

Research Article

Biofloc system applied to Nile tilapia (*Oreochromis niloticus*) farming using different carbon sources: Growth performance, carcass analysis, digestive and hepatic enzyme activity

**Khanjani M.H.^{1*}; Alizadeh M.²; Mohammadi M.²;
Sarsangi Aliabad H.²**

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Abstract

The effect of different carbon sources in the biofloc system on growth performance, body biochemical compositions, digestive and hepatic enzymes of Nile tilapia was investigated in this research. *Oreochromis niloticus* fingerlings with average weight of 1.57 ± 0.12 g were cultured for 37 days in fiberglass tanks (130 L), at a density of one fish per liter. The experiment was designed in five treatments including one control group and four biofloc treatments by adding different carbon sources: molasses (BFTM) and starch (BFTS) (complex carbon sources), barley flour (BFTB) and corn (BFTC) (simple carbon sources). Results showed the lowest dissolved oxygen (5.45 mg. L^{-1}), pH (7.25) and feed conversion rate (0.99) in BFTS treatment ($p < 0.05$), while the latter showed the highest protein efficiency ratio (2.91) and survival rate (98.2%). There were no significant differences in growth performance among various treatments ($p < 0.05$). Somatic indices improved in biofloc treatments compared to the control group. Bioflocs formed in different carbon sources showed different nutritional value ($p < 0.05$) which affected the quality of carcass analysis. The highest amount of amylase (95.86 U/mg protein) and protease (17.77 U/mg protein) activities were obtained in BFTB treatment and the lowest amount of hepatic enzymes activity was observed in the BFTS treatment ($p < 0.05$). Generally, the present study showed that cultured tilapia using in situ biofloc produced by different carbon sources can promote FCR, survival, body composition, digestive and hepatic enzymes compared to the control group. Improved digestive activities are more noticeable in complex carbon sources and hepatic enzymes activities are stronger in simple carbon sources.

Keywords: Biofloc technology, Carbon sources, Growth, body composition, Digestive and hepatic enzymes, *Oreochromis niloticus*

1-Department of Fisheries Science and Engineering, Faculty of Natural Resources, University of Jiroft, Jiroft, Kerman, Iran

2-National Research Center of Saline waters Aquatics, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Bafgh, Yazd, Iran

*Corresponding author's Email: Emails: m.h.khanjani@ujiroft.ac.ir; m.h.khanjani@gmail.com

Introduction

Biofloc technology is based on stimulating the growth of heterotrophic bacteria by the addition of carbonaceous organic matter to the cultivation system and careful regulation the carbon to nitrogen ratio (Khanjani and Sharifinia, 2020). In addition, carbohydrates have been recognized as an effective way to reduce the effects of nitrogenous wastes in systems with either zero or very little water exchange in aquaculture (Emerenciano *et al.*, 2017; Khanjani *et al.*, 2017).

In biofloc system carbohydrate digestibility, protein content and cost per unit must be taken into account in order to select the type of carbon source. Complex carbohydrates (as: wheat flour, barley flour) often contain proteins which must be taken into consideration when calculating the carbohydrate needed to maintain a high C: N ratio (Avnimelech, 2009; Walker *et al.*, 2020). There are many other considerations for carbon source selection, such as availability, digestibility and palatability, uptake efficiency by bacteria and bioavailability, ability to disperse in water, and cost-effectiveness. Carbonaceous organic matter should be either soluble or well powdered to reduce its settling rate and suspended in water to make it more accessible to bacteria (Khanjani *et al.*, 2017).

Numerous studies on the use of different carbon sources in the biofloc system of various species have been carried out including starch for

Oreochromis spp (Crab *et al.*, 2009), wheat flour for *Penaeus monodon* (Anand *et al.*, 2014), molasses for *Oreochromis sp* (Ekasari *et al.*, 2015; Khanjani *et al.*, 2021b), glucose for *O. niloticus* (Long *et al.*, 2015), beet molasses for *Cyprinus carpio* (Najdegerami *et al.*, 2016), corn meal for *Oreochromis niloticus* (Day *et al.*, 2016), rice powder for *O. niloticus* (Maya Gutierrez *et al.*, 2016), molasses, wheat flour, starch and their mixtures for *Litopenaeus vannamei* (Khanjani *et al.*, 2017), poly-b-hydroxybutyric acid and glucose for *O. niloticus* (Luo *et al.*, 2017) and glycerol for *Clarias gariepinus* (Dauda *et al.*, 2018).

The use of different carbon sources in the biofloc system have different effects on water quality, growth performance, body biochemical compositions, immune activities, digestive enzymes, antioxidant activity and produced biofloc (Ahmad *et al.*, 2016, 2019; Khanjani *et al.*, 2017; Bakhshi *et al.*, 2018; Panigrahi *et al.*, 2019; Khanjani *et al.*, 2021a).

Nile tilapia (*O. niloticus*) has been recognized as an important species for cultivation worldwide (Wang and Lu, 2016) due to its characteristics and advantages including the ability to feed on various nutrients (phytoplankton, suspended particles in the water column and microbial masses) (Bosisio *et al.*, 2017), rapid growth, easy adaptation to intensive culture and artificial food intake (Fitzsimmons *et al.*, 2011). The use of the biofloc production system in cultivation of species with characteristics such as tolerance to

moderate levels of oxygen, feeding on detritus, and adaptability to high densities is associated with greater success (Khanjani and Sharifinia, 2020). Tilapia due to the specific characteristics is capable of being produced in an intensive and super intensive condition in the biofloc system (Avnimelech, 2009; Samocha *et al.*, 2017). This species is also able to consume biofloc-dependent microorganisms (Durigon *et al.*, 2019). The type of carbon source used in the biofloc system plays a critical role in how the system is managed (Panigrahi *et al.*, 2019).

Further studies are needed to elucidate the impact of different carbon sources on water quality, growth performance, and physiological activities of cultured species in the biofloc system. In the present study, four types of carbohydrate sources including barley flour and corn (as complex carbohydrates), molasses and starch (as simple carbohydrates) were evaluated in the biofloc system and their effects on growth performance, somatic indices, body biochemical compositions, digestive and hepatic enzymes of Nile tilapia fingerlings as well as biofloc biochemical composition and water quality were measured.

Materials and methods

The present study was carried out in the National Research Center of Saline Waters Aquatics (Yazd province, Iran). Nile tilapia fingerlings were obtained with an average weight of 1.57 ± 0.12 g

and mean length of 4.02 ± 0.09 cm. Altogether, 15 circular polyethylene tanks (300 liters) were designed for this experiment. Each tank was filled with 130 liters of well water at 8 ppt salinity and then 130 fingerlings (1.57 g/L) were stocked in each tank. The experiment was conducted for 37 days. Five treatments were considered for the present study, including control group (with water exchange) that replaced daily 50-60% of fresh water with the same salinity of water before feeding, and four biofloc treatments with limited water exchange (0.4 to 0.6 % was replaced daily by adding various sources of carbon including molasses (dry weight 54.18%; carbohydrate 73.24% D.W), corn flour (dry weight 88.5%; carbohydrate 75.79% D.W), barley flour (dry weight 91.39%; carbohydrate 73.03% D.W) and starch (dry weight 90.81%; carbohydrate 98.67% D.W) (Table 1). Feed was offered three times daily (08:00, 12:00 and 16:00) with the diet containing 35% of crude protein (Manufactured by Mazandaran Animal Feed & Aquaculture Company, Iran MANAQUA). Feeding percentage was calculated based on the body weight percent. This percentage was calculated 8% of body weight at the beginning of the experiment and then decreased to 6% of the body weight during the experiment.

In the biofloc treatments, feeding was 25% less than the control group. In these treatments, feeding was considered 75% artificial diet plus 25% produced biofloc in the rearing tanks.

Also, in the biofloc treatments, 2.5 ml of floc per liter as initial stock was added to the production tanks before stocking the fish. Initial stock of biofloc was prepared from three growing ponds (6 m in diameter and 1.2 m in depth) of Nile tilapia based on biofloc. In this way, some of the water in the ponds

was filtered through a 20-micron mesh and then the obtained floc was added to the BFT treatments at a specified amount as a stock. Natural photoperiod (12 h of light and 12 h of dark) was considered during the experiment.

Table 1: Characteristics of treatments based on the different carbon sources for cultivation of Nile tilapia fingerlings.

Treatments	Type of carbohydrates	Water exchange (daily)
control	without adding carbohydrate	50-60%
BFTM	Molasses	0.4-0.6%
BFTC	Corn flour	0.4-0.6%
BFTB	Barley flour	0.4-0.6%
BFTS	Starch	0.4-0.6%

The source of carbohydrate was added to the treatment tanks to develop biofloc. The carbon sources were added once a day based on the calculation as described by Avnimelech (2009). The C:N ratio was considered 15:1. It was assumed that 50% of the carbon content in the carbonaceous material was absorbed by microbial communities (Avnimelech, 2009).

The carbon sources were mixed in a beaker with the water tank and distributed evenly over the tank surface immediately after feeding at 12:00 hour. Fish was weighed once a week to measure fish growth and estimate the amount of feed and organic carbon (Khanjani *et al.*, 2020a).

Addition of carbon sources to one gram of feed depends on the assimilation and assumption of nitrogen in the system. In this study, carbon sources were used 0.65, 0.48, 0.53, and 0.3 g for molasses, corn flour, barley

flour, and starch, respectively, for one gram of feed (Avnimelech, 2009).

Water quality parameters

Temperature, pH and dissolved oxygen (DO) were determined daily at 8 am and 16 pm. Salinity was measured daily at 9 am by HQ30D Multi Meter HACH. Transparency was measured every five days by a Secchi disk. Determination of settled solids was conducted every five days. For this, one liter of water was poured into a graduated conical funnel and allowed 20 minutes to settle (Avnimelech and Kochba, 2009). To measure total suspended solids (TSS), 100 ml of water was filtered with Whitman 42 filter paper and put in an oven at 105°C for 3 h to dry. Total counts of heterotrophic bacteria (CFU: colony-forming units) were measured according to APHA method (2005) using culture medium (R2-agar).

Ammonia, nitrite and nitrate levels were measured once in a week using a

spectrophotometer (Perkin Elmer lambda 25 UV / Vis) (MOOPAM, 1999).

Growth, nutritional and somatic indices

Measuring fish length and weight were performed at the beginning and end of the experiment. Growth indices:

WG (g) = final weight - initial weight

LG (mm) = final length- initial length

BWI (%) = (final weight- initial weight)/ initial weight \times 100

DWG (g/day) = (final weight - initial weight) (/days of experiment)

SGR (% / day) = [(ln final weight-ln initial weight) \times 100] / days of experiment

SR (%) = (number of individuals at end of rearing period / initial number of individuals stocked) \times 100.

FCR = feed consumed (dry weight) / live weight gain (wet weight)

PER = weight gain (g) / intake protein (g).

Somatic indices Condition Factor (K), Viscerosomatic index (VSI), Hepatosomatic index (HSI), Carcass

including weight gain (WG), length gain (LG), body weight index (BWI), Daily weight gain (DWG), specific growth rate (SGR) and survival rate (SR) and nutrition indices including feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated according to the following formulas (Khanjani *et al.*, 2017).

yield (CY) were also determined based on the following equations (Durigon *et al.*, 2019).

Condition factor, $K = 100 \times (\text{total weight (g)} / \text{total length}^3) \text{ (cm)}$.

$VSI (\%) = 100 \times (\text{visceral weight (g)} / \text{final fish weight (g)})$.

$HSI (\%) = 100 \times (\text{liver weight (g)} / \text{final fish weight (g)})$.

$CY (\%) = 100 \times (\text{fish weight without visceral (g)} / \text{final fish weight (g)})$

Body proximate compositions

To measure body proximate compositions, twenty fish from each replicate were randomly sampled at the end of the experiment. For numbing, these samples were covered by the ice (ethical issues were considered). Next, the abdomen was removed and the remains were homogenized with the meat grinder. Then, the homogenized samples were stored at -18°C until

analysis. Finally, parameters such as protein, lipid, moisture and ash were determined (AOAC, 2005).

Biofloc analysis

At the end of the experimental period, the water was passed through 20-micron nets. The biofloc from each treatment was separated and placed in a container. Then, the samples were dried by an oven at 72°C for 72 h. The dried

bioflocs were stored at -18°C until biochemical analysis. The AOAC (2005) method was used to determine protein, lipid, moisture and ash.

Digestive enzyme activity

At the end of the experiment, five fish were picked from each replicate. They were anesthetized with clove powder (200 ppm) and killed by observing ethical issues (Sloman *et al.*, 2019).

The intestine was separated and visceral fat was placed on ice. Tissues were washed with cold normal saline solution and stored at -80°C . Intestine tissue was thawed and homogenized in 1:5 (w/v) cold 50 mM tris-HCl buffer, pH=7.5 for enzyme extract preparation. The homogenates were centrifuged (10000 g for 20 min at 4°C) and the resulting supernatant separated and frozen at -80°C .

Alkaline protease (APr) activity was determined by the method of Garcia-Carreno and Haard (1993). Azocasein 2% in Tris-HCl, pH = 7.5 was used as the substrate. The lipase (LP) specific activity was measured by Iijima *et al.* (1998) method. Nitrophenyl myristate was used as the substrate. Amylase (AM) activity was determined by the method of Bernfeld (1955). Starch was used as the substrate. The activity of all enzymes was stated as the specific activity being the micromole of hydrolyzed substrate per minute per milligram protein (U / mg) (Jenabi Haghparast *et al.*, 2019).

Hepatic enzyme activity

Five fish from each replicate were randomly sampled, and anesthetized

using clove powder (200 ppm). The fish were killed by observing ethical issues. Liver tissue were removed by dissection and stored at -80°C . Then, liver samples were mashed onto ice using porcelain mortar and pestle. The samples were homogenized in phosphate buffer (0.050 M, pH 7.4) to prepare a10% (w/v) liver homogenate. After that, the homogenates were centrifuged (4°C at 12000 rpm for 15 min) and finally, the supernatant was separated then stored at -80°C .

Determination of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were conducted by the IFCC (International Federation of Clinical Chemistry) method (Bergmeyer *et al.*, 1985). Alkaline phosphatase (ALP) was measured using DGKC, 1972 (Deutsche Gesellschaft fur Klinische Chemie) method. These enzymes were determined according to photometric method by medical diagnosis kits (Pars Azmoon Company, Tehran, Iran).

Data analysis

SPSS (version 21) software was used to analyze data (mean \pm SD, standard deviation). At first, the data were checked for normality and variances for homogeneity by Kolmogorov-Smirnov and Levene's tests, respectively. Then, one-way ANOVA test was used to compare the means. Subsequently, Duncan's test at 5% level was applied to compare between individual treatments.

Results

The values (mean±SD) of water physicochemical parameters during the experiment are presented in Table 2. Based on the measurements, there was a significant difference between treatments in dissolved oxygen and pH ($p<0.05$), so that the lowest DO (5.45 mg/L) and pH (7.25) were found in BFTS treatment (starch as a carbon

source) which is significantly different from other treatments ($p<0.05$). Results showed significant differences between control and biofloc treatments for settled solids (SS), total suspended solids (TSS) and transparency so that the highest SS and TSS were obtained in BFTB treatment (barley carbon source) during the experiment.

Table 2: Water quality parameters in cultivation tanks of *O. niloticus* under different carbon sources during 37 days of experiment.

Parameters	Control	BFTM	BFTC	BFTB	BFTS
DO a.m. (mg/L)	6.90± 0.25 ^a	6.45 ± 0.38 ^b	6.77 ± 0.3 ^a	6.87 ± 0.48 ^a	6.36 ± 0.30 ^b
DO p.m. (mg/L)	6.70± 0.2 ^a	5.56 ± 0.58 ^{bc}	5.82 ± 0.43 ^b	5.83 ± 0.31 ^b	5.45 ± 0.47 ^c
pH a.m.	7.51 ± 0.06 ^a	7.45± 0.045 ^b	7.46 ± 0.042 ^b	7.48 ± 0.066 ^{ab}	7.39 ± 0.052 ^c
pH p.m.	7.40± 0.05 ^a	7.27 ± 0.06 ^b	7.36 ± 0.06 ^a	7.37 ± 0.04 ^a	7.25 ± 0.07 ^b
Salinity (PPT)	8.55± 0.04 ^b	8.91 ± 0.09 ^a	8.93 ± 0.15 ^a	8.88 ± 0.12 ^a	8.84 ± 0.13 ^a
SS (ml/L)	1.59± 0.75 ^b	22.66 ± 13.48 ^a	23.93 ± 16.40 ^a	26.41 ± 18.20 ^a	22.64 ± 13.63 ^a
TSS (mg/L)	77.37± 33.13 ^b	318.64 ± 193.70 ^a	334.14 ± 213.32 ^a	362.83 ± 224.99 ^a	309.04 ± 186.32 ^a
Transparency (cm)	25.74± 1.84 ^a	11.21± 7.80 ^b	11.68± 7.54 ^b	11.19± 6.73 ^b	11.48± 7.12 ^b
TAN (mg/L)	3.36± 1.73 ^b	5.94± 3.81 ^a	6.7± 3.55 ^a	6.81± 3.75 ^a	4.76± 3.45 ^a
NO ₂ (mg/L)	0.35± 0.25	0.40± 0.30	0.43± 0.29	0.44± 0.33	0.39± 0.28
NO ₃ (mg/L)	10.5± 4.66 ^b	19.21± 8.94 ^a	16.45± 7.13 ^a	16.73± 7.09 ^a	20.01± 10.15 ^a

a.m. - before midday; p.m. - after midday

Values are expressed as mean±SD. Values in the same row with different letters are significantly different ($p<0.05$).

BFTM: molasses as carbon source, BFTS: starch as carbon source, BFTB: barley flour as carbon source, BFTC: corn as carbon source

There were significant differences between control group and BFT treatments in total ammonia nitrogen (TAN) and nitrate (NO₃) values during the experiment ($p<0.05$). The lowest values were obtained in the control group.

The values (mean±SD) of the total logarithm of heterotrophic bacteria (CFU) are given in Table 3. The lowest values were observed in control group. The values (mean±SD) of growth parameters for different treatments are shown in Table 4, which showed

significant differences between control group and BFT treatments ($p<0.05$). The highest FCR (1.41) and the lowest PER (2.02) were obtained in control group and showed a significant difference with other treatment ($p<0.05$).

Table 4 shows the survival values of Nile tilapia fingerlings in different treatments. The highest (98.2%) and the lowest (95.38%) survival rates were obtained in BFTS treatment and control group, respectively ($p<0.05$).

The values of somatic indices including K, VSI (%), HSI (%) and CY (%) are shown in Table 4. According to the results, there was a significant difference between control group and

biofloc treatments in somatic indices. The lowest level of condition factor (1.89) and carcass yield (82.81%) were obtained in the control group ($p < 0.05$).

Table 3: Logarithmic values (mean±SD) of total density of heterotrophic bacteria (log cfu/ml) during the experiment in different treatments.

Days of experiment	Control	BFT7M	BFTC	BFTB	BFTS
3	3.81± 0.07 ^b	5.65± 0.04 ^a	5.58± 0.02 ^a	5.59± 0.03 ^a	5.74± 0.07 ^a
18	4.23± 0.08 ^b	6.99± 0.012 ^a	6.88± 0.13 ^a	6.9± 0.14 ^a	6.97± 0.02 ^a
34	4.69± 0.02 ^c	7.47± 0.05 ^a	7.26± 0.014 ^b	7.21± 0.15 ^b	7.46± 0.12 ^a

Table 4: Growth performance and Somatic indices of Nile tilapia *O. niloticus* fingerlings cultivated under different carbon sources at the end of 37 days of the experiment period (mean ± SD).

Parameters	Control	BFTM	BFTC	BFTB	BFTS
Final weight (g)	7.00± 0.48 ^b	7.36± 0.35 ^a	7.21± 0.37 ^a	7.27± 0.48 ^a	7.42± 0.47 ^a
Final length (cm)	7.16± 0.56 ^a	7.15± 0.28 ^a	7.03± 0.32 ^b	7.10± 0.40 ^{ab}	7.17± 0.39 ^a
WG (g)	5.43± 0.48 ^b	5.79± 0.35 ^a	5.64± 0.37 ^a	5.7± 0.48 ^a	5.85± 0.47 ^a
LG (cm)	3.14± 0.56 ^a	3.13± 0.28 ^a	3.01± 0.38 ^b	3.08± 0.45 ^{ab}	3.15± 0.33 ^a
BWI (%)	345.86± 35.6 ^b	368.9± 26.55 ^a	359.71± 22.15 ^a	363.24± 28.71 ^a	372.75± 20.84 ^a
DWG (g/day)	0.146± 0.02 ^b	0.156± 0.012 ^a	0.152± 0.015 ^a	0.154± 0.018 ^a	0.158± 0.011 ^a
SGR (%/day)	3.35± 0.26 ^b	4.16± 0.2 ^a	4.11± 0.21 ^a	4.13± 0.24 ^a	4.19± 0.16 ^a
PER	2.02± 0.24 ^b	2.9± 0.21 ^a	2.8± 0.24 ^a	2.82± 0.29 ^a	2.91± 0.2 ^a
FCR	1.41± 0.22 ^a	1± 0.09 ^b	1.034± 0.1 ^b	1.027± 0.11 ^b	0.99± 0.06 ^b
SR (%)	95.38± 0.76 ^c	97.94± 1.60 ^{ab}	96.41± 1.17 ^b	96.15± 0.76 ^b	98.20± 0.44 ^a
Somatic indices					
K (%)	1.89± 0.2 ^b	2.013± 0.13 ^a	2.075± 0.22 ^a	2.045± 0.21 ^a	2.038± 0.26 ^a
VSI (%)	17.19± 1.25 ^a	15.89± 0.94 ^b	16.04± 1.14 ^b	16.09± 1.23 ^b	15.97± 1.07 ^b
HSI (%)	3.49± 0.48 ^a	3.14± 0.24 ^b	2.78± 0.37 ^c	2.68± 0.36 ^c	3.21± 0.42 ^b
CY (%)	82.81± 1.25 ^a	84.11± 0.94 ^b	83.96± 1.14 ^b	83.91± 1.23 ^b	84.03± 1.07 ^b

Values are expressed as mean±SD. Values in the same row with different letters are significantly different ($p < 0.05$).

Body weight index (BWI), daily weight gain (DWG), specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR).

Condition factor (K), viscerosomatic index (VSI), hepatosomatic index (HSI), carcass yield (CY)

BFTM: molasses as carbon source, BFTS: starch as carbon source, BFTB: barley flour as carbon source, BFTC: corn as carbon source.

The values of biochemical compositions of fingerlings in different treatments are presented in Table 5. The highest dry weight content (26.24%), protein (59.23%), lipid (25.69%) and ash (14.2%) were observed in control, BFTB, BFTC and BFTM, respectively. Also, the results of biochemical

composition of bioflocs are depicted in Table 6, which shows that dry weight, protein, lipid and ash values were affected by different carbon sources, so that the highest values were obtained in BFTM, BFTB, BFTC and BFTM, respectively. Table 6 shows the activity of digestive enzymes in different

treatments. The highest levels of amylase (95.86 U/mg protein), lipase (2.18 U/mg protein) and alkaline protease (17.77 U/mg protein) were observed in BFTB, BFTC and BFTB treatments, respectively, which showed significant differences with other treatments ($p < 0.05$). The activity of hepatic enzymes affected by different

treatments is shown in Table 6. The results show a significant difference between the control group and the biofloc treatments ($p < 0.05$). The highest levels of AST, ALT and ALP (U/mg protein) were observed in the control group.

Table 5: Values of body proximate composition of *O. niloticus* (% dry weight) and biofloc obtained at the end of the experiment period in different treatments.

Treatment	Dry weight (%)	Crude protein (% DW)	Crude lipid (% DW)	Ash (% DW)
Fish				
Control	26.24± 0.24 ^a	57.11± 0.19 ^c	25.03± 0.23 ^c	12.97± 0.42 ^c
BFTM	25.83± 0.15 ^{ab}	57.60± 0.17 ^{bc}	25.21± 0.08 ^{bc}	14.2± 0.18 ^a
BFTC	26.06± 0.02 ^{ab}	58± 0.36 ^b	25.69± 0.17 ^a	13.57± 0.24 ^b
BFTB	26.00± 0.21 ^{ab}	59.23± 0.38 ^a	25.47± 0.06 ^{ab}	13.76± 0.20 ^{ab}
BFTS	25.66± 0.17 ^b	57.42± 0.54 ^{bc}	25.32± 0.07 ^b	13.41± 0.14 ^{bc}
Biofloc				
BFTM	17.69± 0.28 ^a	26.91± 0.25 ^c	2.05± 0.09 ^d	32.04± 0.39 ^a
BFTC	16.00± 0.36 ^b	27.86± 0.22 ^b	3.55± 0.15 ^a	25.17± 0.34 ^c
BFTB	14.42± 0.35 ^c	31.70± 0.46 ^a	3.06± 0.07 ^b	28.23± 0.26 ^b
BFTS	12.99± 0.26 ^d	24.39± 0.37 ^d	2.58± 0.10 ^c	23.89± 0.28 ^d

Values are expressed as mean± SD. Values in the same column with different letters are significantly different ($p < 0.05$).

Table 6: Digestive and hepatic enzymes activities of Nile tilapia fingerlings cultivated under different carbon sources at the end of 37 days of the experiment period. (mean± SD).

Parameters	Control	BFTM	BFTC	BFTB	BFTS
Intestine					
AM (U/mg protein)	59.44± 1.06 ^d	62.76± 0.35 ^c	74.73± 0.31 ^b	95.86± 0.75 ^a	62.26± 0.32 ^c
LP (U/mg protein)	1.63± 0.10 ^c	1.74± 0.14 ^c	2.18± 0.10 ^a	2.02± 0.04 ^b	1.97± 0.05 ^b
APr (U/mg protein)	10.04± 0.31 ^c	13.04± 0.43 ^c	15.07± 0.33 ^b	17.77± 0.14 ^a	11.99± 0.22 ^d
Liver					
AST (U/mg protein)	2.79± 0.03 ^a	2.49± 0.05 ^c	2.68± 0.09 ^{ab}	2.65± 0.08 ^b	2.41± 0.06 ^c
ALT (U/mg protein)	0.57± 0.03 ^a	0.40± 0.015 ^c	0.55± 0.02 ^{ab}	0.52± 0.026 ^b	0.38± 0.01 ^c
ALP (U/mg protein)	0.065± 0.001 ^a	0.045± 0.002 ^c	0.057± 0.001 ^b	0.054± 0.003 ^b	0.042± 0.002 ^c

Different superscripts indicate significant differences in the five treatments ($p < 0.05$).

Amylase (AM), lipase (LP), alkaline protease (APr), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST).

BFTM: molasses as carbon source, BFTS: starch as carbon source, BFTB: barley flour as carbon source, BFTC: corn as carbon source.

Discussion

Water quality Parameters

Water quality parameters are very important for maintaining the health of aquatic species and can act as a limiting factor (Sharifinia *et al.*, 2020). In this study water physicochemical parameters including temperature, pH, DO, salinity, TSS, SS, TAN, nitrite and nitrate were measured in the appropriate range of fish cultivation (Emerenciano *et al.*, 2017). Temperature is one of the factors influencing biofloc formation (Hostins *et al.*, 2015); in the present study the temperature was measured within the appropriate range of floc formation and fish cultivation (Hostins *et al.*, 2015; El-Shafiey *et al.*, 2018).

The observed higher value of salinity in the biofloc treatment rather than control might be due to limited water exchange in biofloc treatments (Khanjani *et al.*, 2020a).

Salinity is also one of the prominent parameters which can influence on heterotrophic bacteria and the nitrification process (Khanjani *et al.*, 2020a). Thus, the biofloc formation, quality and performance of cultured aquatic organisms in BFT can be impacted by this factor.

The observed lower DO and pH in the biofloc treatments particularly in the afternoon compare to the control group might be contributed to the additional carbohydrates to the biofloc tanks, which results in more oxygen consumption by floc-associated organisms (Kim *et al.*, 2014; Khanjani *et al.*, 2017), higher respiration rates

and carbon dioxide production, which leads to acidification of the environment and reduction of pH (Khanjani *et al.*, 2017; Khanjani *et al.*, 2020a).

Observed higher salinity level in the BFT treatments rather than control might be related to the accumulation of salts from the uneaten feed in the biofloc system (Alves *et al.*, 2017). There is also more water evaporation in without/with limited water exchange systems (Khanjani *et al.*, 2017).

The observation of increased SS and TSS in biofloc treatments could be contributed to the additional of carbohydrates to the experimental tanks, which has created favorable conditions for the growth of biofloc-related organisms (Ray *et al.*, 2010; Khanjani *et al.*, 2017).

The average level of SS (22.64 to 26.41 mg/L) and TSS (309.04 to 362.83 mg/L) were obtained in the biofloc treatments during the experimental period, which was in agreement with the results of Panigrahi *et al.* (2019). TSS levels in the biofloc system were reported at 500 mg/L (Ray *et al.*, 2010) and 760 mg/L (Kim *et al.*, 2014). Increasing TSS requires more aeration and more precise management of water physicochemical parameters in the biofloc system (Ray *et al.*, 2010).

Among BFT groups, the BFTS treatment showed the lowest values of TAN and NO₂ and the highest NO₃ level among BFT groups which indicated a higher population of ammonia and nitrite oxidizing bacteria

rather than other biofloc treatments (Correia *et al.*, 2014).

In this study, the level of nitrogen compounds in the biofloc treatments was lower than that reported by Mirzakhani *et al.* (2019). This indicates the proper development of heterotrophic bacteria to control nitrogen compounds. The better and faster absorption of carbon by bacteria from simple carbohydrates such as molasses and starch might result to the increase their population (Khanjani *et al.*, 2017). Also, it was found that the addition of organic carbon sources to the system with very little water exchange prevents the increase in TAN content, which was consistent with the results of other researchers (García-Ríos *et al.*, 2019; Ahmad *et al.*, 2016, 2019). Total ammonia nitrogen decreased more when using simple carbohydrates, but the difference was not significant. The faster reduction of ammonia using simple carbon sources such as molasses and starch is probably due to better absorption and degradation of carbon as a substrate for heterotrophic bacteria that metabolize ammonia and thus improve water quality (Khanjani *et al.*, 2017; El-Shafiey *et al.*, 2018).

Concentrations of TAN and NO_3 were higher in BFT treatments compared to the control group. This is due to the accumulation of organic matter as a result of limited water exchange in biofloc treatments. All carbohydrate sources added to the water column were effective in bio-flocculation resulting in a significant

increase in total heterotrophic bacteria, which is consistent with the results of Burford *et al.* (2003). The highest and lowest amount of total densities of heterotrophic bacteria was obtained in BFTS and BFTB treatments, respectively. These results indicated that simple carbon sources such as starch are better broken down by heterotrophic bacteria than the complex carbon sources such as barley flour. These findings are consistent with the results of the studies by Silva *et al.* (2017) and Panigrahi *et al.* (2019).

Growth performance

The results of present study demonstrated that different carbon sources (simple and complex carbohydrates) do not have a significant effect on the growth performance of tilapia fingerlings, which is consistent with the results of Varghese (2007) and Silva *et al.* (2017).

Growth performance in the biofloc treatments was better than control group. Bioflocs have probiotic properties (Ferreira *et al.*, 2015) and contain essential nutrients (fatty acids, amino acids, minerals) (Ju *et al.*, 2008), a number of organic compounds such as carotenoids, chlorophylls, bromophenols, phytosterols and antibacterial compounds (Crab *et al.*, 2010; Najdegerami *et al.*, 2016) that along with artificial diet, provide a complete diet for farmed aquatic animals (Khanjani and Sharifinia, 2020). According to various researchers, the presence of biofloc in the cultivation system results in

improved growth performance of cultured aquatics (Ahmad *et al.*, 2016; Najdegerami *et al.*, 2016; Panigrahi *et al.*, 2019; Adineh *et al.*, 2019), which might be due to the limited water exchange.

In the present research, the lowest PER and the highest FCR were observed in the control group which showed a significant difference with biofloc treatments ($p < 0.05$). Various studies have reported that the presence of biofloc in the cultivation system improves feed efficiency and protein efficiency ratio (Ahmad *et al.*, 2016; Mirzakhani *et al.*, 2019; Panigrahi *et al.*, 2019). Panigrahi *et al.* (2019), reported 0.87 to 1.6 for shrimp FCR in biofloc system under different carbon sources and 0.96 to 1.21 for tilapia (Mirzakhani *et al.*, 2019). PER values for tilapia were measured 1.8 to 2.6 (Mirzakhani *et al.*, 2019) and 1.79 to 2.33 (Durigon *et al.*, 2019).

Survival rate in the present study was significantly higher in the biofloc treatments than the control group ($p < 0.05$). Survival rates were reported 22.6 to 90.6 for *C. gariepinus* (Dauda *et al.*, 2017) 100% for *O. niloticus* (Mirzakhani *et al.*, 2019) and 81 to 100% for *L. vannamei* (Panigrahi *et al.*, 2019) under the influence of different carbon sources. In the present study survival rate of 95.89 to 98.2% was observed in the biofloc treatments in different carbon sources, which was higher than the control group. It was shown that due to the presence of immunostimulant compounds such as peptidoglycan, beta-glucan and

lipopolysaccharide in the wall of biofloc bacteria as well as the presence of antioxidant in biofloc, the immunity and survival in the cultured species increases (Kim *et al.*, 2014; Walker *et al.*, 2020). Bio-active compounds in the biofloc improve aquatic survival which is possibly because of the amount of essential amino acids, fatty acids and other nutritional compounds (Xu and Pan, 2013).

Somatic indices

There was a significant difference in somatic indices between control group and biofloc treatments. In the control group the lowest level of K (1.89), CY (82.81 %) and the highest value of VSI (17.19 %) were observed. HSI values in treatments with complex carbon sources including BFTB (2.68) and BFTC (2.78) were less than the biofloc treatments with simple carbon sources including BFTS (3.21) and BFTM (3.14).

Different values have been reported in the various studies: K (1.6–1.8) and HSI (3.03–3.5) for *C. carpio* (Bakhshi *et al.*, 2018); K (1.56–1.92) and HSI (1.71–2.51) for *C. carpio* (Adineh *et al.*, 2019); K (1.71–1.80) and HSI (1.15–2.51) for *O. niloticus* (Durigon *et al.*, 2019) and finally, K (1.4–1.70) for *O. niloticus* (Mirzakhani *et al.*, 2019) which might be related to the experimental conditions.

K as a somatic index provides important information regarding the physiological parameters of cultured species (Lima-junior *et al.*, 2002). According our results, the HSI was

influenced by the type of carbon source added to the cultivation system, possibly due to the feeding on the different bioflocs produced under the influence of different carbon sources. Various studies have shown that HSI of cultured species can be influenced by the diet (Gallagher, 1999; Durigon *et al.*, 2019). Studies have also shown that higher energy demand by cultured aquatics reduces hepatic storage (Trenzado *et al.*, 2007; Adineh *et al.*, 2019).

The biochemical compositions of the Nile tilapia body and the biofloc

In the present study the biochemical compositions of Nile tilapia fingerlings were affected by different carbon sources. The highest protein content was observed in BFTB (59.23%), lipid in BFTC treatment (25.69%) and ash in BFTM treatment (14.2%). Various studies have shown that different carbon sources affect the body biochemical composition of *L. vannamei* (Khanjani *et al.*, 2017), *C. carpio* (Bakhshi *et al.*, 2018), *C. gariepinus* (Dauda *et al.*, 2017), *Labeo rohita* (Ahmad *et al.*, 2016) and *O. niloticus* (Mirzakhani *et al.*, 2019) in the biofloc system. The increase in the amount of protein, lipid and ash in the cultured species in the biofloc system is due to feeding on the microbial flocs (Bakhshi *et al.*, 2018; Khanjani *et al.*, 2017), which is consistent with the present study.

Flocs are considered as a high-quality food in aquaculture because they contain essential amino acids,

lipids, minerals, and vitamins (Ekasari *et al.*, 2014; Wang *et al.*, 2015; Bakhshi *et al.*, 2018).

In this study biofloc biochemical compositions were affected by different carbon sources so that the level of protein was measured 24.39-31.70%, and the amount of lipid and ash were obtained 2.05-3.55% and 23.89-32.04%, respectively. Lopez-Elias *et al.* (2015), the values were measured 23.7–25.4% for protein, 2.6–3.5% for lipid, and 33–40.4% for ash and in another study by Becerril-Cortes *et al.* (2018) the levels were obtained as follow: protein 30.2–48%, lipid 2–2.5% and ash 6.7–16.5% for produced bioflocs in tilapia cultivation tanks. The mentioned values are consistent with the current study. The results of the present study are also in agreement with the reports of Crab *et al.* (2010), Ekasari *et al.* (2015) and Bakhshi *et al.* (2018) that different carbon sources in the biofloc system affect the biochemical quality (protein, lipid and ash) of microbial flocs and cultured aquatics.

The type of carbon source (Crab *et al.*, 2010; Dauda *et al.*, 2017; Khanjani *et al.*, 2017), the C:N ratio (Minabi *et al.*, 2020), water salinity (Khanjani *et al.*, 2020b) and feeding rates (Khanjani *et al.*, 2016) play a prominent role in the biochemical composition of bioflocs.

Digestive enzymes

Our results showed that there was a significant difference in digestive enzyme activities of Nile tilapia fingerlings between control group and

biofloc treatments, indicating the effect of biofloc on digestive enzyme activity. Microbial flocs are considered as an effective exogenous enzyme and also a stimulator of endogenous enzymes in fish (Xu *et al.*, 2012; Najdegerami *et al.*, 2016; Zhang *et al.*, 2016; Bakhshi *et al.*, 2018; Adineh *et al.*, 2019).

Also, based on the results of this study, the highest levels of lipase, protease and amylase activity were obtained in BFTC, BFTB and BFTB treatments, respectively, indicating a different effect of carbon sources on digestive enzyme activity, which correspond to the results of Bakhshi *et al.* (2018) and Ahmad *et al.* (2019).

The latter reported that amylase and protease activity in intestine of *L. rohita* were significantly higher in tapioca carbonaceous source in biofloc system. Higher digestive enzyme activities in biofloc systems can enhance utilization of macromolecules which leads to higher nutrient digestibility (De *et al.*, 2015; Ahmad *et al.*, 2019). These differences may be due to the composition of the carbon sources (Ahmad *et al.*, 2019).

Biofloc enhances digestive enzymes activity and improves feed utilization in fish, which results in improving the assimilation of dietary bioactive substances and subsequently stimulating immunity in fish (Long *et al.*, 2015, Promthale *et al.*, 2019).

Fat-soluble vitamins, carotenoids, phytosterols, and taurine are abundant in microbial flocs (Xu and Pan, 2013). Therefore, the biofloc system is

expected to improve the activity of digestive enzymes.

Hepatic enzymes

Liver status is an important pathological indicator for detecting injuries caused by nutritional conditions, because its function is to metabolize compounds that come from the gastrointestinal tract (Abedian *et al.*, 2013).

AST and ALT are pervasive aminotransferases in fish mitochondrion, which are prominent indicators of hepatopancreas function and damage (Zhou *et al.*, 2014), as well as using as stress indicators (Haridas *et al.*, 2017).

In the current study, different sources of carbon had a different effect on the activity of tilapia hepatic enzymes. The lowest activity of AST, ALT and ALP was observed in BFTS (starch carbon source) treatment. Various studies have shown that the presence of biofloc in the cultivation of *O. niloticus* (Liu *et al.*, 2018) and *C. carpio* (Adineh *et al.*, 2019) reduces the activity of hepatic enzymes, which is consistent with the present study.

The higher activity of hepatic enzymes, lower performance of cultured aquatic, and activity of these enzymes also indicates an increase in protein catabolism (Adorian *et al.*, 2019). Our study showed that the presence of biofloc could reduce the destruction of Nile tilapia hepatocytes and the type of produced biofloc under different carbon sources had a different effect on the activity of hepatic

enzymes, so that the hepatic enzyme activity decreased further under the effect of the simple carbon sources (starch and molasses) compared to the complex carbon sources (barley flour and corn). This may be due to the favorable and beneficial effect of produced biofloc in these treatments on the liver function.

Decreased activity of ALT and AST enzymes in fish may indicate inactivation of trans-amination and decrease in amino acid catabolism (Bibiano *et al.*, 2006). The activity of transaminases (ALT and AST) is useful in estimating fish nutritional status (Bibiano *et al.*, 2006). Also, Rehulka and Minarik (2007) showed that increased AST activity is a sign of serious damage to the liver through release of mitochondrial AST. In the present study the highest level of AST was obtained in the control group.

Change in ALP activity is one of the physiological responses that is associated with immune responses, and can be considered as an indicator of fish health (Tahmasebi-Kohyani *et al.*, 2012; Liu *et al.*, 2014). Decreased activity of this enzyme indicates improved immune system (Adorian *et al.*, 2019).

Generally, the present study showed that different carbon sources are effective in biofloculation. The type of carbon source did not show significant effect on the growth performance of Nile tilapia fingerlings. Improving water quality and survival rate was observed in simple carbon sources (starch and molasses) compared to

complex carbon sources (barley flour and corn). Improvement of somatic indices, digestive and hepatic enzymes activities were observed in BFT treatments compared to the control group. Bioflocs composed of different carbon sources had different biochemical quality and the feeding of Nile tilapia fingerlings on these flocs affected the carcass quality.

The activity of hepatic enzymes was also lower in treatments with simple carbon sources (BFTM, BFTS), indicating a better performance of Nile tilapia in these treatments. It is suggested that in future studies a mixture of simple and complex carbon sources in different ratios will be used and evaluated on performance of the Nile tilapia.

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