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Use of different carbon sources for the biofloc system during the grow-out culture of common carp (*Cyprinus carpio* L.) fingerlings

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ABSTRACT

A 10 weeks feeding trial was carried out to evaluate the effects of different carbon sources (sugar beet molasses: SBM + BFT, sugar: S + BFT, corn starch: CS + BFT) on water quality, growth performance, digestive enzymes activity, biofloc and fish microbial community and composition in biofloc based system for common carp fingerlings. Three hundred healthy fingerlings (22.5 ± 0.2 g) were randomly assigned to 12 rectangle tanks (70 L) at a density of 8.02 kg m^{-3}. Fish in BFT treatments were fed at 75% of the daily feeding rate (DFI) of control. Results showed a significant difference in water quality parameters among different culture systems; CS + BFT had the lowest amount of total ammonia nitrogen (TAN) at the end of culture period. No difference was observed between the BFT treatments in case of nitrite-N and nitrate −N after 10 weeks and total suspended solids (TSS) also experienced a decline during the experiment. The fish yield was the highest in CS + BFT, which also had the lowest significant feed conversion ratio ($P < 0.05$). This was coincidental with the highest activity for total protease, lipase and amylase in BFT treatments when compared with the control ($P < 0.05$). A significantly higher body protein and lipid content and lower carbohydrate was seen in CS + BFT treatment ($P < 0.05$). Different carbon sources did not affect on protein and carbohydrate content of flocs but CS + BFT significantly caused an increase in lipid and ash content in microbial flocs ($P < 0.05$).The fingerlings essential fatty acids affected by different carbon sources and the lowest values were found in BFT treatments which differed significantly with control ($P < 0.05$). Microbial community was analyzed by PCR-DGGE as well as conventional method and results indicated the changes in both bacterial intensity and diversity among the different culture systems. Among the different carbon sources used, corn starch has increased the content of total culturable bacteria and *lactobacillus* spp. in tanks and fish hindgut. Overall, this study suggests that microbial flocs formed in corn starch based biofloc can improve common carp growth performance and tanks water quality under zero water exchange and hence ensures sustainability.

1. Introduction

Fisheries and aquaculture are important sources of food, nutrition, income and livelihoods for several hundred millions of people around the world (FAO, 2016). Common carp (*Cyprinus carpio* L.) is perhaps the best-known teleost in the world and is the third most important cultured fish, due partly to a long history of domestication and worldwide introductions (Komen, 1990; Ødegård et al., 2010). The pond farming of this species is important in the inland aquaculture and mostly is reared in earthen ponds (Bauer et al., 2006). Indeed, due to rising population and local market demands, intensive and super intensive common carp culture in concrete ponds have been developed in different countries. The rapid accumulation of organic matter, feed residues, and toxic inorganic nitrogen due to using of high-intensity systems has led to an increase in concerns regarding the development of sustainable aquaculture (Samocha et al., 2004; Avnimelech, 2006; Azim and Little, 2008; Zhao et al., 2012). Therefore, there is an urgent demand for a relatively new and alternative aquaculture system.

Biofloc technology (BFT) has been recently considered to prevent the accumulation of toxic nitrogen metabolites. This system works by

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manipulating the carbon/nitrogen ratio (C/N) and converting these metabolites to heterotrophic microorganisms and organic particles, even in zero-water exchange systems (Avnimelech, 2007; Azim and Little, 2008; De Schryver et al., 2008; Crab et al., 2009). Theoretically, toxic nitrogen metabolites (such as NH3 and NO2-) in biofloc systems are converted into microbial flocs, which are available as an additional diet for aquatic animals (De Schryver et al., 2008; Avnimelech, 2009). Thus, BFT is an economical alternative for use in decreasing the commercial diets of fish in fishponds, while simultaneously reducing potential environmental problems (Bauer et al., 2012).

To date, most studies regarding BFT mainly focused on shrimp culture. For example, a literature review was performed to survey all reported cases and found that BFT improves the water quality in intensive shrimp culture (Megahed, 2010; Crab et al., 2010; Zhao et al., 2012). It also can increase the growth performance through additional microbial flocs and stimulated digestive enzyme activities (Becerra-Dorame et al., 2011; Xu et al., 2012a, 2012b), which improve the immune defense (Xu and Pan, 2014; Kim et al., 2014). Overall, few studies have been performed on fish in BFT system. Positive effects of BFT on growth performance, water quality, digestive enzymes activity, and immune response in tilapia (Avnimelech, 2007; Azim and Little, 2008; Crab et al., 2009; Widanarni and Puspita, 2012), in *Labeo rohita* (Verma et al., 2016) and in African catfish (*Clarias gariepinus*) (Ekasari et al., 2015) have been reported in different studies. Up to now, results regarding the effects of BFT on common carp are limited to the ones performed by Najdegerami et al. (2016), who reported that using sugar beet molasses in BFT improves growth performance, digestive enzymes activities, and liver histology. However, the effects of other carbon sources on the growth performance, water quality, body composition as well as the biofloc and gut microbial flora, which is important for carp aquaculture, have not been well delineated.

2. Materials and methods

2.1. Tank facilities and experimental design

The experiment was conducted at the Urmia Lake Research Institute, Urmia University, Iran. Prior to initiation of the experiment, approximately 300 common carp fingerlings (initial weigh 22.5 ± 0.2 g) procured from a local fish farm (Gilan, Iran) and were acclimatized to experiment conditions in a rectangle tank (Vol. 1000 L) for 14 days. During the adaptation period, the fingerlings were fed with a commercial diet (Faradaneh Co., Shahrekord-Iran) three times daily (8, 13 and 18) at 3.5% of their body weight under a normal light regime (light/dark: 12/12 h). The nutritional composition of the commercial diet consisted of 35% protein, 4% lipid, and 7% ash. The tank was provided with continuous aeration and a water flow system, and the water temperature was maintained at around 22 ± 1 °C.

In the second stage, the experimental design was completely randomized, with four treatments each administered to three replicates in 12 rectangle tanks (Vol. 70 L). The fingerlings were stocked at 8.02 kg m^{-3}(25 fish/tank). In control group, the fish were fed with commercial diets at 3.5% of their body weight, with a flow-through system, whereas in the BFT treatment groups, the fish were fed with BFT and a commercial diet (75% DFI), and there was no exchange of water. The control and BFT treatments were used as follows: control or 100% DFI, sugar beet molasses as carbon source in BFT + 75% DFI (SBM + BFT), sugar as carbon source in BFT + 75% DFI (S + BFT), and corn starch as carbon source in BFT + 75% DFI (CS + BFT). For the formation of microbial flocs stock, 200 L of the first-stage effluent was transferred to four conoid tank, and total ammonium nitrogen (TAN) was measured. Different carbon sources were added based on the calculation of Avnimelech (1999) who assumed that 20 g of carbon source is required to convert 1 g of TAN. The tanks were continuously aerated using an air-stone connected to an air pump. The light regime was maintained at 12:12 (light/dark, artificial luminosity of ~600 lx). Tank aeration was stopped when TAN concentration decreased to almost zero and total suspended solids reached 300 mg L^{-1}, after which the experimental tanks were inoculated with 200 mg L^{-1} of microbial flocs in each treatment. Common carp fingerlings were stocked at the aforementioned density and fed using the treatment schedule described. During the experimental period, carbon sources were added at the rate of 20 times the TAN concentration to maintain a C/N ratio of 20 for optimum production of BFT.

2.2. Water quality parameters

During a 10-week experimental period, water temperature, pH (ELMETRON CP-411), and dissolved oxygen (AZ Instrument 8403, Portable DO Meter) were determined twice daily in the tanks. First, the total ammonia nitrogen (TAN), nitrite (NO_2^-), and nitrate (NO_3^-) were measured. Then, 100 mL of water in each tank was filtered under vacuum pressure through a microfiber glass filter paper (Whatman). Next, these parameters were calorimetrically measured using a specific kit (Palintest®, UK). A filter paper was used to determine the total suspended solid (TSS).

2.3. Sampling and analysis

The survival of the fingerlings over the 10 weeks was calculated according to daily observation in each tank. The initial (W0) and final weights (W) of all the fingerlings from each tank were determined at the beginning and end of the experiment. Weight gain was calculated using the following formula: weight gain = final weight - initial weight. The specific growth rate (SGR) was calculated as follows: SGR (%) = [ln W – ln W0/t] × 100, where W was the average weight after 10-weeks, W0 was the average initial weight (measured at the beginning of the experiment), and t was the experimental period (70 days). The same approach was used to calculate the feed conversion ratio (FCR), expressed as the feed consumption (g) over the weight increase of the fish (g) per treatment. Condition factor was calculated from the equation; K = 100 W/L^3 (Avnimelech, 2012), where W is the weight of fish (g) and L is the total length of fish (cm) (Richard et al., 2006). For measuring viscerasomatic index, hepatosomatic index and gut weight ratio, five fish from each tank were sampled, weighed and then anaesthetized with 200 mg L^{-1} clove oil. Viscera, liver and gut were dissected out, weighed and the aforementioned parameters were calculated using the following standard formula (Palmegiano et al., 2006):

Viscerasomatic index (%) = [viscera weigh (g) / body weight (g)] × 100

Hepatosomatic index (%) = [liver weight (g) / body weight (g)] × 100

Gut weight ratio (%) = [gut weight (g) / body weight (g)] × 100

2.4. Assay of digestive enzymes

Three fingerlings from each (replicate) with a total of twelve fish from each treatment were randomly sampled, euthanized with clove powder (200 mg L^{-1}), dissected to collect the whole digestive tract. They were homogenized in 100 mM Tris-HCl buffer with 0.1 mM EDTA and 0.1% triton X-100 at 9:1 ratio (pH 7.8) in an electric homogenizer (Heidolph, Instruments Switzerland). All these processes were performed on ice. The homogenate were centrifuged at 20000*g* for 20 min at 4 °C, supernatant collected, and then stored at − 20 °C for further analysis.

Total protease activity was assayed at 25 °C using 1% (w/v) casein (Sigma, USA) as a substrate in 0.2 M phosphate buffer at pH 7.0 (Walter, 1984). Tyrosin was used as a standard, and one unit of proteolytic activity and pepsin was defined as the amount of enzyme required for the formation of 1 mg of tyrosin per min. Amylase activity was determined according to Langlois et al. (1987), using 0.3% soluble starch as substrate dissolved in NaH2PO4 buffer (pH 7.4). Amylase