Global Veterinaria 8 (5): 503-506, 2012
ISSN 1992-6197

The Influence of Synbiotic (*Biomin imbo*) on Growth Factors and Survival Rate of Zebrafish (*Danio rerio*) Larvae via Supplementation with Biomar

Hamed Nekoubin, Esmail Gharedaashi, Mohammad Reza Imanpour, Hashem Nowferesti and Alireza Asgharimoghadam

Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract: This study (at 90 days) was carried out to evaluate the effect of synbiotic (*Biomin imbo*) on growth factors and survival rate of Zebrafish (*Danio rerio*) larvae via supplementation with Biomar. The synbiotic were used in three concentrations of 0.5, 1, 1.5 g/Kg of diet (Biomar). The Zebrafish larvae in experimental treatments were fed of the three levels of synbiotic with 4 percent body weight (3 times a day). The larvae in control treatment were fed without supplemented Biomar. The growth factors and survival rate of larvae fed on synbiotic were compared to those fishes in control treatment that fed of unsupplemented Biomar. The results showed that larvae fed the synbiotic had significantly increased final body weight in comparison to control treatment. The synbiotic also had significant positive effects on specific growth rate (SGR) and food conversation efficiency (FCE) in comparison to those fed the control treatment. The food conversation ratio (FCR) and condition factor (CF) were significantly decreased in comparison with the control treatment ($p<0.05$). Also survival rate in experimental treatments in comparison with control treatment, was significantly increased ($p<0.05$).

Key word: Zebrafish · Danio Rerio · Supplementation · Biomar · Specific Growth Rate and Survival Rate

INTRODUCTION

Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism, hence synbiotics, enhancing their isolated beneficial effects. When two nutritional ingredients or supplements are given together; the resulting positive effect generally follows one of three patterns: additivity, synergism or potentiation. Additive effect occurs when the effect of two ingredients used together approximates to the sum of the individual ingredient effects. In case of synergism, it is said to occur when the combined effect of the two products is significantly greater than the sum of the effects of each agent administered alone. The term potentiation is used differently, some pharmacologists use potentiation interchangeably with synergism to describe a greater than additive effect and others use it to describe the effect that is only present when two compounds are concurrently [1].

Synbiotics affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria and thus improving the host "welfare". In humans, probiotics are mainly active in the small intestine while prebiotics are only effective in the large intestine, so the combination of the two may give a synergistic effect [2]. The first application of synbiotics in fish is that of Rodriguez-Estrada *et al.* [3].

The appropriate use of probiotics in the aquaculture industry were shown to improve intestinal microbial balance and also to improve feed absorption, thus leading to increased growth rate [4,5] and also reduced feed conversion ratio (FCR) during the cultural period [6]. Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathenogenic bacteria through the production of inhibitory compounds; improvement of water quality;

Corresponding Author: Hamed Nekoubin, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes [7]. Because *Bacillus* bacteria secrete many exoenzymes [8], these bacteria have been used widely as putative probiotics.

Over the past twenty years, the zebrafish (*Danio rerio*) has attracted considerable attention as an excellent vertebrate model system for studying genetics and development [9] and more recently, for understanding human diseases and for the screening of therapeutic drugs [10-13].

In 2004, Rawls *et al.* [14] made a decisive contribution to our understanding of the role of gut microbiota, by comparing the intestine of zebrafish larvae reared either conventionally, or in microbe-free conditions.

The present study examined the effects of synbiotic (*Biomin imbo*) on growth factors and survival rate in Zebrafish (*Danio rerio*) larvae via supplementation with Biomar.

MATERIALS AND METHODS

The symbiotic (*Biomin imbo*) was prepared from the commercial product Protexin aquatic (Iran-Nikotak). Also Biomar was provided by aquatic foods company. Nutrient compositions of experimental diets (Biomar) are given in Table 1. Proximate composition of diets was carried out using the Association of Analytical Chemists [15] methods. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method; Crude fat was determined using petroleum ether (40-60 Bp) extraction method with Soxhlet apparatus and ash by combustion at 550°C.

This experiment was conducted in a completely randomized design with four treatments (three synbiotic levels and a control) and three replicates per treatment for a total of twelve fiberglass tanks (each with a capacity of 60 liters). Larvae of Zebrafish (initial weight: 0.21±0.09 g) were obtained from the Institute of Ornamental Fish Hatchery in Gorgan, Iran. The density of fish larvae per tank were 50 fish. Zebrafish larvae in control and experimental treatments were fed 4 percent of their body weight for 3 times a day (8.00, 16.00 and 24.00). The control treatment was fed unsupplemented Biomar. Water quality parameters of input water to rearing system were monitored each week throughout the experiment. The water temperature was 19.46±1.23°C, pH was 7.85±0.26 and water oxygen level was maintained above 7.65 ± 0.55 mg L^{-1} during the experiment an electrical air pump (by a single filtration unit).

Table 1: Nutrient composition of experimental diets (%)

Ingredients	%
Protein	54
Lipid	18
Fiber	1.5
Ash	10
Vitamin	2

The fish were weighed individually at the beginning and at the end of the experiment. Before distributing fish to the experimental tanks (in the beginning of exogenous feeding), 30 fish were sampled from the holding tank for biometry. In the termination of experiment, 40 larvae from each tank were sampled and the final weight and length of body were measured. Growth parameters of fish were calculated based on the data of biometry of Zebrafish larvae.

One-way ANOVA and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by using the SPSS program.

RESULTS AND DISCUSSION

The results clearly showed that the synbiotic had beneficial effects on the growth parameters on Zebrafish larvae. The feeding and growth parameters of Zebrafish larvae are presented in Table 2. Effects of synbiotic treatments on growth performance and survival rate of Zebrafish larvae resulted better than control treatment ($p<0.05$). Also, the three different treatments of synbiotic were significantly different for any of growth parameters that, among the three different concentrations of synbiotic supplemented with Biomar fed to Zebrafish larvae, the greatest effect appeared to be obtained in treatments T3 (supplemented with 1.5 g/kg). This is particularly true for specific growth rate, where the highest was obtained in the experimental treatment T3. The food conversion ratio (FCR) in the experimental treatments was significantly decreased in comparison with control treatment ($p<0.05$).

Effects of commercial probiotic on aquaculture has been investigated by researchers and some of this research has not shown any positive effects on growth parameters or survival rate or any promising results on the cultural condition. For instance, Shariff *et al* [16] found that treatment of *Penaeus monodon* with a commercial *Bacillus* probiotic did not significantly increase survival.

Table 2: Growth parameters and survival rate of Zebrafish (*Danio rerio*) larvae in experimental treatments (trial 1-3) and control

	Treatments			
Growth Indices	Control Unsupplemented Biomar	T1 supplemented Biomar with 0.5 g/kg	T2 supplemented Biomar with 1 g/kg	T3 supplemented Biomar with 1.5 g/kg
Initial weight (g)	0.21±0.09	0.21±0.09	0.21±0.09	0.21±0.09
Final body weight (g)	0.41±0.01^{c}	0.44±0.01^{c}	0.48±0.01^{b}	0.59±0.17^{a}
Body weight increased (g)	0.20±0.01^{c}	0.23±0.01^{c}	0.27±0.01^{b}	0.38±0.17^{a}
Specific growth rate for weight (% BW day^{-1})	0.76±0.06^{c}	0.82±0.07^{bc}	0.91±0.13^{b}	1.17±0.15^{a}
Feed Conversion Ratio (%)	5.27±0.32^{a}	5.06±0.11^{b}	4.75±0.19^{c}	3.73±0.12^{d}
Feed Conversion efficiency (%)	0.18±0.01^{c}	0.19±0.01^{bc}	0.21±0.02^{b}	0.26±0.02^{a}
condition Factor	0.59±0.09^{a}	0.53±0.09^{ab}	0.51±0.11^{abc}	0.50±0.12^{abc}
Survival rate (%)	79.99±5.71^{b}	94.28±4.28^{a}	96.18±2.18^{a}	98.09±2.18^{a}

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

These results disagree with our findings, although fish and crustaceans may respond differently to probiotics.

The better body weight and SGR for weight and length were obtained in experimental treatments. Similar finding were observed by Gatesoupe [17] in using *Bacillus toyoi* on turbot (*Scophthalmus maximus* Linnaeus, 1758), where Swain *et al* [18] in Indian carps that improved the growth factors and feeding performance and Ghosh *et al* [19] on the Rohu.

The growth parameters were significantly affected by addition of synbiotics to the rearing tanks ($p<0.05$). The synbiotic had positive effect on growth parameters in all of synbiotic treatments in comparison with control treatment. The maximum of final body weight (FBW) (0.59±0.17 g), specific growth rate (SGR) (1.17 +0.15% body weight/day), Feed Conversion efficiency (FCE) (0.26±0.02%) were observed in treatment T3. The lowest of growth parameters were obtained in control treatment, while the highest condition factor (CF) (0.59±0.09), food conversion ratio (FCR) (5.27±0.32), were obtained in this treatment where the fish larvae fed by unsupplemented Biomar.

Also supplemented Biomar with symbiotic had significant positive effect on survival rate. Bagheri *et al* [20] found that supplementation of trout starter diet with the proper density of commercial bacillus probiotic could be beneficial for growth and survival of rainbow trout fry. This finding agrees with our results. Ghosh *et al* [21] indicated that the *B. circulans*, *B. subtilis* and *Bacillus pamilus*, isolated from the gut of Rohu, have extracellular protease, amylase and cellulose and play an important role in the nutrition of Rohu fingerlings. The photosynthetic bacteria and *Bacillus sp*. (isolated from the pond of common carp) was used in diet of common carp (*Cyprinus carpio* Linnaeus, 1758) by Yanbo & Zirong [22].

REFERENCES

1. Chou, T.C., D. Rideout, J. Chou and J.R. Bertino, 1991. Chemotherapeutic synergism, potentiation and antagonism. In: Dulbecco R (Ed), Encyclopedia of human biology, vol. 2. Academic Press, San Diego, California, pp: 371-379.
2. Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr., 125: 1401-1412.
3. Rodriguez-Estrada, U., S. Satoh, Y. Haga, H. Fushimi and J. Sweetman, 2009. Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydrobutyric acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. Aquacult Sci., 57: 609-617.
4. Fuller, R., 1989. Probiotics in man and animals. J Appl. Bacteriol., 66: 365-378.
5. Rengpipat, S., W. Phianphak, S. Piyatiratitvorakul and P. Menasveta, 1998. Effects of probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture, 167: 301-313.
6. Wang, Y.B., Z.R. Xu and M.S. Xia, 2005. The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. Fisheries Sci., 71: 1036-1044.
7. Verschuere, L., G. Rombout, P. Sorgeloos and W. Verstraete, 2000. Probiotic bacteria as biological control agents in aquaculture. Microbiol Mol Biol Rev., 64: 655-671.
8. Moriarty, D.J.W., 1998. Control of luminous Vibrio species in penaeid aquaculture pond. Aquaculture, 164: 351-358.

9. Fishman, M.C., 2001. Genomics: Zebrafish-the canonical vertebrate. Sci., 294: 1290-1291.
10. Penberthy, W.T., E. Shafizadeh and S. Lin, 2002. The zebrafish as a model for human disease. Frontiers in Bioscience, 7: 1439-1453.
11. Shiels, H.A., C. Cros, H. Dixey, C. Wilson, G. Luxan and F. Brette, 2009. The zebrafish heart- A suitable model for human cardiology? Comparative Biochemistry and Physiology-Part A: Molecular and Integrative Physiol., 153: S84-S89
12. Sumanas, S. and S. Lin, 2004. Zebrafish as a model system for drug target screening and validation Drug Discovery Today: TARGETS, 3: 89-96.
13. Keller, E.T., J.M. Keller and G. Gillespie, 2006. The Use of Mature Zebrafish (*Danio rerio*) as a Model for Human Aging and Disease. Handbook of Models for Human Aging. pp: 309-316.
14. Rawls, J.F., 2004. Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. PNAS, 101: 4596-4601.
15. A.O.A.C. 2000. Official methods of analysis. Association of official analytical chemist. EUA.
16. Shariff, M., F.M. Yusoff, T.N. Devaraja, S. Srinivasa, P. Rao, 2001. The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* (Fabricius), ponds. Aquac. Res., 32: 181-187.
17. Gatesoupe, F.J., 1991. *Bacillus sp*. Spores: A new tool against early bacterial infection in turbot larvae, *Scophthalmus maximus* ln: Larvens P. Jaspers E. Roelands I. (Eds), Larval-fish and crustacean larviculture symposium. European Aquaculture Society, Gent., 24: 409-411.
18. Swain, S.K., P.V. Rangacharyulu, S. Sarkar and K.M. Das, 1996. Effect of a probiotic supplementation on growth, nutrient utilization and carcass composition in merigal fry. Aquaculture, 4: 29-35.
19. Ghosh, K., S.K. Sen and A.K. Ray, 2003. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in Formulated diets for Rohu, *Labeo rohita*, Fingerlings. Aquaculture-Bamidgeh, 55(1): 13-21.
20. Bagheri, T., A. Hedayati, V. Yavari, M. Alizade, A. Farzanfar, 2008. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turkish J. Fisheries and Aquatic Sci., 8: 43-48.
21. Ghosh, K., S.K. Sen and A.K. Ray, 2002. Characterization of Bacillus isolated from the gut of Rohu, *Labeo rohita* fingerlings and its significance in digestion. Appl Aquaculture, 12: 33-42.
22. Yanbo, W. and X. Zirong, 2006. Effect of probiotic for commom carp (*Cyprinus carpio*) based on growth performance and digestive enzymes activities. Animal Feed Science and Technol., 127: 283-292.