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Effect of Effective Microorganisms (Em) and Molasses, Wheat Bran, and their Mixture in a Biofloc System on Microbial Protein Production, Water Quality, Growth Performance and Feed Utilization of Nile Tilapia (*Oreochromis niloticus*) Fingerlings.

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ABSTRACT

The present experiment was carried out to investigate the effect of effective microorganisms, EM; carbohydrate sources as molasses, M; wheat bran, and their mixture in a Bio-floc (BF) system on microbial protein production, water quality, growth performance, and feed utilization of Nile tilapia (*Oreochromis niloticus*) fingerlings. Seven experimental treatments were designed as following: T1: control, fish were fed a basal diet without any addition of carbohydrate source. T2: T1 plus effective microorganisms (EM) in water media. T3: T1 with supplementary molasses (M) in water media. T4: T1 with supplementary wheat bran (WB) in water media. T5: T1 with supplementary of mixture from EM and M in the water media. T6: T1 with supplementary of mixture from EM and WB in the water media, and T7: T1 with supplementary of mixture from M and WB in the water media**.** Fish in each tank were fed with one of the experimental diets containing 22.49 % crude protein and 471.60 Kcal. gross energy /100g DM at feeding rate of 3% of the actual fish live body weight, two times daily (9.00am and 2.00 pm) for six days a week for 90 days experimental period. Fish were distributed randomly into fourteen indoor fiberglass tanks. Each treatment was conducted in two indoor circular fiberglass tanks of 1m diameter filled with 1000 litter of fresh water. Nile tilapia fingerlings with an average initial body weight of 47.5±0.05 (g/fish) were randomized stocked at the rate of 42 fish / tank. The results showed the followings:The water quality parameters showed no significant differences in water temperature (C^0), pH value and DO (mg/l); however, there were marked differences in the values of total ammonia nitrogen (TA-N, mg/l) and Bio-floc (BF) concentrations (mg/l), with high values of TA-N and BF in tanks' water supplemented with different carbohydrate sources than control, especially with T6 which containing mixture of EM and WB in water media followed by T4 (WB), T3 (M), T5 (EM+M), T2(EM), and T6 (M+WB), respectively compared with T1 (control without carbohydrate source).Fish growth performance and feed utilization were significantly ($P \leq 0.05$) increased with EM and carbohydrate source supplementation than control. Also, T6 which containing a mixture of EM and WB gave the highest performance followed by WB (T4) and M (T3), respectively.Finally, the present results recommended the use of a mixture of effective microorganisms and wheat bran as a carbohydrate source in fish water tanks for optimal performance with best impacts on intensive tilapia culture reared under zero water exchange system.

Keywords: Effective microorganisms, carbon sources, Nile tilapia, water quality, growth performance.

INTRODUCTION

Nile tilapia, *Oreochromis niloticus*, represents the main Egyptian aquaculture production (GFARD, 2015). Tilapia production can be done in different farming systems including integrated, intensive and super-intensive system, also the biofloc technology systems (BFT) (Pérez-Fuentes *et al.,* 2016). The BFT system is a stimulation of aerobic and heterotrophic biota via fertilization with organic carbon source and vigorous water aeration which is conducted under zero water exchange or minimal water changes rate (Rurangwa and Verdegem, 2015).

The selection of carbon source depends on several criteria such as, local availability, cost, biodegradability and assimilation efficiency by bacteria. Commonly, the by-products derived from animal or human food industry usually used as carbon source (Labib, 2016). The Most used carbon source could be used is grain, sugar, molasses, sugarcane bagasse, chopped hay and tapioca (Ahmed *et al.,* 2016 and Labib, 2016).

Moreover, the microorganisms in the biofloc communities allow controlling the inorganic nitrogen levels in fish culture water, which in turn produce microbial biomass ingested by fish and reduce the loss of nitrogen in the aquaculture systems (Wang *et al.,* 2015).

Nearly similar technology is effective microorganisms (EM) which was developed previously in University of Ryukyus, Japan, before forty years (Sangakkara, 2002). The EM technology is a multi-culture of aerobic and anaerobic beneficial bacteria, these species produce organic acids, enzymes, antioxidants, and metallic chelates (Zhao *et al.,* 2006).

Therefore the present study was aimed to investigate the effect of EM addition in the rearing water with different carbon sources (molasses, wheat bran, and their mixture) on Water quality, growth performance, and feed utilization of Nile tilapia (*O. niloticus*) fingerlings.

MATERIALS AND METHODS

The present study was performed at the Laboratory of Fish Nutrition, Faculty of Agriculture (Saba Basha) in cooperation with National Institute of Oceanography and Fisheries (NIOF), El Max Research Station , Alexandria , Egypt , throughout the period from 4 August to 5 November 2016 (90 days) to investigate the effect of effective microorganisms, EM; carbohydrate sources (wheat bran, WB and molasses, M and their mixture) in a biofloc system on water quality, growth performance, feed utilization of Nile tilapia, *Oreochromis niloticus*, fingerlings.

Experimental facility

At the start of the experiment, a total number of 588 healthy fish were chosen. Fish were obtained from a private commercial fresh water fish farm sited in Motobas, Kafr-ElSheikh governorate, Egypt. Prior to the start of the experiment, experimental fish were kept for two weeks in circular fiberglass tanks (1 cubic meter) as an acclimation period and fed a control basal diet Table 1.

Table 1. Feed ingredients and proximate chemical analysis of the experimental diet.

Ingredients (g 100 g-[1])	Experimental diet
Soybean meal (46%)	10
Corn gluten meal (60%)	15
Rice bran	14
Wheat bran	10
Yellow corn	46
Sun flower oil	2
L. Lysine	0.5
L. Methionine	0.5
Vitamins[1] and minerals mixture[2]	2
Total	100
Chemical composition (%) on dry matter basis	
Dry matter (DM)	95.30
Crude protein (CP)	22.49
Ether extract (EE)	11.50
Crude fiber (CF)	4.11
Ash	4.56
Nitrogen free extract (NFE) [3]	57.44
Gross energy (GE; kcal/100g DM[4])	471.60
P/E ratio (mg CP/ kcal) [5]	47.65

[1]Vitamin mixture/kg premix containing the following: vitamin A 3300IU, vitamin D3 410 IU, vitamin E 2660mg, vitamin B1 133mg, vitamin B2 580 mg, vitamin B6 410 mg, vitamin B12 50mg, biotin 9330 mg, colin chloride 4000mg, vitamin C 2660 mg, inositol 330mg, para- amino benzoic acid 9330mg, niacin 26.60 mg, and pantothenic acid 220mg.

[2] Mineral mixture/kg premix containing the following: 325 mg Manganese, 200mg Iron, 25mg Copper, 5 mg Iodine, and 5mg Cobalt.

[3] NFE=nitrogen free extract.

[4]GE=Gross Energy: was calculated as 5.65, 9.45 and 4.12 Kcal per gram of protein, lipid and carbohydrate, respectively after NRC (2011).

[5]P/E ratio = protein to energy ratio mg crude protein/Kcal GE.

Fish were distributed randomly into fourteen indoor fiberglass tanks. Each treatment was conducted in two indoor circular fiberglass tanks, each of 1m diameter filled with 1000 litter of fresh water with an exchange rate of 20% / tank daily for T1 only; however, zero water exchange was applied in the other treatments. Nile tilapia fingerlings with an average initial body weight of 47.5±0.05 (g/fish) were randomized stocked at the rate of 42 fish / tank. Continuous aeration was conducted by pumping air into the water in the tank bottom using two sandy diffusers. Rearing water temperature was maintained between 27.0 and 28.0 ºC by using electrical heaters. The light period was maintained for only twelve hours; while in the other twelve hours were maintained dark. Effective microorganisms and carbohydrate sources (WB and M and their mixture) were added daily to water of each tank in order to adjust the C/N ratio of water to 20:1 (T2, T3, T4, T5 ,T6 and T7); however, no carbohydrate source was added to T1 (control) . The EM product is produced in the Ministry of Agriculture, Egypt with the co-operation between the Egyptian government and the Japanese Embassy in Egypt. The EM accelerates the natural decomposition of organic matter using 3 well-known groups of microorganisms: mainly (1) lactic acid bacteria (2) yeasts and (3) phototrophic or photosynthetic bacteria (from green seaweed and in any soil particle). The microorganisms in EM are beneficial and highly efficient; they are not harmful, not pathogenic, not genetically modified and not chemically synthesized. They are well known natural microorganisms; yeasts and lactic acid bacteria (*Lactobacillus*) promote the process of beneficial anti-oxidative fermentation, accelerate the decomposition of organic matter, and promote the balance of the microbial flora (Kolk and Smink, 2005 and Kriangsak, 2001). They are also beneficial microorganisms which are helpful in the aquaculture productive systems. Effective microorganisms and carbohydrate sources (WB and M and their mixture) were added daily to water of each tank in order to adjust the C/N ratio of water to 20:1 (T2, T3, T4, T5 ,T6 and T7); however, no carbohydrate source was added to T1 (control) .

Fish and experimental design

This experiment consists of seven treatments were designed in randomized as the following: T_1: control, fish were fed a basal diet without carbohydrate source was added. T2: as T1 with EM supplementation in water media. T3:T1 with supplementary M in water media. T4: T1 with supplementary WB in water media. T5: T1 with supplementary a mixture of EM and M in the water media. T6: T1 with supplementary a mixture of EM and WB in the water media, and T7: T1 with supplementary a mixture of M and WB in the water media.

Preparation of microbial protein production

Molasses and wheat bran were used based on the promotion of bacteria as the main primary producers by the use of carbon sources and substrates. The sugarcane molasses was used in this study as an external carbon source to improve reactor stability and increase chemical oxygen demand. Carbon was added at the rate of 20 times the concentration of total ammonia nitrogen (TAN) concentration in the tank's water to maintain appropriate C: N ratio for optimum production of microbial protein system (Avnimelech, 1999).

It is possible to theoretically (the following schema) calculate the amount of organic matter needed for an intensive pond, based on the amount of nitrogen excreted by the aquaculture species. Carbohydrate sources were added daily according to nitrogen excreted in the fish for manipulation the C: N ratio in order to raise the C: N ratio to 20:1.

Daily feeding of 2% of fish weight (Craig and Helfrich, 2002)

↓

20 g feed added per kg fish per day

↓ Take a feed with 25% protein

5 g protein added per kg fish per day

↓ 16% of protein is N (Craig and Helfrich, 2002)

0.8 g N added per kg fish per day

↓ On average 75% of the feed-N ends up in the water (ammonification of uneated feed + excretion) (Piedrahita, 2003)

0.6 g N per kg fish per day ends up in water

↓ Microorganisms need a C/N ratio of 10 (Avnimelech, 1999)

6g C per kg fish per day needed for biofloc production

↓ 40% of dry matter for most organic compounds is carbon

Daily dosage of organic compound 15 g dry matter /d.

The bases of calculating the wasted of nitrogen in water and carbon added (Crab *et al*., 2012).

Experimental diet

An experimental diet (22.5 % crude protein and 471.6 kcal GE/100 g DM diet) was formulated using plant protein sources without any addition of fish meal (Table 1). Dietary ingredients were ground and thoroughly mixed and the oil was slowly added at the same time of mixing with warm water (45°C) until the diet began to clump. The diet was then processed by a California pellet mill machine and dried for 48 hours in a drying oven at 70°C. The pellet size was 0.3 mm in diameter and 2 mm in length. Fish were fed two times daily (9.00: 14.00 hrs), 6 days a week for 90 days. Feeding rate was 3% of fresh body weight. Fish were weighed at two weeks interval and the amounts of feed were readjusted according to fish weight. Fish samples were collected at the beginning and the end of the experiment and store frozen at -20°C until whole body chemicals analysis.

Measurements of biofloc volume

Bio-floc volume was determined using Imhoff cones fortnight, the volume taken in by the flocs in 1000 ml of the tank water after 30 min sedimentation were registered (Avnimelech and Kochba, 2009).

Fish performance and feed utilization parameters

Fish growth performance and feed utilization parameters were calculated according to Cho and Kaushik (1985) from following equations:

1) Average weight gain (AWG, g / fish) = [final body weight-initial body weight];

2) Average daily gain (ADG, g / fish / day) = [AWG/time (days)];

3) Specific growth rate (SGR, % / day) = [ln final weight - ln initial weight] × 100 /time (days);

4) Feed conversion ratio (FCR) =feed intake (g)/body weight gain (g);

5) Protein efficiency ratio (PER) = gain in weight (g)/protein intake in feed (g);

6) Protein productive value (PPV, %) = 100 [protein gain in fish (g)/protein intake in feed (g)] ;

7) Energy utilization (EU, %) =100 [energy gain in fish (Kcal) / energy intake in feed (Kcal)]; and

8) Survival rate (SR, %) = 100 x (total number of fish survived/total number of fish stocked).

Chemical analysis

Chemical analysis of experimental diet and fish carcass was conducted in order to determine the percentages of dry matter (DM %), crude protein (CP %), ether extract (EE %), crude fiber (CF %), and ash (Ash %) according to the AOAC (2000) method. Nitrogen free extract (NFE %) was calculated by differences, by deducting the sum of percentages of moisture, CP, EE, CF and ash from 100. Gross energy (kcal/g DM, GE) contents of the experimental diets and fish samples were calculated by using factors of 5.65, 9.45 and 4.12 kcal/g of protein, lipid and carbohydrates, respectively (NRC, 2011).

Statistical analysis

Experimental data were statistically analyzed with one-way ANOVA and Duncan's (1955) multiple range tests and expressed as mean values ± SE. Effects with a probability of $P<0.05$ were considered significant. Statistical analyses were performed using SPSS for Windows (Standard Version 17 SPSS Inc. Chicago, Illinois).

RESULTS AND DISCUSSION

Water quality

The effect of EM and carbon sources in a biofloc system on water quality of Nile tilapia after 90 days rearing period was showed in Table 2. The water quality parameters monitored throughout the experimental period (temperature, pH and DO) were not affected (P ≥ 0.05) by any of the conducted treatments. All the environmental variables during the study period were within the range considered suitable for the culture of Nile tilapia. A temperature in water of all treatment was in optimal condition for the fish culture which ranged from 26.90 to 27.1^{0}C. Tekeliogla (1998) recommended a preferred temperature values for tilapia between 20 to 35^{0}C. The pH were lower in the T2 treatment, suggesting a reducing condition in such treatments, probably due to the activity of heterotrophic bacteria, which release CO_2 to the water column causing a pH decrease (El-Kady *et al*., 2016). Dissolved oxygen remained within the recommended range for growth of tilapia. By aerating the DO, the average was kept above 5 mg/l. This value is within the recommended levels as reported by El-Sayed (2006). The incidents of increased TAN were higher in all treatments compared to group number T1. The differences in TAN concentrations between all treatments as expected, as there is increase in the heterotrophic bacteria activities in BFT treatment which process to decrease TAN by nitrification (El-Kady *et al.,* 2016). Within the BFT treatments, nitrate-N gradually decreased in all treatment, this may be explained by the low dose of nitrogen delivered for the system (Nieuwenhuiz, 2000).

Table 2. Effect of effective microorganisms and carbon sources in biofloc system on water quality and biofloc volume of Nile Tilapia.

Treatment*	Temperature (°C)	pH	DO[1] (mg/l)	TAN[2] (mg/l)	Biofloc volume (mg/l)
T1	26.91 ±0.05	7.90 ±0.10	5.47 ±0.58	1.20 ^{c} ±0.20	1.40 ^{e} ±0.20
T2	26.90 ±0.04	7.80 ±0.01	5.44 ±0.09	2.31^{ab} ±0.10	12.10 ^{c} ±1.70
T3	27.10 ±0.09	7.93 ±0.09	5.55 ±0.09	2.22 ^{ab} ±0.24	17.80 ^{b} ±1.07
T4	26.90 ±0.10	7.94 ±0.08	5.45 ±0.04	1.87 ^{abc} ±0.17	23.00 ^{a} ±1.00
T5	26.90 ±0.07	8.04 ±0.06	5.53 ±0.05	2.30 ^{ab} ±0.20	15.13 ^{bc} ±0.80
T6	27.10 ±0.12	8.00 ±0.05	5.55 ±0.03	1.67 ^{bc} ±0.17	23.90 ^{a} ±1.77
T7	27.10 ±0.13	8.03 ±0.14	5.44 ±0.07	2.45 ^{a} ±0.26	7.87 ^{d} ±0.87

***Means in the same column having different letters are significantly (P≤0.05) different. [1]DO: Dissolve oxygen and [2]TAN=Total ammonia nitrogen**

Growth performance

The effect EM, as well as EM and different carbon sources in biofloc system on growth performance of Nile tilapia after three months rearing period is shown in Table 3. Results showed that initial weights had ranged between 47.55±0.07 and 50.25± 0.25 g/fish with insignificant differences among treatments indicating complete randomization of dividing the fish into experiments groups. Averages of final weight (FW), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) for the experimented groups (T1-T7) were significantly (P≤0.05) affected by additives of T6 (EM+WB) and T4 (WB) in a biofloc system released the highest final body weight, being 121.64±1.88 and 110.08±2.08 g/fish, respectively. In the same trend, ADG and SGR showed significant differences among most treatment and recorded the highest values in treatments T6 and T4, being the values were 0.82 and 0.66 g/fish/day and 1.05 and 0.87 %/day, respectively. On the other hand, the lowest values were recorded with the treatments T1, T2, T3, T5, and T7. Different studies have reported enhanced growth rates of fish raised in ponds with high activity of algae, microbial biofloc, and other natured biota (Moss *et al.,* 2001 and Burford *et al*., 2004).

However, it is not yet known exactly how microbial biofloc enhance growth. But, Lzquierdo *et al*. (2006) suggested lipid contributions of microbial biofloc that are significant. Moreover, Avnimelech, 1999 and El-Kady *et al*. (2016) reported that the microbial protein supplied by the storied fish biomass was enough to supplement the protein provided by the fish feed. In culture system together with microbial biofloc acting as a feed also do play some important ecological values. The deterioration of water quality due to unconsumed feed, fecal matter of cultured organisms or the presence of other organic matter in culture facilities in nullified because the biofloc microbes act as conditioner for water (El-Kady *et al.,* 2016 and Mansour and Esteban, 2017). Also, *O. niloticus* fed different dietary protein (24 and 35%) and reared under biofloc systems did not showed any significant changes in growth performance (Azim, 2008). The improvement in growth afforded by biofloc system in the present study would be due to the abundance of active heterotrophic bacteria which can assimilate the waste nitrogen and produce new cellular protein for fish consumption as previously indicated (Crab *et al*., 2012 and Long *et al*., 2015).

Table 3. Effect of effective microorganisms and carbon sources in biofloc system on growth performance of Nile tilapia.

Treatments*	Initial weight (g/fish)	Final weight (g/fish)	Weight Gain (g/fish)	Average daily gain (g/fish/day)	Specific growth rate (%/day)
T1	49.22 ^{ab} ± 0.88	76.62 ^{d} ± 0.58	27.41 ^{d} ± 0.31	0.30 ^{d} ± 0.01	0.49 ^{d} ± 0.01
T2	49.24 ^{ab} ± 0.91	94.09 ^{c} ±1.51	44.84 ^{c} ± 0.61	0.50 ^{c} ± 0.01	0.72 ^{c} ± 0.01
T3	47.50 ^{b} ± 0.26	97.30 ^{c} ±3.70	49.80 ^{bc} ± 3.96	0.55 ^{bc} ± 0.04	0.80 ^{bc} ± 0.05
T4	50.25 ^{a} ± 0.25	110.08 ^{b} ± 2.08	59.83 ^{b} ± 2.33	0.66 ^{b} ± 0.03	0.87 ^{b} ± 0.03
T5	47.60 ^{b} ± 0.21	96.36 ^{c} ± 5.64	48.76 ^{c} ±5.86	0.54 ^{c} ± 0.07	0.78 ^{bc} ± 0.07
T6	47.55 ^{b} ± 0.07	121.64 ^{a} ±1.88	74.10 ^{a} ±1.81	0.82 ^{a} ± 0.02	1.05 ^{a} ± 0.02
T7	47.62 ^{b} ± 0.00	89.14 ^{c} ± 3.38	41.52 ^{c} ± 3.38	0.46 ^{c} ± 0.04	0.70 ^{c} ± 0.05

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

T1:control,T2 (EM: Effective microorganisms), T3 (M): Molasses, T4:(WB): Wheat bran, T5: EM+M, T6:EM+WB, and T7: M+WB.

Feed utilization

The effect of EM and carbon sources in biofloc system on feed and nutrient utilization of Nile tilapia after three months rearing period is shown in Table 4. Results showed that feed intake had ranged between 79.51 and 102.52 g/fish with insignificant differences among treatments indicating complete randomization of dividing the fish into experiments groups. Moreover, the FCR improved significantly with all studied treatments compared to the control (T1). The best FCR was obtained in T6 (EM+WB) followed by T4 (WB). In the same trend, PER, PPV and EU showed significant differences among most treatment and recorded the highest values in treatments T6 and T4, being the values were 3.22 and 2.55; 56.54 and 34.50 %; and 27.53 and 19.48, respectively. On the other hand, the lowest values were recorded with the treatments T1and T2. The results obtained for FI, FCR, PER, and PPV in this study agree with the findings of Xia *et al*. (2010) and El-Kady *et al.* (2016). Who reported the effect of biofloc, feeding rate and dietary protein level on growth performance and feed utilization of Nile tilapia, flathead grey mullet and thin lipped mullet in poly-culture. The results revealed significant differences for the effect of dietary protein level in biofloc system on growth performance and feed utilization. Overall, the beneficial effects of probiotics on fish performance parameters were in agreement with the findings of Wang *et al.* (2008) who studied the effect of using probiotics in tilapia compared with studies lacking positive effects (Shelby *et al*., 2006).

Table 4. Effect of effective microorganisms and carbon sources in biofloc system on feed and nutrients utilization of Nile tilapia.

Treatments*	Feed intake (g/fish)	FCR¹	PER²	PPV³ (%)	EU⁴ %
T1	79.51^{c} ±1.53	2.90^{a} ±0.09	1.55^{d} ±0.01	15.90^{d} ±1.31	12.63^{cd} ±1.79
T2	85.19^{bc} ±1.21	1.90^{bc} ±0.05	2.36^{bc} ±0.01	45.83^{b} ±0.01	22.93^{ab} ±0.05
T3	88.34^{b} ±3.67	1.78^{c} ±0.07	2.53^{bc} ±0.15	37.16^{bc} ±3.80	18.27^{bc} ±1.54
T4	102.52^{a} ±0.73	1.72^{cd} ±0.05	2.55^{bc} ±0.07	34.50^{bc} ±0. 49	19.48^{b} ±0.44
T5	84.68^{bc} ±0.69	1.76^{c} ±0.23	2.58^{b} ±0.28	45.83^{b} ±0.01	22.05^{ab} ±3.68
T6	100.78^{a} ±1.28	1.36^{d} ±0.05	3.22^{a} ±0.13	56.54^{a} ±2.77	27.53^{a} ±1.66
T7	90.05^{b} ±3.34	2.18^{b} ±0.10	2.06^{c} ±0.14	21.99^{d} ±3.15	9.30^{d} ±1.15

***Means in the same column having different letters are significantly (P≤0.05) different. T1: control, T2 (EM: Effective microorganisms), T3 (MO): Molasses, T4: (WB): Wheat bran, T5: EM+M, T6: EM+WB, T7: M+WB. ¹FCR: Feed conversion ratio, ²PER: Protein efficiency ratio, and ³PPV: Protein productive value and ⁴EU: energy utilization.**

However, there were no significant differences in growth and utilization of treating with *B. subtilis* compared with the control (Body and Tucker, 2009). Biofloc systems therefore represent a suitable culture condition for growth and feed utilization of *O. niloticus* without any obvious negative effect on water quality or fish survival, which reflects previous findings concerning the positive effect of biofloc on the growth performance of cultured fish and shrimp (Arnold *et al*., 2009; Wang *et al*., 2015 and Verma *et al*., 2016). The suitability of using biofloc at 4% level of dietary supplementation for improving growth, FCR and digestive enzyme activities has been demonstrated in *Penaeus monodon* (Anand *et al*., 2013).

Body composition:

The effect of EM and carbon sources in biofloc system on proximate chemical composition of Nile tilapia after three months rearing period is shown in Table 5. Results of this Table reveal that DM contents ranged between 28.16 and 28.70 % with insignificant differences among the dietary treatment groups. The same Table reflects a significant increase in crude protein in T6 compared with T1 (59.00 and 49.30 %, respectively). In the same Table, average of ether extract content was found to be higher at the end of experimented with T4, T1, T2 and T5 and there were no significant differences ($P \geq 0.05$).

Table 5. Effect of effective microorganisms and carbon sources in biofloc system on body chemical composition of Nile tilapia.

Treatments*	DM¹	% on dry matter basis			Energy content
		CP²	EE³	ASH	
T1	28.16^{d} ±0.03	49.30^{f} ±0.56	27.70^{ab} ±0.01	23.00^{a} ±0.56	539.40^{d} ±3.19
T2	28.52^{b} ±0.03	57.75^{b} ±0.35	27.30^{ab} ±0.70	14.95^{d} ±0.35	583.42^{a} ±4.68
T3	28.64^{a} ±0.01	56.60^{c} ±0.01	26.20^{c} ±0.28	17.20^{c} ±0.28	566.55^{b} ±2.67
T4	28.70^{a} ±0.01	53.40^{e} ±0.42	27.95^{a} ±0.07	18.65^{b} ±0.35	565.02 ±1.72^{b}
T5	28.27^{c} ±0.01	55.75^{d} ±0.21	27.40^{ab} ±0.56	16.85^{c} ±0.35	547.52^{cd} ±0.01
T6	28.31^{c} ±0.04	59.00^{a} ±0.14	26.65^{bc} ±0.63	14.35 ±0.49^{d}	584.33^{a} ±5.21
T7	28.25^{c} ±0.04	58.00^{b} ±0.28	23.50^{d} ±0.28	18.50^{b} ±0.56	548.96^{c} ±4.27

***Means in the same column having different letters are significantly (*P*≤0.05) different. T1: control, T2 (EM: Effective microorganisms), T3 (MO): Molasses, T4: (WB): Wheat bran, T5: EM+M, T6: EM+WB, T7: M+WB. ¹DM: Dry matter, ²CP: crude protein, ³EE: Ether extract.**

Also, an average of ash content was found to be higher in the groups T1, T4, and T7; whereas the lowest ash values were found in groups T6, T2, and T3. The higher protein concentration in fish was evaluated in biofloc with wheat bran that may be related to the chemical composition of heterotrophic bacteria and other organisms associated to biofloc and biofilm (Fernández *et al*., 2008 and El-Kady *et al.,* 2016). The effect of a carbon source on the growth of cultured species depends on some characteristics of the biofloc produced such as its volume, chemical composition, and ability to store bioactive compounds, e.g. polymers, catotenoids, phytosterols and extracellular enzymes (De Schryver and Verstraete, 2009 and Zhao *et al*., 2016). In the present study, the development of biofloc not only maintained the water quality suitable for fish culture under zero water exchange but also could supplement protein nutrition, thereby resulting in high survival and similar growth performance and FCR of the fish fed with 25% and 30% dietary protein levels (Kiron, 2012 and El-Kady *et al.,* 2016).

CONCLUSION

The present results recommended the use of a mixture of effective microorganisms and wheat bran as a carbohydrate source for optimum performance in fish water tanks with best impacts on intensive tilapia culture reared under zero water exchange system.

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تأثير إضافة المُخصب الحيوى (الميكروبات الحية الدقيقة) والمولاس ونخالة القمح وخليطهما على إنتاج البروتين الميكروبى وجودة المياه وأداء النمو وكفاءة الاستفادة من الغذاء لاصبعيات أسماك البلطى النيلى

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أجريت هذه التجربة لدراسة تأثير إضافة المخصب الحيوى (الميكروبات الحية الدقيقة) مع مصدرين من الكربوهيدرات (المولاس ونخالة القمح) وخليطهما الى البيئة المائية فى نظام البيوفلوك على معدل إنتاج الكتلة الميكروبية، وأداء النمو والاستفادة من الغذاء لأسماك البلطى النيلى. تم تصميم سبع معاملات تجريبية مختلفة على النحو التالى: المعاملة الأولى: غُذيت الأسماك فيها على العليقة القياسية دون إضافة مصدر كربوهيدراتى. المعاملة الثانية: غُذيت الأسماك فيها على العليقة القياسية مع إضافة المخصب الحيوى (الميكروبات الحية الدقيقة) الى البيئة المائية. المعاملة الثالثة: غُذيت الأسماك فيها على العليقة القياسية مع إضافة المولاس الى البيئة المائية. المعاملة الرابعة:غُذيت الأسماك فيها على العليقة القياسية مع إضافة نخالة القمح الى البيئة المائية. المعاملة الخامسة: غاذيت الأسماك فيها على العليقة القياسية مع إضافة خليط من المخصب الحيوى مع المولاس الى البيئة المائية. المعاملة السادسة: غُذيت الأسماك فيها على العليقة القياسية مع إضافة خليط من المخصب الحيوى مع نخالة القمح الى البيئة المائية. المعاملة السابعة: غُذيت الأسماك فيها على العليقة القياسية مع إضافة خليط من المولاس مع نخالة القمح الى البيئة المائية.غُذيت الأسماك فى كل تانك على عليقة قياسية تحتوى على ٢٢.٤٩٪ بروتين خام و ٤٧١.٦ كيلو كالورى طاقة كلية /١٠٠ جرام مادة جافة، بمعدل ٣٪ من الوزن الكلى للأسماك، مرتان يوميا على مدار ستة أيام في الأسبوع لمدة ٩٠ يوما. وتم توزيع الأسماك عشوائيا على أربعة عشر تانك مصنوع من الألياف الزجاجية، وتم توزيع الأسماك على التانكات (متوسط وزن ٤٧.٥ ± ٠.٠٥ جرام / سمكة) بشكل عشوائي (٢ تانك / معاملة)، بحجم التانك ١٠٠٠ لتر، بواقع ٤٢ سمكة / تانك. وأظهرت النتائج ما يلي:لا توجد فروق معنوية فى معايير جودة المياه، وخاصة فى درجة الحرارة والأس السالب لتركيز أيون الهيدروجين وتركيزالأكسجين الذائب، بينما كانت الفروق ملحوظة فى تركيز الأمونيا الكلية ومعدل إنتاج البروتين الميكروبى (قيم البيوفلوك)، حيث أظهرت النتائج قيم منخفضة فى الأمونيا الكلية، وزيادة فى قيم البيوفلوك فى المعاملة السادسة التى غُذيت فيها أسماك البلطى على العليقة القياسية بالإضافة الى خليط من المُخصب الحيوى مع نخالة القمح فى البيئة المائية، يليها فى الترتيب المعاملة الرابعة التى غُذيت فيها أسماك البلطى على العليقة القياسية بالإضافة الى نخالة القمح فى البيئة المائية على الترتيب. كفاءة النمو وكفاءة الاستفادة من الغذاء تتحسن معنويا عند تغذية أسماك البلطى على العليقة القياسية بالإضافة الى خليط من المُخصب الحيوى مع نخالة القمح فى البيئة المائية، يليها فى الترتيب المعاملة الرابعة التى غُذيت فيها أسماك البلطى على العليقة القياسية بالإضافة الى نخالة القمح فى البيئة المائية على الترتيب مقارنة بالمعاملة القياسية . لذا تُوصى النتائج الحالية بإضافة خليط من المُخصب الحيوى (الميكروبات الحية الدقيقة) مع نخالة القمح الى البيئة المائية، فهى الأمثل لنمو الميكروبات في تانكات أسماك البلطى النيلى تحت نظام الاستزراع المكثف، وبعدم تغير المياه.