Effect of Biofloc-based Diet on Hepatic Enzymes of Ctenopharyngodon idella Fingerlings

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ABSTRACT

The impact of bioflocs on the hepatic enzymes under variable diet and water exchange conditions was investigated in the fingerlings of Ctenopharyngodon idella in this study. For this purpose, the fingerlings of C. idella were fed on different concentrations of bioflocs bacteria enriched on banana peels) under different diet treatments for a period of 50 days. The diet treatments devised in this study were, T1 (bioflocs only with zero water exchange), T2 (bioflocs only with 10 % weekly water exchange), T3 (bioflocs + 50 % commercial diet and with zero water exchange) and T4 (bioflocs + 50 % commercial diet and with 10 % weekly water exchange). A control experiment C (commercial diet with daily water exchange) was run parallel. At the end of the experimental trial, the cultured fish were processed for the determination of hepatosomatic indices, levels of total soluble proteins and hepatic enzymes being marker of hepatic injury for all the treatment and control groups. The highest levels of acid phosphatase (ACP, 11.75 ± 0.23 IU L⁻¹), alkaline phosphatase (ALP, 81.50 ± 1.49 IU L⁻¹), aspartate aminotransferase (AST, 30.25 ± 1.62 IU L⁻¹) and superoxide dismutase (SOD, 19.75 ± 1.78 IU L⁻¹) were recorded in the liver samples of the fingerlings of the treatment group T1, while the lowest corresponding figures were noted for the fingerlings of the treatment group T4. These values were lower than found in the control group having 10.50±0.23, 77.25 ± 1.19, 24.50 ± 0.96 and 18.25± 1.19 IU L−1 levels for the ACP, ALP, AST and SOD, respectively. The hyperproduction of the hepatic enzymes revealed dysfunction of the livers in the fingerlings of the treatment group T1. However, the moderate production of hepatic enzymes, elevated levels of total soluble proteins (8073.00 ± 848 mg mL⁻¹) and high hepatosomatic indices (0.913) for the fingerlings of the treatment group T1, while the lowest corresponding figures were noted for the fingerlings of the treatment group T4, thus, declare the productive utility of bioflocs-amended commercial fish feed with 10 % weekly water exchange to achieve optimum growth of the grass carp.

INTRODUCTION

Increasing demand of proteins to meet nutritional needs of rapidly increasing human population urges economical, innovative and sustainable technologies for the production of proteinaceous diets. Proteins make a very important part of the fish meal for their necessary roles in growth maintenance and reproduction. Essential and non-essential amino acids both are critical in building body tissues of the fish. Non-essential amino acids are synthesized from nitrogen source present in the dietary proteins of the fish, while essential amino acids must be supplied in fish diet. However, protein levels must be managed to raise healthy fish and achieve optimum production (Pelczar et al., 1993).

A major requirement for an efficient fish culture is the assimilation of dietary proteins to the formation of tissue proteins, growth and repair rather than dissimilation to the energy (Weatherley and Gill, 1987; Jauncey, 1998). The activity of the enzymes involved in energy metabolism has been found to be linked to the growth rates in fish (Ahmad et al., 2012). Variation in the concentration levels of liver enzymes-Acid phosphatase (ACP), alkaline phosphatase (ALP); aspartate aminotransferase (ASTO, superoxide dismutase (SOD) is considered as important index of growth and metabolic disturbances (Ahmad et al., 2012; Mir et al., 2016). The effects of dietary nutrients on liver metabolism have been studied in different perspectives. Dietary carbohydrates are found to be directly linked to the lipid contents of the liver (Zhou et al., 2013). For
instance, lipid vacuoles of larger size and peripheral nuclei in hepatocytes were found in livers of juvenile *Labeo rohita* and fry fed on non-gelatinized and gelatinized carbohydrates, respectively (Mohapatra et al., 2003; Kumar et al., 2005; Ahmad et al., 2012). The pathological effects of nutrition may result in structural modifications of nuclei within hepatocytes (Caballero et al., 1999). Limited ability of the fish to digest and metabolize nutrients may result in nutritional problems such as poor feed consumption and hence poor growth when they are taken in excess (Hemre et al., 2002). Maximum activity of ACP enzymes under the effects of dietary proteins in fingerlings of *Labeo rohita* was observed when a diet with optimum (20.54 mg) protein was fed (Ahmad et al., 2012).

The biofloc (BF) technology is an advanced and innovative approach for improved aquaculture production and involves the use of microbial aggregates as a rich source of protein. The technology is mainly based on the principle of waste nutrients recycling, in particular nitrogen, into microbial biomass that can be used in situ by the cultured animals (Avnimelech, 2009; Kuhn et al., 2010). Heterotrophic microorganisms are allowed to grow by maintaining the C/N ratio in the water through the modification of the carbohydrate content in the feed or by the addition of an external C source in the water (Avnimelech, 1999), so that the bacteria can assimilate the waste ammonium for new biomass production. Hence, ammonium/ammonia can be maintained at a low and non-toxic concentration so that water replacement is no longer required (Bossier and Ekasari, 2017).

Beneficial effects of BFT on fish growth, water quality, liver condition, digestive enzymes, and immunity have been studied in tilapia (Avnimelech, 2007), common carp (Najdegerami et al., 2016), rahu (Verma et al., 2016) and catfish (Ekasari et al., 2015). The bioflocs are reported to increase the growth performance by stimulating digestive enzyme activities (Xu et al., 2012), and improving the immune response (Kim et al., 2016).

Nutritional contribution of the BF-based diets to the metabolic functions and enzymes of the fish is very scarce. Pradhan and Das (2015) reported a marked effect of blue green algae (*Microcystis aeruginosa*) supplemented diet on ALP levels of the carp liver and its survival rate. The higher ALP levels indicate the higher reserved energy breakdown utilized for growth and survival of fish. A recent study by Najdegerami et al. (2016) reported significantly improved hepatocellular quantification and health of the liver of common carp fed on diets partially replaced with BF. Thus, the present study was designed to determine the effect of BF utilizing agri-wastes as C source, on the liver functions of the grass carp (*Ctenopharyngodon idella*) and to optimize the BF concentration for healthy liver functions.

**MATERIALS AND METHODS**

**Production of BF**

Microorganisms for the development of BF were enriched on the medium containing banana peels as carbon source. For this purpose, 20 g of dried and finely ground banana peels were weighed on electrical balance and added into 1 L distilled water in screw capped glass bottles to obtain a 2% (w/v) concentration. The pH was adjusted to 8. The medium containing bottle was then autoclaved at 121°C under pressure 15 psi for 15 min. The sterile medium was then inoculated with 1 g of soil sample (obtained from the fish pond) under hygienic conditions. An air pipe provided with a cotton plug to supply sterile oxygen into the medium bottle was used for the continuous aeration of the medium. This medium was incubated at room temperature for 10 days for the enrichment of heterotrophic microorganisms growing on banana peels. All the experiments for enrichment culture development were performed in triplicates.

The experiment was then up-scaled to the fiberglass aquaria (90 L capacity) for the production of BF. Each aquarium was filled with 60 L water and provided with the banana peels’ powder (2%). Then 500 mL of the microbial growth was taken from the previously enriched microbial culture and inoculated to each of the aquaria allocated for the treatments, T1, T2, T3 and T4. The control group was kept uninoculated and without the addition of any feed ingredients. The BF growth was further developed for 30 days in these fiber glass aquaria before stocking the fish. The neutral to alkaline pH was maintained during this period. Two air pipe aerators attached with hollow plastic tubes were used for the rich and continuous aeration of the aquaria. The C/N ratio of 15:1 was maintained for the treatments, T1, T2, T3 and T4. The control group was kept uninoculated and without the addition of any feed ingredients. The BF growth was further developed for 30 days in these fiber glass aquaria before stocking the fish. The neutral to alkaline pH was maintained during this period. Two air pipe aerators attached with hollow plastic tubes were used for the rich and continuous aeration of the aquaria. The C/N ratio of 15:1 was maintained for the treatments, T1, T2, T3 and T4. The control group was kept uninoculated and without the addition of any feed ingredients. The BF growth was further developed for 30 days in these fiber glass aquaria before stocking the fish.

**Experimental trial**

The experiment was conducted to find the effect of BF-supplemented fish feed on the liver metabolic enzymes of the grass carp fingerlings under different experimental conditions. The experiment was performed in Microbiology and Biotechnology Laboratory, Department of Zoology, Government College Women University, Faisalabad, Pakistan. The experiment was comprised of 4 different feeding treatments, T1, T2, T3 and T4 (Table I) on the basis of diet and water exchange and compared against the control group utilizing commercial fish feed. Fingerlings of the *C. idella* were obtained from the Fish
For the estimation of ACP and ALP activity, the methods proposed by the Jaffe and Badansky (1943) were followed. The ACP and ALP activity was calculated as: 

\[ \text{ACP activity (IU L}^{-1}\text{)} = 122 \times A \]

\[ \text{ALP activity (IU L}^{-1}\text{)} = 3300 \times A \]

Where, \( A \) = Change in absorbance per minute (Jaffe and Badansky, 1943).

The AST activity was estimated using Randox kits (Reitman and Frankel, 1957). The AST activity was calculated as:

\[ \text{AST activity (nm min}^{-1}\text{)} = 1746 \times \Delta A \text{ 340 nm min}^{-1} \]

Where; \( \Delta A \text{ 340 nm} \) = Extinction coefficient; \( \Delta A \text{ 340 nm} \) = Change in absorbance per minute for the homogenate sample.

The hepatic SOD activity was measured by the xanthine oxidase method (Fridovich, 1974). The assay is based on the competitive use of superoxide ions (generated by XOD during the conversion of xanthene into hydrogen peroxide and uric acid) by SOD. The level of TSPs in the supernatant was measured by the method as described by Lowry et al. (1951).

### Statistical analysis

Statistical analysis of the data was performed through analysis of variance and students’ t-test using Prism 5 Software. Differences between means were considered significant at \( P \leq 0.05 \).

### RESULTS

#### Production of BF

The microbial counts in the bioflocculated aquaria running under different feeding conditions were

| Table I. Effect of four different diets (T1, T2, T3, T4) and water exchange on total and specific activity (IU L\(^{-1}\)) of different hepatic enzymes in the livers of C. idella fingerlings. |
|------------------|----------------|----------------|----------------|----------------|
|                  | Control       | T1             | T2             | T3             | T4             |
| HSI              | 0.50±0.03     | 0.44±0.07      | 0.54±0.09      | 0.48±0.05      | 0.91±0.06***   |
| ACP              | 10.50±0.23(0.0009) | 11.75±0.23** (0.0013) | 10.50±0.35 (0.0003)| 11.00±0.21 (0.0009) | 10.00±0.12 (0.0015) |
| ALP              | 77.50±1.22 (0.0341) | 81.50±1.49 (0.0776) | 77.25±1.19 (0.0096) | 80.75±1.71 (0.0389) | 75.5±0.94 (0.0497) |
| AST              | 24.50±0.96 (0.0118) | 30.25±1.62* (0.0199) | 24.50±1.21 (0.003) | 26.25±1.78 (0.0116) | 22.00±2.90 (0.0210) |
| SOD              | 18.25±1.29 (0.0120) | 19.75±1.78 (0.0188) | 16.00±2.88 (0.0070) | 19.50±1.98 (0.0024) | 13.25±2.37 (0.0064) |
| TSP              | 2075.04±60 | 1049.99±107**** | 2269.92±777 | 1517.88±405 | 8073.00±848**** |

Values are Mean ± SEM of replicates (n=21) and are significantly different from the control value of respective parameter in the row. Significance levels are *, \( P \leq 0.05 \); **, \( P \leq 0.01 \); ***, \( P \leq 0.001 \); ****, \( P \leq 0.0001 \).

HIS, histosomatic index; ACP (IU L\(^{-1}\)), acid phosphatase (IU L\(^{-1}\)); ALP, alkaline phosphatase (IU L\(^{-1}\)); SOD (IU L\(^{-1}\)), superoxide dismutase; TSP (mg mL\(^{-1}\)), total soluble proteins. Values in parentheses are specific activity (IU L\(^{-1}\)) of the respective enzymes. Composition of feed: Bacteria (CFU mL\(^{-1}\)): 10\(^{10}\) in control and 10\(^{8}\) to 10\(^{9}\) in all treated feeds. Biofloc volume: 0.2 mL L\(^{-1}\) in all treatment groups. Commercial feed (%): 5% of body weight twice daily in control; 2.5% of body weight thrice in a week in T3 and T4. Water exchange (%): 90% every second day throughout the experiment in control; 0% in T1 and T3, 10% in T2 and T4 weekly. Composition of commercial feed (%): Fish meal, 12; Protein, 38; Rice polish, 12; wheat flour, 10, fish oil, 6; vitamin premix, 1; minerals, 1; ascorbic acid, 1; chromic oxide, 1.
The specific activity (U mg⁻¹ of protein) of the ACP is also presented in Table I. Maximum specific activity of ACP was shown by the fingerlings reared in the treatment group T4 showing specific activity of 0.0015 U mg⁻¹ of the TSPs, while the minimum specific activity (0.0009 U mg⁻¹) was given by the fingerlings reared in the control group.

The maximum specific activity of ALP was given by the fingerlings reared in the treatment group T1 with 0.0776 U mg⁻¹ of the TSPs, while the minimum specific activity (0.0096 U mg⁻¹ of protein) was given by the fingerlings reared in treatment group T2. The ALP specific activities of the treatment groups T3 and T4 were 0.0389 and 0.0497 U mg⁻¹ of the protein, respectively (Table I).

The maximum specific activity of AST (0.0210 U mg⁻¹) was shown by the fingerlings reared in the treatment group T4, while the minimum specific activity (0.0030 U mg⁻¹ of protein) was shown by the fingerlings reared in treatment group T2. The AST specific activities of the groups T3, T4 and C were 0.0116, 0.0210 and 0.0118 U mg⁻¹ of the protein, respectively (Table I).

The maximum specific activity of SOD (0.0188 U mg⁻¹ of protein) was shown by the fingerlings reared in the treatment group T1, while the minimum specific activity (0.0024 U mg⁻¹ of protein) was shown by the fingerlings reared in treatment group T3. The SOD specific activities of the study groups T2, T4 and C were 0.0070, 0.0064 and 0.0120 U mg⁻¹ of the protein, respectively (Table I).

**Estimation of TSPs**

The level of TSPs varied significantly among different diet treatments (Table I). The levels of TSPs found in the liver samples of the fingerlings for the study treatments T1, T2, T3, T4 and C were 1049.99 ± 107, 2269.92 ± 777, 1517.88 ± 405, 8073.00 ± 848 and 2075.04 ± 60 mg mL⁻¹ (Table I).

**DISCUSSION**

The impact of BF supplemented feed on the production of hepatic enzymes in *C. idella* fingerlings was evaluated in this study. The higher carbon to nitrogen ratios in the BF system bring about the enrichment of beta-polyhydroxyalkanotes accumulating bacteria. These PHB containing heterotrophic bacteria are not only known for bioflocculant-production but also exert antibacterial activity against pathogens (Russel, 1992; Salehizadeh et al., 2001; Kumaresan et al., 2002; Sinha et al., 2008; Emerenciano et al., 2013). These attributes of the biofloc community support them to be employed in a sustainable aquaculture.

The present study’s results revealed that the values of HSI differed significantly among all the feeding groups.
The highest value of HSI was noted for the treatment group T4. The size of an organ proportional to the body weight is determined by taking the relation of the organ to body weight. Wilber and Gilchrist (1965) suggested the use of these ratios a valuable criterion in evaluating the correlation between certain experimental conditions and the biological response of a particular organism. The liver is the main organ performing various metabolic processes and detoxification. Its ability to regenerate makes it unique among the vital organs of the body. Organ weight is a direct response of an experimental condition thus making it most sensitive indicator. Significant differences in an animal’s organ weight may occur under different experimental conditions even when no apparent morphological changes are observed (Bailey et al., 2004). This makes the organ weight analysis an important parameter to find the effect of an experimental diet. Afuang et al. (2003) correlated HSI range of 1.5–2.7 with incorporation of body lipids which is greatly influenced by the dietary components. Thus, a lipid-rich diet gives a higher HSI owing to its relation to the lipid contents (Ogunji et al., 2008). These reports clearly explain the lower levels of HSI when a protein-rich diet is used as represented by the present study results (0.436–0.913) of the C. idella fingerlings feeding on protein-rich microbial diet.

The ACP and ALP levels in the liver samples of the fingerlings belonging to the different treatment groups were non-significantly different from those of the control groups. These results are consistent with the findings of Mohapatra et al. (2014) where the levels of ACP and ALP were reduced in the probiotics-fed group of Labeo rohita as compared to the non-probiotics-fed group. For endoplasmic reticulum and plasma membrane, ALP is marker enzyme (Muhammad, 2007). Thus, it is an ectoenzyme of plasma membrane which is used to determine the plasma membrane integrity oftenly (Akanji et al., 1993; Shanhjahan et al., 2004). Its high levels in the serum and tissue indicate damage to the external cell boundaries. Elevated levels of ALP may enhance the chances of membrane damage because it is an enzyme bounded to the membrane (Rao, 2006).

The AST activity in the present study ranged between 22-30 IU L⁻¹ in all the study groups. The decreased level of the enzyme in the fingerlings of the treatment group T4 in comparison with the control and the remaining treatment groups is attributed to the balanced nutritional contents of the fish feed as reported by the Metón et al. (1999). Some other researchers, however, relate these differences in the activity of the AST to the fish species rather than diet compositions (Nagai and Ikeda, 1972; Cowey and Walton, 1989). Increased levels of AST and ALT in the Nile Tilapia and other fish species have been reported previously when the fish were fed with the probiotic-supplemented diets (El-Rhman et al., 2009; Harikrishnan et al., 2011). The activities of AST and ALT in the heart and liver tissues are markers for their functions and integrity (Adeniyi et al., 2010). They reorganize the proteins’ building blocks. These enzymes are reported to be released from the damaged liver tissue (Nelson and Cox, 2000). Increased levels in the serum are indications of cellular damage (necrotic tissues). Myocardial infarction and increased risk of cardiovascular disease are attributed to increase of ALT and AST levels (Ioannou et al., 2006).

The SOD levels among different treatment groups ranged between 13.25–19.75 IU L⁻¹ which were non-significantly different from those of the control group. Slightly higher levels of SOD in the fingerlings of the treatment groups T1, T2 and T3 as compared to those fed on BF-supplemented commercial diet indicated a light stress of the live microbial culture on the hepatocytes. Our results are supported by the previous findings where L. vannamei reared in a BF-based system showed improved physiological functions as reported by Becerra-Dórame et al. (2014). This improved performance was indicated by the SOD activity. Similarly, Jang et al. (2011) reported enhanced expression of an oxidase-activating enzyme (WPPAE1) in hemocytes of L. vannamei reared for a long term in a BF-based system. The much higher level of TSPs was noted in the treatment group T4. The enhancement of TSPs in different cells under potentially suitable diet treatments has been reported by many researchers (Gupta and Sharma, 2016; Rajkumar et al., 2016; Kumar et al., 2017).

CONCLUSIONS AND RECOMMENDATIONS

The present study arrived at the conclusion that utilization of BF-based fish diet in aquaculture is a promising approach to achieve much better results of the fish weight gain. Since higher levels of the hepatic enzymes are markers for the tissue damage thus lower levels of the liver enzymes in the treatment group T4 of the present study clearly demonstrate that the mixture of BFs and commercial diet is beneficial and economic for rearing of the grass carp fingerlings and fulfill needs of the nutrients for the carp culture. However, the highest levels of the hepatic enzymes in the treatment group T1 depicted hepatic stress and revealed that only BFs are not enough to support optimum growth of the grass carp. Our findings of the present study will be helpful for developing economical fish farming and valorization of agricultural wastes for the development of nutritional supplements (BFS) in aquaculture. Future studies on different other
agro-industrial wastes like wheat straw, rice husk, rice straw, cotton sticks, maize waste, sugarcane bagasse and fruit wastes, are recommended for their potential utility in developing BFs. The microscopic examinations and the microbial community analyses of the BFs are further needed to be explored in future studies.

ACKNOWLEDGEMENTS

The support of Fish Seed Hatchery, Faisalabad, Pakistan for providing fingerlings of the grass carp (C. idella), is highly acknowledged.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES


Impact of Biofloc on Liver Functioning of Grass Carp


