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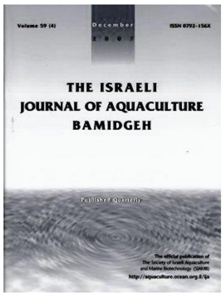

Effect of Biogut on Growth Performance of *Cyprinus carpio* (Linn.) Fingerlings

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Abstract

In this study we evaluated the effect of three levels of Biogut (a probiotic) on growth performance of common carp fingerlings. Biogut was incorporated into a formulated diet (protein level: 35%) at 0.5, 1.0 and 1.5% of the diet and designated as T_1, T_2 and T_3 and T_0, the fourth (control) diet, without biogut. The diets were fed to common carp fingerlings, stocked at 10/tank, with three replicates, for 60 days. Results indicated that the common carp fingerlings fed with the diets containing biogut exhibited increased growth compared to those fed the control diet. The survival rate was 100% in all the groups. The average weight and length of common carp was maximal in the 0.5% biogut treated group. Specific growth rate, protein efficiency ratio, RNA/DNA ratio and protease activity were also highest in the group fed on 0.5% biogut. The results demonstrate that 0.5% biogut supplementation in the diet of common carp fingerlings produces positive effects.

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Introduction

India is the second largest producer of farmed fish in the world with 95% of total fish production deriving from inland aquaculture (4.6 million tons) (FAO, 2013). Owing to the rapid growth of the aquaculture sector, the per capita consumption of fish has increased from 5 kg to 16 kg during the last five decades (Ayyappan and Biradar, 2004). High quality feed with a protein content of 30-40% is important for proper growth and fish health. In addition to the essential nutrients, complementary additives are needed to enhance the growth and survival of fish. Some of the common growth promoting feed additives used in aquaculture include antibiotics, hormones, ionophores and some salts (Fuller, 1992; Gongora, 1998). The main aim of aquaculture is to achieve maximum sustainable production per unit water area.

Probiotics are often used in aquaculture to promote growth and disease resistance of fish and shellfish. According to the WHO, 2001, probiotics are 'live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Feed probiotics are widely used in poultry and piggery; recently use of probiotics in fish is getting much attention due to their multiple modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Verschuere et al., 2000; Carnevali et al., 2006). Biogut is a non-hormonal growth promoter in animals. Information regarding the use of biogut in fish in general, and in common carp in particular, is scarce. In this experiment we studied the effect of biogut on growth, survival, feed utilization, and body composition of *Cyprinus carpio* fingerlings.

Materials and Methods

Experimental system. The experiment was carried out in a closed recirculation system consisting of 12 circular fiberglass tanks (Fig. 1).

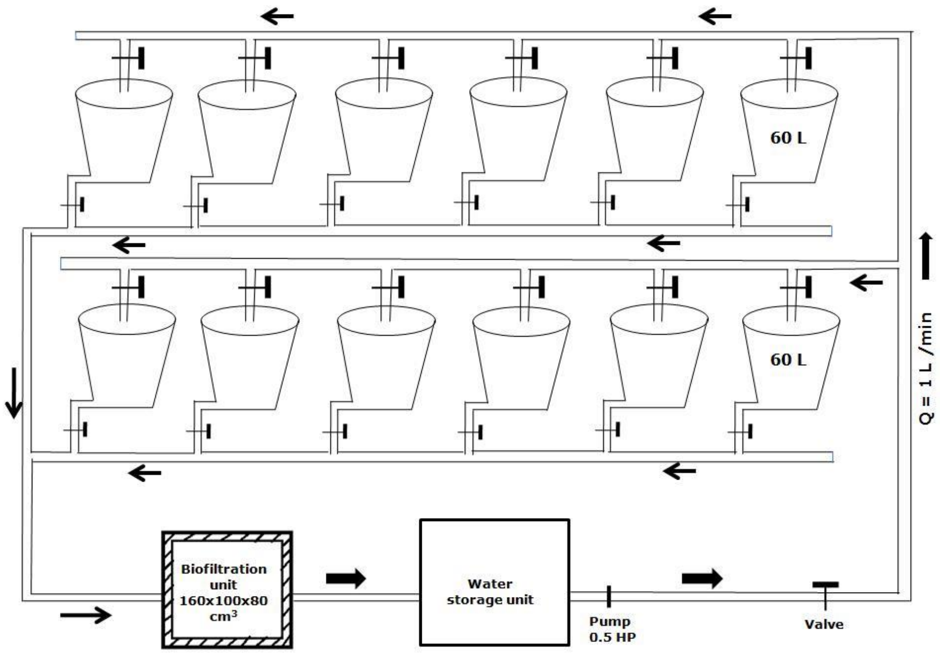

Figure 1: Recirculation system with experimental tanks (Diameter 62 cm).

All the experimental tanks (40 l water) were aerated using a Spencer Vortex air-blower (0.5 HP).

Diet preparation. The ingredients used in preparation of the experimental diet were fishmeal, rice bran, groundnut oil cake, wheat flour, rice flour, ragi flour, vitamin and mineral premix (Table 1).

Table 1: Proportion of various ingredients of the experimental diet

Treatment	*Fish meal (g/kg)*	*Ground nut oil cake (g/kg)*	*Rice bran (g/kg)*	*Wheat flour (g/kg)*	*Ragi flour (g/kg)*	*Rice flour (g/kg)*	*Mineral mix (g/kg)*	*Biogut (g/kg)*
T_0	250	350	200	90	50	50	10	0
T_1	250	350	195	90	50	50	10	5
T_2	250	350	190	90	50	50	10	10
T_3	250	350	185	90	50	50	10	15

All ingredients, apart from biogut, were procured from the local market. Biogut is a commercial product procured from M/s. Varsha Group, Bangalore, India with a combination of probiotic bacteria such as *Lactobacillus sporogens*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Bacillus licheniformis* and *Saccharomyces cervisiae,* and enzymes such as amylase, protease, lipase cellulase, phytase, and beta-galactosidase. All ingredients, apart from biogut, were sieved to particles of uniform size (3 mm). The sieved ingredients were packed in polythene bags and stored at room temperature for further use. Commercially available vitamin and mineral premix (Agrimin) was also added.

All the ingredients were analyzed for proximate composition by employing the standard methods (AOAC, 1975) (Table 2).

Table 2: Proximate composition of the ingredients used for the preparation of experimental diet

Ingredients	*Moisture (%)*	*Dry matter (%)*	*Crude protein (%)*	*Crude fat (%)*	*Crude fibre (%)*	*Ash (%)*	*NFE (%)*
Fish meal	7.53 (0.03)	92.47 (0.14)	62.82 (0.60)	5.99 (0.26)	1.03 (0.02)	28.63 (0.45)	6.0
Ground nut oil cake	11.08 (0.10)	88.92 (0.62)	42.37 (0.04)	8.39 (0.19)	3.51 (0.03)	5.82 (0.14)	28.83
Rice bran	8.22 (0.02)	91.78 (0.05)	13.86 (0.03)	0.97 (0.01)	25.32 (0.22)	29.30 (0.21)	22.33
Wheat flour	12.53 (0.37)	87.47 (0.33)	13.62 (0.43)	2.09 (0.06)	0.3 (0.07)	1.7 (0.49)	69.76
Ragi flour	13.1 (0.07)	86.9 (0.63)	5.83 (0.58)	1.3 (0.21)	2.68 (0.48)	2.7 (0.42)	74.39
Rice flour	11.37 (0.26)	88.63 (0.44)	5.17 (0.12)	0.85 (0.03)	0.2 (0)	0.72 (0.01)	81.69

Values in parenthesis indicate standard deviation

Moisture content was estimated by heating samples at 105^0C for 30 minutes and then cooling and weighing till a constant weight was obtained. Crude protein was analyzed using Kjeltec system (Tecater, 1002), fat content by Soxtech system (Tecater, 1043), fibre content using Fibretech system (Tecater, 1017). The ash content was determined by first charring the sample and then heating it in a muffle furnace at 550 ± 10^0C for 6 hours. Carbohydrate content was calculated as nitrogen free extract (NFE) by Hastings (1976) as follows:

NFE=100 - (%moisture + %crude protein + %crude fat + %crude fiber + %ash)

Three test diets, namely T_1, T_2 and T_3 were prepared by incorporating biogut at 0.5, 1.0 and 1.5% levels, respectively (Table 1). The diet without biogut supplementation served as control (T_0). The required quantities of ingredients were weighed accurately, mixed with water (1:0.8) and hand-kneaded to required consistency. The dough obtained was cooked under steam in a pressure cooker at 105^0C for 20-30 minutes. The cooked feed was cooled to room temperature rapidly by spreading on an enamel tray, then the required quantities of biogut and vitamin and mineral premix were added, mixed and blended. The dough was extruded through a 3 mm diameter pelletizer. The pellets were dried in a hot air oven at 60^0C until the moisture content was reduced to less than 10%. The dried pellets were packed separately in high density polythene bags, labeled and stored at room temperature for further use.

Experimental design. The common carp fingerlings were procured from BRP Fish Seed Farm, Shivamogga, Karnataka, India and acclimatized to laboratory conditions for 15 days. 120 common carp fingerlings were divided into four groups of 30 each, three replications per group. Each replicate tank was stocked with 10 fingerlings and the experiment was conducted for 60 days in the recirculation system. The fish were fed at 10% of their body weight twice a day. Weight and length of fish samples was recorded at 15 day intervals. After each sampling, the quantity of feed given was re-adjusted based on the weight of fish.

Water quality parameters. Water quality parameters were monitored throughout the experimental period. Required water quality was maintained by periodic partial exchange of water. Water samples collected at each sampling were analyzed for pH, temperature, dissolved oxygen, free carbon dioxide, NH_3, and total alkalinity by following standard methods (APHA, 1985).

Growth parameters. Specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), survival rate, daily weight gain, and net production of fish from all the treatments were calculated using the following formula.

$$SGR = \frac{Log\ final\ weight\ (g) - Log\ initial\ weight\ (g)}{No.of\ days} X\ 100$$

$$FCR = \frac{Dry\ weight\ of\ feed\ given\ (g)}{Gain\ in\ wet\ weight\ of\ fish\ (g)}$$

$$PER = \frac{Increment\ of\ body\ weight\ (g)}{Protein\ in\ take\ (g)}$$

$$Survival\ \% = \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} X\ 100$$

$$Daily\ gain\ = \frac{Final\ weight\ (g) - \ Initial\ weight\ (g)}{60\ days}$$

Proximate composition of fish muscle. Crude protein, crude fat, total ash and carbohydrate (NFE) were analyzed from whole flesh of the fish for proximate composition by the standard methods described.

DNA, RNA and enzyme activity in muscle tissue. DNA content (Giles and Meyers, 1965), RNA content (Ceriotti, 1955) and enzyme (protease) analysis (Lowry et al. 1951) were estimated from muscle tissue.

Statistical analysis. The mean growth of fish with different levels of biogut was analyzed statistically by variance technique (ANOVA) and differences between means was carried out to determine differences between treatments (mean at significant level of ($p<0.05$) by Duncan’s multiple range test.

Results

Growth and survival. Data on length and weight of common carp were recorded at fortnightly intervals (Table 3).

Table 3: Length (cm) and weight (g) of common carp recorded during the experimental period

Treatments	*Tank No*	*No. of days*									
		0		15		30		45		60	
T_0		L	W	L	W	L	W	L	W	L	W
	1	4.22	1.45	4.72	2.20	5.29	3.07	5.55	3.59	6.12	4.25
	2	4.32	1.45	4.55	2.15	5.22	2.91	5.73	3.57	6.28	4.15
	3	4.3	1.45	4.7	2.0	5.35	3.06	5.5	3.68	6.08	4.35
	Avg	4.28	1.45	4.65	2.12	5.286	3.01	5.593	3.61	6.16^{a}	4.25^{a}
	±SD	0.052	0	0.092	0.014	0.065	0.089	0.120	0.058	0.105	0.1
T_1	1	4.27	1.45	4.92	2.45	5.44	3.60	5.74	4.03	6.37	4.75
	2	4.28	1.45	4.77	2.3	5.39	3.33	6.34	4.20	7.13	4.95
	3	4.28	1.45	4.88	2.55	5.01	3.58	5.59	3.83	6.74	5.0
	Avg	4.276	1.45	4.856	2.43	5.28	3.50	5.89	4.02	6.746^{c}	4.90^{c}
	±SD	0.005	0	0.077	0.125	0.235	0.150	0.396	0.185	0.380	0.132
T_2	1	4.33	1.45	5.0	2.35	5.46	3.38	5.81	4.0	6.19	4.70
	2	4.34	1.45	4.69	2.25	5.24	3.97	5.81	3.93	6.37	4.60
	3	4.25	1.45	4.77	2.3	5.34	3.28	5.92	3.96	6.44	4.75
	Avg	4.306	1.45	4.82	2.3	5.346	3.54	5.84	3.96	6.33^{ab}	4.68^{b}
	±SD	0.049	0	0.160	0.05	0.110	0.372	0.063	0.035	0.128	0.076
T_3	1	4.39	1.45	4.8	2.25	5.22	3.15	5.73	3.76	6.24	4.4
	2	4.39	1.45	4.79	2.3	5.17	3.19	5.70	3.77	6.17	4.45
	3	4.31	1.45	4.92	2.45	5.13	3.19	5.42	3.83	6.27	4.55
	Avg	4.36	1.45	4.836	2.33	5.173	3.17	5.616	3.79	6.22^{ab}	4.47^{ab}
	±SD	0.046	0	0.072	0.104	0.045	0.023	0.170	0.037	0.051	0.076

Figures in the same column having same superscripts do not differ significantly ($p < 0.05$)

The highest weight gain was recorded in treatment $T_{1,}$ followed by treatments T_2, T_3 and T_0. The final average weight recorded after 60 days of treatment was 4.90g, 4.68g, 4.47g and 4.25g respectively. Final average length of common carp also followed a similar trend (Table 3). Final average length was highest in T_1 (6.74 cm), followed by T_2 (6.33 cm), T_3 (6.22 cm) and T_0 (6.16 cm). SGR of common carp fed T_1 diet (0.5% biogut) was the highest of all the treatments. During the experimental period, no mortality was observed in any of the treatment groups.

SGR, FCR, PER, survival, daily weight gain, and net production of common carp fed with biogut are presented in Table 4.

Table 4: Survival (%), SGR, FCR, PER, weight gain and net production at the end of the experimental period

Treatmnts	*Survival (%)*	*Weight gain (%)*	*SGR (%/day)*	*FCR*	*PER*	*Net production (g/tank/ 60days)*	*Daily weight gain (g/day)*
T_0	100 (0)	193.1 (5.63)	1.79 (0.04)	1.51 (0.01)	0.076 (0.005)	425	0.046 (0.0012)
T_1	100 (0)	237.92 (7.44)	2.02 (0.04)	1.41 (0.01)	0.098 (0.003)	490	0.057 (0.0016)
T_2	100 (0)	222.98 (4.29)	1.94 (0.02)	1.44 (0.01)	0.092 (0.002)	468.3	0.053 (0.0012)
T_3	100 (0)	208.04 (4.30)	1.87 (0.02)	1.47 (0.01)	0.085 (0.002)	446.6	0.049 (0.0004)

Values in parentheses indicate standard deviation.

The data show that the SGR, PER, daily weight gain and net production values were higher in all the treatment groups than the control. FCR values observed were better in probiotic supplemented diet than the control.

Proximate composition of fish muscle. The proximate compositions of common carp muscle recorded are presented in Table 5.

Table 5: Proximate composition of fish muscle recorded at the end of the experiment

Parameter	T_0	T_1	T_2	T_3
Moisture (%)	79.91	77.79	77.99	80.49
Protein (%)	12.97	14.79	14.04	13.59
Fat (%)	3.28	3.24	3.83	2.30
Ash (%)	2.37	2.72	2.66	2.10
NFE (%)	1.47	1.46	1.48	1.52

Maximum moisture content was recorded in T_3 (80.49%), while minimum was found in T_1 (77.79%). The protein content was maximum in T_1 (14.79%), followed by T_2 (14.04%), T_3 (13.59%) and T_0 (12.97%). The crude fat content was highest in T_2 (3.83%) and lowest in T_3 (2.30%); it was 3.24 and 3.28% in T_1 and T_0, respectively. Ash levels were 2.72 % in T_1, 2.66 % in T_2, 2.37 % in T_0 and 2.10 % in T_3. The highest NFE was recorded in T_2 (1.48 %) and the lowest in T_3 (1.52 %).

DNA/RNA and protease activity. Data on muscle nucleic acid content of *C. carpio* is presented in Figure 2.

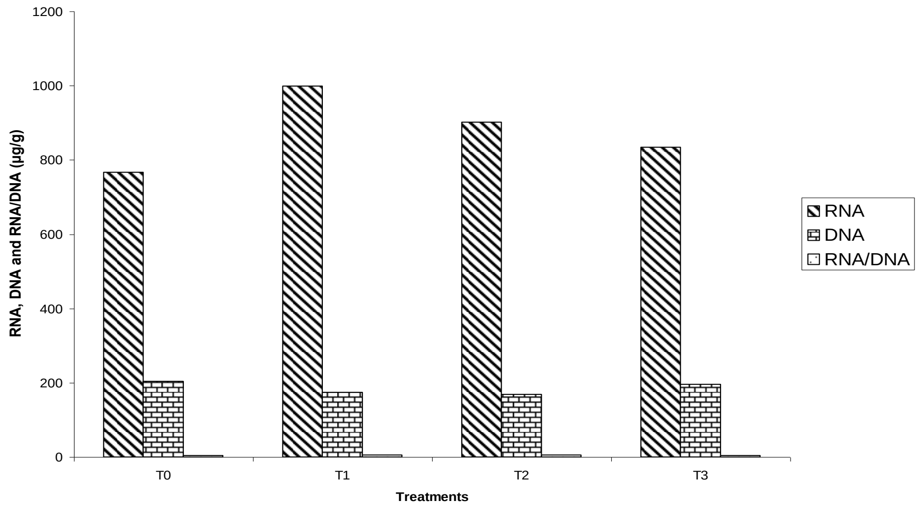

Figure 2: RNA, DNA and RNA / DNA ratio recorded in the common carp

Treatment T_1 showed the highest RNA content of 998.57µg/g, while the lowest value was recorded in T_0 (766.31 µg/g). The DNA content was highest in T_0 (203.16 µg/g) and lowest in T_2 (168.45 µg/g). Figure 3 shows the protease activity recorded in different groups. The protease activity in the intestinal tissue was highest in T_1 (0.0935 units) and lowest in T_0 (0.068 units). The values of protease activity were 0.084 and 0.076 units in T_2 and $T_{3,}$ respectively.

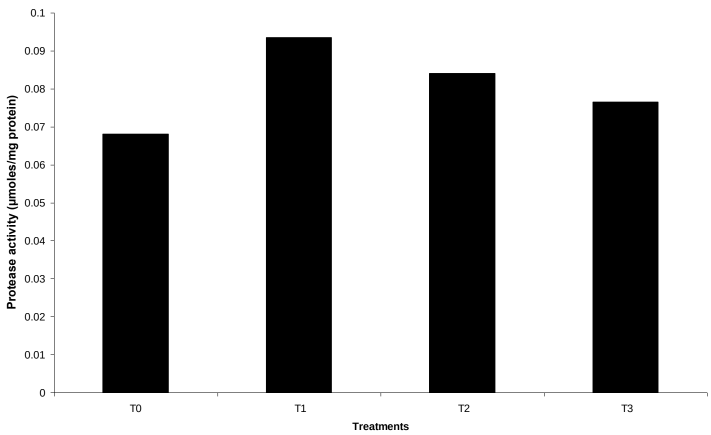

Figure 3. Protease activity recorded in the common carp.

Discussion

All three levels of biogut in the treatment diets resulted in higher growth than the control diet indicating that addition of biogut improved growth of common carp. Biogut contains a mixture of *Lactobacillus* sp., *Bacillus* sp., *Saccharomyces cervisiae,* and different enzymes; this might have improved diet digestibility resulting in better growth. The best diet digestibility occurred at the 0.5% level and it fish growth was higher than in the other treatments. It is also possible that the probiotic bacteria may have optimized the use of dietary protein. Since more than 60% of the cost of fish feed is protein, the effective utilization of protein by these bacteria might reduce feed wastage. In addition, water quality parameters recorded during the experimental period were found to be within the permissible levels supporting fish growth. The SGR and PER were higher in the treatment groups than in the control indicating improved utilization and conversion of the biogut treatment diets by the test species, *Cyprinus carpio*. Similar results were also reported in *Catla catla* (Murthy and Naik, 2000). FCR values observed were better in the biogut supplemented diets than in the control, suggesting that the addition of biogut improves feed utilization and growth of fish. Probiotics have been found to improve growth, protein efficiency ratio, and FCR in rohu *Labeo rohita* (Mohapatra et al. 2012). Probiotics increase the protease, amylase and cellulose activities in grass carp which in turn explains improved growth in fish fed diets supplemented with probiotics (Wang 2011).

In our study the highest level of protein content was found in the 0.5% biogut treated group indicating deposition of more protein and reduction in moisture content in flesh. Probiotics degrade the antinutritional factors in feed thereby improving the flesh quality of fish. A variation in protein and fat content in fish may be attributed to changes in their synthesis and deposition rate (Irianto and Austin (2002). As in biogut content *Bacillus subtilis,* this may have enhanced the protease levels resulting in feed absorption and deposition of protein as reported in *Centropomus undecimalis* (Kennedy et al. 1998). Improved carcass quality of Tilapia by deposition of more protein was due to *L. fermentum* as probiotics (Nwanna et al., 2014). Improvement in the biological value of probiotic supplemented diets enhances conversion of dietary protein to flesh in fish (Gatesoupe, 2002). Digestive enzymes are one of the most important factors influencing the efficiency of feed utilization in fish, and characterization of these enzymes provides some information regarding the digestive capacity of fish to hydrolyze carbohydrate, protein, and lipid contents of feed ingredients (Lemieux *et al*., 1999). The results showed higher levels of enzyme activity in diets containing probiotics; this might have resulted in improved growth observed with the probiotic supplemented diets. Similar results have been recorded in Tilapia and Seabass (Lara-Flores et al. 2003; Tovar-Ramírez et al. 2004).

RNA/DNA is known to provide a dependable indicator of growth trend (Buckley 1980). In the present study, optimum growth and highest RNA/DNA ratio was observed in fish fed 0.5% biogut. Higher RNA/DNA was observed in fish fed with oil supplemented diets (Bazaz and Keshavnath 1993). The role of growth promoters in enhancing RNA/DNA ratio is supported by earlier findings of Manoj Kumar (1994) and Srinivasa (2000) in common carp and rohu fed with G-probiotic. The increase in protein synthesis may be reflected in the RNA/DNA ratio, which is basically an index of growth per cell (Sable, 1974; Mustafa and Jafri, 1977; Shyama and Keshavanath, 1991). In conclusion, supplementation of biogut at 0.5% in the basal diets improved growth, weight gain, FCR, PER, net production, RNA/DNA, and digestive enzyme activity in common carp.

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