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EFFECT OF FROZEN *Daphnia magna* DIET MIXED WITH PROBIOTIC PROTEXIN ON GROWTH AND SURVIVAL OF RAINBOW TROUT (*Onchorhynchus mykiss*) FRY REARED UNDER CONTROLLED CONDITIONS

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ABSTRACT: *Effect of probiotic Protexin was experimentally tested on growth and survival of rainbow trout fry reared under controlled conditions. Experiments to determine the effect of different levels of probiotic (2×10^4 (T_1), 2×10^5 (T_2) and 2×10^6 (T_3) CFU/g^{-1}) on growth and survival rates of rainbow trout in comparing with those of control diet containing no probiotic were carried out under laboratory conditions. In this trail, frozen Daphnia magna was considered as a basal diet for fry feeding. Rainbow trout offered the control diet exhibited the same growth and feed utilization with all experimental treatments, and no significant differences ($P>0.05$) in growth were observed among fish groups fed various levels of the probiotic. There was no effect of probiotic inclusion level on water quality. There was no effect of probiotic supplementation on survival at the end of experiment in T_1 and T_3, but survival rate in T_2 was higher than other groups, significantly ($P<0.05$). Viability against high temperature stress was affected by dietary probiotic inclusion, as supplemented diets by probiotic revealed the better and more efficient results in fish survival. Viability of T_2, T_3 and control in challenging with high salinity was homogenous, while T_1 showed the significant difference ($P<0.05$) with others, properly.*

ORIGINAL ARTICLE

Key words: *Probiotic, growth, survival, rainbow trout (Oncorhynchus mykiss)*

INTRODUCTION

Probiotics are usually live microorganisms which when administered in adequate amounts confer a health benefits on host. Nowadays, probiotics are also becoming an integral part of the aquaculture practices to procure high production. The common probiotics that are used for aquaculture practices include *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, *Carnobacterium*, *Shewanella*, *Bacillus*, *Aeromonas, Vibrio*, *Enterobacter*, *Pseudomonas*, *Clostridium*, and *Saccharomyces* species. The involvement of probiotics in nutrition, disease resistance and other instrumental activities in fish has proven beyond any doubt (Nayak, 2010).

In aquaculture programs, probiotics are used for a quite long time but in last few years probiotics became an integral part of the culture practices for promoting growth and disease resistance. This strategy offers innumerable advantages to overcome the limitations and side effects of antibiotics and other drugs and also leads to high production through enhanced growth and disease prevention (Das et al. 2008; Sahu et al. 2008). In aquaculture, the range of probiotics evaluated for use is considerably wider than in terrestrial agriculture. Several probiotics either as monospecies or multispecies supplements are commercially available for aquaculture practices (Decamp and Moriarty, 2006; Ghosh et al. 2007). Apart from the nutritional and other health benefits (Austin et al. 1995; Gram et al. 1999; Carnevali et al. 2006), certain probiotics as water additives can also play a significant role in decomposition of organic matter, reduction of nitrogen and phosphorus level as well as control of ammonia, nitrite, and hydrogen sulfide (Boyd and Massaut, 1999).

Numerous microbes have been identified as probiotics for aquaculture programs, many of which differ markedly in their mode of action. There are, however, some common mechanisms of action that have been

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reported for the majority of probiotic strains. Probiotics help in feed conversion efficiency and live weight gain (Al-Dohail et al. 2009; Saenz de Rodriguez et al. 2009) and confer protection against pathogens by competitive exclusion for adhesion sites (Vine et al. 2004; Chabrillon et al. 2005), production of organic acids (formic acid, acetic acid, lactic acid), hydrogen peroxide and several other compounds such as antibiotics, bacteriocins, siderophores, lysozyme (Yan et al. 2002; El-Dakar et al. 2007) and also modulate physiological and immunological responses in fish (Khattab et al. 2004; Balcazar et al. 2006).

This study was conducted on investigation the effect of probiotic Protexin on growth and survival of rainbow trout fry and their resistance against environmental stressors.

MATERIALS AND METHODS

Probiotic preparation

The probiotic was prepared from Protexin Co (Iran-Nikotak). The five species of probiotic bacillus as bacterial blend under the commercial title of Protexin aquatic were used for bioencapsulation of *A. urmiana*. The blends of probiotic (mixture of *Lactobacillus plantarum, Lactobacillus delbrueckii bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus Salivarius, Enterococcus faecium, Aspergillus oryzae and Candida pintolopesill*) from suspension of spores with special media were provided. Three concentrations of bacterial suspension, 2×10^4 (T_1), 2×10^5 (T_2) and 2×10^6 (T_3) bacteries per milliliter (CFU ml^{-1}) were provided by Protexin Co and the colony forming unit (CFU) of probiotic were tested by microbial culture in Tryptic Soy Agar (TSA) (Rengpipat et al., 1998).

Daphnia magna provision

The *Daphnia magna* is an important live feed that were used as a vector to carry probiotics (Faramarzi et al. 2011). *D. magna* was harvested from intensive production ground ponds of sturgeon in Marjani center (Iran), and maintained at −30 ◦C prior to use.

Experimental design

Four trials were carried out with rainbow trout (*Onchorhynchus mykiss*). Sixteen plastic tanks (30 l) with four replicates for treatment and control were used. Trails 1, 2 and 3 were fed by *D. magna* which was treated by probiotic. The other three aquaria served as the control and treated with only basal diets, which were absent probiotic.

Healthy fry of the rainbow trout (*Onchorhynchus mykiss*) provided by the Fish Hatchery of Fazelabad, Iran were acclimatized in two fiberglass tanks (200 l), and were fed Biomar feed six times daily for 2 weeks. Then healthy rainbow trout was distributed into 16 tanks (with water circulation) with initial stocking density of 30 fish per tanks in the Laboratory of Aquaculture Department, Gonbad University for 60 days culture. All rainbow trout had similar initial weights (470 mg). The experiment was conducted as a completely randomized design with four treatments (trials 1–3 and control). Each treatment had four replicates of 30 rainbow trout each.

Fishes were fed six times daily with each feed. Daily feeding rate was about 50-70% of total body weight and properly regulated according to actually intake of rainbow trouts. 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.

Temperature range of water was 16–18 °C. For water quality control, temperature and dissolved oxygen (DO) were measured daily, and weekly analyses were done of total dissolved solid (TDS), NO_3, SO_4, PO_4, salinity, electrical conductivity and pH levels. Dissolved oxygen level was maintained above 7.8 mg l^{-1} by setting the air pump.

Thermal and salinity challenges

At the end of experiment, for evaluation of the fish quality 20 fry of each replicate (10 fry for thermal challenge and 10 fry for salinity challenge) were tested by high temperature and salinity. In this propose, fishes were transferred to other tanks and temperature and salinity were increased to 32 °C and 20 g l^{-1}, respectively. Then, survival duration was calculated.

Sampling and analytical methods

Weights of all collected fishes from each tank 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, rainbow trout survival was also determined by counting the individuals in each tank. The weight and length gain (WG and LG) (g d^{-1} and cm d^{-1}) were calculated as (Yanbo and Zirong, 2006): WG = final weight (g) − initial weight (g); LG = final length (cm) − initial length (cm)

The feed conversion ratio (FCR) was expressed as (Yanbo and Zirong, 2006): FCR = total feed consumption (total feed casting − total feed residue) (g)/ total final weight (g) − total initial weight (g) + total morality weight (g); Specific growth rate (SGR) and condition factor (CF) were determined using the following equations (Zargham et al., 2011): $SGR = (\ln w_t - \ln w_0) \times 100/t$; $CF= W/L^3 \times 100$.

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Gastro Somatic Index (GSI) and Thermal Growth Coefficient (TGC) were calculated as (De Silva and Anderson, 1995): Gastro Somatic Index (GSI) = [Digestive system (g)/ body weight (g)] ×100; Thermal Growth Coefficient (TGC %) = [final weight $^{0.333}$ – initial weight $^{0.333}$ / °c (day – degrees)] × 100.

Statistical analysis using one-way ANOVA was performed to find significant difference on various parameters between treated and control trials. Differences among means were determined and compared by Duncan's multiple range tests (SPSS software). A significance level of P<0.05 was used. Data are reported as means ± standard deviations (Ziaei-Nejad et al. 2006).

RESULTS

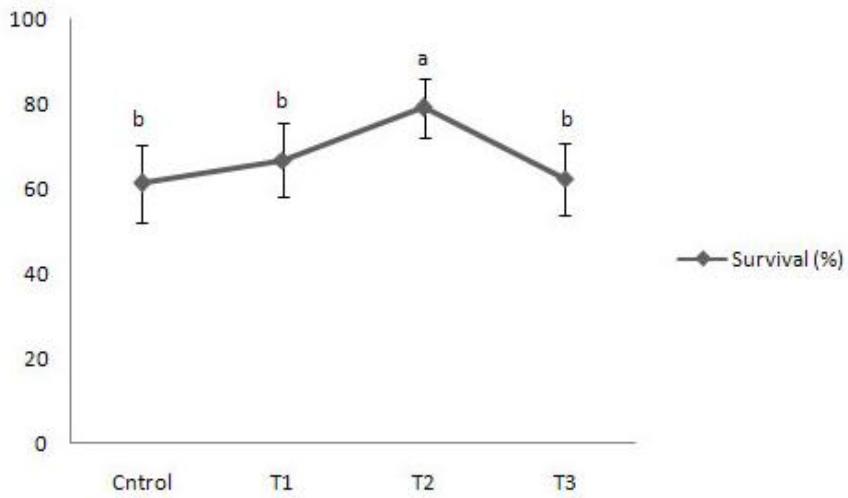
Water quality parameters

There was no obvious effect of probiotic on the water quality in treatments. Total salinity (4.73 g l^{-1}), SO_4 (1216 mg l^{-1}), NO_3 (150.84 mg l^{-1}), PO_4 (150.84 mg l^{-1}), TDS (4180 mg l^{-1}), electrical conductivity (7989.84 μ mos/cm^2) and pH (7.2–7.61) were stable and within acceptable ranges (Boyd and Tucker, 1998).

Survival rate

According to figure 1, the rainbow trout survival rate of T1, T3 and control was the same after 60 days culture ,approximately, but there was a significant difference (P<0.05) between these trails and T_2 which was treated by $2×10^5$ CFU ml^{-1}.

Figure 1. Survival rate (mean ± standard error) of trails at the end of experiment



Growth performance

Data on growth performance including initial weight, weight gain (WG), length gain (LG), Specific growth rate (SGR), condition factor (CF), Gastro Somatic Index (GSI) and Thermal Growth Coefficient (TGC) were reported in Table 1. At the beginning, no significant difference was observed in the initial weight between trials 1–3 and control (P>0.05). Comparably, weight gain of the control did not show a significant difference (P>0.05) with those of the trials 1–3. The values of condition factor (CF) in trials 1-3 treated with the probiotic were significantly lower than the control (P<0.05). Means of the FCR, SGR, TGC and GSI were not significant (P>0.05) in trials 1-3 treated with probiotic compared with control.

Table 1 - Performance of rainbow trout offered diets with various inclusion levels of probiotic

Parameters	Control	T_1	T_2	T_3
W_0 (g)	348±3.5	350±1.5	346±3.8	349±1.5
WG (g)	748.4±118	751.02±132	689.8±120	758.68±145
LG (cm)	4.15±0.15	4.25±0.05	4.15±0.10	4.24±0.10
FCR	1.18±1.27	1.22±1.49	1.24±1.08	1.15±1.25
SGR	2.47±0.05	2.47±0.09	2.20±0.01	2.50±0.08
CF	1.02±0.12^a	0.97±0.19^b	0.96±0.14^b	0.97±0.12^b
GSI	17.77±1.57	15.70±1.22	15.80±1.87	15.49±2.14
TGC	2.46±0.51^{ab}	2.48±0.48^a	2.30±0.53^b	2.49±0.48^a

Values in the same row with multiple superscripts are significantly different

Thermal and salinity challenges

As can be seen in Figure 2, remarkable difference (P<0.05) in fry tolerance against high temperature stress (32 °C) was observed between probiotic treatments and control. Indeed, survival rate of fry was soared by probiotic supplementation.

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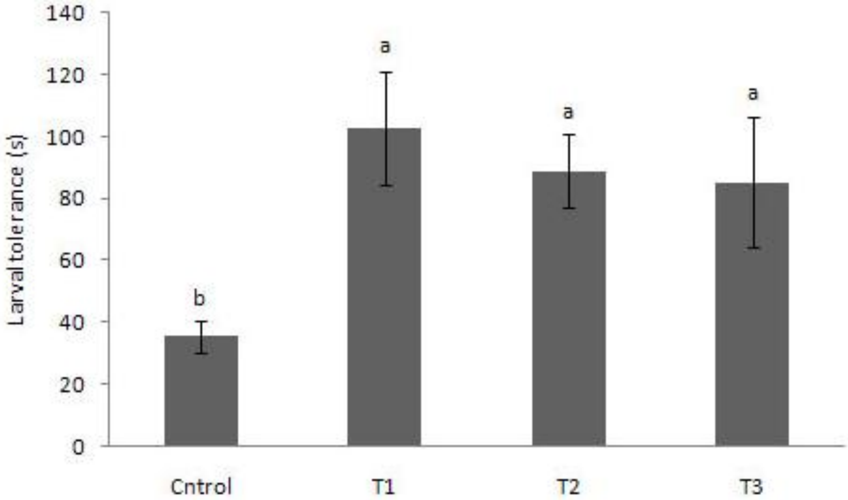

Figure 2. Fry viability (mean ± standard error) against high temperature stress. Scale bars with the same letter in each parameter are not significantly different (P>0.05)

As far as figure 3 is concerned, significant promotion in fry tolerance was obtained in T_1 with regards to high salinity stress (20 g l^{-1}), however, there were any differences among other trails (P>0.05).

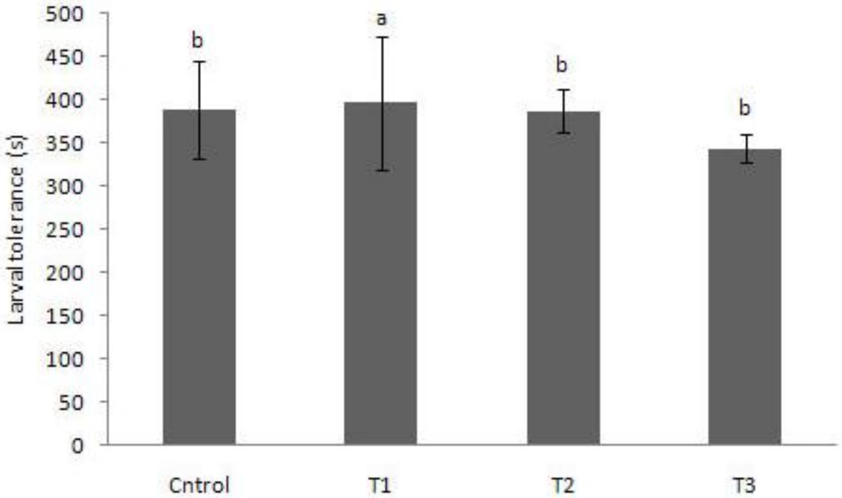

Figure 3. Fry viability (mean ± standard error) against challenge of salinity. Scale bars with the same letter in each parameter are not significantly different (P>0.05)

DISCUSSION

The exact mode of probiotic action has not been fully elucidated and there is continuous argue about its effect on the water quality (Mohamed et al. 2010). It has been reported that use of *Bacillus* sp. improved water quality and increased the health status of juvenile *Penaeus monodon* and reduced the pathogenic vibrios (Dalmin et al. 2001). In the present study, there was no obvious effect of the probiotics added to feeds on water quality. These results agree with the findings of Yanbo and Zirong (2006) and Mohamed et al. (2010).

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 probiotic in trails 1, 3 (2×10^4 and 2×10^6 CFU ml^{-1}) and control had shown inconsistent results. In contrast, the use of the probiotics mixture in the trails 1 and 3 at determinate density caused no significant survival increases when compared to the control. Inversely, survival rate in trail 2 (2×10^5 CFU ml^{-1}) had significant improvement and higher proportion.

All the probiotic supplemented diets and control basal diet revealed the same results in growth parameters. Similar results were observed by Abdelhamid et al. (2002) and Diab et al. (2002), who were reported probiotic addition to fish diets have not affected on growth parameters, in tilapia. Nevertheless, various study proved the positive effects of commercial probiotics lead to growth performance in some species (Noh et al. 1994; Bogut et al. 1998; Ghosh et al. 2003, Yanbo and Zirong, 2006; Farahi et al. 2011).

The equivalent FCR, SGR and GSI values observed with probiotic-supplemented diets and control suggested that addition of probiotics could not improve feed utilization of rainbow trout. Compatibly, Mohammadi Azarm et al. (2004) achieved the same result about FCR in rainbow trout larvae. In practical terms, this meant that probiotic use could not decrease the amount of feed necessary for fish growth which could not result in production cost reductions. Also, having higher and marked amount of CF in the control group, it could be concluded that probiotic-supplemented diets were not efficient related to growth performance.

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Being exposed a variety of conditions and environmental stressors in rainbow trout larvae and fry cultivation, it is crucial to exhibit new techniques for obviating of these issues. Farahi et al. (2011) evaluated the effect of dietary supplementation of probiotic *Bacillus* sp. on maternal performance and larval quality of the angelfish (*Pterophyllum scalare*). They tested the tolerance of angelfish larvae to high temperature exposure. Their results shown larval survival significantly increased in probiotic treatments. Accordingly, in our study the rainbow trout fry which received the perobiotic in their diet exhibited the best survival rate than the control, clearly. Pooramini et al. (2008) reported that challenging with different levels of salinity after 24 hours showed treatments contained yeast as a probiotic had 100% survival and showed significant differences with cod oil treatment (without yeast) and control ($p<0.05$). Moreover, Jafarian et al. (2007) revealed that supplemented diets by probiotic *Bacillus* sp. enhanced the survival rate of Persian Sturgeon (*Acipencer persicus*) Larvae. In the present research, the most significant promotion was observed in trails 1 (2×10^4 CFU ml^{-1}), but other treatments did not show any differences, obviously.

To sum up, though probiotic administration was not efficient for growth parameters in the current experiment, we cannot deny the positive effects of probiotic in improving the fry resistance against environmental stressors. In fact, the larval and fry forms of most fish and shellfish are released in the external environment at an early stage of development. These larvae and fry are highly exposed to gastrointestinal microbiota-associated disorders, because they start feeding even though the digestive tract is not yet fully developed (Timmermans, 1987) and the immune system is still incomplete (Vadstein, 1997). So, it will be effective to add the probiotic for achieving the most survival rate and reduce the financial detriments. On the other side, lucrative usage of frozen *Daphnia magna* as a feed for rainbow trout fry is a great consequence for reducing expenditures and obtaining favorite results in fish farming.

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