Complexity of carbon sources and the impact on biofloc integrity and quality in tilapia (*Oreochromis niloticus*) tanks

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Abstract. Organic carbon sources are the key factor in optimizing the biofloc system conditions. Our concern was to study the effect of different carbon sources on biofloc conditions and tilapia performance. Biofloc treatments with five different organic carbon simple sources (glucose, molasses) and complex sources (starch, wheat bran, cellulose) were conducted in presence of control (clear water). No significant differences ($p > 0.05$) were noticed among different organic carbon sources regarding tilapia performance. Complex carbon source represented in wheat bran and cellulose showed less fluctuation in the values of NH_4^+ and No_2 during the experimental period than the other carbon sources. The precipitated biofloc from both wheat bran and cellulose showed the highest fat content (8.08 and 7.72 respectively). In terms of heterotrophic bacteria production, plankton count and biofloc nutritional content, cellulose seems to be the better choice. From the nutritional and economical points of view, using agriculture by product with high cellulose content as a carbon source in biofloc system is more reasonable, guarantee positive environmental impact and aquaculture sustainability.
Key Words: organic carbon sources, biofloc, Nile tilapia, growth performance.

Introduction. With more expansion of biofloc system application around the world, optimizing the conditions for biofloc system is needed for efficient water utilization and for optimal fish growth performance. These efforts are especially needed under conditions of water shortages and rise in world population. Optimal biofloc conditions guarantee more aquaculture intensification, best profiteering of land, water and feed recourses. Intensification of fish culture causes waste accumulation in forms of solids (e.g. feces and uneaten feed) and nutrients (e.g. nitrogen and phosphorus) which have adverse effect on the environment. Biofloc is considered a waste treatment system under condition of zero water exchange. Carbon sources supplementation are the backbone for initiating the system, where affording Carbon: Nitrogen ratio (C:N ratio) of 10 guarantee the activation of the heterotrophic bacteria (Avnimelech 1999; De Schryver et al 2008). The last have the ability to assimilate the nitrogen wastes and recycle it into microbial protein which considered as secondary feed source for species such as tilapia, carp and shrimp. Consequently, water quality and performance of fish culture could be enhanced (Avnimelech 2009; Crab et al 2010). Previous research work examined carbon sources for accessibility, dissolving rate of carbohydrate in water, bioavailability and costs under biofloc condition, especially for shrimp culture. Molasses, corn flour and wheat bran mixture (3:1:1, respectively) improved shrimp (*Litopenaeus vannamei*) growth performance and floc nutritional value (Wang et al 2016). Rice flower showed better values than molasses regarding growth and immune response of black tiger shrimp *Penaeus monodon* cultured under biofloc system Kumar et al (2017). Recently, Khanjani et al (2017) investigated the effect of molasses, starch, wheat flour and mixture of them on the performance of Pacific white shrimp *L. vannamei* where molasses given the best performance of shrimp. Crab et al (2010) developed biofloc system under different carbon sources acetate, glycerol and glucose to determine the possible use of bioflocs as a feed for shrimp post larvae *Macrobrachium rosenbergii* where glycerol+bacillus was appropriate for shrimp culture. For our knowledge some studies evaluated the effect of supplementing biofloc system with carbon sources of different complexity on tilapia culture. The experiment was designed to determine the effect of organic carbon source with different complexity grade (glucose, molasses, starch, wheat bran, cellulose) on the maintaining of biofloc system condition, tilapia growth performance, water quality parameters, feed utilization, floc chemical composition and fish hematological parameters meanwhile detecting the most efficient and economic carbon sources for tilapia culture under biofloc system.

Material and Method

Fish stocking and rearing techniques. Fingerlings of Nile tilapia, *Oreochromis niloticus* were obtained from El-Fayum governorate and transferred to the farm of El-Kanater El-Khayria, National Institute of Oceanography and Fisheries (NIOF), Cairo. Fingerlings were fed on a diet with a crude protein of 30% and were acclimatized to laboratory condition for two weeks. Eighteen (18) cylindrical plastic tanks (with water capacity of 150 L and filled to 100 L) were stocked with fingerlings at a rate of 10 fish $tank^{-1}$ (100 fish m^{-3}), with an initial weight of 14.00±0.03 g. The six experimental treatments were represented in triplicate. Tanks were supplemented with well water source and were continuously aerated using air pump (SIEMENS, 1LE1002-1BA22-2AA4, 2-pole * Size 112M, Czech Republic) using porous stone with length of 4.7 cm. As for the control (clear system), water was exchanged biweekly, while for experimental biofloc tanks, no water exchange was done (zero water exchange) except to compensate the evaporated water.

Diet preparation. A homogenous mixture was used to blend the dry ingredient of basal diet. The ration was then pelleted using the mill (California Pellet Mill, San Francisco, CA, USA), solar dried and storied at -4C°. Experimental fish were fed six days a week on the basal diet (26% protein) for 85 days (Table 1). The daily ration (3% of stocked biomass) was delivered three times a day (10.00, 12.00 and 14.00 hr) in equal portions. Fish were weighted every 15 days and daily feed ration was adjusted accordingly.

Table 1

Formulation and chemical composition of the basal diet

Ingredients	*Diet formula*
Fish meal	6
Soya bean meal	43
Yellow corn	20
Wheat bran	23
Soya oil	6
Vit. and Min. premix [1]	1.45
CMC [2]	0.5
Vit. C	0.05
Proximate composition	
Dry matter (%)	89.70
Protein (%)	25.93
Lipid (%)	14.48
Total carbohydrate (%)[3]	43.21
Ash (%)	6.08
Gross energy (MJ /kg) [4]	19.26
Metabolizable energy (MJ /kg)[5]	13.84
Protein energy (mg CP/ KJ DE)	74.28

[1] Vitamins and minerals mixture each 3 Kg of mixture contain: 12 m.IU vit A, 12 mIU vit D3, 10 g vit. E, 2 g vit. K, 1 g vit. B1, 5 g vit. B2, 1.5 g vit. B6, 10 mg vit. B12, 30 g niacin, 1000 mg folic acid, 50 mg biotin, 10 g banathonic acid, 50 g zinc, 30g iron, 60 g manganese, 10 g copper, 100 mg cobalt, 100 mg selenium, 1000 mg iodine; [2] CMC: carboxy methyl cellulose; [3] Total carbohydrate = 100-(CP + EE + ash); [4] Calculated using gross calorific values of 23.63, 39.52 and 17.15 KJ g^{-1} for protein, fat and carbohydrate, respectively according to Brett (1971); [5] Calculated metabolizable energy = 70% of determined GE value (Hepher et al 1983).

Managing of biofloc tanks. C:N ratio of 10:1 (De Schryver et al 2008) were maintained in different experimental tanks using different carbon sources: glucose, molasses, starch, wheat bran or cellulose that were examined for optimal biofloc condition. Daily addition of different carbon sources was applied. Carbon sources were completely mixed with water sample of the experimental tanks prior spreading into the whole water body.

Water quality assessment. Water temperature and dissolved oxygen (HI 9146-HANNA interment, USA) were recorded weekly. pH values were recorded twice a week (Orion pH meter, Abilene, Texas, USA). Ammonia, nitrite and nitrate were detected biweekly (APHA 1992). Total suspended solid (TSS) values were measured twice during the experimental period from June to September 2014 using (Multi direct lovibond). Total bacterial count (TBC) was determined according to (AOAC 1995; APHA 1992) then reported as CFU mL^{-1}.

Chemical analysis. At experiment end, diet sample along with random samples of five individual fish was sampled from each tank of different treatment and precipitated flocs were collected from different treatments for determination of proximate composition. Fish and bifloc sample were dried in an oven at 80ºC till constant weight then were grounded and stored at -20ºC for subsequent analysis. By incineration at 550ºC for 6 h, ash content was detected. Crude protein was determined by micro-Kjeldhal method, %N × 6.25 (using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden). Soxhlet extraction with diethyl ether (40-60ºC) was used to estimate crude fat content of different samples, the chemical analysis determined according to AOAC (1995).

Hematological parameters. Tubs coated with 10% EDTA (ethylene diamine tetra acetate) were used to collect blood samples for estimation of hematocrit (Ht). The cyanomet hemoglobin method was used to detect the value of hemoglobin (Hb) and white blood cell (WBCs) was estimated by the method of Martins et al (2004).

Statistical analysis. One-way ANOVA was used to analyze experimental data and to detect the best carbon source under biofloc system using the SAS v9.0.0 (2004) program. Duncan test (1955) at $p \leq 0.05$ level of significant was applied.

Results and Discussion

Water quality parameters. Water temperature, pH, dissolved oxygen (DO), ammonia (NH_4^+), nitrite (NO_2) and nitrate (NO_3) are shown in Table 2. No significant differences ($p > 0.05$) were observed for water temperature, pH, NH_4^+, NO_2 and NO_3 values among different carbon sources during the experimental period. DO values had significant differences ($p < 0.05$) among different treatments. The fluctuations of NH_4^+, NO_2 and NO_3 during experimental period are shown in Figure 1. Values of NH_4^+ and NO_2 were high at the beginning of the experiment then decreased along with degradation of different carbon sources subsequently increase of bacterial count. The changes in NH_4^+, NO_2 and NO_3 values were in the acceptable range for Nile tilapia (Delong et al 2009). Generally, values of chemical water quality parameters decreased over time for all treatments. Supplementing experimental tanks with organic carbon sources enhanced the heterotrophic bacterial growth; subsequently increase of nitrogen fixation and decrease ammonia and nitrite which enhanced the water quality (Luo et al 2013, 2014). Despite no water discharged in biofloc treatments were done, no significant differences among control and biofloc treatments in different water quality parameters were found, these in consonance with Nootong & Pavasant (2011). Application of biofloc technique enhanced the water quality via minimizing the level of NH_4^+, NO_2 in carp ponds by elevating the C:N ratio (Wang et al 2015). Same was recognized by Prajith & Madhusoodana (2011) for shrimp culture. Khanjani et al (2017) stated that there were no significant differences among molasses, starch, and wheat flour regarding water quality parameters ammonia, nitrite and nitrate in shrimp *L. vannamei* tanks under biofloc system. Molasses (simple) or rice flour (complex) carbon sources showed the same effects on water quality parameters in shrimp tanks that fed either 30 or 40% crude protein under condition of no water exchange (Kumar et al 2017). Biofloc treatments recorded significantly lower DO than control, this may be due to (i) the decomposition of organic matter by microbial metabolism process which consumed oxygen from water (Khanjani et al 2017), (ii) increased the microbial load in biofloc tanks in presence of high C:N ratio.

Table 2

Water quality parameters of different treatments during the experimental period

Parameters	*Control*	*Different carbon sources*						*P value*
		Glucose	*Molasses*	*Starch*	*Bran*	*Cellulose*	*±MSE*	
Temperature(°C)	28.75	28.72	28.70	28.74	28.75	28.77	0.33	1.0000
DO ($mg\ L^{-1}$)	5.71[a]	4.90[b]	4.61[b]	4.81[b]	4.86[b]	4.96[b]	0.25	0.0543
PH	8.58	8.25	8.31	8.39	8.30	8.32	0.14	0.6040
NH_4^+ ($mg\ L^{-1}$)	0.96	1.21	1.12	1.22	1.15	0.95	0.34	0.9877
NO_2 ($mg\ L^{-1}$)	0.19	0.24	0.23	0.33	0.34	0.18	0.08	0.5517
NO_3 ($mg\ L^{-1}$)	0.76	1.18	1.22	1.10	1.53	1.15	0.17	0.0583
TSS	-	537[a]	549[a]	359[b]	553[a]	479[a]	38.35	0.0074

Data are presented as means±standard error (SE). Means followed by different letters in each rows are significantly ($p < 0.05$) different.

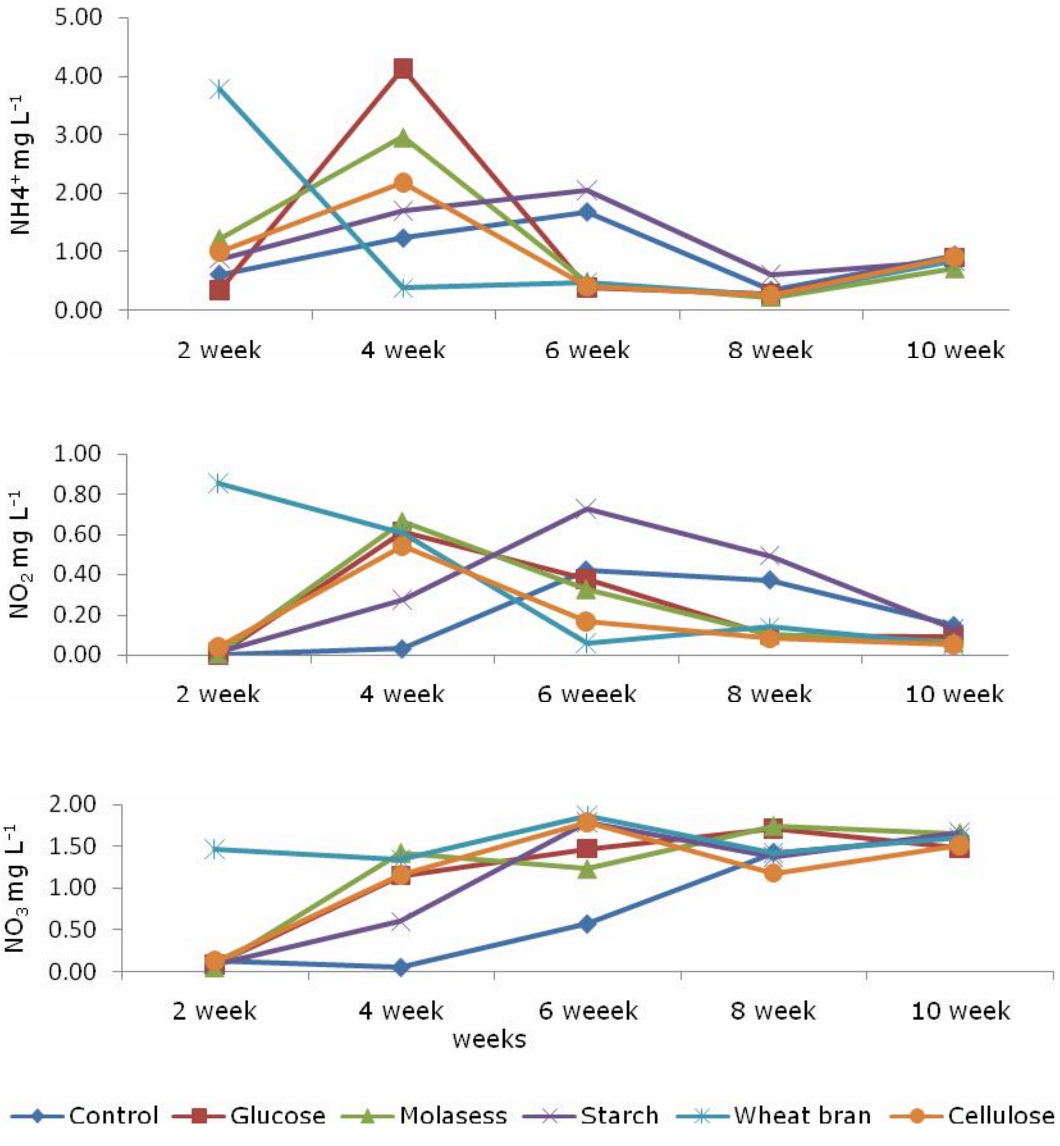

Figure 1. Fluctuations of $NH4^+$, NO_2 and NO_3 during experimental period.

In general, total suspended solids (TSS) values increased gradually during the experimental period, and ranged from 359 to 553 mg L^{-1} among treatment of different carbon sources. The highest TSS value was recorded for wheat bran while, the least value was noticed for starch treatment. Since the recommended level of TSS should not reach 500 mg L^{-1} (Samocha et al 2007), precipitation of biofloc was done after 30 days of the experiment beginning and at the end. Even though, elevations of TSS over optimal level were recorded, thus a procedure of floc precipitation had to be done more frequently to avoid elevation of TSS levels.

Total bacterial counts (TBC) are presented in Figure 2. The TBC counts significantly differed ($p > 0.05$) among different carbon sources. Cellulose, bran and molasses treatments were recorded the highest total count, respectively, compared with the others. TBC count in simple carbon sources treatments were lower than in complex sources treatments. It could be suggested that the last degraded gradually and slowly which led to higher carbon concentration in late stages of the experimental time, subsequently bacterial count increased by the end of the experiment. In contrary, Kumar et al (2017) observed that molasses which is considered as a simple carbon source showed higher microbial load than a complex carbon source as rice flour, under condition of feeding fish on 40% dietary protein. He suggested that this could be due to molasses contain sucrose (disaccharides) in contrast with rice flour, where the last contains polysaccharide. Serra et al (2015) who reported that the speed of carbon degradation rate of simple carbohydrate (molasses and dextrose) is faster than the complex carbohydrate (rice bran), that provide carbon rapidly in water environment as a substrate of bacteria. The positive effect of different complex carbon sources on biofloc shrimp culture was reported by Prajith & Madhusoodana (2011) where the total number of heterotrophic bacteria increased in water (99.9, 74.45, 60.39, 88.7, and 72.9 x 10^3, respectively), without significant differences among different carbon sources (tapioca flour, yam flour, wheat flour, rice flour and potato flour). Generally, slow degradation of complex carbon sources may have higher constant concentration of organic carbon during the experimental period, which gradually increased the count of heterotrophic bacteria. Subsequently, there was less fluctuation in values of NH_4^+ and NO_3. The constantly of last parameters may affect positively the health condition of aquatic organisms.

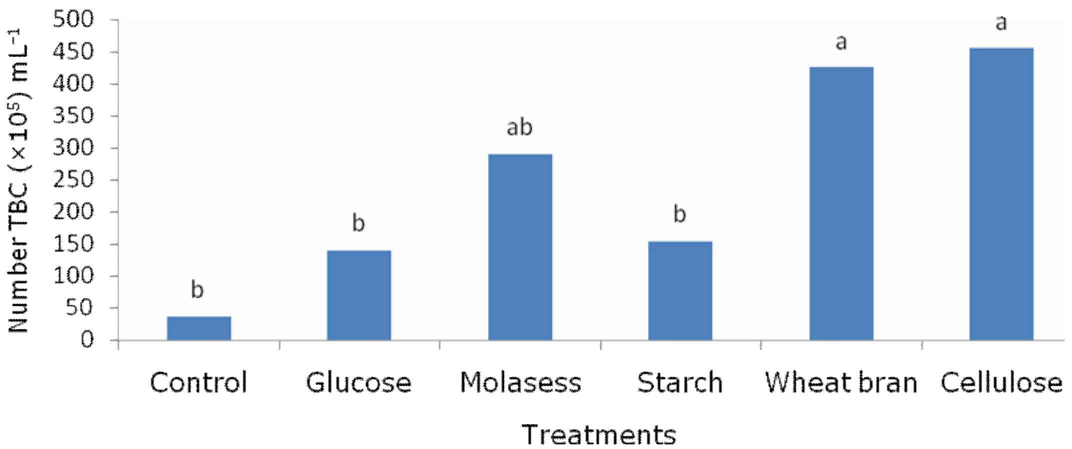

Figure 2. The effect of different organic carbon sources on total bacterial count under biofloc system compared with control.

Growth performance and feed utilization of tilapia. The growth performance (final body weight (FBW), weight gain (WG) and specific growth rate (SGR)) and feed utilization (feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio(PER)) are given in Table 3. No significant differences were noticed ($p > 0.05$) among different carbon sources regarding fish performance. Generally, all organic carbon sources showed superiority in growth performance and feed utilization compared to the control. Addition of different carbon sources revealed a balanced C:N ratio, subsequently activation of the heterotrophic bacteria. This along with micro organisms such as zooplankton protozoa and others acted as secondary feed source (Schneider et al 2005). Luo et al (2014) concluded that WG, SGR and FCR were better in biofloc system than recirculating aquaculture systems (RAS). Long et al (2015) noted the same results for tilapia. Furthermore, Ekasari et al (2015) demonstrated better productive performance of Nile tilapia larva under biofloc condition was gained. Crucian carp *Carassius auratus* showed improved growth performance with increasing of the C:N up to 25:1 (Wang et al 2015).

Table 3

Growth performances and feed utilization of tilapia under biofloc system with different carbon sources compared with control (clear system)

Parameters	*Control*	*Different carbon sources*						*P value*
		Glucose	*Molasses*	*Starch*	*Bran*	*Cellulose*	*±MSE*	
Initial weight	14.06	14.01	13.98	14.01	14.04	13.99	0.03	0.3071
Final weight	22.81	26.69	26.14	24.35	26.24	24.50	1.10	0.1277
Weight gain	7.20^b	10.20^a	11.63^a	10.30^a	12.20^a	10.00^a	0.83	0.0211
SGR (% day)	0.57	0.75	0.73	0.65	0.73	0.66	0.05	0.1234
Feed intake	35.35	36.65	37.85	38.59	40.18	37.48	0.86	0.5034
FCR	5.60^a	4.27^b	3.50^b	3.80^b	3.40^b	3.90^b	0.32	0.0118
PER	0.70^b	0.91^{ab}	1.14^a	1.02^a	1.17^a	0.99^a	0.08	0. 0248

Data are presented as means±standard error (SE). Means followed by different letters in each rows are significantly ($p < 0.05$) different. WG = Final body weight (g) - Initial body weight (g); SGR% = (ln FBW - ln IBW) /t × 100; FCR = Diet intake (g)/weight gain (g); PER = weight gain (g)/protein intake (g).

Growth performance parameters didn't clearly reflect the effect of different carbon sources under biofloc system condition. Kumar et al (2017) suggested that the complex carbon source rice flour positively affected shrimp (*P. monodon*) growth performance than simple carbon source molasses**.** Same trend was recorded by Serra et al (2015) who reported that the best result of shrimp (*L. vannamei*) growth recorded for rice bran treatment as a complex carbohydrate source in comparison with dextrose as a simple carbon source. Many authors suggested no differences among organic carbon sources as a control element in biofloc system were noticed (Prajith & Madhusoodana 2011; Khanjani et al 2017). Then, choosing cheaper carbon sources especially complex sources such as agriculture by product showed to be more economical and eco-friendly.

The poor growth performance and feed utilization that recorded under either control or floc treatments could be due to; low diet stability because of using roughness ingredient, spawning that showed under both floc and control treatments. The same most of reasons were recorded by Azim & Little (2008). Furthermore, elevation of TSS in biofloc tanks may be another reason behind the poor growth performance. It seems that under condition of biofloc, the settling process should be preceded more frequently during the experiment duration. Adverse effect on shrimp performance was recognized by Furtado et al (2011) with the excess of TSS values. Elevation of TSS concentration above recommended level (500 mg L^{-1}) resulted in adverse effect on growth performance and production of tilapia fish (Azim & Little 2008). Low growth performance and feed utilization in present case could be attributed to high values of TSS which exceeded the optimal range in biofloc treatments.

Chemical composition of fish and biofloc. Carcass chemical compositions of tilapia are presented in Table 4. No significant differences ($p > 0.05$) among treatments regarding ash, fat and protein contents were noticed. Significant differences ($p < 0.05$) were showed in dry matter, where the highest values were recorded for wheat bran. Khanjani et al (2017) noticed higher fat content of shrimp *L. vannamei* in complex carbon source treatment (wheat flour) compared to treatments of molasses, starch or their mixture. The highest fat content of tilapia in our results showed in wheat bran as a complex carbon source without significant difference with other treatments. Long et al (2015) found that lipid content increased in fish carcass under the biofloc treatment compared with the control, while no significant differences in crude protein and ash content were recorded. Luo et al (2014) showed no significant differences in protein and fat composition of tilapia muscle between either RAS or biofloc system.

Table 4
Chemical composition of fish under biofloc system with different carbon sources compared with control (clear system)

Parameters	Control	Different carbon sources						P value
		Glucose	Molasses	Starch	Bran	Cellulose	±MSE	
Dry matter	23.11^{bc}	23.24^{bc}	24.07^{ab}	22.50^{c}	25.08^{a}	24.16^{ab}	0.43	0.0428
Ash	19.13	19.12	19.38	20.62	19.24	20.10	0.62	0.5818
Fat	19.83	18.68	19.46	17.34	20.14	19.80	0.66	0.1155
Protein	59.48	59.28	57.7	60.17	57.86	59.21	1.04	0.6892

Data are presented as means±standard error (SE). Means followed by different letters in each rows are significantly ($p < 0.05$) different.

Biofloc chemical composition under different carbon sources are tabulated in Table 5. The heights protein and fat content listed for starch and wheat bran treatment, respectively. Crude protein, lipid, ash and carbohydrate values of previous studies ranged between 18.4-49%; 0.1-4%; 11.8-42% and 19-36.4%, respectively (Azim & Little 2008; Kuhn et al 2010, 2016; Emerenciano et al 2013; Maica et al 2012; Dantas et al 2016). The resulted values in our study lay in the same ranges except for fat content where showed higher values that reached 8.08%. Carbon sources may reflect the biochemical composition of biofloc differently. Same was suggested by Crab et al (2010) where different carbon sources (glycerol+bacillus, glucose and acetate) resulted in different chemical composition of biofloc. Wheat flour resulted in the highest protein and lipid contents (30.73, 2.18, respectively) in the study of Khanjani et al (2017).

Table 5
Chemical composition of biofloc with different carbon source

Parameters	Different carbon sources						P value
	Glucose	Molasses	Starch	Bran	Cellulose	±MSE	
Protein	28.34^{bc}	32.74^{ab}	33.70^{a}	27.07^{c}	29.10^{abc}	1.56	0.0482
Ash	19.27	20.97	21.59	19.21	20.75	0.91	0.1885
Fat	3.93^{c}	5.21^{bc}	6.09^{abc}	8.08^{a}	7.72^{ab}	0.86	0.0239

Data are presented as means±standard error (SE). Means followed by different letters in each rows are significantly ($p < 0.05$) different.

Hematological blood parameters of fish. Hematological parameters including: hemoglobin (HB), red blood cell (RBC), hematocrit (HTC), and white blood cell (WBC) of tilapia under different organic carbon sources compared with the control are presented in Table 6.

Table 6
Hematological parameters of fish with different carbon sources in biofloc system compare with control (clear system)

Parameters	Control	Different carbon sources						P value
		Glucose	Molasses	Starch	Bran	Cellulose	±MSE	
HGB (g dl)	4.67	4.4	5.03	4.17	5.47	5.13	0.62	0.6873
HCT (%)	19.03	18.93	22.67	17.6	23.97	20.77	2.97	0.3550
RBC (10^6/ul)	1.36	1.56	1.71	1.24	1.76	1.7	0.19	0.3550
WBC (10^3/ul)	203.04	266.46	269.11	263.81	365.63	351.34	58.25	0.4184

Data are presented as means±standard error (SE). Means followed by different letters in each rows are significantly ($p < 0.05$) different.

Despite no significant differences ($p > 0.05$) among treatments in all blood parameters were recorded, the highest WBC values were recorded for complex carbon source (wheat bran and cellulose) treatments. The same trend was observed for other blood parameters. It seems that biofloc condition didn't affect fish health negatively compared with the clear water condition represented by the control. The same was reported by Long et al (2015) who suggested that no significant effect of biofloc up on blood hematology parameters, especially on RBC, WBC, Hb, and HTC. Furthermore, Azim & Little (2008) suggested the same range of hematocrit value for tilapia under either biofloc treatment (BFT) or recalculating aquaculture system (RAS).

Conclusions. Biofloc system with different carbon sources achieved superiority over control system in all parameters. There were no significant differences among biofloc treatments regarding tilapia performance. The slow degradation of complex carbon source (wheat bran and cellulose) delayed the improvement of water quality at the beginning of the experiment with less fluctuation values of NH_4^+ and NO_3. The present study suggested that complex carbon sources especially cellulose were proper choice interims of bacterial count, microorganisms diversity and quality of biofloc composition. From the nutritional and economical points of view choosing cheap agriculture by product with high cellulose content as a source of carbon in biofloc system is reasonable, may be resulted in positive impact on the environment and sustainability.

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