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EFFECT OF *BACILLUS* SPP. PROBIOTIC ON GROWTH AND FEEDING PERFORMANCE OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) LARVAE

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Summary

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In this study, we investigated the influence of probiotic supplementation with *Bacillus* spp. on growth parameters and feeding performance of developing rainbow trout (*Oncorhynchus mykiss*) larvae. A commercial preparation of five species of *Bacillus* (Protexin Aquatic Company, London, England) were added to a blend of *Daphnia* meal (50%) and commercial diet (50%) at four concentrations: 1×10^5 bacterial CFU/g (diet D1), 2×10^5 bacterial CFU/g (diet D2), 3×10^5 bacterial CFU/g (diet D3), 4×10^5 bacterial CFU/g (diet D4). The control diet (RD) was not supplemented with the bacteria. The trout larvae of average weight 726±125 mg were fed on 10 percentage of their body weight, 6 times a day for 30 days. The results showed that in all experimental treatments, the growth and feeding parameters significantly increased in comparison to control treatment ($P<0.05$). Diet D2 resulted in significantly better growth, lower feed conversion ratio (FCR) and higher protein efficiency ratio than the control and other experimental treatments. No significant difference among other treatments was found.

Key words: *Bacillus* probiotic, commercial diet, *Daphnia*, feeding performance, growth, rainbow trout larvae

INTRODUCTION

The term probiotic has been defined by Verschuere *et al.* (2000) as a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by insuring improved use of the feed or enhancing its nutritional value. However probiotics are usually defined as live microbial feed supplements that are administered in such a way as to enter the gastrointestinal tract and remain alive, benefiting the host animal (Gatesoupe, 1999). The use of probiotics has a long tradition of widespread use in animal husbandry (Stavric & Kornegay, 1995), but little has been done to incorporate this method into aquaculture (Lara-Flores *et al.*, 2003). The main strategy in use today is supplementation of the probiotic bacteria into the feed of immature fishes, in order to increase the growth parameters (Ghosh *et al.*, 2003). The positive effect of probiotics depends on both the action mechanisms and the capacity of colonisation, that is to say, its ability to reach, remain, or reproduce in the place where the effect is required (Planas *et al.*, 2004). The application of probiotics in aquaculture is increasing rapidly (Gatesoupe, 1999) and some papers associated with the effect of probiotics in fish have been published (Sharma & Bhukhar, 2000). The appropriate use of probiotics in the aquaculture industry has been shown to improve intestinal microbial balance, and also to improve feed absorption, thus leading to increased growth rate (Rengpipat *et al.*, 1998; Fuller, 1989) and reduced feed conversion ratio (FCR) during the cultural period (Wang *et al.*, 2005).

Bacillus spp. are Gram-positive, spore forming bacteria, commonly used commercially as probiotics. *Bacillus* can act positively on cultured organisms by enhancing survival and growth (Gomez-Gil *et al.*, 2000). Many studies indicate that growth performance and feeding efficiency of fish larvae were promoted by the use of probiotic *Bacillus* spp. The sum of positive effects of probiotics on fish larvae by bioencapsulation of live food (Gatesoupe, 1991; Makriedis *et al.*, 2001) and supplementation by diets (Ghosh *et al.*, 2003; Lara-Flores *et al.*, 2003) have been studied. Ghosh *et al.* (2002) indicated that *Bacillus circulans*, *B. pumilus* and *B. cereus* had an important role in the feeding efficiency of cultivation fishes. Many probiotic bacteria have been proposed to improve growth performance and health in rainbow trout. *Daphnia magna* is one of the most important live food sources for fish, and is well-studied by fish nutritionists around the world. The use of *Daphnia* meal as possible feed substitute to reduce the cost of fish feed is employed in this research.

The present study was designed to evaluate the influence of dietary supplementation of an endospore *Bacillus* as probiotic in four isonitrogenous diets for rainbow trout (*Oncorhynchus mykiss*) larvae.

MATERIALS AND METHODS

Bacillus preparation

The probiotic *Bacillus* spp. was obtained from the Protexin commercial product (Iran- Nikotak), which was a blend of five *Bacillus* species. The blend of probiotic bacilli (*B. licheniformis, B. subtilis, B. circulans, B. laterosporus* and *B. polymyxa*) from suspension of spores with special growth media was provided. The spores of bacteria were grown in their special media at 37 ºC for 8 h. Four concentrations of bacterial suspensions, 1×10^5, 2×10^5, 3×10^5 and 4×10^5 CFU.mL^{-1} were provided. The colony forming unit (CFU) of *Bacillus* sp. were tested by microbial culture in Tryptic Soy Agar (TSA) (Rengpipat *et al.,* 1998).

Supplementation of diet and Daphnia meal with Bacillus spp. blend

The *Daphnia* species used in this study was *Daphnia magna*, which we obtained from Shadid Marjani Sturgeon fish hatchery (Agh-ghala, Golestan, Iran). Five isocaloric and isonitrogenous diets (labelled with D letter) were prepared containing similar ingredient composition blend of commercial diet (50%) and *Daphnia magna* meal (50%) (Table 1). Experimental diets (D1–D4) were supplemented with probiotic at four different concentrations: 1×10^5 (diet D1), 2×10^5 (diet D2), 3×10^5 (diet D3), 4×10^5 (diet D4) CFU/g. A control diet (without supplementation) was also prepared. The experimental diets were mixed with designed quantities of bacterial cells in broth suspension culture.

Table 1. Approximate composition of *Daphnia* and commercial diets

Approximate composition	*Daphnia*	Commercial diet
Protein (%)	39.68±2.14	54.00
Lipid (%)	24.99±3.70	18.00
Dry matter (%)	4.99±0.25	90.67
Moisture (%)	95.01±7.45	2.60
Ash (%)	28.15±2.60	9.70

The homogenous mixtures of different diets were spread over aluminum foil and dried at 40 ºC in a hot air oven. The dried mixture was powdered and sieved to obtain micro pellets (Ghosh *et al.*, 2003).

Experimental design

Forty-five rainbow trout larvae with initial body weight of 726.7±125.24 mg were obtained from a local cold water hatchery (Golestan, Iran). Fish larvae were acclimatised to laboratory conditions for five days and fed a commercial diet (French Co.). This experiment was conducted in a completely randomised design with five treatments (trials 1–4 and control) replicated three times for a total of 15 circular fiberglass tanks (each with a capacity of 13 L). The trout larvae were fed on the base of the 10 percent of their body weight 6 times per day (2.00, 6.00, 10.00 AM; 2.00, 6.00, 10.00 PM) for a period of 30 days.

Water quality measurements

The water quality parameters of each tank were monitored each week throughout the experimental period. The water quality parameters were: temperature 19.5±0.45 ºC, pH 7.61±0.18, salinity 4.65±0.35 ppt and electrical conductivity 7589.4±496.32 mmhos.cm^{-1}. Dissolved oxygen level was maintained above 8.2± 0.5 mg/L by using an air pump. The water flow in each tank was 0.6 L.min^{-1}.

Fish sampling

The fish larvae were weighed individually at the start and the end of the experiment. At the beginning and end of study, all larvae were seined from each tank and anaesthetised with *Eugenia caryoplyllatathen* extract. The fish larvae were weighed by digital scales with precision of 0.1 mg and total length was measured with a caliper to 0.1 mm.

Calculations and statistical analysis

The growth performance was calculated using mathematical growth models as followed:

$$SGR\ (\%/day) = 100\times [(LnW_f - LnW_i)/ T]$$

$$FCR = TFI/ (FB - IB)$$

$$FCE\ (\%) = [(FB - IB)/TFI] \times 100$$

$$CF = 100 \times (W/TL^3)$$

$$TGC\ (\%)=[FB^{0.333}-IB^{0.333}/\sum^{oC}{}_{(day\text{-}degrees)}] \times 100$$

$$ADG = 100\times [(W_f - W_i)/(W_i) \times T]$$

$$RFI = 100\times[(TFI)/0.5(W_f - W_i)\times T]\times 100$$

$$PER = (FB - IB)/PI$$

$$LER= (FB - IB)/LI$$

where: SGR = specific growth rate; FCR = feed conversion ratio; FCE = food conversion efficiency; CF = condition factor; TGC = thermal growth coefficient; ADG = average daily growth; RFI = relative food intake; PER = protein efficiency ratio; LER = lipid efficiency ratio; W_f and W_i = final and initial body weight (g); T = time of rearing (days); TL = total length (cm); FB and IB = final and initial fish biomass (g); TFI = total feed intake (g), $\sum^{oC}_{(day\text{-}degrees)}$ = daily mean temperature; PI = protein intake (g); LI = lipid intake (g).

Results were analysed using one-way ANOVA followed by Duncan's multiple range tests to examine which of them varied significantly (SPSS v. 19 software). In all statistical tests, $P \leq 0.05$ was taken as level of significance.

RESULTS

The effects of probiotic supplementation of *Daphnia* meal and commercial diet on the growth performance of rainbow trout (*Oncorhynchus mykiss*) are presented in Table 2. The growth parameters were significantly changed in experimental trials fed with a diet supplemented by suspension probiotics ($P<0.05$) when compared to the controls. Weight gain and growth rate of trout larvae, were positively affected by bacterial supplementation in experimental treatments ($P<0.05$, Table 2). The maximum body weight was observed with D2 ration (2×10^5 CFU/g). The specific growth rate (SGR) was significantly higher in the experimental treatments when compared to the controls ($P<0.05$). However, no significant difference in experimental treatments and control were obtained for condition factor (CF). Total growth gain (TGN) and average daily growth (DG) were significantly higher in the experimental treatments when compared with the control ($P<0.05$). The *Bacillus* spp. probiotic decreased the relative food intake (RFI) of rainbow trout larvae fed diet D2 in comparison with the control diet ($P<0.05$). However, the best results of growth obtained in fish larvae which were fed experimental trial by diet supplemented with 2×10^5 *Bacillus* per litre.

The probiotic effects on rainbow trout (*Oncorhynchus mykiss*) larvae feed utilisation is presented in Table 3. The results showed that in all experimental treatments, the feeding parameters were significantly increased ($P<0.05$). The use of probiotics significantly increased the growth performance of rainbow trout larvae in comparison with the control subjects ($P<0.05$). This was particularly true for food conversion efficiency (FCE), for which the highest value was obtained with the experimental D2 diet of 2×10^5 CFU/g (62.83). It should be noted that food conversion ratio (FCR) in the experimental treatments was nearly half that of the control treatment ($P<0.05$). The maximum protein efficiency ratio and lipid efficiency ratio (0.90 and 3.22, respectively) were obtained when rainbow trout larvae were fed on 2×10^5 CFU/g (diet D2). There was no significant difference in growth parameters between D1, D3 and D4 ($P>0.05$).

Overall, the analysis of results showed that the best growth performance and feed utilisation were observed with the experimental D2 diet containing 2×10^5 CFU/g. A positive significant correlation between the concentration of *Bacillus* spp. in experimental diets and body weight gain ($r=0.515$, $P<0.05$) was obtained. There were also positive significant correlations between the different concentrations of probiotic supplementation and some growth parameters and feeding performance parameters, including SGR, FCE, PER and LER (Fig. 1). In contrast, relative food intake (RFI) was significantly negatively correlated with the concentration of *Bacillus* in experimental diets (Fig. 1) ($P<0.05$). The results clearly showed that *Bacillus* spp. had beneficial effects on the food conversion ratio (FCR)

Table 2. Growth parameters in rainbow trout larvae fed diets with different levels of *Bacillus* spp. probiotic (mean±SD; n=45)

	Diets				
	RD (non-supplemented)	D1 (1×10^5 CFU/g)	D2 (2×10^5 CFU/g)	D3 (3×10^5 CFU/g)	D4 (4×10^5 CFU/g)
Initial body weight (mg)	726.7±125.24	726.7±125.24	726.7±125.24	726.7±125.24	726.7±125.24
Final body weight (g)	2.23 ± 0.04^{b}	2.32 ± 0.08^{ab}	2.44 ± 0.17^{a}	2.35 ± 0.09^{ab}	2.36 ± 0.06^{ab}
Specific growth rate	3.41 ± 0.49^{b}	3.53 ± 0.1^{ab}	3.68 ± 0.27^{a}	3.57 ± 0.13^{ab}	3.58 ± 0.1^{ab}
Condition factor	1.11 ± 0.07^{a}	1.16 ± 0.114^{a}	1.21 ± 0.12^{a}	1.15 ± 0.05^{a}	1.12 ± 0.03^{a}
Thermal growth coefficient	0.64 ± 0.01^{b}	0.66 ± 0.02^{ab}	0.70 ± 0.05^{a}	0.67 ± 0.03^{ab}	0.67 ± 0.02^{ab}
Average daily growth	6.28 ± 0.15^{b}	6.64 ± 0.32^{ab}	7.16 ± 0.7^{a}	6.80 ± 0.44^{ab}	6.83 ± 0.35^{ab}
Relative food intake (%)	15.65 ± 0.38^{a}	14.81 ± 0.72^{ab}	13.83 ± 1.37^{b}	14.50 ± 0.86^{ab}	14.41 ± 0.73^{ab}

Groups with different superscripts differ significantly at $P<0.05$ (ANOVA).

Table 3. Feed utilisation parameters in rainbow trout larvae fed diets with different levels of *Bacillus* spp. probiotic (mean±SD; n=45)

	Diets				
	RD (non-supplemented)	D1 (1×10^5 CFU/g)	D2 (2×10^5 CFU/g)	D3 (3×10^5 CFU/g)	D4 (4×10^5 CFU/g)
Feed conversion ratio	1.74 ± 0.29^{a}	1.68 ± 0.56^{ab}	1.59 ± 0.11^{b}	1.65 ± 0.07^{ab}	1.64 ± 0.58^{ab}
Food conversion efficiency	57.43 ± 0.94^{b}	59.68 ± 1.98^{ab}	62.83 ± 4.36^{a}	60.62 ± 2.72^{ab}	60.82 ± 2.17^{ab}
Protein efficiency ratio	0.79 ± 0.02^{b}	0.84 ± 0.04^{ab}	0.90 ± 0.09^{a}	0.86 ± 0.06^{ab}	0.86 ± 0.04^{ab}
Lipid efficiency ratio	2.94 ± 0.05^{b}	3.06 ± 0.10^{ab}	3.22 ± 0.22^{a}	3.10 ± 0.14^{ab}	3.12 ± 0.11^{ab}

Groups with different superscripts differ significantly at $P<0.05$ (ANOVA).

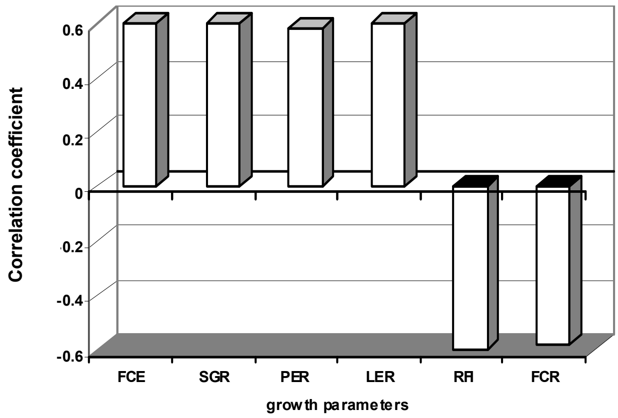

Fig. 1. Correlation coefficients between the dietary levels of the *Bacillus* probiotic and growth parameters in rainbow trout larvae

in *Oncorhynchus mykiss* larvae ($P<0.05$) as this variable was in negative significant correlation with the concentration of probiotic in experimental diets.

DISCUSSION

The best performance of fish in terms of growth performance and feed utilisation efficiency was recorded at the bacterial supplementation of 2×10^5 CFU/g in diet. Probiotic diet supplementation resulted in better growth performance and feed utilisation than in controls. The nutritive value of *Daphnia* meal as an alternate protein source has been demonstrated in the present study. Similar results were reported by Ghosh *et al.* (2003) and Bairagi *et al.* (2004) about Indian carp. Yanbo & Zirong (2006) suggested that the addition of probiotic reduced the culture cost of fishes in cultivation systems which was confirmed by our results. Probiotics induce useful microflora into larval intestine and cause high growth performance. In our study, *Daphnia* meal was supplemented with probiotic to increase its efficiency. Similar results were reported by Bairagi *et al.* (2002), using intestinal bacterial strains (*Bacillus* spp.) for fermentation of duckweed (*Lemna polyrhiza*) leaf meal for feeding of Rohu (*Labeo rohita*). In their study, the growth and feed utilisation efficiency of Rohu were higher than the control group and similar results were observed regarding *Daphnia* meal in our study. Our results indicate the suitable concentration of probiotic in this experiment, for bacterial supplementation of diet was 2×10^5 CFU/g. Similarly, Ghosh *et al.* (2003) indicated that *Bacillus circulans* supplementation in diets of Rohu had the best growth performance at about 2×10^5 cells per 100 g of feed. Using *Bacillus subtilis* and *Bacillus circulans* in formulated diets for Rohu fingerlings led to an increase in PER and LER, and a decrease in FCR in experimental treatments (Bairagi *et al.*, 2004).

Fish growth parameters were significantly increased by probiotic supplementation in our study, which is consistent with the findings of Gatesoupe (1991) using *Bacillus toyoi* on turbot (*Scophthalmus maximus*) and Swain *et al.* (1996) measuring the improvement of growth factors and feeding efficiency of Indian carp. Bairagi *et al.* (2004) showed that inoculation of *Leucaena* leaf meal with two specific strains of fish intestinal bacteria which have extracellular cellulolytic and amylolytic activities, *Bacillus subtilis* and *B. circulans*, have the best effect on growth and feeding efficiency of Rohu fingerlings. This study also showed that different levels of probiotic *Bacillus* spp. could had different effects on growth parameters in rainbow trout larvae. Based on these results, inoculation of 2×10^5 CFU/g in diet is recommended to improve utilisation of *Daphnia* meal and stimulate productive performance of rainbow trout larvae. The results of this investigation will be helpful in formulation of practical diets for trout culture.

In conclusion, the experiments showed that the probiotic *Bacillus* highly increase the growth performances and feeding efficiency in trout larvae. Furthermore, different levels of probiotics *Bacillus* resulted in different performance.

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