

Effects of Probiotic Bioplus 2B on Growth Indices and Survival of Zebra Fish (*Danio rerio*)

¹Safoura Sedaghat, ²Mohammad Reza Imanpoor and ¹Hamide Kordi

¹University of Agricultural Sciences and Natural Resources, Gorgan, Iran

²Department of Fisheries, University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: The main aim of this work was to investigate the effects of probiotic BioPlus 2B on growth indices and survival of zebra fish (*Danio rerio*). In this experiment, Probiotic was introduced in diet at three different levels (T₁: 500 mg/kg, T₂: 1000 mg/kg, T₃: 2000 mg/kg) and their effects were studied compared to those of control containing no probiotic. Each treatment had 3 replicates and used 10 fish with average weights of 0.21±0.03 g at every aquarium during 60 days. Feeding varied during the experiment. First, it was 5-8 percent of body weight. Then, with increasing fish weight, percentage of the food decreased. At the end of culture period, growth performance and survival rate of giant zebra fish were compared. The results showed that, the average of weight, body weight increase percent and survival ratio in fishes that fed by diets containing T₃ probiotic was significantly ($P<0.05$) higher than control group, Food conversion ratio in fishes that fed by diets containing T₃ probiotic was significantly ($P<0.05$) lower than control groups. Specific growth rate differences were not significant between groups ($p>0.05$). Based on these data, it was concluded that probiotic Bioplus 2B in high level could serve as functional feedstuffs in the diet of by enhancing growth performance and survival.

Key words: Probiotic • Bioplus 2B • Growth • Survival • Zebra Fish

INTRODUCTION

Zebrafish (*Danio rerio*) are an important laboratory model to study development, genetics and human disease, as they undergo rapid development, they display genetic similarities to humans and can develop many disease pathologies by induction of major concern, however, is the lack of nutritional control due to the absence of a standardized reference diet. Within the existing variability of (*Danio rerio*) feeding protocols, few studies have evaluated the effect of diet on adult *D. Rerio* growth and survival [1].

It is widely demonstrated that farmed fish are more susceptible to disease agents than their wild counterparts due to the artificial conditions posed by intensive rearing [2]. Recent concerns regarding antibiotic resistance and the use of probiotic agents in livestock has resulted in a demand for alternative strategies to improve animal production and health without the need of antibiotics [3]. Lactic acid bacteria, including *Bacillus* spp. are widely used as probiotics in humans and their use has reportedly led to health benefits against gastrointestinal disorders including diarrhea, inflammatory bowel disease, lactose

intolerance and Salmonella or Shigella infections [4]. Although various *Bacillus* spp. are used as probiotics for humans and animals [5], their mechanism of action is not yet fully understood.

BioPlus 2B contains a combination of naturally-occurring bacteria strains, *Bacillus subtilis* and *Bacillus licheniformis*, which improve the intestinal microflora of swine. The improved intestinal balance helps increase live weight gain and improves feed conversion. The effects of probiotics on fish and shrimp were reported in previous papers. The genus *Bacillus* contains some of the bacteria used as probiotics by the aquaculture industry to improve feed digestion and absorption and water quality. Many researches on probiotic for aquaculture have been donning [6, 7] with competitive exclusion of potential probiotics on rainbow trout [8, 9]. Stimulation of immune system in rainbow trout with several candidate probiotics has also been evaluated by some researchers [9-11].

Therefore, this experiment was conducted to assess the effects of supplementation the diets with BioPlus 2B on growth performance and survival of zebra fish (*Danio rerio*).

MATERIALS AND METHODS

Rearing Conditions and Experimental Design: Zebra fish, *Danio rerio* (average weight = 0.21 ± 0.03 g) were obtained from ornamental fish breeding centre in Iran and maintained in 12 Aquaria with continuous fresh water supplied from Aquaculture Centre (temperature = 27 ± 0.06) for a period of 60 days. Three treatments were conducted to evaluate the effect of probiotic BioPlus 2B administered to zebra fish, each treatments, in triplicate, was stocked with 30 fish. The fish were fed at three different level (T_1 :500mg/kg, T_2 :1000mg/kg, T_3 :2000 mg/kg) and control diet groups served as well.

Feeding and Probiotic Supplement Preparation: Biomar starter food was taken as a basal diet for the supplementation of probiotic. The commercial probiotic used in this experiment (Bioplus 2B, RazakCo. Iran) contained spores of two species of *Bacillus* (*B. subtilis* and *B. licheniformis*). Probiotics prepared as described in its original manual. The proper amounts of probiotic suspension were sprayed into the feed slowly, mixing part by part in a mixer. Then, the feed was air dried under sterile conditions for 12 h and stored at 20°C. The commercial feed sprayed with sterilized diluents alone served as the control diet.

Determination of Nutritional Effects and Survival: Every 15days, fishes were taken to determine wet weight and total length. The numbers of mortality were recorded as well. Digital calliper to measure the length of the fish with 0.01 mm and digital scale to weight of fish with 0.01 g were used. Indicators of growth include: body weight increase (BWI), present of body weight increase (PBWI %), specific growth rate (SGR), Feed conversion ratio (FCR) and Survival ratio (SR) were expressed as following:

$$BWI = W_t - W_0$$

$$PBWI(\%) = 100 * \frac{W_{(t)} - W_{(i)}}{W_i}$$

$$SGR = 100 * \frac{\ln W_{(2)} - \ln W_{(1)}}{T2 - T1}$$

$$FCR = \frac{\text{Dry weight of ingested food}}{\text{Wet weight of produced tr}}$$

$$SR = N * W$$

Where t is the period of culture in days, $\ln W_0$ is the natural logarithm of the weight of the fry at the beginning of the experiment and $\ln W_t$ is the natural logarithm of the weight of the fry at day t . (W_0 and W_t are in gram).

Statistical Analysis: Data were subjected to one-way ANOVA and significant difference between the treatments was determined by Duncan's test. Data are presented as treatment mean $\pm$ SD. The values of $P < 0.05$ were considered significantly different. All analyses were performed using statistical software SPSS v. 16.

RESULTS

In this research, there were significant differences ($P < 0.05$) in initial mean weight (0.21 ± 0.03 g) among treatments (Table 1). Survival ratio just in T_3 was significantly ($P < 0.05$) higher than control (Fig.1). Average weight was recorded higher in fish fed with probiotic supplement. Only in T_3 treatment the average body weight increase (BWI), was significantly ($P < 0.05$) higher than control group (Fig. 1). In all probiotic treatments, food conversion ratio (FCR) was significantly ($P < 0.05$) higher than control groups (Fig. 1) and the differences of specific growth ratio (SGR) between the treated fish and control groups was not significant ($P > 0.05$) (Fig. 1).

DISCUSSION

Probiotic had significance influence on final mean weight, percentage of survival and feed conversion rate. According to Ringo and Storm [12], high proportion is probably related to an increase in suitable attachment sites as a result of histological and functional development of fry and improved internal environmental conditions for bacterial growth [13]. Growth rate throughout the experiment was improved in T_3 , it can be certainly suggested that the more probiotic cells in diets and host intestine would necessarily result in the more improved growth and survival. Better growth, as observed in T_3 , may establish better health conditions in zebra fish and therefore, decrease mortality. BioPlus 2B produces several peptide antibiotics, including subtilin and bacitracin produced by *B. subtilis* and *B. leicheniformis*; respectively, which were present in the probiotic we used. Moreover, there are a number of other substances with bio control activities isolated from species of *Bacillus* [14]. Iturins, cyclic lipoproteins isolated from *B. subtilis* are toxic to a wide range of fungi and yeast [7]. Therefore, administered *Bacillus* gave rise to the fry resistance to

Table 1: growth indices and survival (mean± S.E) of zebra fish (*Danio rerio*)

Treatment	BWI	PBWI	SGR	FCR	SR
T1	0.12±0.015 ^b	53.91±4.68 ^b	2.54±0.2 ^a	9.1±0.77 ^{ab}	2.55±0.09 ^b
T2	0.12±0.015 ^b	61.28±7.93 ^{ab}	2.74±0.36 ^a	8.31±0.65 ^b	2.65±0.58 ^b
T3	0.15±0.006 ^a	69.17±6.29 ^a	2.56±0.17 ^a	7.03±0.25 ^c	3.56±0.49 ^a
Control	0.11±0.012 ^b	51.19±3.92 ^b	2.60±0.28 ^a	9.58±0.15 ^a	2.36±0.56 ^b

Letters with different superscripts at the same row differ significantly by $P<0.05$ by means separation using the Duncan multiple ranges test.

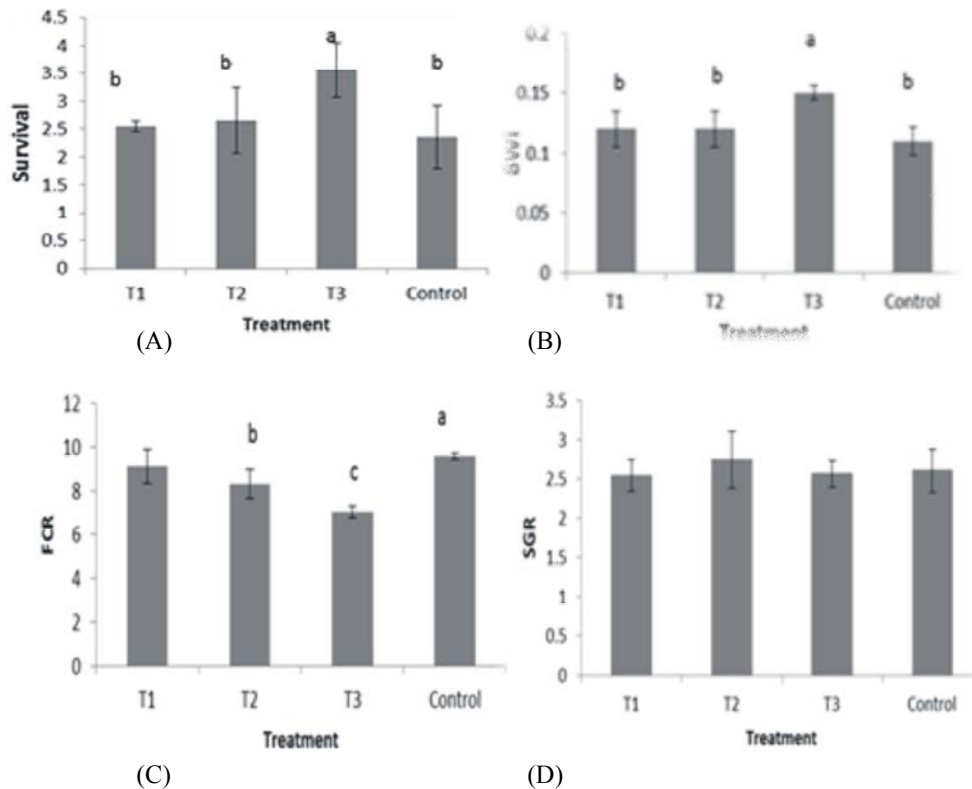


Fig. 1: Survival ratio (A); body weight increased (B); Food conversion ratio (C) and Specific growth rate (D)

*Significantly ($P<0.05$) different

pathogens and enhanced survival by producing inhibitory substances to other microorganisms. The *Bacillus* species produce proteases (for example, subtilin), which helps in digestion [15]. They are also said to produce vitamin K and B12 [14]. Gram-positive bacteria, including members of the genus *Bacillus*, secrete a wide range of exoenzymes [7], which might have supplied digestive enzymes and certain essential nutrients to promote better growth. *Bacillus subtilis* and *B. leicheniformis* can break down proteins and carbohydrates [14]. So it can be suggested that administration of *Bacillus* bacteria to trout fry results in enhanced digestion of food and improved growth, including low food conversion ratio (FCR) and

high specific growth rate (SGR). High protein efficiency ratio (PER) as well as greater protein values of carcass in probiotic treatments may be due to proteins secreted by members of genus *Bacillus* [14].

We found that supplementation of trout starter diet with the proper density of commercial BioPlus 2B probiotic could be beneficial for growth and survival of Zebra fish, especially in fast growing conditions, where it would be essential to stimulate the precocious maturation of digestive system [16]. No clear negative effect of probiotic on Zebra fish was detected. So we suggest the effects of BioPlus 2B probiotic to be tested in other locations.

ACKNOWLEDGEMENTS

We are grateful to all the staff working at the rearing and Central Laboratories of Agricultural and Natural Resources University of Gorgan for their help and providing facilities during our trial.

REFERENCES

- Goolish, E.M., K. Okutake and S. Lesure, 1999. Growth and Survivorship of larval zebra fish *Danio rerio* on processed diets. N. Am. J. Aquaculture, 61: 189-198.
- Irianto, A. and B. Austin, 2002. Probiotics in aquaculture: Review. J. Fish Diseases, 25: 633-642.
- Wang, J.H., Y.J. Cho, J.S. Chen, Y. Yoo, H.J. Huang, Kim and I.H. Kim, 2009. The effect of probiotic BioPlus 2B on growth performance, dry matter and nitrogen digestibility and slurry noxious gas emission in growing pigs. J. Applied Ichthyology, 120: 35-42.
- Madsen, K.L., 2001. The use of probiotics in gastrointestinal disease. J. Gastroenterology, 15: 817-822.
- Hong, H.A., I.H. Duc and S.M. Cutting, 2005. The use of bacterial spore formers as probiotics. Of FE Microbial Science, 29: 813-835.
- Ahilan, B., G. Shine and R. Santhanam, 2004. Influence of probiotics on the growth and gut microflora load of juvenile Gold fish (*Carassius auratus*). J. of Asian Fisheries Science, 17: 271-278.
- Ghosh, K., S.K. Sen and A.K. Ray, 2002. Growth and survival of Rohu (*Labeo rohita*) spawn fed diets supplemented with fish intestinal microflora. J. Acta. Ichthol, 32: 83-92.
- Irianto, A. and B. Austin, 2003. Use of dead probiotic cells to control furunculosis in rainbow trout (*Oncorhynchus mykiss*): A short communication. J. of Fish Diseases, 26: 59-62.
- Nikoskelainen, S., A.C. Ouwehand, G. Bylund, S. Salminen and E.M. Lilius, 2003. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). J. Fish and Shellfish Immunology, 15: 443-452.
- Irianto, A. and B. Austin, 2002. Probiotics in aquaculture: Review. J. Fish Diseases, 25: 633-642.
- Panigrahi, A., V. Kiron, T. Kobayashi, J. Puangkaew, S. Satoh and H. Sugita, 2004. Immune responses in rainbow trout (*Onchorhynchus mykiss*) induced by a potential probiotic, *Lactobacillus rhamnosus*. 1999. J. of Veterinary Immunology and Immunopathology, 102: 379-388.
- Ringo, E. and E. Storm, 1994. Microflora of free living fish and effect of diet and salinity on intestinal Microflora. J. of Aquaculture Research, 25: 623-629.
- Vine, N.G., W.D. Leukes and H. Kaiser, 2006. Probiotics in marine aviculture: Review. J. FE Microbial, 30:404-427.
- Rosovitz, M.J., M.I. Voskuil and G.H. Chambliss, 1998. Bacillus. Balows and B.I. Duerden (Eds), Systematic Bacteriology. Arnold Press, London, 30: 709-720.
- Sanders, M.E., L. Morelli and T.A. Tompkins, 2003. Spore formers as human probiotics: Bacillus, Sporolactobacillus and Brevibacillus: Comprehensive Reviews. J. Food Science and Food Safety, 2: 101-110.
- Wache, Y., F. Auffray, F.J. Gatehouse, J. Zambonino, V. Gayet, L. Labbe and C. Quentel, 2006. Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal micro biota and digestive enzymes in rainbow trout (*Onchorhynchus mykiss*) fry. J. Aquaculture Research, 258: 470-478.