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Effects of Probiotics and Spirulina on Survival and Growth of Juvenile Common Carp (*Cyprinus carpio*)

C. Muthu Ramakrishnan[1], M.A. Haniffa[1], M. Manohar[2], M. Dhanaraj[1], A. Jesu Arockiaraj[1]*, S. Seetharaman[1], and S.V. Arunsingh[1]

[1] *Center for Aquaculture Research and Extension (CARE), St. Xavier's College (Autonomous), Palayamkottai 627002, Tamil Nadu, India*

[2] *Department of Microbiology, K.R. College, Kovilpatti, Tamil Nadu, India*

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Abstract

A 45-day feeding trial was conducted to investigate the effects of probiotics and spirulina on survival, growth, feed conversion ratio (FCR), protein efficiency ratio (PER), and total heterotrophic microbial count in common carp (*Cyprinus carpio*). Two probiotic organisms (the bacteria *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae*) and a single cell protein (*Spirulina maximus*) were incorporated into diets at concentrations of 1%, 2%, or 3%. The control diet contained no supplement. *Spirulina maximus* at 3% produced the best and statistically significant ($p<0.05$) survival, growth (3.69±0.10 g), specific growth rate (1.27±0.02%/d), FCR (0.71±0.08), and PER (1.96±0.03). In general, *L. acidophilus* produced better growth than *S. cerevisiae*. The highest FCR (1.93±0.05) was obtained in the control. The total heterotrophic microbial count was highest in *S. cerevisiae* treatments, followed by *L. acidophilus* and *S. maximus*. The present investigation shows that incorporation of a probiotic or spirulina in diets for common carp results in increased growth rate.

Introduction

Aquaculture diets are conventionally based on expensive feedstuffs such as fish and fishmeal. Development of aquaculture will be greatly enhanced by finding alternative and less expensive ingredients. Spirulina, a freshwater microalgae of the class Cyanophyceae, is a good source of protein and energy (Harel et al., 2002). It can partially replace fishmeal protein in fish feeds and can be manipulated to produce essential amino acids, vitamins,

* Corresponding author. Present address: Bengis Center for Desert Aquaculture, Albert Katz Department of Dryland Biotechnologies, Jacob Blaustein Institute for Desert Research, Ben Gurion University of the Negev, Sede Boker Campus 84990, Israel, E-mail: murreltvl@gmail.com

natural β-carotene, and antibacterial substances of better quality and quantity. A recent study of *Synechococcus elongates* and *Spirulina rebselse* showed growth-promoting activity in ruminants (Manohar, 2005). Spirulina enhanced growth in the sturgeon, *Acipenser baeri* (Palmegiano et al., 2005).

Probiotics also can be used to replace fishmeal. Probiotics are live microbial cells that are administered to the gastrointestinal tract of the host as a feed supplement, improving its intestinal microbial balance and health (Fuller, 1989), yet effects may vary in different digestive systems. The addition of probiotics reduced culture costs of *Cyprinus carpio* (Ghosh et al., 2003) and Indian major carps (Swain et al., 1996) while studies of the commercial probiotics, *Streptococcus faeccium* and *Saccharomyces cerevisiae* (yeast), and antibiotics obtained better growth when these ingredients were included in carp feed (Noh et al., 1994; Bogut et al., 1998).

Microbial strains such as *Lactobacillus* sp. (Lara-Flores et al., 2003), *Corynebacterium divergens* (Gildberg et al., 1997), *Vibrio alginolyticus* (Austin et al., 1995), *Pseudomonas fluorescens* (Smith and Davey, 1993), *Streptococcus thermophilus* (Gatesoupe, 1991), and *S. cerevisiae* (Scholz et al., 1999) are used as biological control agents in aquaculture. They are non-pathogenic, non-toxic, and can survive in the gut and remain stable and viable for long periods under storage and field conditions. In *Penaeus monodon*, application of appropriate probiotics improved intestinal microbial balance, leading to better growth by improving food absorption and digestive enzyme activities (Surajit Das et al., 2006). Similarly, *Artemia nauplii* enriched with *Lactobacillus* resulted in better growth and survival in *Macrobrachium rosenbergii* (Babitha et al., 2006). The most important factor is that the probiotic agent be beneficial and harmless to the host.

The aim of the present study was to evaluate the effects of two probiotics (the bacteria *Lactobacillus acidophilus* and the yeast *S. cerevisiae*) and spirulina (*Spirulina maximus*) on survival and growth of juvenile common carp (*C. carpio*).

Materials and Methods

Cyprinus carpio fingerlings (4.21±0.73 g) were collected from the Fish Farmers Development Agency (FFDA) in Manimuthar, India, and transported to the Center for Aquaculture Research and Extension (CARE) in Palayamkottai, India. They were stocked in an earthen pond (12 x 10 x 3 m) and fed a commercial pellet feed (CP Aquafeed, Chennai) for 10 days of acclimatization. The acclimatized fingerlings (4.59±0.95 g) were randomly selected and distributed into 50-l plastic troughs filled with well water at a rate of 20 fingerlings per trough. Three replicates were maintained for each of the ten treatments.

Ten diets were prepared: an unenriched control diet plus nine diets containing either *L. acidophilus*, *S. cerevisiae*, or *S. maximus*, each at a concentration of 1%, 2%, or 3% (Table 1). The *L. acidophilus* and *S. cerevisiae* were obtained from the Department of Microbiology at the K.R. College of Arts and Science in Kovilpatti, India. The cultures were thoroughly checked and purified before use in the diet. The microbe concentrations in the probiotics were 10^7-10^8 colony forming units (CFU)/g. The spirulina was obtained from Parry Nutraceuticals in Chennai, India. The supplements were cultured in laboratory conditions, harvested, and maintained at -20°C until use in diet preparation. The bio-chemical analyses of the feeds were analyzed by standard methods (AOAC, 1980).

The fingerlings were fed 3% of their body weight twice a day for 45 days. Every third day, tanks were partially cleaned and water was partially changed. The temperature averaged 28±1.5°C, dissolved oxygen 7.4±0.6 mg/l, and total ammonia 0.5±0.2 mg/l. Fingerlings were weighed at 15-day intervals to determine weight gain, specific growth rate (SGR), feed conservation ratio (FCR), survival, protein efficiency ratio (PER), and feeding rate. Gut samples were well homogenized and serially diluted in aseptic conditions. Heterotrophic bacteria were counted on MRS agar plates (Himedia) for the bacteria treatments and malt extract agar plates (Himedia) for the yeast treat-

Table 1. Ingredients and proximate composition of diets.

Ingredient (%)	*All diets*									
Fishmeal	35.0									
Soybean meal	17.0									
Rice bran	11.0									
Groundnut oil cake	10.0									
Tapioca flour	10.0									
Mineral premix	1.5									
Vitamin premix	0.5									
	Control	*Bacterial probiotic*			*Yeast probiotic*			*Spirulina*		
		1%	*2%*	*3%*	*1%*	*2%*	*3%*	*1%*	*2%*	*3%*
Wheat flour	15.0	14.0	13.0	12.0	14.0	13.0	12.0	14.0	13.0	12.0
Lactobacillus acidophilus	-	1.0	2.0	3.0	-	-	-	-	-	-
Saccharomyces cerevisiae	-	-	-	-	1.0	2.0	3.0	-	-	
Spirulina maximus	-	-	-	-	-	-	-	1.0	2.0	3.0
Proximate composition (%)										
Crude protein	36.2	37.2	38.0	38.3	37.8	39.5	37.6	40.6	41.4	43.2
Crude lipid	7.6	8.1	7.2	8.2	9.3	7.5	9.2	8.1	9.1	10.1
Crude carbohydrate	21.2	20.8	21.6	20.7	19.8	21.5	21.5	18.7	20.2	22.6
Ash	8.4	9.2	8.1	8.9	9.1	8.2	9.2	8.8	9.1	11.2

ments (Pelczar et al., 1982). Concentrations are expressed in number of colony forming units (CFU)/ml.

Means and standard deviations were compared by one-way ANOVA. Duncan's multiple range test using SPSS (version 7) software was performed to find significant (p<0.05) differences in growth parameters.

Results

The fish readily accepted all ten diets. The control fish had statistically lower growth and survival than fish fed enriched diets (Table 2). All factors were best in the 3% spirulina diet while all spirulina diets performed better than the probiotic diets. There were no statistical differences between groups in carcass composition although the highest protein level was obtained with the 3% spirulina diet (Table 3). The total heterotrophic bacteria count significantly differed among treatments.

Discussion

Spirulina was an adequate and nutritious food source that increased growth in *C. carpio*. This finding corresponds well with earlier studies that showed that *Synechococcus elongates* and *Spirulina rebselse* promote growth in ruminants (Manohar, 2005) and that spirulina is a valuable feed supplement for *C. carpio* (Nandeesha et al., 1993). The spirulina-incorporated diets produced better SGR and FCR than the probiotic diets. The PER indicates that supplementing diets with

Table 2. Growth performance of *C. carpio*. Values are given in mean ± standard deviation.

	Diet									
	Control	Bacterial probiotic			Yeast probiotic			Spirulina		
		1%	2%	3%	1%	2%	3%	1%	2%	3%
Initial wt (g)	4.59±0.95^{a}	4.59±0.95^{a}	4.59±0.95^{a}	4.59±0.95^{a}	4.59±0.95^{a}	4.59±0.95^{a}	4.59±0.95^{a}	4.59±0.95^{a}	4.59±0.95^{a}	4.59±0.95^{a}
Final wt (g)	5.10±0.18^{b}	5.45±0.83^{a}	5.93±0.13^{a}	6.01±0.48^{a}	5.58±0.16^{a}	5.35±0.75^{ab}	6.25±0.26^{a}	5.79±0.92^{a}	7.05±0.74^{a}	8.28±0.81^{a}
Net wt gain (g)	0.51±0.07^{b}	0.86±0.07^{a}	1.34±0.13^{a}	1.42±0.09^{a}	0.99±0.08^{a}	0.76±0.13^{ab}	1.66±0.15^{a}	1.20±0.13^{a}	2.46±0.14^{a}	3.69±0.10^{a}
SGR (%/day)[1]	0.23±0.52^{b}	0.38±0.11^{a}	0.58±0.2^{a}	0.60±0.32^{a}	0.44±0.27^{a}	0.33±0.12^{ab}	0.68±0.31^{a}	0.51±0.22^{a}	0.95±0.02^{a}	1.31±0.02^{a}
Survival (%)[2]	76^{b}	81^{a}	85^{a}	85^{a}	80^{a}	79^{a}	87^{a}	82^{a}	95^{a}	98^{a}
FCR[3]	1.93±0.05^{b}	1.25±0.01^{a}	0.94±0.0^{a}	0.91±0.05^{a}	1.18±0.20^{a}	1.42±0.22^{a}	0.84±0.12^{a}	1.125±0.01^{a}	0.75±0.01^{a}	0.71±0.08^{a}
PER[4]	1.15±0.04^{b}	1.91±0.02^{a}	2.91±0.01^{a}	3.13±0.8^{a}	2.20±0.02^{a}	1.69±0.008^{ab}	3.62±0.9^{a}	2.59±0.02^{a}	5.13±0.0.9^{a}	7.68±1.5^{a}
Feed rate[5]	28.32	37.46	38.97	39.12	38.15	38.01	41.49	38.88	43.36	47.51

[1] Specific growth rate = 100($\log_{\text{final wt}}$ - $\log_{\text{initial wt}}$)/duration
[2] Feed conservation ratio = dry food consumed in g/wet wt gain
[3] Survival = 100(no. fish at end of experiment/no. fish stocked)
[4] Protein efficiency ratio = 100(growth in mg/protein consumed)
[5] Feed rate = total feed consumed/fish wt

Table 3. Carcass composition and gut microbial load of *Cyprinus carpio* fingerlings fed different diets (means±SD; n = 3).

		Diet									
		Control	Bacterial probiotic			Yeast probiotic			Spirulina		
	Initial		1%	2%	3%	1%	2%	3%	1%	2%	3%
Carcass composition (%)											
Protein	43.4±1.05	44.2±0.98	45.0±0.97	45.9±0.88	45.3±1.15	44.9±0.98	45.0±0.73	45.8±0.62	46.3±0.71	47.9±0.73	48.0±1.26
Lipid	10.66±0.49	11.15±0.56	11.83±0.48	11.68±0.54	11.98±0.53	10.78±0.39	10.95±0.32	11.20±0.78	11.95±0.42	12.10±0.53	12.26±0.66
Ash	9.36±0.12	10.13±0.32	10.30±0.53	10.33±0.39	10.87±0.68	9.66±0.73	9.85±0.66	10.31±0.21	10.39±0.23	13.93±0.33	13.33±0.15
Microbial load (CFU/ml)											
Initial	-	1.6±0.03^{a} x 10^{1}	1.5±0.07^{a} x 10^{2}	2.4±1.3^{a} x 10^{1}	2.7±0.51^{a} x 10^{2}	7.5±1.23^{a} x 10^{1}	5.9±1.47^{a} x 10^{2}	2.5±0.63^{a} x 10^{2}	8.3±2.71^{a} x 10^{1}	3.5±1.07^{a} x 10^{2}	7.1±2.75^{a} x 10^{2}
Final	-	1.5±0.07^{a} x 10^{2}	3.7±1.02^{a} x 10^{3}	8.2±1.58^{ab} x 10^{4}	5.3±0.12^{ab} x 10^{6}	2.1±0.75^{ab} x 10^{5}	7.3±1.84^{ab} x 10^{7}	8.1±1.38 ^{a} x 10^{7}	5.3±1.0^{a} x 10^{2}	5.5±2.4^{a} x 103	2.7±0.5^{bb} x 10^{4}

spirulina, followed by yeast and bacteria, significantly improves carp performance.

Similar results were reported by Ghosh et al (2003) for Indian carp (*Labeo rohita*), Ziaei-Nejad et al (2006) for Indian white shrimp (*Fenneropenaeus indicus*), and Lara-Flores et al. (2003) for Nile tilapia. Diets with low probiotic supplements performed more efficiently in stress situations (Ringo and Gatesoupe, 1998). In sturgeon, PER was more favorable in spirulina-based diets than in the control diet (Palmegiano et al., 2005). A similar observation was made using *Spirulina plateuris* at different levels in *C*. *carpio* (Manohar, 2005). In our study, the yeast diets performed better than the control, similar to the findings of Matty and Smith (1978) that diets containing 2%, 7%, and 10% yeast produced higher PER in rainbow trout.

The final microbial count in the carp gut was highest in the fish fed the probiotic diets. Similarly, probiotics promoted colonization of bacteria in the fish gut for a prolonged period and had the capacity to adhere and grow well *in vitro* in the intestinal mucus from turbot (Makridis et al., 2000). In our study, the total heterotrophic bacteria count was greatest in fish fed the 3% yeast diet, similar to earlier findings that dietary incorporation of *S. cerevisiae* increases the gut microflora of *C*. *carpio* (Manohar, 2005). As supplementary components in aquaculture feeds, probiotics have strong adhesive and growth abilities (Mukhopadhyay and Paul, 1996). Yeast has great potential to adhere to and colonize in the intestine of fish (Gatesoupe, 2007).

In conclusion, the incorporation of spirulina and probiotics in common carp diets improves growth performance and total heterotrophic microbial load. The spirulina diets were most effective in stimulating fish growth.

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References

AAOC, 1980. *Official Methods of Analysis*. Assoc. Official Analytical Chemists, Washington DC. pp. 30-120.

Austin B., Stuckey L.F., Robertson P.A.W., Effendi I. and D.R.W. Griffith, 1995. A probiotics strain of *Vibrio alginolyticus* effective in reducing disease caused by *Aeromonas salmonicida, V. anguillarum and V. ordalii*. *J. Fish Dis*., 18:93-96.

Babitha Rani A.M., Reddy A.K. and N.P. Sahu, 2006. Growth enhancement and survival of *Macrobrachium rosenbergii* larvae fed *Artemia* nauplii enriched with cod liver oil and/or *Lactobacillus. Israeli J. Aquac. - Bamidgeh*, 58(3):183-190.

Bogut I., Milakovic Z., Bukvic Z., Brkic S. and R. Zimmer, 1998. Influence of probiotics (*Streptococcus faecium* M74) on growth and content of intestinal micro flora in carp (*Cyprinus carpio*). *Czech. J. Anim. Sci.,* 43: 231-235.

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

Gatesoupe F.J., 1991. The effect of three strains of lactic bacteria on the production rate of rotifers, *Brachionus plicatilis*, and their dietary value for larval turbot, *Scophthalmus maximus*. *Aquaculture*, 96:335-342.

Gatesoupe F.J., 2007. Live yeasts in the gut: natural occurrence, dietary introduction and their effects on fish health and development. *Aquaculture*, 267:20-30.

Ghosh K., Sen S.K. and A.K. Ray, 2003. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, *Labeo rohita*, fingerlings. *Israeli J. Aquac. - Bamidgeh*, 55:13-21.

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

Harel M., Koven W., Lein L., Bar Y., Beherens P., Stubblefield J., Zohar Y. and A.R. Place, 2002. Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture*, 213:347-362.

Lara-Flores M., Olvera-Novoa M.A., Guzman-Mendez B.E. and W. Lopez-Madrid, 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.

Makridis P., Fjellheim A.J., Skjermo J. and O. Vadstein, 2000. Control of bacterial flora of *Brachionus plicatilis* and *Artemia franciscana* by incubation in bacterial suspensions. *Aquaculture*, 185:207-216.

Manohar M., 2005. *Probiotic and Spirulina as a Source of Immunostimulants and Growth in Common Carp*. Ph.D. thesis, Manonmaniam Sundaranar Univ., Tamilnadu, India.

Matty A.J. and P. Smith, 1978. Evolution of yeast, bacterium and an algae as a protein source for rainbow trout: effect of protein level on growth, gross conversion efficiency and protein conversion efficiency. pp. 24. In: *Symp. Fin Fish Nutr. Feed Technol.* June 20, Hamburg. E.I.F.A.C./78/symp.E/7. FAO, Rome, access no. 41385.

Mukhopadhyay P.K. and B.N. Paul, 1996. Value addition components in aquaculture feeds. *Fishing Chimes*, 16:15-16

Nandeesha M.C., De Silva S.S. and D. Krishnamurthy, 1993. Evaluation of mixed feeding schedules in two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*). pp. 753-765. In: S.J. Kaushik, P. Luquet (eds.). *Fish Nutrition in Practice*. INRA, Biarritz, Paris.

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

Palmegiano G.B., Agradi E., Forneris G., Gai F., Gasco L., Rigamonti E., Sicuro B. and I. Zoccarato, 2005. Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*). *Aquac. Res*., 36:188-195.

Pelczar M.J., Reid R.D. and E.C.S. Chan, 1982. *Microbiologia*. McGraw-Hill, Mexico. pp. 89-115.

Ringo E. and F.J. Gatesoupe, 1998. Lactic acid bacteria in fish: a review. *Aquaculture,* 160:177-203.

Scholz U., Garcia Diaz G.D. and J. Latechford, 1999. Enhancement of vibriosis resistance in juvenile *Penaeus monodon* by supplementation of diets with different yeast products. *Aquaculture*, 176:271-283.

Smith P. and S. Devey, 1993. Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress inducible furunculosis by a fluorecent pseudomonad. *J. Fish Dis*., 16:521-524.

Surajit Das, Lyla P.S. and S. Ajmal Khan, 2006. Application of streptomyces as a probiotic in the laboratory culture of *Penaeus monodon* (Fabricius). *Israeli J. Aquac. - Bamidgeh*, 58(3):198-204.

Swain S.K., Rangacharyulu P.V., Sankar S. and K.M. Das, 1996. Effect of a probiotics supplement on growth, nutrient utilization and carcass composition in mrigal fry. *J. Aquac.*, 4:29-35.

Ziaei-Nejad S., Rezaei M.H., Takami G.A., Lovett D.L., Mirva-Ghefi A.R. and M. Shakouri, 2006. The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. *Aquaculture*, 252:516-524.