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INFLUENCE OF PROBIOTICS ON GROWTH PERFORMANCE AND DIGESTIVE ENZYME ACTIVITIES AMONG COMMON CARPS (*CYPRINUS CARPIO*)

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Abstract: The study envisaged to know the effect of probiotics on common carp (*Cyprinus carpio)* with respect to growth performance, feed utilization (feed conversion ratio - FCR, protein efficiency ratio- PER) and digestive enzyme (protease, amylase and lipase) activities. In this particular experiment the probiotics in the form of Bacillus circulans isolated from the gut of rohu was included in carp basal diets at different concentrations (2 x 102; 2 x 104; 2 x 106) per 100g feed for 60 days and fed at 3% of the body weight. Twelve aquaria with replicates for treatment and control were used. After feeding experiment, the diets supplemented with probiotics showed significantly better results of growth performance, FCR, PER than those with the basal diet (Control diet). Diet ED2 resulted in significantly better growth, a lower feed conversion ratio and a higher protein efficiency ratio than the other experimental diets. In conclusion the results indicated that the probiotics highly increased the growth performances and digestive enzyme activities and decreased FCR.

Keywords: Probiotics, Common carp, Growth performance, Protease, Amylase, Lipase.

INTRODUCTION

Effective utilization of aquatic resources is always a concern for scientific community which they would try to achieve through improving the health and growth of aquatic creatures. Adding probiotics which are the live microbial cells that beneficially affect the host animal is one such stratergy used in farm animals as well as aquatic farming. Extensive review of literature indicates the tremendous scope of probiotics utilization in farm animals. In the past, the importance of application of probiotics as growth promoter in fish farming has been discussed by several authors (Gatesoupe, 1991; Storm and Ringo, 1993; Swain et.al., 1996; Gildberg et.al., 1997).

Appropriate probiotic applications were shown to improve intestinal microbial balance, thus leading to improved food absorption (Parker, 1974; Fuller, 1989), digestive enzymes activities (Tovar-Ram´ırez et al., 2004) and reduced pathogenic problems in the

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gastrointestinal tract (Lloyd et al., 1977; Pivnick et al., 1981; Cole and Fuller, 1984; Goren et al., 1984). The digestive tract of fish contains a much larger number. of micro-organisms than the surrounding water, as many as 10^8cells g^{-1} (Ringo et.al., 1998). Gomez – Gill et.al., (2000), Riquelene et.al., (2000), and Verschuere et.al., (2000) reported a range of probiotics that can be used in aquaculture like Gram negative and Gram positive bacteria, bacteriophases, yeasts and unicellular algae. According to Gildberg et al., (1997), the main strategy of using probiotics in fish is to isolate intestinal bacteria with favourable properties from mature fish and mix it in the feed of immature fish of the same species. Further the probiotics application as the environment friendly treatments was gaining much attention (Gatesoupe, 1999) and in some papers effect of probiotics in fish was also discussed (Mohanty et al., 1993, 1996; Sharma and Bhukhar, 2000).

In the present study, an attempt has been made to isolate the potent bacteria *Bacillus* sp. from the gastro intestinal (GI) tracts of freshwater fish, *Cyprinus carpio* and *Labeo rohita* for inclusion in the experimental diets to test their efficiency in terms of growth enhancement and disease resistance.

Materials and Methods

Experimental design:

Four trials were carried out with common carp (*Cyprinus carpio*). Twelve aquaria (250 l) with three replicates for treatment and control were used. The basal diet formulation and proximate composition is shown in Table 1. These ingredients procured from the local fish market were mixed, extruded and air-dried at room temperature. Then this diet was stored at -20^0C until used.

Bacterial strains:

Bacterial strain was isolated from the intestines of ten healthy common carp fish (average weight 100 g) collected from local fish pond. These fish were starved for 24 h and the ventral surface of the fish was scrubbed with 1% iodine solution (Trust and Sparrow, 1974). Intestines from all the fish were dissected out aseptically and homogenised with 0.9% NaCl solution (10:1) (Das and Tripathi, 1991). The homogenate was used as inoculum. One ml of homogenized sample was spread on sterilized soybean-casein digest agar (Tryptone Soya Agar) plates and incubated at 37°C for 24h in duplicate. Colonies with different morphological appearance were isolated and streaked separately on tryptone soy agar (TSA) plates to check their purity. Isolated colonies were characterized and identified (Ghosh et al., 2002a). Among them, *Bacillus circulans* was selected for incorporation into diet as it was the

predominant species isolated and supposed to be a suitable bacterial species with excellent protease and moderate cellulase producing capacity.

Experimental diets:

Four isocaloric and isonitrogenous diets (approximately 32% crude protein) were prepared with similar ingredient composition (fish meal, 25%; rice bran, 30%; groundnut oil cake, 25% and fish meal (8%). The feed ingredients were finely powdered and fortified with vitamin-mineral premix. Experimental diets (ED_1, ED_2 and ED_3) were supplemented with the isolated bacterial strain of *Bacillus circulans* cells in a tryptone soya agar suspension culture at different concentrations *Viz.,* 2 x 10^2 (Diet ED_1), 2 x 10^4 (Diet ED_2) and 2 x 10^6 (Diet ED_3). The control diet (CD) was not supplemented with bacterial cells. . Before pelleting chromic oxide (1% w/v) was added to each formulated diet as an external marker And then the mixtures were passed through the pelletizer (pellet size 2.0 mm diameter). The pellets were dried at 40^0C in BOD incubator for 72 hours, and packed in air tight bags and stored in refrigerator until used. The percentage composition of ingredients and proximate composition of the diets are presented in Table 1.

Experimental design:

Healthy juveniles of the common carp (*Cyprinus carpio*) procured from the local fish farm and were fed basal feed twice daily for 2 weeks. A sample of fingerlings was sacrificed to determine the initial carcass composition. Then healthy common carps were distributed into 12 aquaria with initial stocking density of 15 carps per aquarium in the Laboratory for 60 days culture. All common carp fingerlings had similar initial weights (2.0–2.5 g). The experiment was conducted as a completely randomized design with four treatments (trials 1–3 and control) and each treatment had three replicates of 15 common carps each. Every day 50% of the water from each aquarium tank was replenished. Carps were fed twice daily at 8:00 and 18:00 with each feed and daily feeding rate was about 3% of total body weight. The daily ration was adjusted at fortnight intervals on the basis of the weight increment. Every day the diet remains of each aquarium were collected by siphoning before the second daily feeding to further analysis and minimize leaching. A daily record of feed offered and remains was kept. Fecal samples were collected by pipetting (Spyridakis et al., 1980). They were oven dried (60^0C) and analyzed for digestibility determinations. At the end of the experiments, fish from all treatments were weighed, sacrificed and treated for subsequent proximate analysis.

Chemical analyses and data collection:

Experimental diets and fecal samples were analyzed for proximate composition (AOAC,1990) as follows: moisture content by oven drying at 105^0C for 24 hours: protein (N x 6.25) by the micro-kjeldhal technique using the Kjeltec system (Tecator, Sweden): lipid by extracting the residue with 40-60^0C petroleum ether for 8 hours in a soxhlet apparatus: Crude fibre using the Fibertec system (Tecator, Sweden) and Nitrogen Free Extract (NFE) was computed by subtracting the values for crude protein, lipid, ash, fiber and moisture from 100 (Maynard, et al., 1979). Chromic oxide in the diets and in the feces was estimated spectrophotometrically (Bolin et al., 1952). The proximate analysis of the carcasses were done before initiation and after termination of the experiment following the similar procedures used for diets. Water quality parameters were monitored following methods outlined by APHA, (1995).

Growth Parameters:

Growth increment was monitored at fortnight intervals by sampling 10 specimens from each raceway. Final sampling was done after 60 days by weighing all the surviving fish from raceways. Specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE) and protein efficiency ratio (PER) were assessed according to Das et al. (1991). The ratio of RNA to DNA was used as an index to growth. To estimate DNA and RNA contents, aliquots were prepared from 100 mg of fish tissue (Munro and Fleck, 1966) and DNA and RNA contents were estimated following the methods of Burton (1956) and Markham (1955), respectively.

The daily gain (g d−1) (DG) was calculated as:

$$\frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Days}}$$

The relative gain rate (%) (RGR) used the following formula:

$$\frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{initial weight (g)}} \times 100\%$$

And the feed conversion ratio (FCR) was expressed as:

$$\frac{\text{Total feed consumption (total feed casting} - \text{total feed residue) (g)}}{\text{Total final weight (g)} - \text{total initial weight (g)} + \text{total morality weight (g)}}$$

Enzyme analysis:

Fish from each experimental set were dissected on an ice tray at the beginning and end of the experiment to study the effect of probiotics based on digestive enzyme activities. The intestine was removed to determine the digestive enzyme activities. The intestinal tissues were removed, thoroughly washed with chilled distilled water to remove blood and mesenteries, collected in ice-cooled petri dishes, weighed and cut into small pieces. 50% of homogenate was prepared with an ice-cooled 0.1M phosphate buffer (pH 7.0) and centrifuged in a refrigerated centrifuge at 2,500 rpm for 15 min. The supernatant was used for enzyme assay. α-amylase was quantitatively determined following the method described by Bernfeld (1995). Protease activity was measured according to the method described by Kunitz (1947) using Bovine Serum Albumin as the substrate. Lipase activity was estimated by Bier's (1962) titrimetric method. All enzymatic assays were conducted within 24 h after extraction.

The Apparent digestibility of nutrients was calculated according to De Silva and Anderson (1995) using the following formula:

$$\text{AD\%} = 100 - 100 \times \frac{\%\text{Cr2O3 in diet}}{\%\text{Cr2O3 in feces}} \times \frac{\%\text{nutrient in feces}}{\%\text{nutrient in diet}}$$

RESULTS:

Growth performance:

There was no obvious effect of probiotics on the water quality in the four feed treatments. Total ammonium (0–0.2 mg l–1), nitrite (0–0.1 mg l–1) and pH (7.0–7.4) were stable and within acceptable ranges (Boyd and Tucker, 1998). Cent percent survival was observed with all groups after 60 days of culture and there was no difference (P>0.05) between trials 1 and 3 treated with the probiotics and control.

The observed parameters on growth performance of fingerlings are depicted in Table 2. The highest weight gain was observed in the group fed ED_2 when compared to the fish fed other experimental diets ED_1, ED_3 and control diet (CD). Diet 2 (ED_2) resulted in significantly higher (p>0.05) growth, production, FCR, FCE and SGR followed by diet ED_1. Diets ED_1 and ED_2 resulted in better performance than the control diet (CD), but with a higher inclusion level (ED_3) resulted in poorer performance. Higher growth was recorded in terms of increment in both, weight and length of the fingerlings. Significantly higher specific growth rate was observed with more suitable condition factor in the case of probiotic incorporated diets.

On biochemical analysis, it has been observed that fish fed with experimental diet had higher protein percent in body muscles (13.12%) over the control group (11.84%) (Table 3). The carcass protein in fish fed with diets ED_1, ED_2, was higher than in those fed the control diet, but there is no significant difference between those fed with diet ED_3 and control diet. Carcass lipid content decreased with increased level of inclusion, same trend was observed even regarding ash content also. Further, RNA-DNA contents in the test specimens were measured after the termination of the experiment and increased RNA-DNA ratio in the treated groups indicates better growth increment and higher protein synthesis which could be attributed to supplementation of the probiotic bacterium (Table 4).

Enzyme activities:

After 60 days, mean digestive enzyme activities of all probiotic treatment groups (trials 1–3) were significantly higer ($P<0.05$) with that of the control group (Tab 4). The protease activity was remarkably higher ($P<0.05$) in group 2 fed with ED_2 (0.219 µmol min^{-1} mg $protein^{-1}$) and group 1 fed with ED_1 (0.195 µmol min^{-1} mg $protein^{-1}$) compared with those fed with ED_3 (0.174 µmol min^{-1} mg $protein^{-1}$) and control diets (0.142 µmol min^{-1} mg $protein^{-1}$). However, there was no difference ($P>0.05$) between trials 1 and 3 although the average value of protease activity in trial 2 presented the increasing trend. As for amylase and lipase, assays showed higher activity in trial 2 fed with ED_2 ($P<0.05$) as compared to the rest. Amylase assays revealed significantly higher activity ($P<0.05$) in trial 2 as compared to other trials and control. However, all the enzyme activities were significantly higher than in the control group, highest in ED_2 group, followed by ED_1 and ED_3.

Digestibility:

A significant difference was observed in apparent digestibility of dry matter, protein and ash among groups. The digestibility of protein and dry matter was gradually increased from fish fed CD to ED_2 and then decreased with increased level of inclusion level of bacteria from ED_2 to ED_3.

DISCUSSION:

The *Bacillus* sp used as probiotics for terrestrial livestock have telluric origins. They are not autochthonous in the gastro intestinal tract but may be active during intestinal transit (Gournier – Chatean et al., 1994). There are many reports of the isolation of *Bacillus* strains from fish. In the present study, an extracellular enzyme producing strain of *B. circulans* was isolated from the gastrointestinal tract of common carp and used as probiotic supplement in experimental diets.

All the probiotics supplemented diets resulted in growth performances and feed utilization better than that of the control basal diets (Table 2), suggesting that the addition of probiotics reduced the culture cost of common carp (*Cyprinus carpio*). Of all the diets, diets supplemented with 2 x 10^4 bacteria cells (ED_2) resulted in the best performance in terms of percent weight gain, SGR, FCR and PER and those with a higher level of inclusion did not perform better.

Improvement of the nutritional efficiency of diet observed in this experiment may be due to the probiotic effect of the bacteria used. The intestinal tract of fish fingerlings is much more simple and shorter than that of the adults (Stroband and Dabrowski, 1979), which is associated with low production of digestive enzymes. The fingerlings therefore, do not have the necessary enzymes to digest the feed at the optimal level. Exogenous enzymes extracted from probiotic bacteria might have supplied digestive enzymes and certain essential nutrients to promote better growth and survival as indicated by Douillet and Langdon (1994), who observed faster growth and higher survival in Pacific oyster fed on bacterium.

Similar results were reported by Ghosh et al. (2003) and Swain et al. (1996) in Indian carps. Noh et al. (1994) and Bogut et al. (1998) also proved that the commercial probiotics preparations of *Streptococcus faecium* improved the growth and feed efficiency of Israeli carp. Similar effects have been demonstrated in developing mammals, particularly in pigs (Bertin et al., 1997). However, the possibility of species differences, as suggested by Noh et al. (1994) and Bogut et al. (1998) cannot be ruled out. They studied the effect of supplementing Israeli carp feeds with different additives, including antibiotics, yeast (*S. cerevisiae*) and bacteria (*S. faecium*) and observed better growth response with probiotic-supplemented diets, but obtained the best growth with a bacterium, not with yeast. But their conclusion in Israeli carp was in contrast to that of in Nile tilapia (*Oreochromis niloticus*) (Lara-Flores et al., 2003).

The beneficial roles of bacterial enzyme on fish growth have been reported by Ghosh et al., (2001). The experimental evidence in this particular study, therefore, suggest that the strain of *Bacillus circulans* might have induced growth in common carp fingerlings by producing essential nutrients not present in the formulated diets, or by improving digestion by supplying digestive enzymes to the fish. Similar observations were also made by Ghosh et al., (2002, 2003) with rohu fingerlings and spawn.

Increased RNA-DNA ratios noticed in common carp fingerlings corresponding to growth increment are indicative of higher protein synthesis which could be attributed to probiotic effect of the bacterium used.

The activities of the digestive enzymes (protease & α-amylase) in the intestines of the common carp fingerlings were higher than the initial value in all the dietary treatments. Apparent digestibility of ash did not significantly change due to the bacterium supplementation. Protein and lipid digestibilities tend to be higher than the control diet in fish fed with the probiotic upto ED_2 and thereafter, it was lower. Apparent protein digestibility was positively correlated to the growth performance of the fish. Same trend was observed with lipid digestibility.

CONCLUSION

It could be concluded that the addition of probiotics in common carp basal diets has improved the growth performance, feed utilization and digestive enzyme activities. These observations clearly indicate that *B. circulans* may be used as an supplement in formulated diets for common carp fingerlings for better utilization of nutrients by increasing the endogenous level of enzymes.

Table 1: Ingredients (% dry weight) and proximate composition (on dry matter basis; n=3) of the experimental diets.

Ingredients	**%**
Rice bran	40
Ground nut oil cake	25
Fish meal	8
Soya bean oil cake	25
Vitamin-mineral premix	1
Chromic oxide	1

***Bacillus circulans* concentration (2 x**	**Control diet (CD)**	**Experimental diets**		
		ED_1	**ED_2**	**ED_3**
	No supplement	**10^2**	**10^4**	**10^6**
Proximate composition (%)				
Moisture	8.10	8.30	8.20	8.40
Dry matter	91.90	91.70	92.10	91.60

Crude protein	32.06	32.25	32.43	32.56
Crude lipid	9.75	9.52	9.97	10.10
Ash	13.50	14.28	13.78	14.65
Crude fibre	12.87	12.95	13.20	13.10
NFE	23.72	22.70	22.72	21.19
Organic matter	78.40	77.42	78.32	76.95

Table 2: growth parameters of fish fed experimental diets for 60 days. Results are means ± SD of three determinations.

Parameter	**Control diet (CD)**	**Experimental diets**		
		ED_1	**ED_2**	**ED_3**
Initial weight (g)	2.13	2.15	2.15	2.15
Final weight (g)	4.47	5.15	5.42	4.98
Average weight gain (%)	109.86	140.00	152.09	131.63
Specific growth rate (SGR)	2.29	2.65	2.86	2.44
PER	1.41	1.68	1.85	1.57
FCR	1.25	1.20	1.10	1.22
Survival rate (%)	100	100	100	100
Muscle RNA/DNA	3.62	3.95	4.29	3.77

Table 3: Proximate carcass composition (% wet weight) of common carp fingerlings fed commercial probiotic incorporated diets for 60 days.

Parameter	**Initial values**	**Control diet (CD)**	**Experimental diets**		
			ED_1	**ED_2**	**ED_3**
Protein	10.25	11.84	12.78	13.12	12.45
Lipid	5.84	6.18	6.24	6.26	6.19
Ash	3.21	3.95	4.37	5.12	4.25
Moisture	65.71	65.98	65.32	64.87	65.16
NFE	14.99	12.05	11.29	10.63	11.95

Table 4: Digestive enzyme activity (µmol product liberated min^{-1} mg $protein^{-1}$ at 37^0C) and apparent digestibility (%) of the test diets.

Diets	Amylase	Protease	Lipase	Apparent digestibility	
				Protein	Lipid
Initial values	0.865	0.115	0.005	82.30	85.14
CD	0.912	0.142	0.009	85.16	88.74
ED1	1.135	0.195	0.016	86.85	90.25
ED2	1.258	0.219	0.019	87.50	90.42
ED3	1.196	0.174	0.012	85.12	90.10

References

[1] AOAC., 1995. *Official methods of analysis*, 16th edn., Association of official Analytical chemists, USA.

[2] APHA., 1995. Standard Methods for the Examination of water and Waste water. 19th ed. Am. Water Works Assoc. and water Pollut. Control Fed., Am. Public Health Assoc., New York.

[3] Bogut, I., Milakovic, Z., Bukvic, Z., Brkic, S. and Zimmer, R., 1998. Influence of probiotic *Streptococcus faecium* M74 on growth and content of intestinal microflora in carp *Cyprinus carpio*. Czech J. Anim. Sci. 43: 231-235.

[4] Das, K.M. and Tripathi S.D., 1991. Studies on the digestive enzymes of grass carp, *Ctenopharyngodon idella* (Val.). *Aquaculture,* 92:21-32.

[5] Douillet, A and Langdon, J., 1994. Use of probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas* Thunberg). Aquaculture, 119: 25-40.

[6] Fuller, R., 1989. Probiotics in man and animals. *J. Appl. Bacteriol.,* 66:365-378.

[7] Gatesoupe, F.J., 1999. Review: The use of probiotics in aquaculture. *Aquaculture,* 180: 147-165.

[8] Ghosh, K., Sen S.K. and A.K. Ray., 2002a.Characterization of bacilli isolated from gut of rohu, *Labeo rohita*, fingerlings and its significance in digestion. *J. Appl. Aqua.* 12(3):33- 42.

[9] Ghosh, K., Sen S.K. and A.K. Ray., 2002b. Growth and survival of rohu, *Labeo rohita* (Hamilton) spawn fed diets supplemented with fish intestinal microflora. *Acta Ichthyol. Piscat.*, 32(1):83-92.

[10] Ghosh, K., Sen, S.K. and Ray, A.K., 2003. Supplementation of an isolated fish gut bacterium, *Bacillus circulance,* in formulated diets for rohu, *Labeo rohita*, fingerlings. The Israeli J. of Aquaculture. 55 (1): 13-21.

[11] Gildberg, A., Mikkelsen H., Sandaker E. and E. Ringo., 1997. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). *Hydrobiologia,* 352:279-285.

[12] Gomez- Gil, B., Roque, A. and Turnbull, J. F., 2000. The use and selection of probiotics bacteria for use in culture of larval organisms. *Aquaculture,* 191: 259-270.

[13] Gournier-Chateau N., Larpent J.P., Castellanos I. and J.L. Larpent., 1994. *Les Probiotiques en Alimentation Animale et Humaine. Technique et Documentation Lavoisier.* Paris, 192 pp.

[14] Lara-Flores, M., Olvera-Novoa, M.A., Guzm´an-M´endez, B.E., 2003. Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 216, 193–201.

[15] Noh, S.H., Han, K., Won, T.H. and Choi, Y.J., 1994. Effect of antibiotics, enzyme, yeast culture and probiotics on the growth performance of Israeli carp. Korean J. Anim. Sci., 36: 480-486.

[16] Swain, S.K., Rangacharyulu, P.V., Sarkar, S. and Das, K.M., 1996. Effect of a probiotic supplement on growth, nutrient utilisation and carcass composition in mrigal fry. *Aquaculture*, 4: 29-35.

[17] Tovar-Ram´ırez, D., Zambonino, I.J., Cahu, C., Gatesoupe, F.J., V´azquez-Ju´arez, R., 2004. Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. Aquaculture 234, 415–427.