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Effect of biofloc technology on growth performance and intestine histology of grass carp (*Ctenopharyngodon idella*) fingerlings

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Abstract

Biofloc technology is the "blue revolution" in aquaculture and a recent technology for enhancing the utilization of feed wastes in aquaculture. The effects of different biofloc systems on growth rates and histological changes in the intestines of grass carp (*Ctenopharyngodon idella*) fingerlings were investigated using three carbon sources: molas, starch, and wheat flour. over a period of three months. Four experimental groups, control, molas, starch, and wheat flour, were set in parallel triplicates and growth performance parameters, including final body weight, total weight gain, survival rate, specific growth rate (SGR), and feed conversion ratio (FCR), The current findings demonstrate that all diet-treated groups exhibit significant changes in the measured growth parameters ($p< 0.05$). The lowest final weight (77.3 g), weight gain (75.6 g), and SGR (2.5%/day) were achieved for fish raised on a wheat flour-supported diet. Histo-morphometric analysis of the anterior and posterior segments of the intestine showed a significant increase in the length of the intestine. intestinal villi width as well as an increase in the number of goblet cells in the BFT groups compared to the control. In summary fish farming with the BFT using feed supplemented with various carbon sources might be very beneficial to fish farmers because it can reduce feed costs and improve yield.

Keywords: Biofloc technology, carbon sources, grass carp, gut histology, growth parameters.

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1. Introduction

The production of aquaculture is consistently rising and is an important contribution to ensuring food security. Aquaculture is globally the fastest-growing division of agriculture and therefore the most eminent part to feed and nurture the world's expanding population, but growth must be sustainable (FAO, 2020). In order to fulfill sustainability developmental goals, it is still essential to create and shift aquaculture towards sustainability to meet the rising demand for aquatic foods because aquatic food production systems can provide powerful sustainable solutions. However, a significant challenge to the expansion and development of aquaculture, particularly in developing nations, is the cost of high-quality fish feed (Hamidoghli *et al.,* 2018; Yassir *et al.*, 2010). Aquaculture has benefited from the enormous scientific progress made in the last 5 decades. Tilapias are robust and globally very popular fish and have been already cultivated since ancient times in Egypt, However, their perfect development and performance depend on the type of protein source, the amount of energy in the diet, their physiological state, their size, the level of production, and the environment (Yassir *et al.*, 2010). Due to their great robustness and high feed efficiency, several approaches have led to technological advancements that are more ecologically friendly. For instance, biofloc technology (BFT) has recently been hailed as an effective method for addressing sustainability and improving aquaculture (Khanjani and Sharifinia, 2020; Zablon *et al.*, 2022). The biofloc technology is profitable because, on the one hand, it enhances the efficiency of feed and fish growth while, on the other hand, it minimizes inorganic nitrogen waste. By adding organic carbohydrates to the culture medium, biofloc technology can be used to boost the carbon/nitrogen (C/N) ratio and promote the growth of heterotrophic bacteria (Crab *et al.*, 2007). Aggregates of bacteria, algae, and protozoa, as well as small amounts of organic matter, such as feces and uneaten food, are referred to as "bioflocs". The interactions between these biological elements in BFT aquaculture systems can be characterized as competing and complementary, depending on environmental factors and water quality. Aquatic animals' growth performance, digestion and absorption function, immunity, and antioxidant capacity are all improved by the probiotics and bioactive ingredients found in biofloc, which also contains carotenoids, polysaccharides, and polyhydroxybutyrate. Probiotics also function as immunostimulants, increase feed conversion ratios, inhibit the growth of pathogenic bacteria, produce antibiotics, and improve water quality (Kesarcodi-Watson *et al.*, 2008; Panigrahi *et al.*, 2019; Ren *et al.*, 2019; Tierney and Ray, 2018). Moreover, BFT has been determined to have the potential to enhance intestinal health, including the capacity for digestion and absorption, immunological response, and antioxidant capacity (Liu *et al.*, 2018; Long *et al.*, 2015; Yu *et al.*, 2020). Globally, the most produced fish is the grass carp (*Ctenopharyngodon idella*), which is an important species in freshwater pond culture. According to a report by the

People's Republic of China's Ministry of Agriculture, the yield of grass carp in China was highest in 2017 (5,533,083 t) (Xie *et al.*, 2018). The scientific database on biofloc nutritional requirements is well established, but not much information is available concerning biofloc system requirements and nutritional function for grass carp. Hence, the current experiment was conducted to assess the effects of dietary carbon sources (molas, starch, and wheat flour) on the growth performance and gut histology of grass carp (*C. idella*) reared in a biofloc system, without water change (biofloc technique; BFT).

2. Materials and methods

The current experiment was carried out at the Aquaculture Lab, Department of Zoology, Faculty of Science, Al-Azhar University (Assiut Branch), Assiut, Egypt. The effects of dietary carbon sources (molas, starch, and wheat flour) were determined in a BFT system on growth performance and gut histology of grass carp (*C. idella*).

2.1 Experimental design

A total of 240 young grass carp (*C. idella*) with an average body weight of 1.8±0.2 g were obtained from Ahywa hatchery, Sohag Governorate, Egypt. The temperature of the fish tank was thermostatically maintained at 23 ± 2 °C to ensure the best possible water quality. Twelve circular tanks (each 250 L volume) filled with 200 L of dechlorinated water were used for the current study. Prior to the experiment, fish were acclimated for six weeks under controlled lab conditions. They were then divided into four equal groups, in three replicates each (20 fish/tank). The first group was considered the control and fed the basal control diet containing 25% crude protein (CP). The second, third, and fourth groups were fed on the basal diet containing 25% CP, which was supplied with molas, starch, and wheat flour, respectively, as sources of carbohydrates. Individually, fish received twice-daily feedings (5%) of their body weight, at 9:00 AM and 3:00 PM, six days a week. The current experiment runs for three months, from 1 August to 29 October 2020. A basal control diet was formulated to fulfill the nutritional requirements of the fish and contained 25% crude protein (CP) and 448.3 kcal/100 g. Biofloc groups had an additional carbohydrate diet composed of molas, starch, and wheat flour (1:1) to achieve a C:N ratio of 15:1 according to Avnimelech (1999) with only weekly additions of evaporative water loss, while control groups were reared without any additional diets and 50–70% water replacement weekly to achieve tolerable conditions.

2.2 Growth performance

Growth measurements of *C. idella* were done by randomly sampling. The growth performance was evaluated using the following parameters: initial weight (IW g), initial length, final body weight (FW, g), total weight gain (TWG, g), final length, survival rate (SR%), specific growth rate (SGR% /day), and feed conversion ratio (FCR).

SR% = (number of fish at the end/number of fish at the start) ×100 (Ricker, 1975)

SGR = 100 [(FW)-(IW)]/number of experimental days

FCR = feed fed (g) (dry weight)/WG (g)

2.3 Histological examination

For histological examination, three fish from each treatment were sacrificed by decapitation. The intestine was immediately dissected out, fixed in 10% neutral buffered formalin for 24 hours, processed by the conventional method, sectioned at 5 µm thickness using a rotary microtome, and then stained with Haematoxylin & Eosin, H&E (Bancroft and Stevens, 1996). Slides were examined by light microscopy (Motic microscop BA310 LED FL). Each slide was photographed with a DVC digital camera (HDCE-50 B). Twenty measurements for height and width of villi (µm) were taken from each intestine slide using Image J (1.46) software. To count goblet cells in each segment, the method of Baeverfjord and Krogdahl (1996) was applied.

2.4 Statistical analysis

A database was created using Excel 2013 software to perform the first analysis using descriptive statistics. Data were presented as mean ± SD (standard deviation). The results were subjected to a one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance. Data were analyzed using the SPSS (1997) program, Version 16. Differences between means were compared using Duncan's (1955) multiple range test at a significance level of $p < 0.05$.

3. Results

3.1 Growth performance parameters

The growth performance parameters were investigated monthly during the experiment. The initial weight of control fish was an average of 1.8±0.02 g and was roughly 1.7 g for the other groups. Statistically significant ($P<0.05$) differences in final weight, weight gain, survival rate, and FCR were found in all treatments compared to the control (Table 1).

Table (1): Growth performance of grass carp (*C. idella*) following three months of rearing in different diets of the biofloc system.

Items	(Treatment) At 90 days			
	Control	Molas	Starch	Wheat flour
IW (g/fish)	1.8±0.02	1.7±0.05	1.7±0.1	1.7±.0.7
FW (g/fish)	79.3±0.3^{a}	81.1±0.8^{c}	79.5±1.3^{a}	77.3±1.0^{b}
TWG (g/fish)	77.5±0.9^{a}	79.4±1.6^{b}	78.0±1.4^{a}	75.6±0.5^{b}
Survival rate (%)	94.2±0.02^{a}	96.3±0.1^{b}	95.2±0.2^{b}	94.1±0.01^{a}
SGR (%/d)	2.7±0.3^{a}	2.6±0.2^{a}	2.6±0.2^{a}	2.5±0.2^{a}
FCR	1.6±0.2^{a}	2.0±0.1^{c}	1.7±0.0^{b}	2.1±0.1^{bc}

IW: initial weight, FW: final weight, TWG: total weight gain, SGR: specific growth rate, FCR: feed conversion ratio. Means in the same row with different superscript letters are significantly different at p <0.05.

Fish reared with wheat flour showed the lowest final weight (77.3 g), weight gain (75.6) and SGR (2.5%/day) and the highest FCR (2.1) being significantly different from all other treatments. Table (1) displays the survival rates, which are, in general, very good ranging from 94.1% (wheat flour treatment) to 96.3% (molas treatment).

3.2 Histological examination

At the end of the experiment, the height and width of villi and the number of goblet cells were measured in the anterior and posterior intestines for both control and biofloc diet–treated groups. The results are shown in Table (2) and Figures (1 and 2). The present results showed remarkable variations among treatments. The morphometric analysis of the anterior and posterior parts of the intestine revealed a significant increase in the intestinal villi length and width, as well as an increase in goblet cell count in the biofloc groups compared to the control group. Significant increases in villi length and width were recorded in the starch and wheat flour groups compared to the control. The highest intestinal villi length was reported in the starch group (132.2 µm), while the lowest intestinal villi length was in the control group (26.1 µm). There was a significant reduction in villi length and width in the molas and starch groups (40.8 µm and 23.5 µm), respectively, compared to the control group. The highest number of goblet cells was recorded in the starch group (44.5), and the lowest number of goblet cells was recorded in the flour group (20.1) compared to the control group (Table 2 and Figures 1, 2).

Table (2): Measurements of anterior-posterior gut morphology of grass carp (*C. idella*) reared in the biofloc system.

Items		Control	Molas	Starch	Wheat flour
Goblet cells	Anterior	18.2±2.3^{a}	31.6±4.5^{b}	44.5±6.7^{c}	35.4±0.5^{b}
	Posterior	14.2±2.6^{a}	40.2±1.2^{c}	33.2±1.1^{ab}	20.1±1.1^{b}
Villi length (µm)	Anterior	88.2±3.9^{a}	122.1±7.0^{b}	132.2±6.5^{c}	124±3.1^{b}
	Posterior	26.1±2.1^{a}	40.8±3.1^{b}	52.4.5±5.2^{c}	55.3±4.1^{c}
Width of villi(µm)	Anterior	26.3±3.4^{ab}	26.6±1.7^{a}	28.3±2.1^{b}	30.2±3.1^{c}
	Posterior	23.0±1.6^{a}	27.1±1.4^{b}	23.5±4.1^{ab}	26.2.1±2.1^{c}

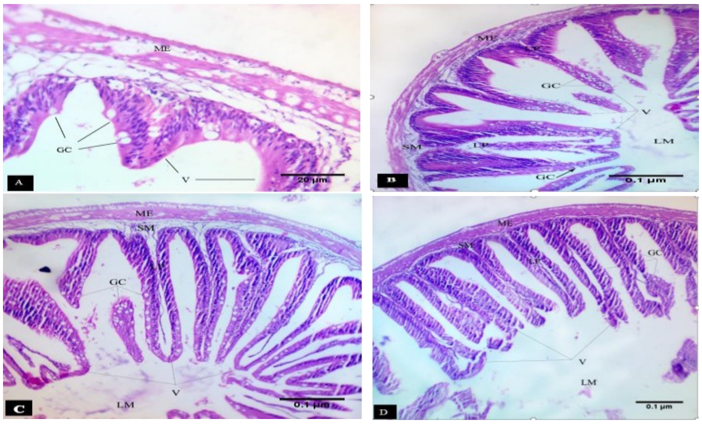

Figure (1): Photomicrographs of the anterior intestine of grass carp (*C. idella*) reared in a biofloc system (control, molas, starch, and wheat flour) showing (A, B, C, and D), respectively well-developed, less developed, and non-branched and short mucosal villi (V), less developed muscular (ME) and sub-mucosal layers (SM) with clear degradation of the numbers of distributed goblet cells (GC) along the entire length of mucosal villi. (H&E. bar=20μ) (cf. Table 2).

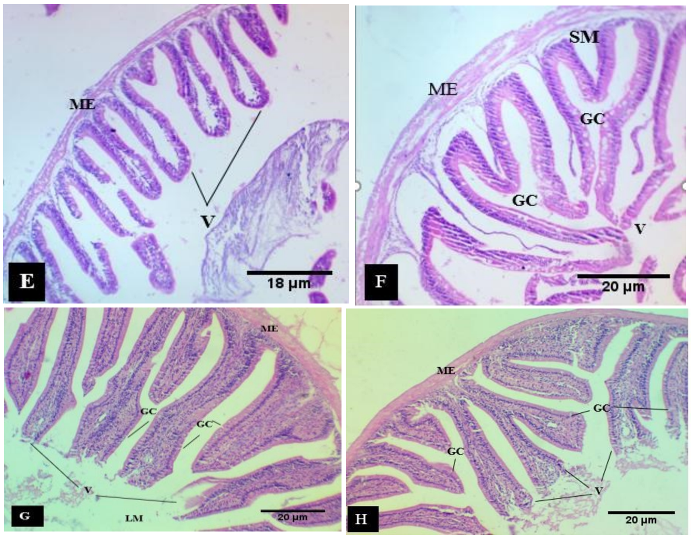

Figure (2): Photomicrographs of the posterior intestine of grass carp (*C. idella*) reared in the biofloc system, (control, molas, starch, and wheat flour) showing (E, F, G, and H), respectively, well-developed and branched mucosal villi (V), muscular layer (ME), sub-mucosal layer (SM) , and lamina propria (LP) with distribution of goblet cells (GC) along the distal portion of mucosal villi. (H&E. bar=20μ) (cf. Table 2).

4. Discussion

One proposed hypothesis is that biofloc is defined as a supplemental food source that is continuously available *in situ* and can provide additional protein (essential amino acids), polyunsaturated fatty acids, vitamins, and minerals for the fish (Avnimelech, 1999; 2007; Azim and Little, 2008; Luo *et al.,* 2014; De Schryver *et al.*, 2008; Wasielesky *et al.*, 2006). Many previous studies have indicated the presence of biofloc organisms in aquaculture, particularly ciliates and nematodes, that can make proteins, lipids, and vitamins available for the farmed species, promoting growth and lowering the need for these substances in commercial feed (Becerril-Cortés *et al.*, 2017; Focken *et al.*, 2006; Khanjani and Sharifinia, 2020; Loureiro *et al.,* 2012). Additionally, knowing the makeup of biofloc organisms can be used to optimize BFT applications to reveal higher productivity and lower environmental impacts (Abakari *et al.*, 2022; Emerenciano *et al.*, 2017; Kumar *et al.*, 2021). When the C/N ratio in these systems reaches 15:1, the addition of additional carbon sources prompts organisms already present in the water to utilize the accumulated nitrogenous waste (in the form of unconsumed feed or feces), which accelerates their growth and results in the creation of flocs (Khanjani and Sharifinia, 2022; Khanjani *et al.*, 2022). By using BFT, these tiny communities have been used to improve the environment and to feed fish and crustaceans (Khanjani *et al.*, 2020; Khanjani *et al.*, 2021). The current study confirmed the beneficial effects of promoted bioflocs on growth performance and histological features in common grass carp fingerlings without water exchange. Based on these results, replacement or supplementation of the commercial diet with carbohydrates such as molas, starch, and wheat flour in the BFT system promotes heterotrophic bacterial populations, resulting in improved growth performance of fish. The current study revealed that the final weight, weight gain, and FCR were significantly increased ($P < 0.05$) compared to the control group except for wheat flour. The results of the present study are in accordance with several investigations on *Oreochromis mossambicus* (Avnimelech, 1999; 2007), *Macrobrachium rosenbergii* (Asaduzzaman *et al.*, 2008), *Litopenaeus vannamei* (Burford *et al.*, 2004; Xu *et al.*, 2013), *Marsupenaeus japonicas* (Zhao *et al.*, 2012), and *Carassius auratus* (Wang *et al.*, 2015). In terms of the positive effects of BFT on growth performance. These results indicated that grass carp fingerlings can adapt well to new nutritional conditions and that microbial flocs stimulated nutrient availability and the production and/or activity of digestive enzymes, resulting in improved digestion of nutrients in the gut, which might imply the improved feed utilization and growth performance due to enhanced morphological appearance of intestinal villi (Moss *et al.*, 2001; Xu *et al.*, 2012a,b; Xu and Pan, 2012). FCR (2.00) in the molas group are in accordance with those obtained by Sagar *et al.* (2009) for *M. rosenbergii*. Ballester *et al.* (2017) also reported a FCR of 2.25 for *M. rosenbergii* cultured in a brown sugar biofloc system after 30 days. However, the better FCRs (0.89–1.76) observed for *L. vannamei* cultured in biofloc systems might also be also due to the different nutritional physiology of these invertebrates that can even better utilize bioflocs (Khanjani *et al.*, 2017; Serra *et al.*, 2015; Peixoto *et al.*, 2018). These authors used a lower initial feeding rate of 12–15% of total body weight that is gradually decreased to 4–9%. In the current study, the higher FCR was probably related to the higher feeding rate. In contrast, El-Shafiey *et al.* (2018) stated that the worst values for the last parameters were noted for Nile tilapia under molas treatment. Supplementing the biofloc system with molas, starch, wheat flour, or a mixture of them had no significant effect on shrimp growth (Khanjani *et al.*, 2017). On the other hand, growth performance of *Peneus monodon* was higher with the addition of jaggery in biofloc systems instead of other carbon sources like cane sugar and molas (Sakkaravarthi and Sankar, 2015). Meanwhile, a mixture of different carbon sources (60% molas + 20% corn flour + 20% wheat bran) positively affected the growth of *L. vannamei* in comparison to molas as a single carbon source (Wang *et al.*, 2016). Generally, the source of carbon did not drastically affect the growth of grass carp fish raised in BFT. Likewise, molas, starch, and wheat flour groups have provided similar growth for *L. vannamei* postlarvae (Khanjani *et al.*, 2017). Ekasari *et al.* (2014) also reported similar growth performance using molas, tapioca, tapioca by-products and rice bran for *L. vannamei* in a grow-out stage. Lobato *et al.* (2019) and Vilani *et al.* (2016) also demonstrated similar growth performance for molasses and cassava flour, and molas and rice bran during white leg shrimp production. Nevertheless, Ahmad *et al.* (2016) reported that maize starch ensures a higher growth in *Labeo rohita* than sugarcane bagasse and tapioca. In the present study, growth was non-variable in the different sources of carbon and control, increasing the power of statistical tests to result in non-significant differences. However, the results suggest that the use of molas gave the best result for the growth of grass carp juveniles in BFT system. Although Hosain *et al.* (2021) recently reported that survival of *M. rosenbergii* post-larvae was higher in 10 and 15%-based BFT, using maize starch carbon source with a carbon/ nitrogen ratio of 10. The application of biofloc organism's biomass as a complementary food source has been shown to be effective at improving growth rate, survival, and food conversion rate. In a study on Nile tilapia (*Oreochromis niloticus*), Azim and Little (2008) reported an increase of 45% in production in the BFT system compared to a conventional system. Burford *et al.* (2004) claimed that 29% of the food consumed by *L. vannamei* can be supplied by biofloc and furthermore, discovered that biofloc culture might replace the demand for marine protein in shrimp diets without compromising growth. In a BFT system, Hargreaves (2013) noticed a 20–30% boost in shrimp growth. In a BFT system using waste as natural feed for the cultured species, the rate of protein and fat assimilation was also higher, and the food conversion ratio also revealed improvements (Khanjani and Sharifinia, 2022; Zhao *et al.*, 2012). The histological analysis of a fish's digestive system is thought to be a reliable indicator of its nutritional state (Caballero *et al.*, 2003; Hall and Bellwood, 1995), and the structural changes are useful for studies on the nutritional development of fish (Rotta, 2003). Depending on the species and foodstuffs used in the tests, different histopathological alterations in the intestine may occur (Dayal *et al.*, 2013). The role of the intestine in nutrient digestion and absorption is well known in herbivorous fish such as tilapia and carp (Grosell, 2010; Magouz *et al.*, 2022). Additionally, a healthy gut can be determined by the thickness of the muscular layer and the height of the intestinal villi (Khojasteh, 2012; Magouz *et al.*, 2022). This experiment examined several parameters (appearance of goblet cells, height and width of villi) of the anterior and posterior gut morphology of fish raised with BFT compared to control. In the present study, the intestine of fish reared in the biofloc system exhibited a remarkable increase in the height and width of villi. Because longer villi in fish cultured in a biofloc system imply increased absorptive efficiency, villi length is a significant histological parameter (Caballero *et al.*, 2003; de Silva *et al.*, 2012). Therefore, the increased intestinal absorptive area with a subsequent increase in nutrient absorption and retention highlights the observed improvement in growth performance in grass carp reared in the biofloc system. The present findings are consistent with German and Horn (2006) and Magouz *et al.* (2022), who found that intestine lengths of herbivores were longer than those of omnivores, and these were longer than those of carnivores. Furthermore, the number of goblet cells could vary with food habits or starvation (de Silva and Anderson, 1995). In the current investigation, more goblet cells were found in the gut of the molas group than in other groups. The immune system's goblet cells serve as lubricants and produce the mucus that lines the brush border, and therefore an increase in their numbers may be an indication of irritation (de Silva *et al.*, 2012) or might be an immune response against anti-nutrients (Marchetti *et al.*, 2006).

5. Conclusion

The results of the current study confirmed the positive effects of BFT on growth performance and histomorphology of the intestine in grass carp without water exchange. The supplementation of the carbon sources molas and starch revealed enhanced growth performance, whereas wheat flour did not change in comparison to the control and molas and starch group. Understanding the benefits and drawbacks of BFT as a source of nutrition for farmed fish in more detail requires expanding the histological evaluation of important organs utilizing ultrastructure analysis.

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