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Effects of Probiotic on the Growth and Survival of Zebra fish (*Danio rerio*)

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Abstract: ***The objective of the study was to understand the effect of probiotic such as fish were divided into two groups; the first one was supplemented with the probiotics and commercial food, while the other group was supplemented with only commercial food. The sample size for each group was 10 which were measured for body weight gain every week during 60 days experiment. At the end of the experiment, the growth performance and survival rate was compared. The results showed that, the average of weight, body weight increase percent and survival ratio in fishes that fed by diets containing probiotic was significantly (P<0.05) higher than control group, Food conversion ratio in fishes that fed by diets containing probiotic was lower than control group. Specific growth rate differences were not similar to control group. Based on these data, it was interpreted that probiotic with lactic acid bacteria and yeast in high level could be useful as functional feed in the diet of Zebra fish which helps in enhancing growth performance and survival rate.***

Keywords: Probiotics, Growth, Survival, Zebra fish

1. Introduction

Zebra fish (*Danio rerio)* is a popular model which is used in laboratory to study the development, human pathology and also genetics as they have a tendency to grow faster; it also has genetic similarities with the human being which makes it easy to study the human disease which is of major concern, but there is shortage in the nutritional knowledge due to non-availability of standard diet for fish. Though, in the current distinctive diets, few studies have analyze the effect of diet on the zebra fish growth performance as well as survival rate (1).

It has been studied that the fish which are farmed are more likely to get affected by the pathogenic diseases than the wild ones due to artificial conditions possessing stress rearing conditions. (2)In the recent times there is a huge concern for the antibiotic resistance and use of probiotics for livestock which has led to development of alternative methods to enhance the growth as well as survival without use of antibiotics.(3) Lactic acid bacteria, which includes the *Bacillus* spp. are used in huge amount as human probiotics which has shown health benefits towards various gastrointestional disorders like inflammatory bowel disease, lactose intolerance, diarrhea and Salmonella or Shigella infections. (4) Even if the *Bacillus* spp. is used widely as a probiotics for animals and humans (5), their mode of action is not yet completely understood till today.

The probiotic combination which contains yeast and lactic acid bacteria helps in increasing the intestional micro flora resulting in increased body weight and survival ratio. The effect of probiotic on fish and shrimp culture was investigated in some papers. There is huge amount of research which is been focused in the field of aquaculture nowadays (6, 7) with the mechanism of competitive exclusion of effective probiotics on the rainbow trout (8,9). Probiotics has also been said to stimulate the immune response in rainbow trout which is been studied by several researchers. (9-11)

Therefore, this experiment was carried out to study the effects of probiotics such as lactic acid bacteria and yeast on the growth rate and survival ratio in zebra fish (*Danio rerio*).

2. Materials and Methods

Rearing Conditions and Experimental Design: Zebra fish, Danio rerio of different weights were obtained from the aquarium shop in Pune and maintained in 2 aquaria with fresh water maintenance (Temperature = 28^{o}C) for a period of 60 days. Two groups consisting of 10 individuals each, probiotics group supplemented with 50 mg of probiotics(Yeast and lactic acid bacteria) and 30 mg of commercial food was supplied, while non-probiotics was supplied with the 50mg of commercial food.

Feeding and Probiotic Supplement Preparation: The diet consisting of lactic acid bacteria was used for feeding in first month which was isolated from the dairy products, the bacteria was isolated using the pure culture techniques, dry live yeast cells were purchased commercially which was used for feeding in the second month. The probiotic supplement with the appropriate amount was sprayed into the feed slowly. The lactic acid bacteria culture was stored in refrigerator while the yeast cells were kept at room temperature. The commercial food was used for non-probiotics group was also sprayed in the same way.

Determination of Nutritional Effects and Survival:
Every week the fish were taken for wet weight measurement. The number of mortality was also recorded. Electronic weighing machine was used for wet weight measurement which was calibrated at 0.00 g. Indicators of growth includes: body weight increase (BWI), specific growth rates (SGR), feed conversion ratio (FCR) and survival rate (SR) were expressed as follows:

BWI=$W_T - W_0$;

SGR= $100*\ln(W_2) - \ln(W_1) / T2-T1$;

FCR=Dry weight of feed consumed by fish/Wet weight of fish (g)

SR=Final no. of fish (g)/Initial no. of fish (g) *100
Where, t is the culture period in days, ln W_0 is the natural logarithm of the wet weight of the fish in the beginning of experiment and ln W_t is the natural logarithm of wet weight of fish at day t.(W_0 and W_t are in gram).

Statistical Analysis: The collected data was subjected to one way ANNOVA and the significant difference between the treatments was determined by Duncan's test. The data is presented as treatment mean ± Standard deviation. The values of $P < 0.05$ were considered significantly different.

3. Results

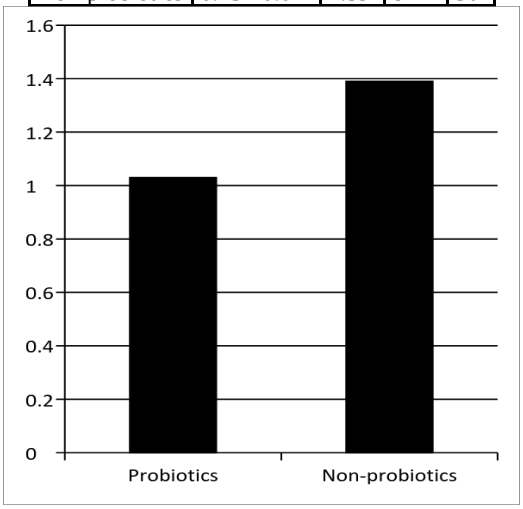
In this research there were significant differences ($P<0.05$) in initial mean weight (0.42 ± 0.13^a g) among the treatments (Table 1).Survival ratio in the probiotic group was higher than the control group i.e. Non-probiotic group. Average weight was recorded high in the fish fed with probiotic supplement. In the probiotic group average body weight increase (BWI), was significantly ($P<0.05$) higher than control group. (Figure 1).In the probiotic group the feed conversion ratio was higher than the non-probiotic groups i.e. control group and the specific growth rate (SGR) between the treated fish and control fish were different.

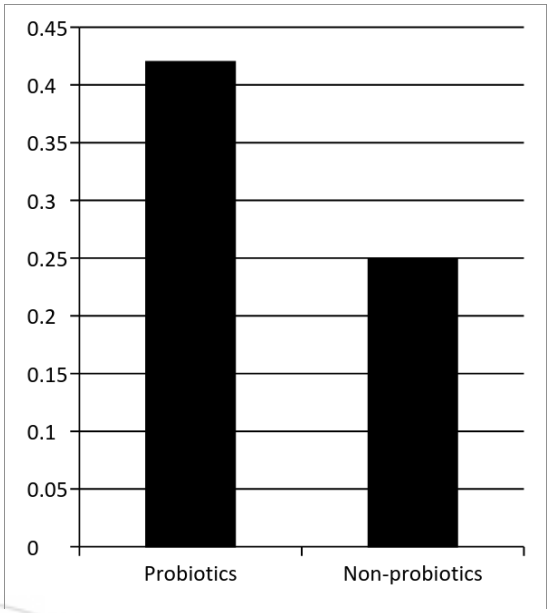
4. Discussion

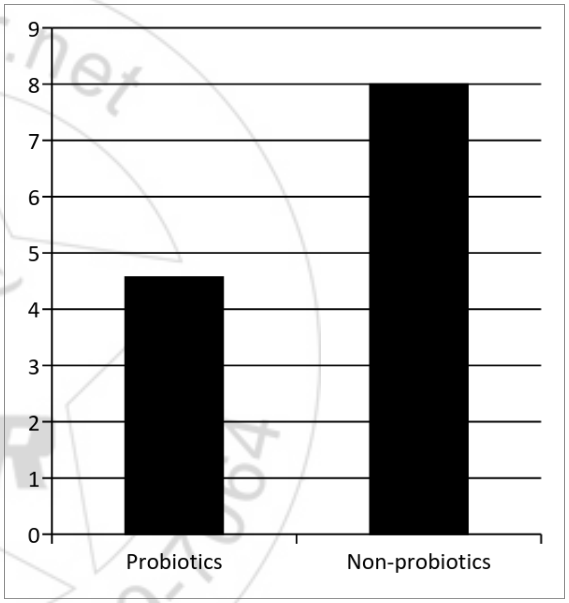
The probiotic had a significant influence on the survival rate, feed conversion ratio as well as on the final mean weight of the treated fish. According to Ringo and Storm, the number is due to development of suitable attachment sites which is result of production of functional as well as histology. The internal environment conditions are also said to improve for the growth of bacteria. In the experiment, more improvement of growth and better survival rate was observed in the probiotic supplemented group which must be a result of more probiotic cells in the diet and also in the intestine of host. As better growth and survival conditions were observed in the zebrafish, it will help in improving the health and also reduce the rate of mortality.

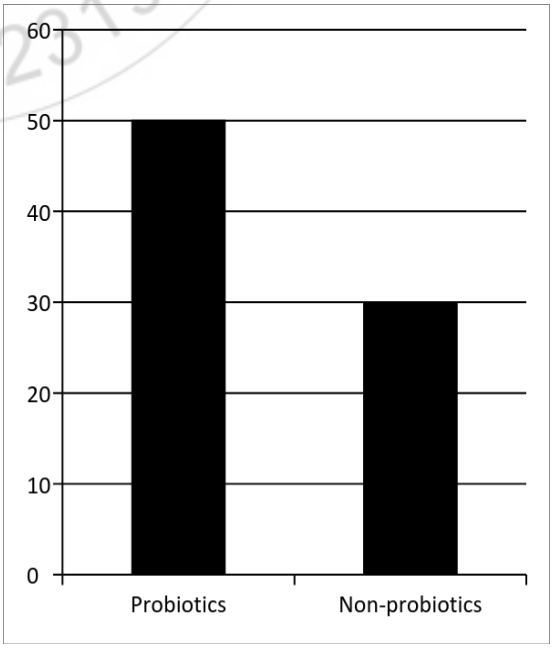
Table 1: Growth indices and survival (mean± S.E) of zebra fish

Treatment	BWI	SGR	FCR	SR
Probiotics	0.42 ± 0.13^a	1.03	4.57	50
Non-probiotics	0.25 ± 0.07^a	1.39	8	30









Further, some of the yeast species and their components, such as β-glucans and mannoproteins, can help in stimulation of the immune and antioxidant systems of the host cells. If the contribution of yeast microbiota in fish health, survival, growth and nutrition is well understood then this may improve both the production, performance of fish as well as the sanitary conditions. (13)

It was found that the use of Lactic acid bacteria as well as yeast cells has proved to effective on the growth, survival of the zebrafish. There was no such negative effect observed on zebrafish. So, it is suggested to test the effect of these two probiotics on other species and different locations.

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