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Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities

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Abstract

Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance, feed utilization (feed conversion ratio, FCR) and digestive enzyme (protease, amylase and lipase) activities was investigated. The photosynthetic bacteria and *Bacillus* sp. isolated from common carp ponds were added to carp basal diets as the probiotics in three forms: 1 g kg^{-1} lyophilized photosynthetic bacteria cells (PSB), 1 g kg^{-1} lyophilized *Bacillus* sp. (B) and their mix. Twelve aquaria with replicates for treatment and control were used. After a 60-day feeding experiment with probiotics supplemented and non-supplemented control diets, the diets supplemented with probiotics showed significantly better results of growth performance and FCR than those with the basal diet (control). Mean digestive enzyme activities of all probiotics treatment groups were significantly different ($P<0.05$) with that of the control. The protease activity was remarkably higher in the mix and *Bacillus* sp. compared with PSB and control. However, there was no difference between the mix and *Bacillus* sp. As for amylase and lipase, assays showed higher activity in the mix as compared to the rest. In conclusion, it showed that probiotics highly increased the growth performances and digestive enzyme activities, and decreased FCR. Furthermore, different probiotics forms indicated different performances and the mix produced the best results.

Keywords: Probiotics; Common carp; Growth performance; Protease; Amylase; Lipase

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1. Introduction

Appropriate probiotic applications were shown to improve intestinal microbial balance, thus leading to improved food absorption (Parker, 1974; Fuller, 1989), digestive enzymes activities (Tovar-Ramírez et al., 2004) and reduced pathogenic problems in the gastrointestinal tract (Lloyd et al., 1977; Pivnick et al., 1981; Cole and Fuller, 1984; Goren et al., 1984). With some trials, growth promotion was clearly demonstrated in poultry (Alder and Damassa, 1980) and pigs (Pollman et al., 1980) compared with control groups. Those results were most promising and gave confidence that further improvements in probiotic applications were possible. The application of probiotics in aquaculture as the environment friendly treatments was also increasing rapidly (Gatesoupe, 1999) and some papers were associated with the effect of probiotics in fish (Mohanty et al., 1993, 1996; Sharma and Bhukhar, 2000).

In human and agriculture application, probiotics research had enjoyed much more attention through history and several modes of action had been supported by unambiguous experimental data (Fuller, 1989). It was clear that the experience obtained with terrestrial animals has been used in aquaculture, especially with regard to the use of lactic acid bacteria. The modes of action were as follows: production of inhibitory compounds; competition for chemicals or available energy; competition for adhesion sites; enhancement of the immune response; improvement of water quality; interaction with phytoplankton; source of macro- and micronutrients; enzymatic contribution to digestion (Verschuere et al., 2000). And there were some papers associated with several modes of probiotics action (Fredrickson and Stephanopoulos, 1981; Lemos et al., 1991; Pybus et al., 1994; Montes and Pugh, 1993; Söderhall and Cerenius, 1998; Fukami et al., 1997). However, little had been done to incorporate probiotics into common carp (*Cyprinus carpio*) based on growth performances and digestive enzyme activities. Thus, this study was designed to evaluate the use of photosynthetic bacteria, *Bacillus* sp. and their mix, as probiotics supplements in diets for common carp (*Cyprinus carpio*), which was one of the most valuable freshwater fish species in Chinese.

2. Materials and methods

2.1. Bacteria strain

The photosynthetic bacteria and *Bacillus* sp. were isolated from the pond of common carp in Zhejiang province of China. They were preserved at the Aquaculture Department of Animal Science College, Zhejiang University, Hangzhou, China. We routinely checked its purity during this investigation. Three forms of bacteria were included in three common carp formulations, including photosynthetic bacteria, *Bacillus* sp. and their mixture. Fresh cells including photosynthetic and *Bacillus* sp. ($\sim 10^{10}$ and 10^{11} colony forming units (CFU) per 1 g of cells wet weight, respectively) were harvested and maintained at $-20\,^{\circ}$C prior to use. Cell aliquots were freeze-dried using a lyophilizer (Virtis Advantage EL, Serial No.: 215009, SP Industries Company, USA) and kept in a sterilized container at $-20\,^{\circ}$C before use.

Table 1
Formulation and proximate composition of experimental diets

Ingredients	%	Proximate composition	% Wet weight
Casein	32	Crude protein	36.44
Gelatin	8	Crude fat	3.52
Dextrine	28	Crude ash	12.74
Cellulose	19	Gross energy (MJ kg^{-1})	15.15
Fat[a]	6	Moisture	6.68
Carboxy methyl cellulose	2		
Mineral premix[b]	4		
Vitamin premix[b]	1		

[a] The mixture of fish oil and lard (1:1).
[b] Lovell (1989, 1998).

2.2. Diets and 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. Ingredient and chemical composition of the basal diets used in the experiment were according to Lovell (1989, 1998). The basal diet formulation and proximate composition is shown in Table 1. These ingredients were mixed, extruded and air-dried at room temperature. Then this diet was kept at −20 °C until used. All ingredients and chemicals used were purchased from Sangon and East China Pharmaceuticals Company, Shanghai, China.

In trial 1, experimental basal diets of three aquaria contained 1 g kg^{-1} lyophilized photosynthetic bacteria cells (PSB) as probiotics by wet weight. Trial 2 including three aquaria was treated with lyophilized *Bacillus* sp. (B) as probiotics and the concentration of supplement was also 0.1% by wet weight. In trial 3, the mix including 0.1% lyophilized photosynthetic bacteria and 0.1% *Bacillus* sp. by wet weight was added to the basal diets. The basal diet and probiotics were mixed at the moment of feeding. The other three aquaria served as the control and treated with only basal diets, which were absent probiotics. Table 2 shows the experimental design.

Healthy juveniles of the common carp (*Cyprinus carpio*) provided by the Fish Hatchery of Hangzhou, China were accalimatised in two concrete tanks (each measuring 400 cm × 150 cm × 100 cm), and were fed basal feed two times daily for 2 weeks. Then healthy common carps were distributed into 12 aquaria with initial stocking density of 10 carps per aquarium in the Laboratory of Aquaculture Department, Zhejiang University for 60 days culture. All common carp had similar initial weights (5.9–7.1 g). The experiment

Table 2
The experimental design

Group/treatment	Trial 1	Trial 2	Trial 3	Control
Probiotics category	PSB[a]	B[b]	PSB + B	–
Additive quantity (g kg^{-1})	1.0	1.0	1.0 + 1.0	0.0

[a] PSB = photosynthetic bacteria.
[b] B = *Bacillus* sp.

was conducted as a completely randomized design with four treatments (trials 1–3 and control). Each treatment had three replicates of 10 common carps each.

Carps were fed three times daily at 6:00, 12:00 and 18:00 with each feed. Daily feeding rate was about 3% of total body weight and properly regulated according to actually intake of common carps. 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 was kept of feed offered and remains. Every third day, each aquarium was partially cleaned including the common carp feces and the water partially changed (about 50%).

The aquaria were supplied with running fresh water which had been filtered through the special cotton filter (flow rate: 1 l min^{-1}), then passed successively through a tungsten heater and degassing column packed with plastic rings (Zhenhua Electric Industrial Co., Ltd., China). Temperature range of aquaria water was 24–26 °C. The air-condition was installed in the environment-controlled laboratory maintained at 28 °C, with a photoperiod of 12 h light and 12 h darkness. For water quality control, temperature and dissolved oxygen (DO) were measured daily, and weekly analyses were done of total ammonium, nitrite and pH levels using the Hach kit model DREL 2400 (Hach Company, Colorado, USA). Dissolved oxygen level was maintained above 6 mg l^{-1} by setting the air pump (ADP-2200, Jinlai Pump Factory, China).

2.3. Sampling and analytical methods

The proximate composition including crude protein, crude fat, crude ash, gross energy and moisture of basal diets was determined using the standard procedures of China according to Zhang and Zhu (1998). Crude protein was determined using the Kjeltec Analyzer Unit (2300, Sweden) and crude fat was measured using the Soxtec Auto Extraction Unit (2050, Sweden). Gross energy was determined with an adiabatic bomb calorimeter (PARR 1281, USA). Weights of all collected common carps from each aquarium were determined at initial and the end during the 60 days experiment, which treated as initial weight and final weight, respectively. At the same time, common carp survival was also determined by counting the individuals in each aquarium. The daily gain (g d^{-1}) (DG) was calculated as:

$$\frac{\text{final weight (g)} - \text{initial weight (g)}}{60\,\text{d}}$$

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:

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

For enzymatic analysis, six common carps starved for 24 h were collected from each aquarium at the end of the trials and anesthetized in diluted MS-222 (ethyl 3-aminobenzoate methanesulfonate, Tricaine; Sigma) (1:2500) in order to study the effect of probiotics based on digestive enzyme activities. Dissection produced a crude mixture of intestine of each

segment by operating at 4 °C following the method of Huang et al. (1996, 1999). The samples of intestines were separated and rinsed with cold distilled water. Total intestine content was then homogenized in phosphate buffer (pH 7.5; PBS) (1 g per 10 ml) using a hand held glass homogenizer at 4 °C. The homogenate was then centrifuged at 4 °C at 15 000 × g for 15 min. The supernatant was then stored at 4 °C prior to analysis. All enzymatic assays were conducted within 24 h after extraction.

Total protein content of supernatant was assayed according to Bradford (1976) using bovine albumin as a standard. Protease activity was evaluated according to Lowry et al. (1951) using Folin-phenol reagent and amylase activity was measured according to Jiang (1982) and Worthington (1993) using iodine solution to reveal non-hydrolyzed starch. Lipase activity was determined based on measurement of fatty acids release due to enzymatic hydrolysis of triglicerides in stabilized emulsion of olive oil (Borlongan, 1990; Jin, 1995). Enzyme activities including protease and amylase were both expressed as specific activity (U mg^{-1} protein) and lipase activity was expressed as U g^{-1} intestine content.

Statistical analysis using one-way ANOVA (Ming, 2002; Statistical Analysis System, SAS, Version 6.03) was performed to find significant difference on various parameters between treated and control trials. A significance level of $P<0.05$ was used.

3. Results

3.1. 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). The common carp survival rate of all the feed treatments was 100% after 60 days culture and there was no different ($P>0.05$) between trials 1 and 3 treated with the probiotics and control.

Data on growth performances and feed utilization including initial weight, final weight, daily gain (DG), relative gain rate (RGR) and feed conversion ratio (FCR) were reported in Table 3. At the beginning, no significant difference was observed in the initial weight between trials 1–3 and control ($P>0.05$). However, the mean final weight of the control

Table 3
Growth performances and feed utilization of common carp fed with (trials 1–3) or without (control) diets supplemented with probiotics

Group/treatment	Trial 1	Trial 2	Trial 3	Control
Initial weight (g)	6.47 ± 0.29a	6.49 ± 0.24a	6.46 ± 0.22a	6.48 ± 0.30a
Final weight (g)	11.48 ± 0.54b,c	11.44 ± 0.44b	11.67 ± 0.36c	9.78 ± 0.48a
DG (g d^{-1})	0.0835 ± 0.0056b	0.0824 ± 0.0038b	0.0868 ± 0.0029c	0.0550 ± 0.0076a
RGR (%)	0.7746 ± 0.0462b	0.7619 ± 0.0231b	0.8068 ± 0.0226c	0.5109 ± 0.0776a
FCR	2.27 ± 0.05b	2.16 ± 0.03c	2.11 ± 0.06c	2.46 ± 0.08a

DG, daily gain; RGR, relative gain rate; FCR, feed conversion ratio. Results relating to growth performances of common carp including initial weight, final weight, DG, RGR and FCR were presented as means ± S.E. of triplicate observations. Total number of common carp per aquarium was 10 ($n = 30$ per treatment). Means in the same row with different letters were significantly different ($P<0.05$).

(9.78 ± 0.48 g) was lower (P<0.05) than that of the trials 1–3 (11.48 ± 0.54, 11.44 ± 0.44 and 11.67 ± 0.36, respectively).

The values of daily gain (DG) and relative gain rate (RGR) in trial 3 treated with the probiotics mix were significantly higher than the rest (P<0.05). Means of the DG and RGR were not significant (P>0.05) in trial 1 treated with 1 g kg^{-1} photosynthetic bacteria cells (PSB) compared with trial 2 treated with 1 g kg^{-1} *Bacillus* sp. (B). The addition of probiotics to diets also produced the best FCR, with values statistically better than the control (P<0.05) and no difference in FCR between trials 2 and 3 was observed (Table 3). It showed that the probiotics highly increased the growth performances and decreased the feed conversion ratio (FCR). Furthermore, different probiotics indicated different performances and the probiotics mix produced the best results.

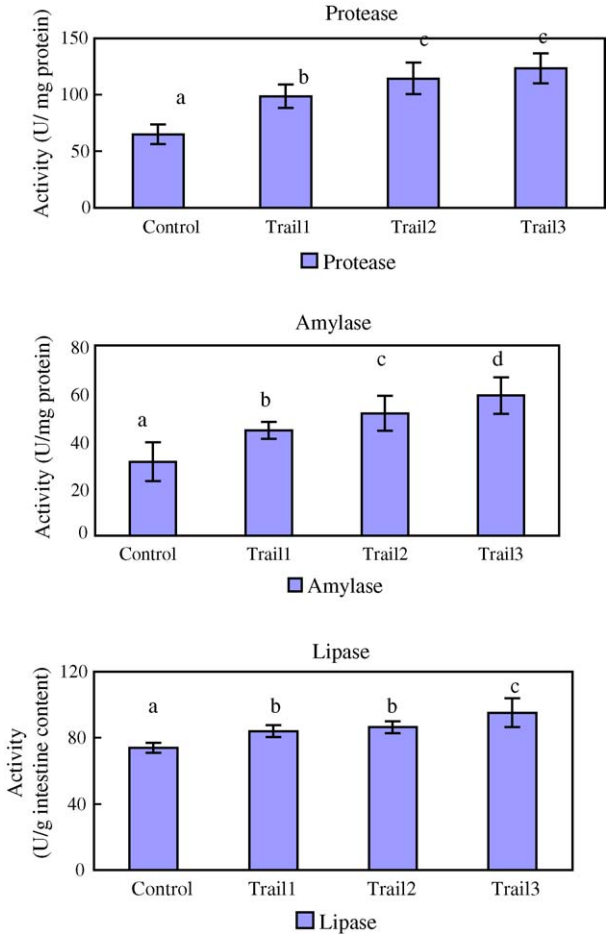

Fig. 1. Specific activity of protease, amylase and lipase in intestine content of common carp fed a basal diet (control) and three diets containing probiotics (trials 1–3) at the end of the 60 days culture. Means with different superscript were significantly different (P<0.05).

3.2. Enzyme activities

After 60 days, mean digestive enzyme activities of all probiotics treatment groups (trials 1–3) were significantly different ($P<0.05$) with that of the control (Fig. 1).

The protease activity was remarkably higher ($P<0.05$) in trial 3 (123.54 ± 13.44 U mg^{-1} protein) and trial 2 (114.50 ± 13.90 U mg^{-1} protein) compared with trial 1 (98.82 ± 10.38 U mg^{-1} protein) and control (65.01 ± 8.77 U mg^{-1} protein). However, there was no difference ($P>0.05$) between trials 3 and 2 although the average value of protease activity in trial 3 presented the trend of increase. As for amylase and lipase, assays showed higher activity in trial 3 ($P<0.05$) as compared to the rest. Amylase assays revealed significantly higher activity ($P<0.05$) for this enzyme in trial 2 as compared to trial 1 and control. However, although lipase in trial 2 treated with lyophilized *Bacillus* sp. had a relatively higher activity, there was not significantly different ($P>0.05$) from that of trial 1 treated with lyophilized photosynthetic bacteria cells (Fig. 1).

4. Discussion

All the probiotics supplemented diets resulted in growth performances and feed utilization better than that of the control basal diets (Table 3), suggesting that the addition of probiotics reduced the culture cost of common carp (*Cyprinus carpio*). Similar results were observed 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. These effects have been demonstrated also in developing mammals, particularly in pigs (Bertin et al., 1997). It is also necessary, however, to consider the possibility of species differences, as suggested by Noh et al. (1994) and Bogut et al. (1998). 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 a yeast. But their conclusion in Israeli carp was in contrast to that of in Nile tilapia (*Oreochromis niloticus*) (Lara-Flores et al., 2003). According to present study, the results indicated that there exited definite difference in various probiotics species. Although the diet appended photosynthetic bacteria as probiotics (trial 1) produced higher values of final weight, DG and RGR than trial 2 fed diet containing *Bacillus* sp. as probiotics, there was no significant difference ($P>0.05$) between them (Table 3). However, trial 3 treated with mix probiotics including photosynthetic bacteria and *Bacillus* sp. obtained the best DG and RGR, which showed both of them could promote each other.

Moriarty (1998) noted an increase of prawn survival in ponds where probiotics including some strains of *Bacillus* sp. were introduced. Rengpipat et al. (1998) also showed the effects of a probiotic bacterium on black tiger shrimp (*Penaeus monodon*) survival and had the similar results. Currently, the use of the probiotics including photosynthetic bacteria, *Bacillus* sp. and their mixture in aquaria had shown inconsistent results. In contrast, the use of the probiotics mixture in the basal diets (trial 3) at determinate density caused no significant survival increases when compared to the control and other treatments (trials 1

and 2). The common carp survival rate of all the feed treatments was 100% after 60 days culture in this study. This result might be explained by the fine experimental condition and the bigger experimental animals used in this study in contrast to theirs.

The better FCR values observed with probiotic-supplemented diets suggested that addition of probiotics improved feed utilization of common carps. Similar results had been reported for probiotics use in diets for weaned piglets (Close, 2000; Matijasic et al., 2004) and Nile tilapia (*Oreochromis niloticus*) (Lara-Flores et al., 2003). In practical terms, this meant that probiotics use could decrease the amount of feed necessary for animal growth which could result in production cost reductions.

Moreover, a marked effect on digestive enzyme activities of common carp was noted (Fig. 1). The better enzyme activities obtained with the supplemented diets suggested that the addition of probiotics improved diet digestibility including protein, starch and fattiness, which might in turn explain the better growth performances and feed efficiency (FCR) seen with the supplemented diets. Similar effects had been reported for other fishes in which digestibility was shown to increase considerably with the use of a probiotic in the diet (Tovar-Ramírez et al., 2004; Lara-Flores et al., 2003).

Results of this study also showed that different probiotics could cause the different effect on enzyme activities. And the mix probiotics with lyophilized photosynthetic bacteria and *Bacillus* sp. produced a relatively better result than the others. Data on protease, amylase and lipase activities in trial 2 treated with *Bacillus* sp. showed higher results than trial 1 treated with photosynthetic bacteria (Fig. 1). This indicated that *Bacillus* sp. more significantly increased the digestive ability of common carp, which might explain the FCR of trial 2 was better than that of trial 1.

It could be concluded that the addition of probiotics in common carp basal diets improved growth performances, feed utilization and digestive enzyme activities. The different bacterial strains used in the present study as probiotics were effective in stimulating fish performance, though the mixture bacteria produced the best results. Based on these results, use of a 2 g kg^{-1} supplement of probiotics (1 g kg^{-1} photosynthetic bacteria and 1 g kg^{-1} *Bacillus* sp.) in common carp diet was recommended to stimulate productive performance.

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