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EFFECT OF DIFFERENT CARBON SOURCES FOR THE PRODUCTION OF BIOFLOC SYSTEM ON DIGESTIVE ENZYME ACTIVATES AND GROWTH OF *CATLA CATLA* FINGERLINGS

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Abstract
The present study was conducted to check the effect of different carbon sources used in bioflocs on the growth and digestive enzymes activities of *Catla catla* fingerlings. For this study 15 experimental fiber glass tanks filled with 1500 liters of water and designated as control, T1, T2, T3 and T4 each with three replicates. 100 individuals of similar average weight (1.20±0.50). Four treatments of bioflocs tank fertilized with different carbon sources, i.e, wheat bran T1 (BFTW), T2 rice bran (BFTR), T3 molasses (BFTM) and T4 sugar baggas (BFTS). All the treatments and control were fed with commercially available feed Supreme aqua feed (CP 25%) (Pakistan) Pvt. Ltd, twice a day. Treatment 1 shows significantly increased in Weight gain and SGR among all treatments. Best FCR among all treatments was calculated for treatment 1 with average value (1.61±0.02). FER among all the treatments with highest value was recorded in TI and T2 as 0.63±0.01 and lowest value was recorded as 0.15±0.00. Results for PER shows significantly increased in Treatment 2 among all treatments. Among all treatments, Treatment 1 shows significant increased for all digestive enzyme activities. Treatment 1 declared as most diverse treatment having 9 phyla, 10 classes and 19 genera of different planktons among all treatments.

INTRODUCTION

Aquaculture is one of the fastest-growing food-producing sectors, accounting for over 40% of global fish output.[1] China is the world's largest aquaculture producer, with 51.5 million tons of fish products produced in 2006.[2] Aquaculture employs 520 million people worldwide,[2] with 98 percent living in poor countries.[3] For a 20% intake of protein about one-third of the world, populations depend upon aquatic products.[4] The need for food production is increasing day by day to meet the demand of increasing population. Human tries to increases the quality and quantity of food especially high protein food such as aqua culture.[5] About 103 million tons of fish are consumed by humans per year as 16.3 kg per capita in developing countries and only 2.0 kg in Pakistan. Depletion of aquaculture resources is a problem for most of the countries same as in Pakistan and therefore, every nation is

trying to find new ways to increase production.

Biofloc technology is microbial-based zero water exchange technology as a new trend in aquaculture. This system converts waste into useful products for aquaculture.[6, 7] It reduces environmental impact by providing sustainable alternatives without damaging the growth and health of species.[8, 9] This system was started to develop in 1980,[10] and attempts were made to improve the water quality of shrimp culture.[11, 12] Since then, zero water exchange, no impact on the environment, feed recyclability, effective utilization of carbon sources, and high fish biomass production are the key areas to research in the biofloc system.[7, 13]

Several biofloc systems are available for use in research and commercial aquaculture. Depending on the exposure to natural light, two systems exposed (outdoor, lined ponds or tanks) and unexposed (indoor biofloc) biofloc systems are available.[14, 15] The unexposed system is an indoor system and if only bacterial processes control water quality; it is referred to as "brown water".[15] However, in the outdoor system, bacterial processes and a complex mixture of algal controls water quality and termed "green water".[14] Both systems consist of microbes and dead organisms.[16, 17]

The microbes play a very important role in the recycling of nutrients, water quality control pathogen control in culture systems.[18, 19] The microbes may also act as a primary food source for cultured species and save a significant amount of commercial food. Different carbon sources are used for enhancing the growth of microbes in biofloc systems.[20] These microbes produce a protein which can replace fishmeal or other protein sources in fish and shrimp feed in omnivorous cultured species.[21] In substitute of fishmeal, alternative plant protein sources are proposed. [22, 23] When plant by-products were included in fish diets, it was discovered that they produced and grew better [24, 25]. Zero water exchange system is achieved by maintaining an appropriate carbon-nitrogen ratio which reduces nitrogen metabolites by transferring metabolites to biofloc.[26, 27]

MATERIALS AND METHODS

The experiment was conducted Integrated Aquaculture research unit at Department of Fisheries and Aquaculture, UVAS Lahore.

BF culture and experimental design

For the purposed study 15 experimental fiber glass tanks having capacity of 1500 liter water. Four treatments each having 100 individuals with a control in triplicates fish were set up using completely randomized design (CRD). Four treatments of bioflocs tank fertilized with different carbon sources, i.e, wheat bran T1 (BFTW), T2 rice bran (BFTR), T3 molasses (BFTM) and T4 sugar baggas (BFTS). All the treatments and control were fed with commercially available feed Supreme aqua feed (CP 25%) (Pakistan) Pvt. Ltd, twice a day. Experimental tanks were aerated (7mg/L) by a centralized aeration unit through air stone with a regulator to control the pressure of air in the tanks.

Monitoring of Water Quality Parameters

Water quality parameters measures such as turbidity, TDS, Total Hardness, alkalinity, pH, and temperature, DO, Phosphate, Sulphate, Nitrite, Nitrate, Calcium, Magnesium, Chloride and Biofloc volume were monitored during the experiment. A portable DO meter was used to measure temperature and dissolved oxygen (DO, mg L^{-1}) twice daily. The pH, salinity (mg L-1), nitrite (NO2-N mg L-1), nitrate (NO3-N, mg L-1), TAN (mg L-1), Hardness and alkalinity were all calculated using an automated multimeter spectrophotometrically following APHA (2005).

Identification of plankton

10 ml of water sample was taken and filtered with a mesh net. Then took 1 ml of water sample on a glass slide with the help of a dropper, added a drop of glycerin, covered with a glass slide, and observed under the light microscope for plankton's identification.

Growth parameters

Growth parameters like, feed conversion ratio (FCR), specific growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER), and body weight increase were determined.[28]

WG = final weight - initial weight

Feed Conversion Ratio (FCR) = Feed applied

Live weight gain

Specific growth rate (SGR) = ln final weight - ln initial weight ×100/ Days of experiment per ¼ weight gain (g) =protein fed (g)

Determination of digestive enzyme activities

For the analysis of enzymatic activities 5 fish from each treatment and control were randomly selected and after dissection a gastro-intestinal tract was excised, homogenized and centrifuged at 5000 rpm at 4°C for about 20 minutes. For further study, the supernatant was collected and deposited at -20 C. A pH 7.0 phosphate buffer of 0.2 M was used to assess the total protease activity at 25°C at the temperature of 1 percent casein (w/v).[29] The response started with pepsin at 37°C and a protein substratum of 2% hemoglobin at 0.06N HCl. The amylase activity was measured in the NaH2PO4 buffer by 0.3% soluble starches (pH 7.4). [30] The lipase behavior was then tested with P-nitrophenylmyristate at 0.25 m Tris–HCl (pH 9.0). Then 4-nitrophenylphosphate (PNPP) was measured in the 30 mm NaHCO3 buffer at 37°C, dissolved by the substratum (pH 9:8). The overall protein concentration was calculated using the Bradford method with reference to bovine serum albumin.[31] Activity of Alkaline phosphatase was assessed by using 4-nitrophenylphosphate (PNPP) at 37^0 C in 30mM $NAHCO_3$ buffer as a substrate at pH (9.8).[32]

Statistical Analysis

Statistical Analysis of various parameters was conducted by using SAS 9.4 version (SAS institute Inc.). Comparison between variables was made through Duncan's different range test.[33] The degree of significance was recorded at a 5% level.

RESULTS

Water quality parameters

Different water quality parameters were assessed in this study which are described in Table 1.Water quality parameters such as Turbidity, TDS, DO, EC, Alkalinity, Total Hardness, pH, Temperature, Sulphate, Nitrite, Nitrate, Calcium, Chloride, Magnesium and Biofloc volume were assessed from our study. The highest value of turbidity among all groups was recoded as 1.50±0.30 and the lowest value was recorded as 0.79±0.03 in T1. TDS with highest value among all groups was recorded as 1239.50±4.50 in control group while with lowest value was recorded in T2. DO in all treatments with highest value was recoded in T2 as 5.62±0.00 while minimum value was noted in the control group as 5.30±0.09. EC among all the groups with highest value was observed in control group and T4 as 1761.50±0.50 and with lowest value as 1753.50±1.50 in T3.

Alkalinity among various groups in all treatments with highest value was recorded in T4 as 472.00±0.00 while the lowest value was recorded in the T1 as 454±1.00. Total hardness in different groups with highest value was recorded in the T3 as 465±4.00 and the lowest value was determined in T1 as 447±0.50. Among all groups the highest value of pH was recorded in T4 and the lowest value was recorded in the T1 as 7.09±0.00. Temperature shows highest value as 28.89±0.11 in T4 among all groups while the lowest value was recorded in T1 as 26.00±1.00. Sulphate among all treatments with highest value was recorded in T4 as 76.70±1.30 and the lowest value was recorded in T3 as 72±1.00.

Nitrite among all groups with highest value was recorded in T4 as 1.63±0.00 while lowest value was recorded in control group as 0.34±0.01. Nitrate in various groups having highest value was recorded in the T1 as 12.45±0.34 and the lowest value was recorded 1.31±0.05 in control group. Calcium among all groups with highest value was recorded in T4 as 117.00±2.00 while the lowest value

60.61±59.39 was recorded in control group. Chloride in all group have highest in T1 as 237±6.00 and the lowest value T2 as 228.80±0.20. Among all groups magnesium shows highest value in T1 as 44±1.00 while the lowest value was recorded in T2 as 39.60±0.40. Bioflocs volume among all treatments with highest value was recorded in T1 as 29.50±0.30 and the lowest value was in T4 as 18.96±0.85.

The flocculated aggregates of suspended organic particles were seen as bioflocs in brown color consisting colonies of microalgae, protozoans and heterotrophic bacteria. Throughout experiments in all treatments, all the water quality parameters were found in suitable ranges for the culture of thaila. We found non significance values between the treatments in terms of turbidity, total hardness, temperature and calcium. In all the other water quality parameters show significant results between the treatments.

Table-1 Water quality parameters during present study.

Parameters	Control	T1	T2	T3	T4	P-Value
Turbidity	1.50±0.30^{a}	0.79±0.03^{b}	0.86±0.06^{b}	0.90±0.04^{b}	0.97±0.02^{b}	0.0730
TDS	1239.50±4.50^{a}	1227.50±1.00^{b}	1223±2.00^{b}	1227.50±1.50^{b}	1225.50±2.50^{b}	0.0372
DO (mg/l)	5.30±0.09^{c}	5.45±0.04^{abc}	5.62±0.00^{a}	5.54±0.02^{ab}	5.44±0.01^{bc}	0.0290
EC	1761.50±0.50^{a}	1756.00±6.00^{a}	1755±3.00^{a}	1753.50±1.50^{a}	1761.50±3.50^{a}	0.4195
Alkalanity	466.00±1.00^{ab}	454±1.00^{c}	464.50±3.50^{b}	466±1.00^{ab}	472.00±0.00^{a}	0.0063
Total Hardness	451.50±0.50^{b}	447±0.50^{b}	454±5.00^{ab}	465±4.00^{a}	454.00±3.00^{ab}	0.0745
Ph	7.72±0.10^{ab}	7.09±0.00^{b}	7.68±0.10^{ab}	7.55±0.25^{b}	8.48±0.39^{a}	0.0473
Temperature	26.45±0.45^{b}	26.00±1.00^{b}	27.15±0.15^{ab}	28.60±0.60^{a}	28.89±0.11^{a}	0.0507
Sulphate	76.50±0.50^{a}	72.50±0.50^{b}	72±1.00^{b}	72.50±0.50^{b}	76.70±1.30^{a}	0.0212
Nitrite	0.34±0.01^{c}	1.34±0.00^{ab}	1.46±0.21^{ab}	1.26±0.02^{b}	1.63±0.00^{a}	0.0012
Nitrate	1.31±0.05^{c}	12.45±0.34^{a}	11.26±0.06^{b}	12.44±0.21^{a}	12.37±0.05^{a}	<.0001
Calcium	60.61±59.39^{a}	111.50±0.50^{a}	115.50±3.50^{a}	114±1.00^{a}	117.00±2.00^{a}	0.5616
Chloride	234.50±2.50^{a}	237±6.00^{a}	228.80±0.20^{a}	230.50±0.50^{a}	232.00±2.00^{a}	0.4363
Magnesium	41.99±0.01^{ab}	44±1.00^{a}	39.60±0.40^{b}	43±2.00^{ab}	41.50±0.50^{ab}	0.1668
Biofloc volume	0.00^{c}	29.50±0.30^{a}	19.36±0.14^{b}	28.60±0.30^{a}	18.96±0.85^{b}	<.0001

Mean values in a single row with different superscripts are significantly different at ($p < 0.05$)

Growth parameter

Different growth parameters like total weight gain, SGR, FCR, FER and PER were assessed in this study which are described in Table 2. In the various treatments highest value of weight gain among all treatments was recorded in treatment T1 as 5.42±0.04 while the lowest value was recorded as 0.85±0.02 in the control group. SGR shows highest value among all treatments in T1 as 8.70±0.17 while the lowest value was recorded in the control group as 0.85±0.02.

FCR was recorded with highest value as 6.75±0.04 in control group and with lowest value in T1as 1.61±0.02 among all groups. FER among all the treatments with highest value was recorded in TI and T2 as 0.63±0.01 and lowest value was recorded as 0.15±0.00. PER among all the groups with highest value was recorded as 2.33±0.02 in T2 and the lowest value was recorded in control group as 1.18±0.02.

In terms of weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio, all treatments showed a substantial difference. Treatment T1 had the highest weight increase, SGR, and FER values, whereas treatment T4 had the lowest weight gain, SGR, FER, and PER values. In comparison to the other three treatments, Treatment T1 had a high FCR value. There was no significant change in FER significant values across all treatments.

Table-2 Growth parameters recorded during present study.

Parameters	Control	T1	T2	T3	T4	P-Value
Weight gain	0.85±0.02^{d}	5.42±0.04^{a}	5.27±0.07^{a}	4.85±0.04^{b}	3.89±0.09^{c}	<.0001
SGR	1.33±0.01^{e}	8.70±0.17^{a}	8.15±0.03^{b}	7.63±0.00^{c}	5.59±0.20^{d}	<.0001
FCR	6.75±0.04^{a}	1.61±0.02^{d}	1.64±0.01^{d}	1.77±0.02^{c}	1.95±0.05^{b}	<.0001
FER	0.15±0.00^{b}	0.63±0.01^{a}	0.63±0.01^{a}	0.54±0.02^{a}	0.54±0.07^{a}	0.0007
PER	1.18±0.02^{d}	2.22±0.01^{b}	2.33±0.02^{a}	2.19±0.00^{b}	1.85±0.02^{c}	<.0001

Mean values in a single row with different superscripts are significantly different at ($p < 0.05$)

Digestive enzyme activities

Different enzyme activity levels were assessed in this study which are described in Table 3. The highest values for total proteases among various treatments were recorded in T1 as 8.49±0.29 while lowest value was assessed in control group as 0.68±0.08. The highest value for pepsin was recorded in control group as 2.07±0.02 and the lowest value was recorded as 0.98±0.01 in T2. Lipase shows highest value among all treatments in T1 as 2.72±1.49 while the lowest value of lipase among various treatments was recorded in control group as 0.08±0.0. The highest value of amylase was analyzed in various treatments as 3.43±0.03 and the lowest value was recorded as 2.85±0.05 in T2. Alkaline phosphate shows remarkable highest values as 0.03±0.00 in control group, T1 and T4 while the lowest values were recorded in T2 and T4 as 0.02±0.00. A significant difference was observed in the activity of total protease and pepsin enzyme, while nonsignificant differences were observed in the activity of rest of three enzymes (Lipase, Amylase, Alkaline phosphatase). Highest enzyme activity was found in treatment T1, while lowest in treatment T2.

Table-3 Digestive enzyme activities recorded during present study.

Parameters	Control	T1	T2	T3	T4	P-Value
Total Protease	0.68±0.08^{e}	8.49±0.29^{a}	5.53±0.03^{d}	7.70±0.10^{b}	6.66±0.06^{c}	<0.0001
Pepsin	2.07±0.02^{b}	2.06±0.30^{a}	0.98±0.01^{c}	1.94±0.04^{b}	1.91±0.01^{b}	<.0001
Lipase	0.08±0.01^{b}	2.72±1.49^{a}	0.07±0.03^{b}	1.51±0.01^{ab}	0.06±0.00^{b}	0.1141
Amylase	2.94±0.04^{c}	3.43±0.03^{a}	2.85±0.05^{c}	3.41±0.01^{a}	3.22±0.02^{b}	0.0002
Alkaline Phosphatase	0.03±0.00^{a}	0.03±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.03±0.01^{a}	0.4832

Mean values in a single row with different superscripts are significantly different at ($p < 0.05$)

Plankton's community

Planktons community measured in the various treatments indicate that in T1 richest level of phytoplankton and zooplankton discussed in Table 4-7. Community of Phytoplankton include various genera of different species as Oscillatoria, Cosinodiscus, Cyclotella Licmophora, Navicula, Melosira, Gomphosperia, Oscillatoria, Gloeocapsa, Alexandrium, Protoperidinium, Scenedesmus, Borodinellopsis, Chlorella, Tetraselmis. Zooplanktons include genera Branchius. Protozoan genera are Radiolarian, Euglena and Cilliate.

Table-4 Plankton communities identified in treatment 1 during present study.

Parameter	Phylum	Class	Genera
Phytoplankton	Cynophyta	Cynophyceae	Oscillatoria
	Ohcrophyta	Coscinodiscophyceae	Cosinodiscus
		Bacillariophyceae	Cyclotella
			Licmophora
			Navicula
			Melosira
	Cynophyta	Cynophyceae	Gomphosperia
			Oscillatoria
			Gloeocapsa
	Dinophyta	Dinophyceae	Alexandrium
			Protoperidinium
	Chlorophyta	Chlorophyceae	Scenedesmus

			Borodinellopsis
			Chlorella
			Tetraselmis
Protozoa	Ciliophora	Ciliatea	Cilliate
	Euglenoidea	Euglenaceae	Euglena
	Sarcomastigophora	Radiolaria	Radiolarian
Zooplankton	Rotifera	Brachionidae	Branchius

Table-5 Plankton communities identified in treatment 2 during present study.

Parameter	Phylum	Class	Genera
Phytoplankton	Chlorophyta	Chlorophyceae	Chlamydomonas
			Borodinellopsis
			Tetraselmis
			Chlorella
	Ohcrophyta	Bacillariophyceae	Cymbella
			Radiolarian
			Cyclotella
			Amphora
			Licmophora
Protozoa	Euglenoidea	Euglenaceae	Euglena
	Ciliophora	Vorticellidae	Vorticella
	Ciliophora	Parameciidae	Paramecium
Zooplankton	Rotifera	Brachionidae	Brachionus

Table-6 Plankton communities identified in treatment 3 during present study.

Parameter	Phylum	Class	Genera
Phytoplankton	Cynophyta	Cynophyceae	Oscillatoria
			Gomphosperia
			Oscillatoria
	Chlorophyta	Chlorophyceae	Borodinellopsis
			Chlorella
	Dinophyta	Dinophyceae	Alexandrium
			Protoperidinium
	Ohcrophyta	Bacillariophyceae	Cyclotella
			Leptocylindrus
			Nitzschia
			Navicula
Protozoa	Ciliophora	Euplotidae	Euplotes
		Parameciidae	Paramecium
		Vorticellidae	Vorticella
	Euglenoidea	Euglenaceae	Euglena
Zooplankton	Gastrotricha	Chaetonotida	Gastrotrich
	Arthropoda	Copepoda	Copepod

Table-7 Plankton communities identified in treatment 4 during present study.

Parameter	Phylum	Class	Genera
Phytoplankton	Ohcrophyta	Coscinodiscophyceae	Cosinodiscus
	Chlorophyta	Chlorophyceae	Tetraselmis
			Chlorella
	Dinophyta	Dinophyceae	Protoperidinium
			Alexandrium
Protozoa	Sarcomastigophora	Radiolaria	Radiolarian
	Euglenoidea	Euglenaceae	Euglena
	Ciliophora	Parameciidae	Paramecium
Zooplankton	Rotifera	Brachionidae	Brachionus
	Gastrotricha	Chaetonotida	Gastrotrich

DISCUSSION

Current study reveals significant results in the activity of total protease and pepsin enzyme, while a non-significant difference was recorded in the activity of rest of three enzymes (Lipase, Amylase, Alkaline phosphatase Treatment T1 had the highest enzyme activity, whereas treatment T2 had the

lowest. The effect of biofloc on Golden crucian carp digestive enzyme activity in zero-water exchange biofloc systems was investigated.[34] They found that C/N 20 and C/N 25 had greater protease activity than the biofloc system control (p 0.05). In contrast to our findings, comparable treatment groups had greater protease, lipase, and amylase activity in the liver. Similarly, the C/N 20 group had significantly higher lysozyme (LSZ), acid phosphatase (ACP), and alkaline phosphatase (AKP) activity than the control group (p 0.05).

Furthermore, there was a substantial variation in weight increase, specific growth rate, feed conversion ratio, and protein efficiency ratio across all treatments (Table 3.1.2). Treatment T1 had the highest weight increase, SGR, and FER values, whereas treatment T4 had the lowest weight gain, SGR, FER, and PER values. In comparison to the other three treatments, Treatment T1 had a high FCR value. There was no significant change in FER P-values across all treatments. Treatment T1 showed effective FCR value as compared to other three treatments. P-value of FER in all treatments showed no significant difference. In a study carried out on *Cyprinus carpio* in biofloc system with four different carbon sources including coffee, moringa, macroalgae and yucca.[35] The highest weight was attained with Moringa as carbon source and with coffee. In another study, the influence of diets supplemented with biofloc meal on survival and production of GIFT tilapia was investigated.[36] They found that diets with 32% crude protein and inclusion level with 20% biofloc yielded had more body weight gain, FCR, SGR, PER, and FER. They concluded that biofloc meal is a potential ingredient for GIFT tilapia diet at 20% level for better growth performance.

CONCLUSION AND RECOMMENDATIONS

This research highlights the efficiency of various carbon sources in refining the biofloc systems, nitrogen dynamics, hematological parameters, and digestive and metabolic enzyme activities of *Catla catla* species fingerlings. The findings demonstrate that the water quality parameters in the BFT system do not change but the microbial community dynamics do. The findings also suggest that carbon sources in biofloc dependent fingerlings have a favorable effect on growth and digestive enzyme function. These findings may persuade farmers to consider carbon sources as viable in the BFT system's intensive culture of common carp. From the results of this analysis, it can be inferred that treatment T1 performed significantly better than all other treatments.

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