THE EFFECT OF ADDING MOLASSES IN DIFFERENT TIMES ON PERFORMANCE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) RAISED IN A LOW-SALINITY BIOFLOC SYSTEM

Mohammad Hossein Khanjani^{1*}, Morteza Alizadeh², Mohammad Mohammadi²,
Habib Sarsangi Aliabad²

¹Department of Fisheries Sciences and Engineering, Faculty of Natural Resources, University of Jiroft, Jiroft, Iran

²Saline Water Aquatic National Research Center, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Bafgh, Iran

*Corresponding author: m.h.khanjani@ujiroft.ac.ir, m.h.khanjani@gmail.com

Abstract

This study aimed to evaluate the effect of adding molasses in different times on water quality, growth performance, body biochemical composition, digestive and hepatic enzymes of Nile tilapia in the biofloc system. Tilapia fingerlings (1.53 ± 0.14 g) were distributed in five treatments including control, BFT24 (adding molasses to the tanks every 24 h), BFT48 (48 h), BFT72 (72 h), and BFT96 (96 h) and reared for 37 days in fiberglass tanks (130 L), with a stocking density of one fish per litre. The results showed that highest increases in biomass (740.12 g) and survival (98.97%) were obtained in BFT24 treatment ($P < 0.05$). The body composition was affected by the experimental treatments so that the highest protein content was obtained in the BFT72 ($P < 0.05$). Digestive enzymes activities were significantly ($P < 0.05$) higher in BFT treatments than the control group. The current study showed higher biomass and survival ratio for Nile tilapia were observed in BFT24 treatment. The liver and digestive enzymes of Nile tilapia were affected by the different addition times of molasses to the rearing tanks.

Key words: Nile tilapia, biofloc, molasses, growth performance, liver and digestive enzymes

The increasing development of the aquaculture industry as one of the leading sources of human food supply has led to the adoption of advanced and environmentally friendly technologies. In recent years, biofloc technology has been recognized as an efficient system in the aquaculture industry (Khanjani and Sharifinia, 2020). Environmental compatibility (Emerenciano et al., 2017; Khanjani and Sharifinia, 2020), reducing the need for natural resources (García-Ríos et al., 2019; Avnimelech, 2012), increasing the stocking density of cultured species (Lima et al., 2018)

and controlling nitrogenous waste (Emerenciano et al., 2017) are mentioned as features of the biofloc system. Bioflocs are composed of abiotic (food debris, feces, and organic particles) and biotic (heterotrophic and chemoautotrophic bacteria) components (Khanjani and Sharifinia, 2020) that are available 24 hours a day as a rich food source for filter feeder aquatic species and improve fish production (Hu et al., 2015). Various studies have examined biofloc as a source of protein supplement in the diet (Xu and Pan, 2014; Khatoun et al., 2016; Khanjani et al., 2020 a, b).

Nile tilapia (*Oreochromis niloticus*) is one of the main species that has gained worldwide popularity due to the white meat with a firm texture and the absence of spines within the muscle (Wang and Lu, 2016). This species is fast-growing (El-Sayed, 2006), resistant to various environmental conditions, adapted to high stocking densities (Avnimelech, 2007) and the second-largest freshwater species grown in the world (Menaga et al., 2019). Moreover, tilapia species is one of the most suitable and compatible species in the biofloc system and its potential has been investigated in various studies. Cultivation of tilapia species in biofloc systems has been examined from different aspects: effects of various stocking densities (Haridas et al., 2017; Lima et al., 2018), the performance of growing period and overwintering (Crab et al., 2009), the larval stage (Ekasari et al., 2015), fingerling period (García-Ríos et al., 2019), broodstocks (Ekasari et al., 2015), carbon to nitrogen ratio and different carbon sources (Pérez-Fuentes et al., 2016; Mirzakhani et al., 2019; Khanjani et al., 2021 a), combining with the aquaponic system (Pinho et al., 2017), cultivation in brackish water under the influence of protein and digestible energy (Durigon et al., 2020).

Tilapia is a brilliant group of fish for cultivation in freshwater and brackish water since they are tolerant of salinity between 0 and 12 g L⁻¹ (El-Sayed, 2006). Moreover, freshwater fish cultivated in brackish water can save energy leading to higher growth rate (Lima et al., 2018). Increased salinity may also reduce the toxicity of nitrogen compounds (Colt, 2006).

The biofloc in the production system affects water quality (García-Ríos et al., 2019; Ren et al., 2019), growth performance (Khanjani et al., 2020 b; Durigon et al., 2020), digestive enzyme activity (Najdegerami et al., 2016; Xu and Pan, 2012), immunity (Long et al., 2015; Menaga et al., 2019), antioxidant status (Bakhshi et al., 2018; Liu et al., 2018) and body composition of the cultured species (Mirzakhani et al., 2019; Khanjani et al., 2020 a).

Microbial communities in the biofloc systems act like probiotics (Aguilera-Rivera et al., 2014) and positively affect digestive and hepatic enzymes (Adorian et al., 2019). Different conditions include temperature (Apun-Molina et al., 2009), pH (Márquez et al., 2012), feeding habits (Seixas Filho et al., 2000), diet composition (Durigon et al., 2019; Hakim et al., 2006), nitrogen conversion pathway (heterotrophic or autotrophic) (Becerra-Dórame et al., 2012) and production system as cage or pond (Santos et al., 2016) affects growth performance and digestive enzymes' activity in cultured species.

Various studies have been done in the context of adding different carbon sources to the biofloc system (Pérez-Fuentes et al., 2016; Deng et al., 2018; Mirzakhani et al., 2019; Khanjani et al., 2021 a). Molasses as an organic carbon source is successfully

used in the biofloc system for growth of heterotrophic bacteria in tilapia cultivation ponds (Khanjani et al., 2021 a, b).

It is important to determine the most appropriate time to add carbon to the biofloc system and better system management, in terms of environmental issues and development of sustainable aquaculture. There are no studies to date on the influence of adding carbonated organic matters (molasses) in different times to the system with limited water exchange. Therefore, the present study was conducted to evaluate the effects of adding molasses in different times on water quality, growth performance, somatic indices, liver and digestive enzymes, and body biochemical composition of Nile tilapia in the biofloc system with limited water exchange.

Material and methods

Experimental design

Nile tilapia fingerlings with an average weight of 1.53 ± 0.14 g and length of 3.98 ± 0.1 mm (Mean $\pm$ SD) were obtained from National Research Center of Saline Water Aquatics (Bafgh city, Yazd province, Iran). The experiment was performed at the mentioned research center. Altogether, 15 tanks (with a total volume of 300 liters) were considered for this experiment. Each tank was filled with 130 liters of well water at a salinity of 8 ppt, and then 130 fingerlings of Nile tilapia (1.53 g L^{-1} biomass) were stocked in each tank. The experimental period was 37 days.

Five experimental treatments were considered for the present study, including a water exchange treatment (control group) where 50 to 60% of the water in the rearing tank was replaced daily with fresh water with the same salinity before feeding; and other four biofloc treatments by adding carbonaceous molasses to the cultivation tanks (adding molasses to the tanks was done 24 h as BFT24, 48 h as BFT48, 72 h as BFT72, and 96 h as BFT96) with 0.4 to 0.8% daily water exchange from the bottom of the tanks.

In the biofloc treatments, 2.5 mL biofloc per liter was added to the tanks as the initial stock (Khanjani et al., 2021 a). The initial stock was collected from three growing ponds of Nile tilapia (6 m in diameter and 1.2 m in height) based on biofloc. Feeding was performed three times a day (08:00, 12:00 and 16:00) with a diet containing 35% protein, 9% lipid, 4% fibre, 12% ash, C/N ratio 8.9 (manufactured by Mazandaran Animal and Aquatic Feed Company, MANAQUA, Iran) and based on body weight. At the beginning of the trial, the feeding rate was considered 8% and the rate of feeding decreased with an increase in the growth. In biofloc treatments, feeding was 25% lower than control. The fish were fed in biofloc treatments with 25% of the produced flocs and 75% of the artificial diet.

For aeration and oxygen supply, three air stones were placed at the tanks that were connected to the aerator. Aeration was performed continuously throughout the day and night to supply oxygen. In addition, it causes microbial flocs to remain suspended and be made available to fish as a complementary food.

The amount of molasses (dry matter: 55.18%, crude protein: 9.16%, lipid: 0.36%, fiber: 0.88%, ash: 16.36% and carbohydrate: 73.24%) was calculated based

on the amount of feed and the amount of nitrogen which is released into the water, which was approximately 0.6 g of feed for the carbon to nitrogen ratio of 15. It was added to the biofloc treatments after noon meal according to Avnimelech (2009). The amount of carbonaceous material added to the system was considered equal in all treatments. After weighing, the molasses was poured into a one-liter plastic container and mixed well with the water of the growing tanks and spread evenly across the tank surface (In various treatments: as per their treatment times).

Physicochemical parameters

Water quality factors including temperature, pH and dissolved oxygen were measured twice daily at 8 am and 16 pm. Salinity was determined daily at 9 o'clock using HQ30D Multi Meter HACH. Transparency, settled solids (SS), and total suspended solids (TSS) were measured every after five days' interval.

Transparency was determined with a Secchi disk. To determine the amount of settled solid, one liter of rearing water was poured into Imhoff funnel (scaled conical hopper) and held for 20 minutes to settle (Avnimelech and Kochba, 2009).

To measure total suspended solids, Whatman filter paper 42 (No 1442) was first numbered and weighted for each treatment and then 100 ml of tank water was filtered and placed in the oven at 105°C for 3 hours to dry. Then, it was placed in a desiccator to absorb moisture, and finally, its weight difference was calculated (Khanjani et al., 2017).

Total counts of heterotrophic bacteria (CFU: colony-forming units) were performed using culture medium (R2-agar) according to the APHA (2005) method. Total ammonium nitrogen (TAN), nitrite and nitrate levels were measured weekly with a spectrophotometer (Perkin Elmer Lambda 25 UV/Vis) according to MOOPAM (1999).

Measurement of growth, nutritional and somatic indices

Twenty fish per each replicate were used to measure growth, nutritional and somatic indices. Fish biometrics (length and weight) were done at the beginning and end of the experiment to calculate growth indices including weight gain (WG), body weight index (BWI), daily weight gain (DWG), biomass and specific growth rate (SGR). The number of fish at the beginning and the end of the experiment were recorded to calculate the survival rate (SR). Nutrition indices including feed conversion ratio (FCR), feed efficiency (FE) and protein efficiency ratio (PER) were calculated based on the following equations (Khanjani et al., 2016):

$WG (g) = \text{final fish weight} - \text{initial fish weight}$,

$BWI (\%) = WG (g) / \text{initial fish weight (g)} \times 100$,

$DWG (g) = WG (g) / \text{days of rearing}$,

$\text{Biomass (g)} = WG (g) \times SR (\%) \times \text{initial number of fish}$,

$SGR (\%/day) = [(\ln (\text{final fish weight}) - \ln (\text{initial fish weight}) \times 100) / \text{days of rearing}]$,

$SR (\%) = (\text{number of individuals at end of rearing period} / \text{initial number of individuals stocked}) \times 100$,

$FCR = \text{feed consumed (g)} / WG (g)$,

$FE (\%) = WG (g) / \text{feed consumed} (g) \times 100$,

$PER = WG (g) / \text{protein intake} (g)$.

Somatic indices including condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), carcass yield (CY) were also determined based on the following equations (Durigon et al., 2020):

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

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

Hepatosomatic index (%), $HIS = 100 \times \text{liver weight} (g) / \text{final fish weight} (g)$,

Carcass yield (%), $CY = 100 \times \text{total fish weight} - \text{visceral weight} (g) / \text{total fish weight} (g)$.

Proximate analysis of the carcass biochemical composition

Twenty fish from each replicate were randomly separated from the tanks at the end of the experiment. They were put in the ice until they became numb (ethical issues were followed to kill the fish). Then, the contents of the abdomen were completely removed and the rest of the body was homogenized with the meat grinder. After that, the samples were stored at -18°C until the analysis. Subsequently, protein, lipid, moisture, and ash were measured using the AOAC (2005) method.

Biofloc analysis

At the end of the experimental period, the water of each treatment was passed through 20 μm nets, and the produced biofloc from each treatment was placed in pre-numbered containers. Then, the samples were kept in an oven at 72°C for 72 h until dried. After that, the dried bioflocs were stored in the freezer at -18°C until biochemical analysis. The chemical factors of protein, lipid, moisture, and ash were measured based on AOAC (2005) method.

Digestive enzyme activity

At the end of the experiment, the feeding was cut off 24 h before sampling. Five fish were randomly removed from each replicate and anesthetized with clove powder (200 ppm) and killed by observing ethical issues.

The intestine was removed by abdominal opening and visceral fat was placed on ice. Tissues were washed by 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 (10,000 g for 20 min at 4°C) and the resultant supernatant was separated and frozen at -80°C until analyses.

Alkaline protease activity was measured according to Garcia-Carreno and Haard (1993). Azocasein 2% in Tris-HCl, pH= 7.5 was applied as the substrate. The lipase specific activity was determined by the method described by Iijima et al. (1998); in this method nitrophenyl myristate was used as the substrate. Amylase activity was measured according to Bernfeld (1955); in this measurement starch was used as the substrate. The activity of all enzymes was expressed as the specific activity being micromole of substrate hydrolyzed per minute per milligram protein (u/mg) (Jenabi Haghpars et al., 2019).

Hepatic enzyme activity

At the end of the experiment, 5 fish from each replicate were randomly selected, and then anesthetized. Subsequently, they were killed by observing ethical issues. Livers were removed and kept at -80°C . At the time of assays, liver samples were mashed on ice using porcelain mortar and pestle. Then, the samples were homogenized in phosphate buffer (0.050 M, pH 7.4) to prepare a 10% (w/v) liver homogenate. Next, the homogenates were centrifuged (4°C in 12000 rpm for 15 min) and after that, the supernatant was separated and kept at -80°C .

Measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST) were done according to the IFCC (International Federation of Clinical Chemistry) method (Bergmeyer et al., 1986). Alkaline phosphatase (ALP) was measured using the DGKS, 1972 (Deutsche Gesellschaft für Klinische Chemie) method. These enzymes were measured according to the photometric method by medical diagnosis kits of Pars Azmoon Company (Iran, Tehran).

Statistical analysis

All data were presented as means $\pm$ standard deviation. Before analysis, the data were checked for normality and variances for homogeneity by Shapiro-Wilk and Levene's tests, respectively. The data were analyzed by one-way analysis of variance using the statistical software program SPSS version 21 (Chicago, Illinois, USA). Duncan's multiple range test (DMRT) was used to determine the differences among treatments at a significance level of $P < 0.05$. All graphs were done with Excel version 2013 (Microsoft Corporation, Redmond, WA, USA).

Results

Water quality

Water quality parameters during the experiment are presented in Table 1. The amount of DO, pH, salinity, amount of SS, TSS and transparency were significantly different among treatments. The highest level of DO (7.1 mg L^{-1}) was obtained in the control group ($P < 0.05$). The lowest level of DO (5.92 mg L^{-1}) and pH (7.28) were measured in the treatment which molasses was added daily (BFT24) ($P < 0.05$).

The lowest levels of salinity (8.59 ppt) and TSS (73.59 mg L^{-1}) were obtained in the control group. There was no significant difference in salinity, TSS, and transparency among BFT treatments.

TAN and nitrate showed a higher level in BFT treatments compared to the control group. The lowest levels of TAN (1.59) and NO_2 (7.78 mg L^{-1}) were obtained in the control group ($P < 0.05$).

The fluctuations in TAN, NO_2 , and NO_3 during the experimental period are represented in Figure 1. The average numbers of heterotrophic bacteria in different treatments are given in Table 2. The lowest (4.28 log CFU/ml) number was obtained in the control group ($P < 0.05$).

Table 1. Water quality parameters in tanks of Nile tilapia cultivated under the effect of adding molasses in different times (Mean $\pm$ SD)

Parameters	Control	BFT24	BFT48	BFT72	BFT96
T a.m. ($^{\circ}$ C)	25.24 $\pm$ 0.31 a	25.39 $\pm$ 0.34 a	25.40 $\pm$ 0.76 a	25.4 $\pm$ 0.52 a	25.5 $\pm$ 0.76 a
T p.m. ($^{\circ}$ C)	25.93 $\pm$ 0.49 a	26.10 $\pm$ 0.66 a	26.11 $\pm$ 0.73 a	26.14 $\pm$ 0.64 a	26.21 $\pm$ 0.72 a
DO a.m. (mg L ⁻¹)	7.10 $\pm$ 0.21 a	6.48 $\pm$ 0.32 c	6.81 $\pm$ 0.40 b	6.93 $\pm$ 0.33 b	6.92 $\pm$ 0.35 b
DO p.m. (mg L ⁻¹)	6.90 $\pm$ 0.146 a	5.92 $\pm$ 0.42 c	6.32 $\pm$ 0.40 b	6.29 $\pm$ 0.39 b	6.25 $\pm$ 0.48 b
pH a.m.	7.465 $\pm$ 0.015 c	7.49 $\pm$ 0.016 b	7.53 $\pm$ 0.061 a	7.526 $\pm$ 0.04 a	7.53 $\pm$ 0.053 a
pH p.m.	7.39 $\pm$ 0.017 c	7.28 $\pm$ 0.058 e	7.31 $\pm$ 0.047 d	7.43 $\pm$ 0.049 b	7.46 $\pm$ 0.048 a
Salinity (ppt)	8.59 $\pm$ 0.07 b	8.89 $\pm$ 0.19 a	8.86 $\pm$ 0.2 a	8.84 $\pm$ 0.18 a	8.83 $\pm$ 0.18 a
SS (ml L ⁻¹)	1.53 $\pm$ 0.72 b	20.75 $\pm$ 11.7 a	15.11 $\pm$ 7.91 a	19.88 $\pm$ 12.3 a	21.4 $\pm$ 15.31 a
TSS (mg L ⁻¹)	73.59 $\pm$ 31.67 b	281.45 $\pm$ 149.97 a	222.93 $\pm$ 104.59 a	251.91 $\pm$ 143.78 a	240.25 $\pm$ 157.14 a
Transparency (cm)	25.77 $\pm$ 1.76 a	11.32 $\pm$ 6.34 b	12.91 $\pm$ 6.88 b	12.61 $\pm$ 7.56 b	12.31 $\pm$ 7.49 b
TAN (mg L ⁻¹)	1.59 $\pm$ 0.48 b	3.83 $\pm$ 1.18 a	4.76 $\pm$ 1.37 a	5.28 $\pm$ 1.01 a	5.53 $\pm$ 1.77 a
NO ₂ (mg L ⁻¹)	0.31 $\pm$ 0.17 a	0.30 $\pm$ 0.14 a	0.22 $\pm$ 0.10 a	0.27 $\pm$ 0.13 a	0.27 $\pm$ 0.10 a
NO ₃ (mg L ⁻¹)	7.78 $\pm$ 2.23 b	18.64 $\pm$ 6.64 a	17.63 $\pm$ 5.23 a	15.97 $\pm$ 4.81 a	15.95 $\pm$ 4.56 a

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

Values in the same row with different letters are significantly different ($P<0.05$).Temperature = T; Dissolved oxygen = DO; Settled solids = SS; Total suspended solids = TSS; Total ammonium nitrogen = TAN; Nitrite = NO₂; Nitrate = NO₃.Table 2. Total density of heterotrophic bacteria (log cfu/ml) in different treatments (Mean $\pm$ SD)

BFT96	BFT72	BFT48	BFT24	Control	THB
5.99 $\pm$ 0.12 b	6.16 $\pm$ 0.60 ab	6.47 $\pm$ 0.55 ab	6.77 $\pm$ 0.55 a	4.28 $\pm$ 0.30 c	THB

Total heterotrophic bacteria: THB.

Values in the same row with different letters are significantly different ($P<0.05$).

Table 3. Growth performance and somatic indices of Nile tilapia fingerlings in different treatments (Mean $\pm$ SD)

Parameters	Control	BFT24	BFT48	BFT72	BFT96
Final weight (g)	7.17 $\pm$ 0.74 a	7.28 $\pm$ 0.44 a	7.03 $\pm$ 0.71 a	6.37 $\pm$ 0.66 b	6.46 $\pm$ 0.69 b
Weight gain (g)	5.64 $\pm$ 0.74 a	5.75 $\pm$ 0.44 a	5.5 $\pm$ 0.71 a	4.84 $\pm$ 0.66 b	4.93 $\pm$ 0.68 b
BW1 (%)	368.83 $\pm$ 59.59 a	375.92 $\pm$ 29.16 a	359.63 $\pm$ 46.38 a	316.41 $\pm$ 43.304 b	322.34 $\pm$ 44.95 b
DWG (g/day)	0.152 $\pm$ 0.024 a	0.155 $\pm$ 0.012 a	0.149 $\pm$ 0.02 a	0.13 $\pm$ 0.018 b	0.133 $\pm$ 0.018 b
SGR (%/day)	4.15 $\pm$ 0.35 a	4.21 $\pm$ 0.16 a	4.108 $\pm$ 0.283 a	3.84 $\pm$ 0.283 b	3.88 $\pm$ 0.29 b
Biomass gain (g)	686.36 $\pm$ 29.45 b	740.12 $\pm$ 22.41 a	674.90 $\pm$ 18.21 b	605.10 $\pm$ 11.956 c	599.84 $\pm$ 28.53 c
PER	2.006 $\pm$ 0.34 c	2.85 $\pm$ 0.22 a	2.73 $\pm$ 0.352 a	2.40 $\pm$ 0.33 b	2.445 $\pm$ 0.34 b
FCR	1.43 $\pm$ 0.25 a	1.008 $\pm$ 0.080 c	1.066 $\pm$ 0.155 c	1.213 $\pm$ 0.172 b	1.191 $\pm$ 0.17 b
FE (%)	70.20 $\pm$ 11.87 c	99.80 $\pm$ 7.741 a	95.48 $\pm$ 12.312 a	84.00 $\pm$ 11.50 b	85.577 $\pm$ 11.934 b
Survival rate (%)	94.43 $\pm$ 0.4 c	98.97 $\pm$ 1.05 a	94.36 $\pm$ 1.05 c	96.15 $\pm$ 0.69 b	93.59 $\pm$ 0.79 c
Somatic indices					
CF (%)	1.85 $\pm$ 0.18 b	1.98 $\pm$ 0.11 a	1.99 $\pm$ 0.14 a	2.03 $\pm$ 0.18 a	1.98 $\pm$ 0.25 a
VSI (%)	17.45 $\pm$ 0.87 a	15.75 $\pm$ 0.65 b	15.96 $\pm$ 0.46 b	16.03 $\pm$ 0.57 b	16.16 $\pm$ 0.67 b
HSI (%)	3.22 $\pm$ 0.36 a	2.64 $\pm$ 0.32 b	2.69 $\pm$ 0.24 b	2.79 $\pm$ 0.19 b	2.77 $\pm$ 0.33 b
CY (%)	82.55 $\pm$ 0.87 b	84.25 $\pm$ 0.65 a	84.04 $\pm$ 0.46 a	83.97 $\pm$ 0.57 a	83.84 $\pm$ 0.67 a

Values in the same row with different letters are significantly different ($P<0.05$).
Body weight index = BW1, daily weight gains = DWG, specific growth rate = SGR, protein efficiency ratio = PER, feed conversion ratio = FCR, feed efficiency = FE.
Condition factor = CF, viscerosomatic index = VSI, hepatosomatic index = HSI, carcass yield = CY.
BFT24: molasses was added every 24 h. BFT48: molasses was added every 48 h. BFT72: molasses was added every 72 h. BFT96: molasses was added every 96 h.

Figure 1. Values of (Mean $\pm$ SD) TAN (A), NO₂ (B) and NO₃ (C) in tanks of Nile tilapia cultivated at different treatments during the experiment period

Growth performance and somatic indices

The values of growth and nutrition parameters during the experiment are presented in Table 3, which indicates a significant difference among some of the treatments ($P < 0.05$). The growth performance was significantly lower in BFT72 and BFT96 compared to other treatments. The highest increase in biomass (740.12 g) was obtained in BFT24. The highest level of FCR (1.43) and the lowest FE (70.2%) were observed in the control group ($P < 0.05$).

The values of the survival rate of Nile tilapia fingerlings in different treatments are presented in Table 3. The highest percentage of survival rate (98.97%) was obtained in BFT24. Table 3 shows the results of somatic indices including CF, VSI, HSI, and CY. According to the somatic indices, there were significant differences between BFT treatments and the control group.

Fish body proximate composition and biofloc

The results of the body biochemical composition of Nile tilapia in different treatments are demonstrated in Table 4, which shows the lowest level of protein and ash content in the control treatment. The biofloc biochemical compositions are also presented in Table 4, which show significant differences between the treatments in the amounts of ash, lipid, and protein. The highest level of protein (32.14%), lipid (2.35%) and ash (31.92%) were obtained in BFT48, BFT48 and BFT24 treatments, respectively ($P < 0.05$).

Table 4. Values of body proximate composition of Nile tilapia (% dry weight) and biofloc obtained at the end of cultivation period in different treatments (Mean $\pm$ SD)

Treatment	Dry matter (%)	Crude protein (% DW)	Crude lipid (% DW)	Ash (% DW)
Fish				
Control	26.16 $\pm$ 0.23 b	56.56 $\pm$ 0.32 d	25.33 $\pm$ 0.06 a	12.69 $\pm$ 0.19 c
BFT24	26.13 $\pm$ 0.15 b	57.17 $\pm$ 0.39 c	25.26 $\pm$ 0.07 ab	13.83 $\pm$ 0.10 a
BFT48	26.06 $\pm$ 0.12 b	57.51 $\pm$ 0.22 bc	25.24 $\pm$ 0.05 ab	13.70 $\pm$ 0.19 a
BFT72	26.8 $\pm$ 0.03 a	58.49 $\pm$ 0.21 a	25.16 $\pm$ 0.04 b	13.51 $\pm$ 0.12 a
BFT96	26.73 $\pm$ 0.18 a	57.80 $\pm$ 0.11 b	25.17 $\pm$ 0.05 b	13.11 $\pm$ 0.30 b
Biofloc				
BFT24	18.28 $\pm$ 0.57 a	26.64 $\pm$ 0.13 d	2.17 $\pm$ 0.07 b	31.92 $\pm$ 0.22 a
BFT48	17.11 $\pm$ 0.26 b	32.14 $\pm$ 0.31 a	2.35 $\pm$ 0.025 a	27.39 $\pm$ 0.18 d
BFT72	15.76 $\pm$ 0.14 c	27.81 $\pm$ 0.33 c	2.01 $\pm$ 0.03 c	29.34 $\pm$ 0.40 b
BFT96	14.49 $\pm$ 0.35 d	29.89 $\pm$ 0.28 b	1.92 $\pm$ 0.15 c	28.00 $\pm$ 0.26 c

Values in the same column with different letters are significantly different ($P < 0.05$).

Table 5. Digestive and hepatic enzymes activities of Nile tilapia at the end of the experiment in different treatments (Mean $\pm$ SD)

Parameters	Control	BFT24	BFT48	BFT72	BFT96
Intestine					
AM (U/mg protein)	42.52 $\pm$ 1.5 c	46.66 $\pm$ 2.08 b	46.27 $\pm$ 1.73 b	53.02 $\pm$ 2.05 a	51.87 $\pm$ 2.20 a
LP (U/mg protein)	1.47 $\pm$ 0.015 c	1.5 $\pm$ 0.045 b	1.58 $\pm$ 0.012 a	1.49 $\pm$ 0.075 b	1.52 $\pm$ 0.026 b
APr (U/mg protein)	9.55 $\pm$ 0.4 c	10.74 $\pm$ 0.53 b	12.56 $\pm$ 0.25 a	10.69 $\pm$ 0.53 b	11.71 $\pm$ 0.59 b
Liver					
AST (U/mg protein)	2.43 $\pm$ 0.13 b	2.03 $\pm$ 0.09 c	2.40 $\pm$ 0.05 b	2.71 $\pm$ 0.13 a	2.73 $\pm$ 0.04 a
ALT (U/mg protein)	0.4 $\pm$ 0.02 b	0.34 $\pm$ 0.011 c	0.37 $\pm$ 0.01 b	0.38 $\pm$ 0.02 b	0.42 $\pm$ 0.009 a
ALP (U/mg protein)	0.063 $\pm$ 0.004 a	0.046 $\pm$ 0.009 b	0.048 $\pm$ 0.002 b	0.052 $\pm$ 0.003 b	0.051 $\pm$ 0.005 b

Different letters 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).

BFT24: molasses was added every 24 h. BFT48: molasses was added every 48 h. BFT72: molasses was added every 72 h. BFT96: molasses was added every 96 h.

Digestive and hepatic enzymes

The results of the analysis of factors related to digestive and hepatic enzymes are presented in Table 5. The lowest digestive enzyme activity was obtained in the control group. The highest levels of lipase (1.58) and protease (12.56 U /mg protein) were determined in BFT48 ($P<0.05$). Also, the lowest activity of AST, ALT and the highest activity of ALP were observed in BFT24 and control group, respectively.

Discussion

Water quality

Water quality including physicochemical and biological parameters are very important for maintaining the health of aquatic species and can act as a limiting factor (Kamrani et al., 2016; Yeganeh et al., 2020). According to the results, obtained physicochemical parameters of water are in the proper range for Nile tilapia cultivation (El-Sayed, 2006). The decrease in DO and pH is a prominent characteristic of biofloc systems (Luo et al., 2014; Khanjani et al., 2020 a, b). In the current research, the level of DO and pH were observed lower in the afternoon, probably due to the addition of organic carbonaceous molasses to the biofloc system. As microbial communities develop, carbon dioxide concentration increases due to a higher respiration rate and as a result the level of pH will reduce (Panigrahi et al., 2018; Khanjani et al., 2020 a). The DO concentration in the control group was higher than the other treatments, possibly due to the daily water exchange and the limited amount of microbial communities (Mirzakhani et al., 2019; Long et al., 2015). The lowest amount of DO and pH was determined in BFT24 treatment due to the higher density of heterotrophic communities.

The salinity level in the BFT treatments was higher compared to the values obtained in the control group; this is probably due to the accumulation of salts from the uneaten feed in the biofloc system (Alves et al., 2017). There is also more water evaporation in limited water exchange systems (Emerenciano et al., 2012).

In the present study, the mean values of SS and TSS in BFT treatments were consistent with the results of other studies (Avnimelech, 2012; Long et al., 2015; Lima et al., 2018). The higher level of SS (43.66 ml L^{-1}) was measured in BFT96 treatment and the higher amount of TSS (498.8 mg L^{-1}) in BFT24 on day 28 of the experiment, indicating that biofloc produced in BFT96 treatment is lighter and more porous than that in BFT24 treatment. It has been shown that adding molasses daily to the biofloc tanks results in a more efficient heterotrophic system, producing the microbial communities that are more compact and with more suspended solids. In the Lima et al. (2018), the level of SS was reported 13.51 to 26 ml L^{-1} and TSS from 45.74 to 677.17 mg L^{-1} . The amount of TSS in biofloc-based tilapia rearing ponds can reach up to 1000 mg L^{-1} (Avnimelech, 2012). Too much amount of SS is not suitable for optimal fish growth, which results in increased oxygen consumption and may eventually lead to the accumulation of organic matter and closure of the fish gills (Khanjani and Sharifinia, 2020).

SS and TSS were maintained at the optimum level so as not to negatively affect growth performance. The amount of SS also impacted negatively the level of transparency, in a way that the amount of transparency was decreased with an increase in SS level (Khanjani et al., 2017).

Higher concentrations of TAN and NO_3 were obtained in BFT treatments than the control group. The higher level of TAN is possibly due to the limited water exchange and as a result, the accumulation of organic matter in the biofloc treatments compared to the control group. Besides, the higher level of nitrate in biofloc treatments could be due to the nitrification process, resulting in a higher concentration of inorganic nitrogen forms (Christopher et al., 2015; Mirzakhani et al., 2019).

The values of TAN, NO_2 , and NO_3 in the treatments were determined in the optimum range of Nile tilapia cultivation, which were consistent with the results of other studies (Luo et al., 2014; Mirzakhani et al., 2019). TAN fluctuations may be due to the frequent circulation between soluble NH_3 and solid flocs because of the microbial cells decomposition and the release of nitrogen in the form of NH_3 (Luo et al., 2014).

The highest density of heterotrophic bacteria was obtained in BFT24 treatment and showed a significant difference with BFT96 treatment. Various studies have shown that adding molasses daily increases the density of heterotrophic bacteria during the culture period (Emerenciano et al., 2012; Khanjani et al., 2020 a).

Growth performance and Somatic indices

Based on the results, the growth performance in BFT24 and BFT48 was better than BFT72 and BFT96. The highest amount of biomass was observed in BFT24 which was higher than the control group.

Studies by various researchers have reported that the biofloc system improves the growth performance of cultured aquatics (Avnimelech and Kochba, 2009; Luo et al., 2014; Wang et al., 2015; Mirzakhani et al., 2019; Khanjani et al., 2020 b). The increase in growth performance in BFT24 treatment is possibly because of maintaining optimal water quality and continuous and fresh production of biofloc (Ekasari et al., 2010; Toledo et al., 2016). Bioflocs contain poly-beta-hydroxybutyrate organic compounds (De Schryver et al., 2010) and bioactive compounds such as carotenoids, chlorophylls, and phytosteroids (Ju et al., 2008) that have a positive effect on the growth performance of cultured species. In the current study, the highest increase in biomass was observed in BFT24 treatment, indicating that tilapia fed better on the bioflocs that were affected daily by the addition of molasses.

The highest FCR and the lowest PFR were observed in the control group. Studies have shown that the presence of biofloc with a commercial diet improves the FCR and increases feed efficiency (Khanjani et al., 2020 a; Mirzakhani et al., 2019). Bioflocs exhibit probiotic properties, which contribute to the digestion and absorption of a commercial diet and lead to improved feed efficiency (Aguilera-Rivera et al., 2014).

In the present study, the highest survival rate for tilapia fingerlings was obtained in BFT24 treatment, which was significantly affected by the different addition times of molasses to the cultivation system. In the different studies of tilapia in the biofloc system, the survival rates were reported 100%, 80.4–97.6% and 67–98% by Mir-

zakhani et al. (2019), Crab et al. (2009) and Ekasari et al. (2015), respectively. The lower survival rate was observed in the BFT96 treatment which is probably because of the higher fluctuations of environmental parameters (especially pH and TAN). Fluctuations of environmental parameters such as DO, pH, deionized ammonia, NO₂, and TAN caused stress to cultured aquatics and ultimately decreased growth and survival (Santacruz-Reyes and Chien, 2012; Avnimelech, 2012).

Somatic indices showed significant differences between BFT treatments and control group. The lowest levels of CF (%) and CY (%) and the highest level of VSI (%) and HSI (%) were observed in the control group. CF in the different studies was reported 2.1–2.3% (Crab et al., 2009) 1.95–2.17% (Santos et al., 2016), and 1.17–1.80% (Durigon et al., 2020) for tilapia which is in the range of the results of the present study. CF level in this study was affected by the presence of biofloc, which resulted in the improvement of CF.

Feeding, age, and growth rate can be monitored by CF factor (Kumolu-Johnson and Ndimele, 2010). This factor changes with the amount of food and nutrients, so that more nutrients lead to an increase in CF levels (Morado et al., 2017).

The values of HSI, VSI, and CY were reported 2.17–2.51%, 16.21–17.44% and 82.56–83.79%, respectively (Durigon et al., 2020). The control group in the present study showed the highest level of HSI which was similar to the study of Durigon et al. (2020). They reported that the high rate of HSI is because of the compensation phenomenon in a situation of lack of access to dietary protein in which the liver size increases to metabolize muscle protein so as to meet the body's protein requirement. Biofloc as a natural and nutritious diet that is constantly available for tilapia has a positive effect on the somatic indices (Durigon et al., 2020).

Proximate composition of fish body and biofloc

In the current study, significant differences were observed among different treatments in the biochemical composition of Nile tilapia body and produced biofloc, which indicates that different addition times of carbonaceous material to the cultivation tank affect the biochemical compositions of fish body and biofloc. Mirzakhani et al (2019) reported as follows: protein 56–66%, lipid 4.5–18.6%, and ash 18–25.9% and García-Ríos et al. (2019) determined as follows: protein 63.9–71%, lipid 11.3–16.1%, ash 11.6–14.1%, for Nile tilapia in the biofloc system. The difference in reported values is probably due to different experimental conditions between different studies. The lipid contents of fish cultured in the BFT treatments were lower than the control group. This is possibly because of a very low level of lipid in the biofloc (Emerenciano et al., 2012; Luo et al., 2014; Long et al., 2015; Mirzakhani et al., 2019). It has been reported that changes and higher levels of body biochemical compounds in the BFT treatments compared to control group may be due to amino acid compounds, fatty acids (PUFA, HUFA), and other nutrients found in flocs (Ju et al., 2008; Khanjani et al., 2020 a).

In the present study, the amount of protein (32.14–26.64%), lipid (2.35–1.92%) and ash (31.92–27.39%) in the flocs were obtained, which was in accordance with the reported values by Becerra-Dórame et al. (2012) and Mirzakhani et al. (2019). It was also found that adding molasses in addition times to the cultivation tanks had

a significant effect on the quality of biofloc, as the lowest ash content and the highest protein and lipid content were measured in BFT48 treatment. These changes are probably due to differences in the composition of microbial communities and the conditions for the production of bioflocs. The lipid content of bioflocs is usually at the low level, but the presence of essential fatty acids has significantly increased their nutritional value (Ju et al., 2008; Khatoon et al., 2016). The type of carbon source (Khanjani et al., 2017), C: N ratio (Minabi et al., 2020), water salinity (Ekasari et al., 2010; Khanjani et al., 2020 b), feeding level (Khanjani et al., 2016), the source of light and its intensity (Coyle et al., 2011), pH (Martins et al., 2019) and the type of microbial community (Ahmad et al., 2017) are considered as parameters affecting the biochemical composition of bioflocs.

Digestive and hepatic enzymes

In the present study, adding molasses in addition times to the cultivation tanks affected the activity of digestive enzymes (lipase, amylase and alkaline protease) and hepatic enzymes (AST, ALT and ALP).

Digestive enzymes activity in the BFT treatments was higher than the control group, probably due to the use of biofloc and heterotrophic bacteria acting as probiotics.

These microbial enzymes help break down proteins, carbohydrates, and other nutrients and break down the feed into smaller units and facilitate digestion and absorption (Xu and Pan, 2012). After the food was eaten with biofloc, biofloc acts as a complementary external enzyme in the diet and positively affects the function of digestive enzymes (Lin et al., 2007). Other studies have also shown that the presence of exogenous bacteria can stimulate the production of endogenous enzymes by cultured fish (Ziaei-Nejad et al., 2006; Zhou et al., 2009). The activity of fish digestive enzymes can vary depending on the conditions of the cultivation environment, nitrogen conversion pathway (autotrophic or heterotrophic) and production system (pond or cage) (Apún-Molina et al., 2009; Becerra-Dórame et al., 2012; Santos et al., 2016; Durigon et al., 2019).

The highest amount of lipase and protease was observed in BFT48 treatment, which was affected by the amount of lipid and protein of biofloc. Amylase levels were higher in BFT72 and BFT96, which may be owing to the mixotrophic system in these treatments. The biofloc produced in these treatments is likely to have higher carbohydrates, which affects the activity of amylase.

In this study, hepatic enzymes activity was also affected by the presence of biofloc, so the highest levels of AST, ALT and ALP were observed in BFT96 and control group, respectively, which may be due to increased anabolism or decreased catabolism (Shahsavani et al., 2010). Hepatic enzymes such as transaminases (AST, ALT) are used as general indicators to evaluate vertebrate liver function because increased permeability of damaged hepatocytes causes the secretion of these enzymes into the bloodstream (Suárez et al., 2015). Due to higher environmental fluctuations in BFT96 treatment, tilapia fish may have been more stressed and leads to the secretion of more liver enzymes. Chen et al. (2004) reported a significant increase in AST and ALT enzymes due to some infectious and environmental factors in Nile tilapia. The

ALT enzyme is high in the hepatocellular cytoplasm and when damaged, crosses the cell membrane and enters the bloodstream. This enzyme has been identified as a specific marker of liver injury (Hao et al., 2009). The lowest amount of this enzyme was observed in BFT24 treatment, which indicates a reduction in stress in this treatment and a positive effect of biofloc on the decrease in the secretion of this enzyme. Environmental stresses are factors that increase hepatic enzymes (Hao et al., 2009). Significant differences were observed in ALP enzyme levels between the biofloc treatments and control group, indicating that the presence of biofloc had a positive effect on the reduction of this hepatic enzyme.

Alkaline phosphatase is possibly related to the immune activity (Adorian et al., 2019). It has been found that physical or chemical stress or bacterial and parasitic infections increase alkaline phosphatase activity (Qi et al., 2009), which plays an important role in the immune system. Decreased activity of this enzyme in fish in biofloc treatments indicates a better immune status.

The present study showed that the addition of molasses to the biofloc system every 24 h improves the growth and development of heterotrophic bacteria, thereby reducing DO and pH levels. Biomass and survival performance of fish in BFT24 treatment were also improved compared to the other treatments. The protein and ash content of the body of Nile tilapia increased under the influence of biofloc. The activity of digestive enzymes in the biofloc treatments was better than in the control group so that they were affected by the biochemical compositions of produced biofloc. It showed the lowest amount of AST and ALT activity in BFT24 treatment. Generally, it can be concluded that adding molasses every 24 h to the cultivation tanks of Nile tilapia fingerlings leads to better performance.

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