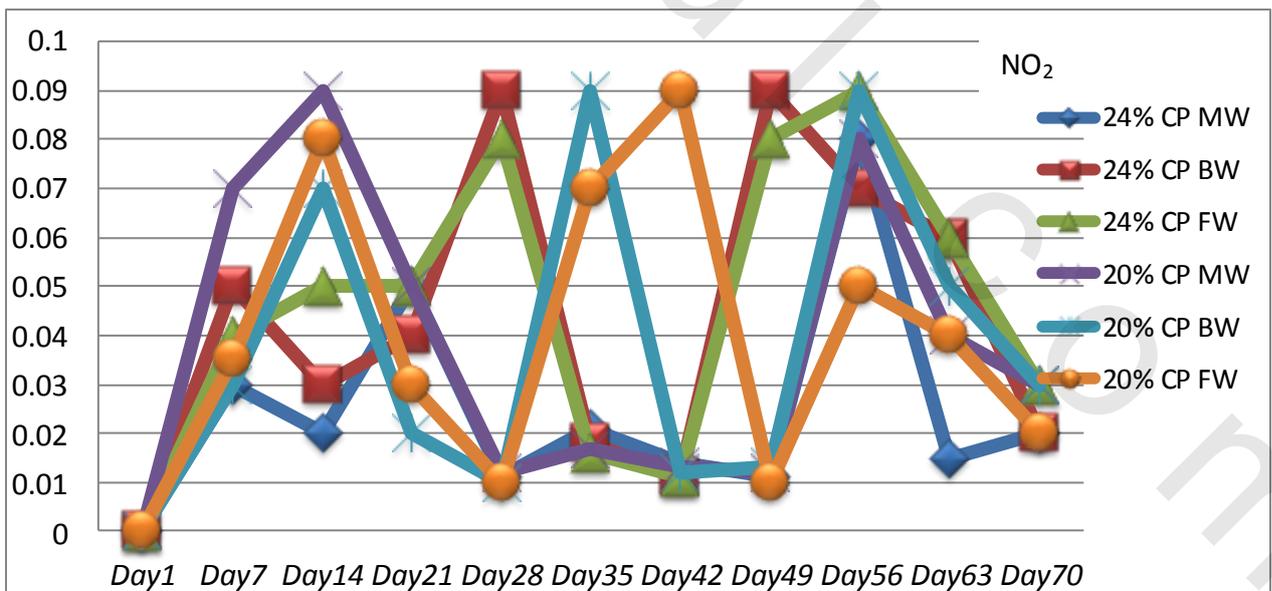


Fig. (20).Total nitrogen nitrite (NO₂) values for biofloc treatments under different Salinities with (24%,20% CP, and 60%starch).

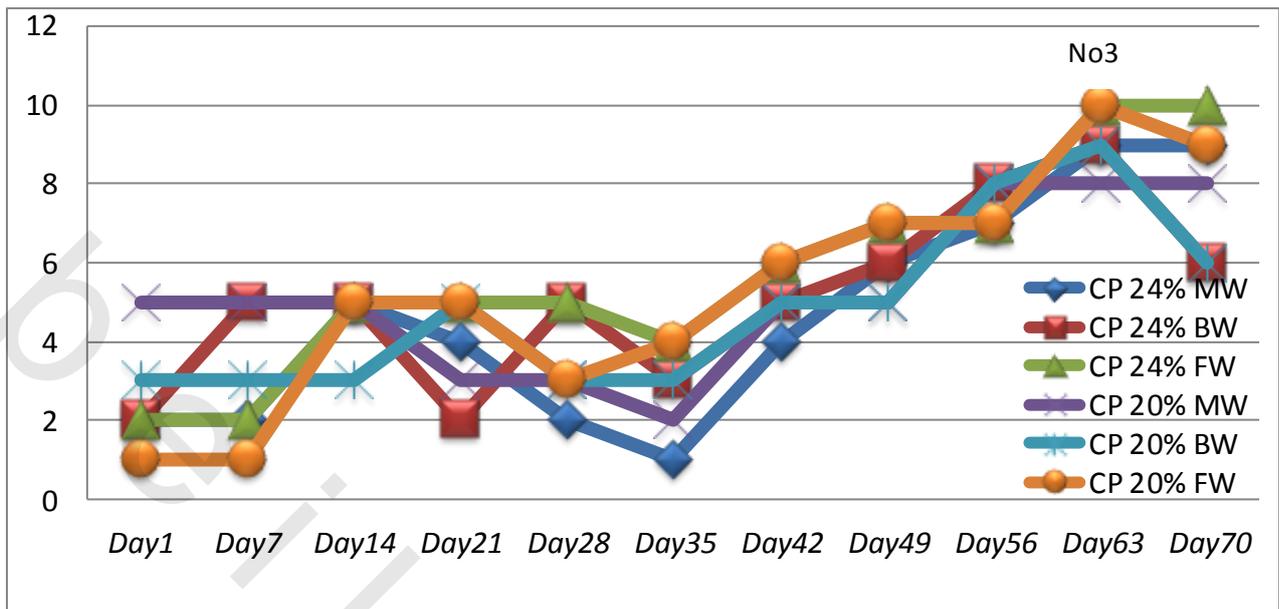


Fig. (21). Total nitrogen nitrate values for biofloc treatments under different Salinities with (24%, 20% CP, and 60% starch), where (MW): marine water, (B W): brackish water, (F W): fresh water. (CP): crude protein.

Growth of euryhaline species such as flathead grey mullet is often affected by salinity because the energy used for osmoregulation is not available for growth (Brett, 1979; Wootton, 1990). Consequently, many of these species have an optimal salinity level at which the growth rate is highest and the cost of osmoregulation lowest, which may affect fish distribution in the wild (Blaber, 1997). Although mullets classified as a marine fish and always spawn at the sea, they are highly euryhaline and thrive in a wide range of salinities (McDowall, 1988). Due to their euryhalinity, they are often stocked in brackish coastal lagoons to improve fish yield (Ravagnan, 1992) and are introduced into fresh water lakes and reservoirs to create new fisheries (Ben Tuvia *et al.*, 1992), so in this experiment, salinity was never a problem for mullet.

In this experiment, application of bio-flocs technology (BFT) in mullet culture under three different levels of salinity offered a solution to avoid environmental impact of high nutrient discharges, and excess of nutrients converted into microbial biomass, which were controlled in water criteria, so we noticed that all ponds characterized no transparent because of their high turbidity, and also noticed no differences in water quality under BFT system due to salinity especially pH, DO, and nitrogen contents which had no significant differences among (fresh, brackish, and marine water). Significant differences were observed in total dissolved solids (TDS), organic phosphors among three levels of salinity due to use of tap water which has a slim

content of TDS, and organic phosphors in two treatments (fresh water<1ppt) and (brackish water 14-17ppt) to reduce salinity in brackish water ponds, but all measurements were in safe levels for fish .In contrast, Azim *et al.*(2008) recorded similar results for inorganic nitrogen (TAN, NO₂-N and NO₃-N) concentrations and dissolved oxygen throughout the experimental period which had implemented on Nile tilapia indoor tanks, but in contradiction with us, they recorded a decrease in PH values throughout the experimental period.

Also, results were recorded close to water parameters (pH, DO, TAN and NO₂-N) by Braga *et al.* (2013), when they designed an experiment for production of *litopenaeus vannamei* in biofloc-dominated zero-exchange raceways (RWs) by using a mixture of fresh water and sea water. They added freshwater weekly to maintain salinity, and RWs were maintained with no water exchange. Mean water temperature, salinity, DO, and pH were 29.6 °C, 29.3 ppt, 5.5 mg/l, and 7.1, respectively. Mean TAN and NO₂-N were <0.4 mg/l, and final NO₃-N levels averaged 309 mg/l. Mean TSS and SS levels were 292 mg/l and 12 ml/l, respectively.

4.2.2. Phytoplankton and Zooplankton count:

As the same case in the first experiment, direct manual quantification of total bacteria was carried out to determine the total bacterial counts, and the observations were as a next. All biofloc treatments under fresh water, brackish water, and marine water had gradually increased in floc volume through the seventy days of experiment. No significant differences among treatments in floc volume but the high floc volume was obtained from biofloc treatments (20% CP with 60% starch, and 24%CP with 60% starch) under fresh water, (20%CP with 60% starch and 24%CP with 60% starch) under brackish water, and (20%CP with 60% starch, 24%CP with 60% starch) under marine water respectively. The absence of a significant difference between treatments is due to the possibility of growth for biofloc organisms in any salinity in case of balance in C/N ratio. The changes of biofloc volume are presented in Fig. (22).

Also, the other second sub-sample (50 mL) which was removed from the original composite water sample that was collected from each pond had been analyzed, in microbiology laboratory in Faculty of Veterinary Medicine using a binocular research microscope with (150×magnification) , to identify and enumerate algae, zooplankton and all microorganisms. The microscopic evaluation which had been performed to the pond water revealed the presence of several species of diatoms, Chlorophyta, Rotifer, Copepod, Cyanophyta, Bacillariophyta, Euglenophyta, Protozoa, Micro green algae, presented in Table (15). No undesirable species of

cyanobacteria (e.g., colonial such as potential toxin-producing *Microcystis* spp. or filamentous types such as *P. perornata*) were observed to be present in the pond water.

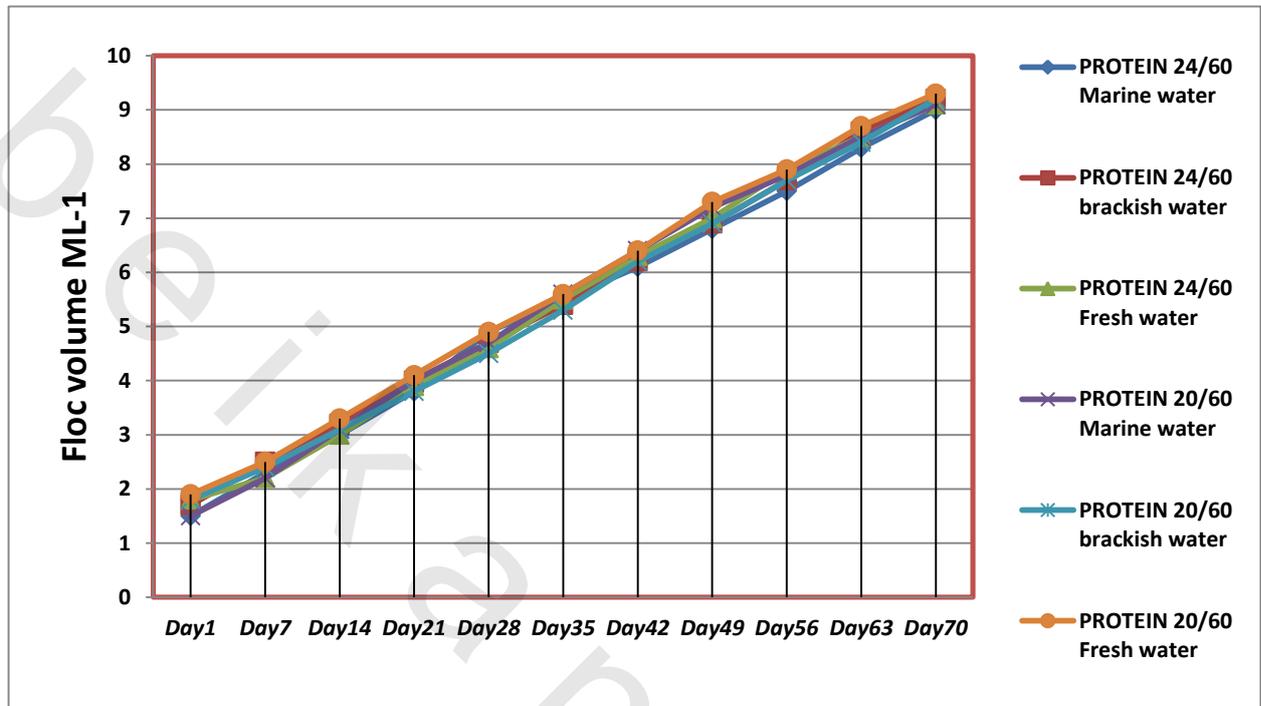


Fig (22): Changes in floc volume (BFV) in the bioflocs treatments with two levels of protein (24%, 20% with 60% starch) under different salinities during the 70-days experimental period. Values are means (\pm S.D.) of three replicate ponds per sampling time in each treatment.

The identification and enumeration of the species in different experimental treatments are presented in Table (15). As described in this table the highest total account of phytoplankton and zooplankton was recorded for (20%CP with 60% starch) under fresh water, while no significant differences were recorded among the treatments in floc enumeration but some species such as Rotifer *Brachinous plicatilis*, and micro green algae species *Skeletonema Costatum*, *Tetrathelmis Tetrathele* existed in marine and brackish water, but were not seen in fresh water. Also the species of *Cyanophyta Coelosphaerium sp.* and *Bacillariophyta* Centric diatoms existed in marine, but were not seen in fresh water and brackish water. Also we noticed in this table that increasing level of protein with different salinities did not cause any increase in final floc enumeration.

Table: (15): Zooplankton and phytoplankton count and species identification in experimental treatments with dietary crude protein levels (24, 20 with 60 % starch) under different salinities (fresh, brackish, and marine water).

		System conditions and count (natural unit/ml)								
		24/60M.W	24/60B.W	24/60F.W	20/60M.W	20/60B.W	20/60F.W			
Division	Genus or species									
Chlorophyta	<i>Ankistrodesmus sp.</i>	5500	9000	180000	5000	45000	190000			
	<i>Gloeocystis sp.</i>	20000	12000	22000	15000	5000	23000			
	<i>Scenedesmus sp.</i>	4000	5000	9000	0	5000	13000			
Copepoda	<i>copepotidae sp.</i>	1000	13000	55400	5000	20000	150000			
Rotifera	<i>Brachinous plicatilis</i>	13000	17000	0	8000	21000	0			
	<i>paramecium sp.</i>	9000	49000	31000	14000	0	40000			
	<i>Euchlanis sp.</i>	12000	36000	29000	108000	0	0			
	<i>Colurellaadriatica(Ehrenberg)</i>	0	1300	2500	1000	3000	4000			
	<i>Colurellaobtusa(Gosse)</i>	0	1000	2000	0	0	0			
	<i>Cephalodellasp.</i>	1000	3000	50000	0	4000	6000			
	<i>Tokophyraquadripartita(Goodr)</i>	0	0	10000	1000	0	9000			
	<i>Trichocerca sp.</i>	18000	90000	62000	19000	3000	22000			
Cyanophyta	<i>Coelosphaerium sp.</i>	3100	0	0	1000	0	0			
Bacillariophyta	<i>Centric diatoms</i>	0	0	0	3000	0	0			
	<i>Pennate diatoms</i>	5000	35000	27000	14000	6000	29000			
Euglenophyta	<i>Trachelomonas sp.</i>	6000	8000	6000	4500	3200	4000			
	<i>Phacus sp.</i>	3000	7000	9000	0	0	0			
Diatomes	<i>Chaetoceros</i>	30000	65000	0	29000	12000	0			
	<i>Liptocylindrus</i>	31000	99000	71000	39000	2000	50000			
	<i>Melosira</i>	22000	81000	63000	17000	0	4000			
	<i>Rhzosolenia</i>	5000	24000	14000	12000	4000	0			
Protozoa	<i>Arcella vulgaris</i>	1000	0	2000	0	4000	0			
	<i>Didiniumsp.</i>	0	1000	1000	1000	500	0			
	<i>Diffflugia corona(Bovee)</i>	0	0	0	6000	0	6000			
	<i>Vorticella campanula(Ehrenber)</i>	122000	65000	45000	70000	50000	110000			
	<i>Centropyxioculeatea</i>	4000	6000	5000	5000	15000	1000			
	<i>Lepadellaovalis(O.F.Mulle)</i>	0	45500	140000	5000	0	150000			
	<i>Philodena sp.</i>	0	23000	150000	0	62000	175000			
Micro Green Algae										
	<i>Tetraselmis tetrathele</i>	220000	180000	0	287000	246000	0			
	<i>Skeletonema costatum</i>	450000	110000	0	316000	475000	0			
Total count		985600	985800	985900	985500	985700	986000			
pooled means		<i>Cl</i>	<i>Co</i>	<i>Ro</i>	<i>Cy</i>	<i>Ba</i>	<i>Eu</i>	<i>Di</i>	<i>Pr</i>	<i>M G A</i>
24		88833.3333	23133	145600	1033	22333	13000	168333	203500	320000
20		100333.333	58333	87667	333.33	17333	3900	56333.3	220167	441333.33
	M W	24750	3000	104500	2050	1500	6750	92500	107000	636500
	B W	40500	16500	114150	0	0	9100	131500	134000	505500
	F W	218500	102700	128750	0	0	9500	101000	264500	0

Where: (Mw) marine water, (Bw) brackish water, (Fw) fresh water,(Ch) *Chlorophyta*, Ro (*Rotifer*),Co (*Copepod*), Cy (*Cyanophyta*), Ba (*Bacillariophyta*), Eu (*Euglenophyta*), Pr (*Protozoa*), Di (*Diatomes*), MGA (*Micro green algae*).

Our identification and enumeration of the species agree with Schrader *et al.* (2011) who cited that the microscopic evaluation of the pond water which was performed revealed the presence of several species of diatoms (division Bacillariophyta) and green algae (division Chlorophyta) including chlorophytes in the following genera: Ankistrodesmus, Chlamydomonas, Closterium, Quadrigula, and Selenastrum, and he did not notice undesirable species of Cyanobacteria (e.g., colonial such as potential toxinproducing Microcystis spp. or filamentous types such as P. perornata) which observed to be present in the pond water. Also Mohamed Souady (2013), confirmed the species of *Lepadella ovalis* (O.F.Muller), *Monostyllaclosterocerca* (Schmarda), *Philodenasp*, *Rotifer* Genus including *Trichocerca* sp. *Colurella adriatica* (Ehrenberg), *Colurella obtusa* (Gosse), *Trichocerca* sp. *Cephalodella* sp. *Euchlannis* sp. *Paramecium* sp. *Tokophyra quadripartita* (Goodrich & Jahn). Protozoa group including *Vorticella campanula* (Ehrenberg), *Centropyxis oculeata* (Stein.), *Arcella vulgaris* (Ehrenberg), *Diffflugia corona* (Bove) *Didinium* sp. And group copepod including *Copepodidae*.

As well as we noticed that the increasing in feed intake showed by fish in fresh water and brackish water with the two levels of protein elevated the fish output (feces) and in the presence of starch, a suitable environment for microorganisms (biofloc) and zooplankton growth established. Meanwhile, active growth of biofloc under these conditions exceeds the fish ability of consuming. Carb *et al.* (2007) concluded that the biological flocs can be considered as a kind of fast growing microbial mixed culture in which the waste nitrogen is recycled to young cells, which subsequently are grazed by the fish.

4.2.3. Mullet growth performance and survival rate:

4.2.3.1. Mullet growth performance

Growth performances of flathead grey Mullet (*Mugil cephalus*) fed two dietary protein (DP) levels 20% and 24% with 60% starch in different salinities (fresh water <1ppt, brackish water 14-17 ppt, and marine water >30ppt) under biofloc (BFT) system are presented in table (16). The results in this table showed that a significant difference in mullet growth performances among different salinities was recognized. The highest final body weight (FBW), weight gain (WG), and specific growth rate (SGR) values recorded for fish fed (20% CP with 60% starch), and (24% CP with 60% starch) at fresh water under BFT system with no significant difference, followed by (24% CP with 60% starch), and (20% CP with 60% starch) at brackish water under BFT system where no significant difference, then (20% CP with 60% starch), and (24% CP with 60% starch) at marine water, respectively.

Table (16): Mean \pm standard error (SE) of initial and final body weight (BW), weight gain specific growth rate (SGR), and survival rate of flathead grey mullet (*Mugil cephalus*) fingerlings as affected with dietary crude protein levels (24, and 20 %) with starch ratios 60 % of daily diet under different salinities.

Protein levels% and 60% starch	Kind of water	Initial BW (g)	Final BW (g)	Gain (g)	SGR (%/d)	Survival %
24	Marine	10.86 \pm 0.10 ^a	24.27 \pm 0.04 ^b	13.16 \pm 0.14 ^b	1.130 \pm 0.01 ^b	91.26 \pm 1.58 ^b
	Brackish	10.75 \pm 0.07 ^a	24.25 \pm 0.12 ^b	13.37 \pm 0.09 ^{ab}	1.146 \pm 0.01 ^a	95.23 \pm 1.37 ^a
	Fresh	10.9 \pm 0.13 ^a	24.37 \pm 0.06 ^a	13.46 \pm 0.20 ^a	1.149 \pm 0.02 ^a	91.27 \pm 4.19 ^b
20	Marine	10.95 \pm 0.066 ^a	24.2 \pm 0.16 ^b	13.25 \pm 0.22 ^b	1.130 \pm 0.01 ^b	91.27 \pm 4.82 ^b
	Brackish	10.94 \pm 0.156 ^a	24.24 \pm 0.17 ^b	13.29 \pm 0.02 ^{ab}	1.130 \pm 0.01 ^b	94.44 \pm 1.58 ^a
	Fresh	10.95 \pm 0.126 ^a	24.45 \pm 0.05 ^a	13.50 \pm 0.09 ^a	1.148 \pm 0.01 ^a	91.27 \pm 2.86 ^b
Pooled means						
24		10.83 \pm 0.00 ^h	24.21 \pm 0.00 ^h	13.33 \pm 0.00 ^g	1.14 \pm 0.00 ^g	92.59 \pm 0.17 ^g
20		10.94 \pm 0.00 ^g	24.29 \pm 0.00 ^g	13.35 \pm 0.00 ^g	1.14 \pm 0.00 ^g	92.32 \pm 0.02 ^h
	Marine	10.90 \pm 0.21 ^x	24.43 \pm 0.47 ^x	13.20 \pm 0.25 ^z	1.130 \pm 0.02 ^v	91.26 \pm 1.80 ^v
	Brackish	10.84 \pm 0.21 ^v	24.24 \pm 0.57 ^v	13.33 \pm 0.26 ^v	1.138 \pm 0.02 ^y	94.83 \pm 1.86 ^x
	Fresh	10.92 \pm 0.21 ^x	24.41 \pm 0.07 ^x	13.48 \pm 0.26 ^x	1.148 \pm 0.05 ^x	91.27 \pm 0.80 ^v

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

Although, there were no significant differences between fresh and brackish water, freshwater showed superiority compared with marine water in all growth performance parameters of fish fed both protein levels. Regarding interaction results, fish fed (20% CP with 60% starch) under fresh water BFT system demonstrated the highest FBW, WG and SGR but the lowest results were noticed for (24% CP, and 20 % CP with 60% starch) under marine water BFT system.

These results agree with the finding of Cardona, (2000) who conducted a laboratory experiments which revealed that the metabolic rate of Mediterranean flathead grey Mullet (*Mugil cephalus*) young specimens was negatively affected by high salinity levels and that an improved growth performance was achieved in fresh water and oligohaline water. Also he demonstrated that through a stratified study on microhabitat use, which was carried out on the island of Minorca (Balearic archipelago), juvenile specimens, shorter than 200 mm (total length), concentrated all year round in fresh water or oligohaline sites. Mesohaline areas were usually

avoided, except in summer. Immature fish, with a total length between 201 and 300 mm, show a similar pattern while in some seasons avoided freshwater sites. The habitat selection pattern of adults, i.e., fish longer than 301 mm, changed seasonally due to their offshore migration during the spawning season (from late summer to early winter). However, they usually showed a greater preference for polyhaline areas and strongly avoided freshwater sites. Euhaline areas were also avoided in autumn and summer. These results suggest that the young of this species are highly dependent on areas of low salinity and any factor which reduces the availability of such areas will, in turn, affect their fishery. Adults depend on polyhaline areas, although the avoidance of freshwater areas might be due to their shallowness.

Similar results had been reported by Barman, *et al.* (2005) when they had implemented two experiments to investigate the effect of inland water salinity on growth performance, feed conversion efficiency and intestinal enzyme activity in grey mullet. In experiment I, a 90 day monoculture of grey mullet at different salinity levels (0, 10, 15, 20 and 25%) was carried out. The fingerlings were stocked at 5000 per hectare and fed on a supplementary diet at 5% BW d⁻¹. This Study revealed that fish growth mean body weight (90.5 ± 4.5 g) and mean length (21.6 ± 0.4 cm), SGR (4.70%) and growth per day (0.99 g d⁻¹) were significantly ($P < 0.05$) enhanced in fish maintained at 10% salinity in comparison with other treatments. Nutrient levels, phytoplankton population, NPP and chlorophyll all decreased with an increase in salinity (>10%). In addition, zooplankton populations increased with an increase in the salinity level. Most of the other hydro chemical characteristics remained at optimal levels in all other treatments. Fish weight gain showed a significant positive correlation with productivity indicating parameters viz. Also, they stated that significant ($p < 0.05$) high growth, (SGR and per cent increase in body weight), feed conversion efficiency and intestinal enzyme activity were observed in the group maintained at 10‰ salinity in the second experiment (Experiment II), when mullet fry were exposed to five different salinity levels (10, 15, 20, 25 and 30%) and maintained for 70 days in the laboratory, and carcass composition, muscle and liver glycogen levels were also significantly ($P < 0.05$) affected by salinity changes.

Comparison between habitat availability and habitat use showed that the distribution of all grey mullet species in the estuaries of Minorca was strongly affected by salinity. *M. cephalus* and *L. ramada* are good osmoregulators, as they maintain a stable internal osmolality in a wide range of external salinity levels, including fresh water (Lasserre and Gallis, 1975; Nordlie and Lefler, 1975; Nordlie *et al.*, 1982; Thomas, 1984; Kulikova *et al.*, 1989). In these two species,

the cost of osmotic regulation is the lowest within the oligomesohaline range (Nordlie and Lefler, 1975; Cardona, 1994, 2000). Therefore growth is expected to be highest within that range, although experimental evidence is available only for *M. cephalus* (Cardona, 2000). Thus, it is not surprising to find that these two species showed a strong preference for sites with a salinity level under 15, although adults may prefer more saline areas (Cardona, 2000; Chang *et al.*, 2004). However, Chang *et al.*, (2004) have revealed the existence of individual differences in the habitat preference of *M. cephalus* in Taiwan, as most specimens avoid freshwater sites throughout their life whereas others spend long periods there.

Generally, we can conclude that growth parameters for flathead grey mullet improved in all salinities under BFT conditions. These results agree with the finding of (Burford *et al.* 2003) who suggested that adding starch helps to develop and control of dense heterotrophic microbial biofloc in the water column but biofloc conditions (BFT) in fresh water showed superiority over BFT in marine water on account of the cost of osmoregulation is lowest compared to marine water, and this available metabolic energy used for growth.

4.2.3.2. Survival rate and Condition Factor (K).

Survival was high in all treatments. It was above 91% under BFT conditions at three salinities (<1ppt, 15.5ppt, and underground marine water). Similar survival rates had been recorded for shrimp under biofloc system by Krummenauer *et al.* (2011) and Otoshi *et al.* (2009) who reported that, survival ranging from 82.3 to 91.8% in 75m² recirculation aquaculture system stocked at densities ranging from 301 to 408 shrimp/ m².

Condition factor is a measure of the suitability of the environment and food for fish growth and survival (Lagler 1956). The data reported in Table (17) and Fig (23) shows the effect of feeding crude protein (24%, 20% with 60% starch) at different salinities under BFT system on condition factor of *M. cephalus* fingerlings.

Data indicated that, although condition factor of *M. cephalus* fingerlings had no significant difference among the treatments at beginning of the experiment, the treatment of (20%CP with 60% starch) in fresh water gained a significant factor affected ($P < 0.05$). The highest mean condition factor was recorded in (20%CP with 60% starch) and it was (0.29 ± 0.02) g and the lowest value (0.025 ± 0.00) g was recorded by fish in two treatments (24%CP with 60% starch) in fresh water and (20%CP with 60% starch) in brackish water.

Table (17).Condition Factor values for flathead grey mullet (*Mugil cephalus*) fed two levels of proteins (24%, 20% and 60% starch) under BFT condition in different salinities.

Items		survival	Initial total length	Condition factor	Final total length	Condition Factor
24, and 20% Cp Starch 60%	kind of water	%				
24Cp	Marine	91.27 ^b	10.77±0.24 ^a	0.87±0.06 ^b	16.10±0.26 ^{ab}	0.58±0.01 ^c
	Brackish	95.23 ^a	10.40±0.43 ^{ab}	0.97±0.06 ^a	15.96±0.26 ^b	0.59±0.03 ^c
	Fresh	91.27 ^b	10.44±0.46 ^{ab}	0.97±0.14 ^a	16.30±0.52 ^a	0.56±0.02 ^d
20Cp	Marine	91.27 ^b	10.20±0.32 ^b	1.04±0.09 ^a	15.83±0.50 ^b	0.61±0.02 ^b
	Brackish	94.44 ^a	10.63±0.26 ^a	0.91±0.05 ^{ab}	16.26±0.20 ^a	0.56±0.00 ^d
	Fresh	91.27 ^b	10.76±0.67 ^a	0.91±0.14 ^{ab}	15.53±0.41 ^c	0.65±0.02 ^a
Pooled means						
24 CP		92.59	10.53±0.37	0.93±0.09	16.12±0.34	0.57±0.03
20 CP		92.32	10.53±0.41	0.95±0.09	15.87±0.36	0.60±0.01
Marine		91.27	10.48±0.28	0.95±0.08	15.96±0.25	0.59±0.01
Brackish		94.83	10.51±0.34	0.94±0.05	16.11±0.23	0.57±0.01
Fresh		91.27	10.60±0.56	0.94±0.14	15.91±0.31	0.60±0.02
LSD		3.45	0.81	0.18	0.82	0.4

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

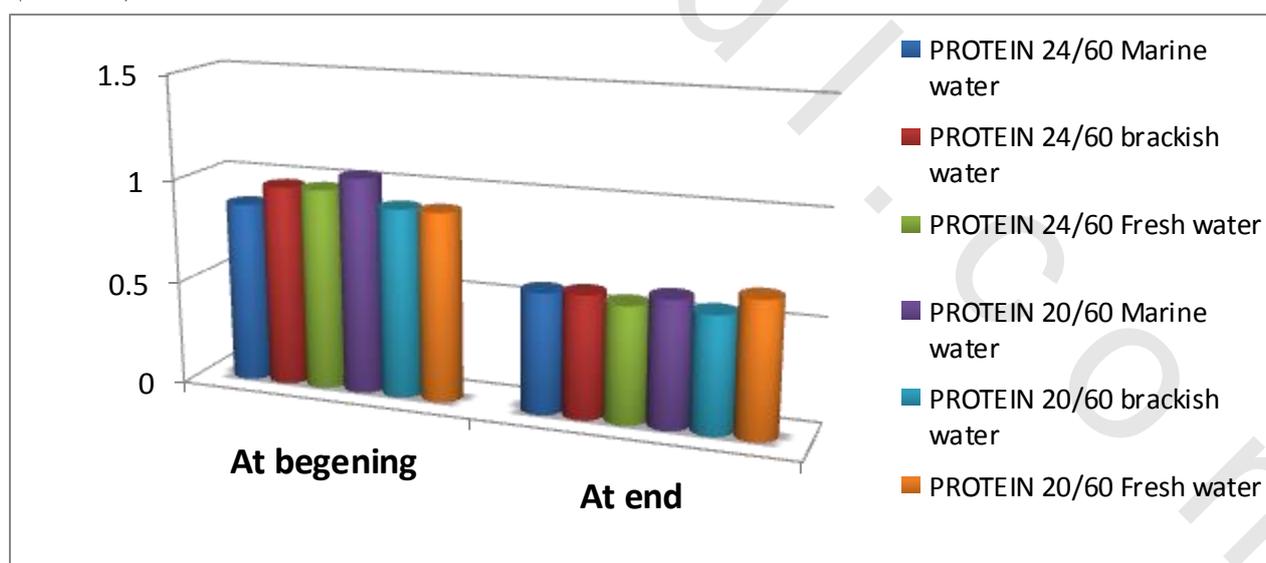


Fig (23).Condition Factor values for flathead grey mullet (*Mugil cephalus*) fed two levels of proteins (24%, 20% and 60% starch) under BFT condition in different salinities. where (MW): marine water, (B W): brackish water, (F W): fresh water. (CP): crude protein.

4.2.4. Feed Utilization.

Feed utilization of different treatments is presented in Table (18). Regarding dietary protein, mullet fed (24, and 20% CP with 60% starch) under fresh water BFT conditions showed high values compared to the treatments under marine and brackish water BFT conditions. Both treatments (20%CP with 60% starch), and (24% CP with 60% starch) under fresh water BFT conditions had semi equal results in feed intake (FI), FCR, and PER, while the treatment of (20%CP with 60% starch) outperformed the treatment (24% CP with 60% starch) in PPV, and ER. The same trend was in brackish water, and marine water without significant differences but CP 20% showed support for PPV with all salinities.

With no significant differences among all treatments, the best treatment which showed highest values for feed intake (FI), and feed conversion ratio (FCR), was the treatment (20% CP with 60% starch) under fresh water BFT conditions. It also showed the highest values for protein efficiency ratio (PER), and energy retention (ER) but the highest value of protein productive value (PPV) was recorded by the treatment (24% CP with 60% starch) under marine water at BFT conditions. Regarding the interaction, mullet fed (20% CP with 60% starch) in fresh water under BFT system recorded the highest feed intake FI, PER, ER, and the best FCR values, so we can conclude that feed utilization of the flathead grey mullet fingerlings was improved in all salinities under BFT conditions, while fresh water and brackish water showed a kind of support for feed utilization parameters compared with marine water under biofloc (BFT) conditions.

These results are close to that recorded by De Silva, and Perera (1976) in their experiment which conducted to study the effects of different levels of salinity (30‰, 20‰, 10‰ and < 1‰) on the growth, food intake and food conversion efficiency of young grey mullet. The daily food intake was found to be very variable at all four experimental salinities, and the intake was found to be salinity-dependent, when food was presented in excess. There was no appreciable difference in the rate of growth when fed 8% of the body weight or in excess. The percentage conversion efficiency of fish fed on an excess diet at 10‰ was the highest. When a constant ration was given, the percentage conversion efficiency was found to decrease with increasing salinity.

Table (18) : Mean \pm standard error (SE) of offered feed and feed conversion ratio (FCR), protein energy ratio (PER), protein productive value (PPV), and energy retention of flathead grey mullet (*Mugil cephalus*) fingerlings as affected with dietary crude protein levels (24, 20 and 60 % starch) with three levels of salinity under BFT conditions.

CP 20, 24% and 60% starch in different salinities		Feed intake	Gain (g)	FCR	PER %	PPV %	ER %
24	Marine	24.87 \pm 0.06 ^a	13.16 \pm 0.14 ^b	1.88 \pm 0.01 ^a	2.20 \pm 0.01 ^a	36.56 \pm 1.25 ^a	66.00 \pm 0.57 ^b
	Brackish	24.60 \pm 0.13 ^b	13.37 \pm 0.09 ^{ab}	1.85 \pm 0.01 ^b	2.24 \pm 0.01 ^a	36.19 \pm 0.80 ^b	64.65 \pm 0.36 ^c
	Fresh	24.97 \pm 0.02 ^a	13.46 \pm 0.20 ^a	1.85 \pm 0.02 ^b	2.24 \pm 0.03 ^a	36.20 \pm 1.99 ^b	66.46 \pm 0.91 ^b
20	Marine	24.92 \pm 0.07 ^a	13.25 \pm 0.22 ^b	1.88 \pm 0.03 ^a	2.21 \pm 0.04 ^a	43.00 \pm 2.57 ^a	56.7 \pm 1.09 ^e
	Brackish	24.81 \pm 0.04 ^{ab}	13.29 \pm 0.02 ^{ab}	1.86 \pm 0.00 ^{ab}	2.22 \pm 0.00 ^a	43.04 \pm 0.18 ^a	61.56 \pm 0.05 ^d
	Fresh	24.96 \pm 0.02 ^a	13.50 \pm 0.09 ^a	1.84 \pm 0.01 ^b	2.24 \pm 0.01 ^a	43.71 \pm 0.98 ^a	69.03 \pm 0.44 ^a
Pooled means							
24		24.80 \pm 0.00 ^h	13.33 \pm 0.00 ^e	1.86 \pm 0.00 ^e	2.22 \pm 0.00 ^e	36.31 \pm 0.11 ^e	65.71 \pm 0.05 ^e
20		24.90 \pm 0.00 ^e	13.35 \pm 0.00 ^e	1.86 \pm 0.00 ^e	2.22 \pm 0.00 ^e	43.25 \pm 0.11 ^h	62.43 \pm 0.21 ^h
	Marine	24.89 \pm 0.06 ^x	13.20 \pm 0.25 ^z	1.88 \pm 0.01 ^x	2.20 \pm 0.04 ^z	39.78 \pm 2.87 ^x	61.35 \pm 1.21 ^z
	Brackish	24.70 \pm 0.48 ^y	13.33 \pm 0.26 ^y	1.85 \pm 0.00 ^y	2.23 \pm 0.04 ^y	39.61 \pm 2.80 ^z	63.10 \pm 1.24 ^y
	Fresh	24.96 \pm 0.012 ^x	13.48 \pm 0.26 ^x	1.84 \pm 0.03 ^z	2.24 \pm 0.02 ^x	39.95 \pm 2.86 ^y	67.74 \pm 1.33 ^x

Means within each comparison in the same column with different superscript differ significantly (P<0.05).

A similar results were recorded where Barman, *et al.* (2005) found that Fish weight gain showed a significant positive correlation with productivity at <10% salinity. Also they stated that Significantly (P < 0.05) feed conversion efficiency and intestinal enzyme activity were observed in the group maintained at 10% salinity when mullet fry were exposed to five different salinity levels (10, 15, 20, 25 and 30%) and maintained for 70 days in the laboratory.

The same thing had been confirmed by De Silva, and Perera (1978) in their study on yearling puffer fish, where the study included the effect of salinity on the food intake, growth,

food conversion ratio and survival. Within the salinity regimes of 0 (freshwater), 8, 18, and 35, the food intake levels were 0.97%, 1.43%, 1.19% and 1.01%, respectively; and food conversion ratios were 1.31, 1.93, 1.61 and 1.36, respectively. The data series were reduced with increasing salinity. However, the survival rates did not show the same tendencies, which were 80%, 100%, 100% and 67%, respectively. There were significant differences among the treatments. In conclusion, the yearling puffer fish optimal culture salinity condition was about 8.0 ppt but they also cited the opposite with young grey mullet in De Silva, and Perera (1978), when they had carried out an experiment to study the effect of body size and salinity on the rate of digestion of young grey mullet, *Mugil cephalus* L., it had been performed using the “sacrifice” method. The rate of digestion was found to be salinity-dependent, being slower at lower salinities than at higher salinities. This is correlated to higher food intake at the lower salinities. Rate of digestion was also found to be dependent on body size, increasing with increasing body weight.

4.2.5. The whole body composition.

The whole body chemical composition of flathead grey mullet (*M. cephalus*) fingerlings are summarized in Table (19). Although there are no substantial differences in mullet chemical composition reared at different salinities under BFT system, mullet protein and ash content increased with marine water BFT conditions under crude protein 24%, and 20% respectively. The opposite was reported for dry matter and lipids, as it were increased in brackish water and fresh water, respectively. These observations agree with (Abedel-Tawab *et al.* 2006) who reported that the changes in protein and lipid content in fish body could be linked with changes in their synthesis and/or deposition rate in the muscle.

Chemical analysis of the bio-flocs in this experiment showed that the crude protein contents differ significantly with a range of 35.97-38.13%, but it was high in all salinities. This suggested that there was no effect of salinity on the crude protein content of the bio-flocs. Regardless of the origin of bioflocs inoculums (freshwater tilapia farm), similarity in the crude protein content as well as other parameters (crude lipid, fatty acids profile and ash content) in different salinity tested was expected, as several studies showed that salinity has no direct effect on the bacterial growth and it was assumed that increased or lowered salinity will select for new physiological types that are able to tolerate the given salt levels, while possessing the same metabolic capabilities (Del Giorgio and Cole 1998 and Nielsen *et al.* 2003).

RESULT AND DISCUSSION

Table (19): Mean \pm standard error (SE) of chemical composition of flathead grey mullet (*Mugil cephalus*) fingerlings as affected with dietary crude protein levels (24, 20 and 60 %starch) with three levels of salinity under BFT system.

CP Levels and 60% starch	Kind of water	Dry Matter %	Protein %	Fat %	Ash %
24	Marine	27.29 \pm 0.00 ^a	64.32 \pm 0.25 ^a	19.55 \pm 0.30 ^{ab}	15.25 \pm 0.20 ^d
	Brackish	27.56 \pm 0.00 ^a	63.76 \pm 0.25 ^b	19.83 \pm 0.20 ^a	15.62 \pm 0.41 ^c
	Fresh	27.15 \pm 0.00 ^{ab}	63.87 \pm 0.13 ^b	19.64 \pm 0.17 ^{ab}	15.16 \pm 0.10 ^d
20	Marine	27.24 \pm 0.00 ^a	64.04 \pm 0.32 ^{ab}	17.36 \pm 1.10 ^d	18.28 \pm 0.09 ^a
	Brackish	27.11 \pm 0.00 ^{ab}	63.97 \pm 0.25 ^{ab}	18.83 \pm 0.36 ^c	16.75 \pm 0.36 ^b
	Fresh	27.45 \pm 0.00 ^a	63.91 \pm 0.15 ^b	19.02 \pm 0.92 ^b	15.49 \pm 0.66 ^c
Pooled means					
24		27.33 \pm 0.00 ^g	63.98 \pm 0.01 ^g	19.67 \pm 0.01 ^g	15.34 \pm 0.01 ^h
20		27.26 \pm 0.00 ^g	63.97 \pm 0.01 ^g	18.57 \pm 0.06 ^h	17.08 \pm 0.05 ^g
	Marine	27.26 \pm 0.53 ^x	64.36 \pm 1.26 ^x	18.45 \pm 0.36 ^z	16.76 \pm 0.13 ^x
	Brackish	27.23 \pm 0.53 ^x	63.86 \pm 1.25 ^y	19.33 \pm 0.38 ^y	16.18 \pm 0.32 ^x
	Fresh	27.30 \pm 0.53 ^x	63.89 \pm 1.25 ^y	19.66 \pm 0.38 ^x	15.32 \pm 0.30 ^y

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

Table (20): Mean \pm standard error (SE) of chemical composition of bioflocin the same column with different superscript are significantly different ($P < 0.05$).

CP levels% and 60 %starch	Kind of Water	Protein %	Fat %	Ash %
24	Marine	36.00 \pm 0.23 ^c	7.16 \pm 0.17 ^c	8.53 \pm 0.17 ^c
	Brackish	37.08 \pm 0.37 ^b	7.53 \pm 0.17 ^b	12.76 \pm 0.75 ^a
	Fresh	38.13 \pm 0.14 ^a	9.05 \pm 0.45 ^a	10.58 \pm 0.39 ^b
20	Marine	35.97 \pm 0.34 ^c	7.13 \pm 0.08 ^c	8.03 \pm 0.12 ^c
	Brackish	37.11 \pm 0.12 ^b	7.16 \pm 0.17 ^b	12.96 \pm 0.18 ^a
	Fresh	37.99 \pm 0.19 ^a	8.96 \pm 0.07 ^a	11.03 \pm 0.20 ^b
Pooled means				
24		37.07 \pm 0.03 ^g	7.91 \pm 0.03 ^g	10.62 \pm 0.07 ^g
20		37.02 \pm 0.03 ^g	7.75 \pm 0.03 ^g	10.67 \pm 0.08 ^g
	Marine	35.98 \pm 0.70 ^z	7.14 \pm 0.13 ^v	5.52 \pm 0.16 ^v
	Brackish	37.09 \pm 0.72 ^v	7.34 \pm 0.14 ^v	5.57 \pm 0.25 ^v
	Fresh	38.06 \pm 0.74 ^x	9.00 \pm 0.17 ^x	7.20 \pm 0.21 ^x

Means within each comparison in the same column with different superscript differ significantly ($P < 0.05$).

The average crude protein content of the bio-flocs in saline water was 36%, and it was in correspondence with other studies in saline water, where the protein content was in the range of 32 to 38% DW (Tacon *et al.* 2002; Ju *et al.* 2008). The average crude protein content of the bio-flocs in brackish water was 37% DW, and the average crude protein content of the bio-flocs in freshwater was 38% DW; it was similar to Vanstechelman (2008) and Ekasari (2010) studies, which were 33 to 37% DW. Also, Ekasari (2010) concluded that there was no effect of carbon source as well as salinity on the content of crude protein and lipid in bio-flocs.

The crude lipid content in this study ranged from 7 to 9% on the DW. This was higher than that in other studies, where it ranged from 2 to 2.5% on the DW in freshwater (Azim *et al.* 2007) and 1.2 to 2.6% on the DW in marine water (Tacon *et al.* 2002; Ju *et al.* 2008). Salinity as well as carbon source did not affect the crude lipid content of the bio-flocs. Russel *et al.* (1995) suggested that one of the major osmoregulatory responses to salt concentration in bacteria is the alteration of the membrane lipid composition.

The average ash content was in the range of 8-13% on the DW, which agrees with other studies, where the ash content ranged from 7 to 32% on the DW (Tacon *et al.* 2002; Azim *et al.* 2007; Ju *et al.* 2008). Furthermore, Tacon *et al.* (2002) suggested that the high ash content in the bio-flocs probably is related to the presence of acid-insoluble oxides and mixed silicates. It is also suggested that the bio-flocs are a good source of essential minerals and trace elements (Tacon *et al.* 2002).

4.2.6. Economic efficiency and evaluation:

Calculation of economic efficiency of the tested diet based on the cost of feed cost of one kg weight gain is shown in table (9). The diet of (20% CP and 60% starch) under BFT system with fresh water was the lowest in total cost and the highest in total return. As described in this table feed, cost per kg gain (LE) which fed 24% CP and 20% CP with 60% starch in different salinities (fresh, brackish, and marine) water under BFT system. In table (9) shown that the diet of 20% CP with 60% starch in fresh water was the lowest one in the relative feed cost/kg compared to the other diets. So the rearing of flathead grey mullet fingerlings with crude protein level 20% and starch level 60% from daily diet seemed to be economic at (*Mugil Cephalus*) fingerlings.

Table (21): Cost of feed required for producing one Kg gain of *M cephalus* fingerlings as reared in different salinities under BFT conditions.

Treatments	Feed cost per kg	FCR	Cost/kg fresh fish	Relative Feed cost/kg
24% CP+60% starch(Marine water)	4.29	1.88	4.39	111.9
24% CP+60% starch(brackish water)	4.29	1.85	4.26	108.6
24% CP+60% starch(fresh water)	4.30	1.85	4.29	109.4
20% CP+60% starch(Marine water)	3.88	1.88	3.96	101
20% CP+60% starch(brackish water)	3.87	1.86	3.96	101
20% CP+60% starch(fresh water)	3.87	1.84	3.92	100

$$\text{Relative feed cost/kg fresh fish} = \frac{\text{Values of feed cost/kg fresh fish}}{\text{The minimum value of ths same parameter}}$$