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

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Abstract: The main aim of this work was effect of the probiotic BioPlus 2B on growth indices and survival of zebra fish (*Danio rerio*). In this experiment Probiotic was introduced in diets at three different levels (T_1: 500 mg/kg, T_2: 1000 mg/kg, T_3: 2000 mg/kg) and their effects compared with those of control diet containing no probiotic. Each treatment had 3 repetition and used 10 fish with means weight 0.21±0.03 g in every aquarium in during 60 days. Feeding was different during experiment and it was 5-8 body weight percent that with increased fish weight the food percent became less. At the end of culture period, growth performance and survival rate for giant Zebra fish compared. The results showed that difference in average of weight, body weight increase percent and survival ratio of fishes fed by diets containing T_3 probiotic significantly (P<0.05) higher than control group. Difference food conversion rate in fishes fed by diets containing T_3 prebiotic significantly (P<0.05) lower than control group. specific growth ratio differences were not significant than control group of Zebra fish (p>0.05). Based on these data, it is concluded that prebiotic Biopelos 2B in high level can serve as functional feedstuffs in the diet of by enhancing growth performance and survival.

Key words: Probiotic · Biopelos 2B · Growth · Survival · Zebra fish

INTRODUCTION

Zebrafish (*Danio rerio*) are an important laboratorymodel to study development, genetics and human disease, as they undergo rapid development, display genetic similarities to humans and can develop many disease pa- thologies 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 intensiverearing [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 for 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 done [6,7]. 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 [2,9,10].

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

MATERIALS AND METHODS

Rearing Conditions and Experimental Design: Zebrafish, *Danio rerio* (average weight = 0.21±0.03g) were obtained from ornamental fish breeding center in Iran and maintained in 12 Aquarium with continuous fresh water supplied from Aquaculture Centre (temperature =27±0.06, mean±standard error) 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 with30 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, fishs were taken to determine wet weight and total length. The numbers of Mortality were recorded as well. Digital caliper 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: increased body weight (BWI), percent increased body weight (PBWI%), specific growth rate (SGR), Feed conversion ratio (FCR) and Survival ratio (SR) were expressed as following:

$$BWI = W_t - W_0$$

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

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

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

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: One way analysis of variance (ANOVA; SPSS,16.0) was used to determine whether significant variation between the treatments existed. Difference between means were determined and compared by LSD test. All tests used a significance level of $P<0.05$. Data are reported as means ± standard errors.

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). Higher mean weight were recorded in fish fed with probiotic supplement, Although mean increased body weight(BWI) just in T_3 was significantly($P<0.05$) higher than control (Fig. 1). In all probiotic treatments, food conversion ratio (FCR) was significantly($P<0.05$) higher than in controls (Fig. 1). Specific growth ratio (SGR) of the treated fish was also determined and showed no significantly($P>0.05$) rate than control($P>0.05$) (Fig. 1).

DISCUSSION

Probiotic had significance influence on final mean weight, percentage of survival and feed conversion rate. According to [11], 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 [12]. growth rate through out the experiment was improved in T_3, it can be certainly suggested that the more probiotic cells in diets and host intestine necessarily does result in the more improved growth and survival. Better growth, asobserved 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 was present in the probiotic we used. Moreover, there are a number of other substances with biocontrol activities isolated from

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}

abcValues with different superscripts differ by P<0.05 by means separation using the Duncan multiple range test.

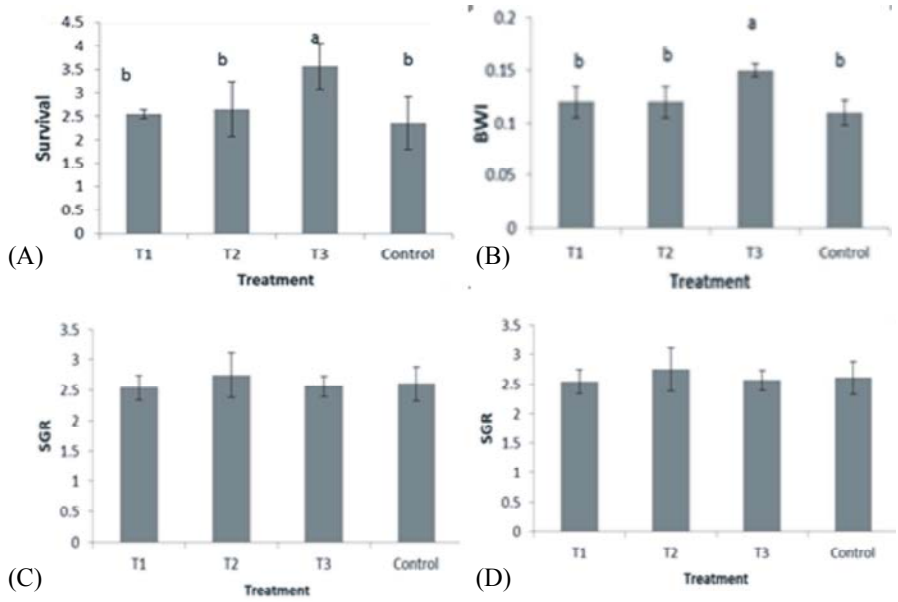

Fig. 1: Survival ratio (A); increased body weight (B); Food convertion ratio (C) and Specific growth rate (D)
*Significantly (P<0.05) different

species of *Bacillus* [13]. 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 pathogens and enhanced survival by producing inhibitory substances to other microorganisms. The*Bacillus* species produce proteases (for example, subtilin), which helps in digestion [14]. They are also said to produce vitamin K and B12 [13]. Gram-positive bacteria,including members of the genus *Bacillus*, secret a wide range of exoenzymes [13], 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 [13]. 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 membres of genus Bacillus [13].

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 [15]. No clear negative effect of probiotic on of Zebta fish detected. so we suggest the effects of BioPlus 2B probiotic to be tested in other locations.

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