International Journal of Pure and Applied Zoology ISSN (Print) : 2320-9577
Volume 1, Issue 3, pp: 249-254, 2013 ISSN (Online): 2320-9585

http://www.ijpaz.com

Research Article

EFFECT OF PROBIOTICS ON REPRODUCTIVE PERFORMANCE IN FEMALE LIVEBEARING ORNAMENTAL FISH *POECILIA SPHENOPS*

G. Chitra[1] and N. Krishnaveni[2]

[1]Departmentof Zoology, Nirmala College for Women, Coimbatore-641 018, Tamil Nadu, India
[2]Departmentof Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641 043, Tamil Nadu, India

Article History: Received 6th September 2013; Accepted 30th September 2013; Published online 30th September 2013

ABSTRACT

A study was carried out to determine the effect of varying dietary probiotic levels on the reproductive performance of a fresh water ornamental species, the black molly (*Poecilia spenops*). The commercial probiotic, Vibact were mixed thoroughly with the artificial feeds at concentrations of 0.2% (T_1), 0.4% (T_2), 0.6% (T_3) and 0.8% (T_4) and fed to healthy fish for a period of 60 days. The obtained results showed that supplementation of probiotic feed increased significantly ($P<0.05$) the fecundity, fry production, fry survival and gonado somatic index (GSI). Fishes fed with 0.4% (T_2) of probiotic supplementation showed significantly ($p<0.05$) better results when compared to all the test diets and control. Based on these data, it is concluded that female black molly brood stocks benefit from inclusion of Vibact in diet during their reproductive stages. Further, study is needed to know the mechanism of action for probiotics such as Vibact and their applications.

Keywords: Probiotic, vibact, *Poecilia spenops*, relative fecundity, gonodo somatic index.

INTRODUCTION

Ornamental fish keeping is one of the most popular hobbies in the world today. The commercial production of ornamental tropical fish is gaining momentum as a global component of international trade fisheries and aquaculture development. The last four decades have witnessed considerable growth and diversification in the international trade of ornamental fish. The live bearing category of the ornamental fish are the most popular of all ornamental fish with the fish hobbyists and entrepreneurs because of the fact that they are brightly colored, accept all kinds of food and breed prolifically to produce living free swimming young ones. Among livebearers, the black mollies (*Poecilia sphenops*) are a very popular group of ornamental fish species due to existence of the variety of body colors and fact that they are easy to breed and keep (Dernekbasi *et al.,* 2010). *Poecilia* species demonstrate viviparous strategy with female storing transferred sperms within the ovary following by internal egg fertilization and hatching of young ones (Chong *et al.,* 2004).

In aquaculture nutrition lipid, protein, fatty acids, vitamin E and C and carotenoids influencing various reproduction process such as fertilization, larval development and fecundity (Izquierdo *et al.,* 2001). Moreover, in contrast to the culture of edible fish, information on the dietary requirements and feeding ornamental fish is limited (Langer, 2011). Nutrition has an important influence on growth and reproductive potential of aquarium fish, and various live feeds have been used for fish rearing. Hormones, nutrient mixture, antibiotics, chemotherapeutants and herbal products are used as nutrient supplements for brood stock of ornamental fish. But there are major limitations to the general use of these agents such as increased risk of suppression of the beneficial microbial activity in the intestinal tract of the breeders. Hence, the breeders easily become prone to disease by the opportunistic pathogens. Moreover the indiscriminate use of antibiotics and chemotherapeutants for improved health and nutrition has been criticized because their use has created problems with drug resistance bacteria,

*****Corresponding author e-mail: e-mail: venisekar1958@gmail.com

toxicity and accumulation both in fish and environment (Ghosh *et al.,* 2008).

In recent years probiotics have been increasingly used in the biological control to prevent diseases in aquaculture. Currently, commercial products are available in liquid or powder presentations, and various technologies have been developed for improvement. Knowledge of probiotics has increased; currently it is known that these microorganisms have an antimicrobial effect through modifying the intestinal microbiota, secreting antibacterial substances (bacteriocins and organic acids), competing with pathogens to prevent their adhesion to the intestine, competing for nutrients necessary for pathogen survival, and producing an antitoxin effect. Probiotic bacteria used as dietary additives seem to offer an attractive choice including overall health benefits to the host organism (Lombardo *et al.,* 2011).

Dharmaraj and Dhevendran (2010) evaluated the efficacy of *Streptomyces* as a probiotic feed for the growth of ornamental fish, *Xiphophorus helleri.* Hernandez *et al.* (2010) analyzed the effects of the commercial probiotic, *Lactobacillus casei* on the growth, and protein content of skin mucus and stress resistance of juveniles of the Porthole live bearer *Poecilia gracilis* (Poecilidae). A study was conducted to examine the effects of the probiotic *Lactobacillus rhamnosus*, as a feed additive, on zebrafish (*Danio rerio*) fecundity (Gioacchini *et al*., 2010). Effect of diet (fatty acid and protein) content during spawning season on fertility, eggs and larvae quality of common porgy was studied by Abrenouch *et al.* (2010). Effect of probiotic immunogen on reproductive performance in female platy (*Xiphophorus maculatus*) was carried by Abasali and Mohamad (2011). Mansa and Allah (2011) studied the effect of dietary vitamin C on reproductive performance of a fresh water ornamental species the platy (*Xiphophorus maculatus*). Nekoubin *et al.* (2012) studied the effect of symbiotic (*Biomin imbo*) on fecundity and reproduction factors of zebra fish (*Danio rerio*).

Dosta *et al.* (2012) to isolated and identified the 16Sr DNA, bacteria with probiotic capabilities from the digestive tract of *Pterophyllum scalare* and evaluate their ability to adhere to the intestinal epithelium using immunohistochemical techniques and bacteriological analysis. Martínez Cruz *et al.* (2012) listed the in applications of probiotics in aquaculture. In this context the present investigation was designed to evaluate the efficacy of commercial probiotic (Vibact) on reproductive performance of female live bearing ornamental fish, *Poecilia sphenops.*

MATERIALS AND METHODS

Experimental Animal

P. sphenops (black molly) is an omnivorous ornamental fish was chosen for the present study as an experimental animal. It belongs to the genus *Poecilia*, known under the common name molly. It is sometimes called short-finned molly or common molly. It inhabits in fresh water and successfully establishes in a variety of environmental conditions. It can grow to a maximum length of 3 inches.

Procurement and Acclimatization

About 4 months old juveniles of black molly (*P. sphenops*) were purchased from a commercial fish farm (Bema fish farm), in Coimbatore. The collected fishes were transported to laboratory in a polythene bag with oxygen. *P. sphenops* having a length 3.5-4 cm approximately were selected for the study. The chosen fishes were transferred to a plastic water tub and acclimatized to the laboratory conditions for a period of 15 days. The fish were kept in water tub and the water was changed daily in order to maintain sufficient amount of oxygen and to get rid of toxic ammonia in the trough. They were fed with pellet diet regularly.

Probiotics

A commercial probiotic vibact has been selected for the present investigation. The vibact tablet contains mixture of bacterial culture including *Streptococcus faecalis* (30 million), *Clostridium butyricum* (2 million), *Bacillus mesentericus* (1 million), *Lactic acid bacillus* (50 million).

Probiotic Feed Preparation

The selected probiotic vibact tablet was supplemented at the levels of 0.2, 0.4, 0.6 and 0.8 g to 100 gm of basal diet. The basal diet was prepared by using (wheat flour, soybean, groundnut oil cake, fish meal and corn flour. The experimental diets were prepared by thoroughly mixing the dry ingredients with water, steam sterilized and after cooling 0.2, 0.4 and 0.6 and 0.8 gm of probiotic tablets were added to the basal diet and soft dough was prepared. This was

then passed through a mincer and the obtained pellets were dried properly. These pellets were packed in an air tight polythene bags to avoid fungal infection. The proximate composition (moisture, protein, lipid and ash) of all probiotic feeds and control feed were determined using standard procedures of AOAC (1990).

Experimental Condition

The feeding trial was conducted in laboratory in circular plastic tubs for 60 days. Before feeding the experimental feed the initial weight of all the fishes were noted. Fishes were divided into four and the groups were fed with different concentrations of 0.2 (T_1), 0.4 (T_2) and 0.6 (T_3) and 0.8 gm (T_4) of probiotic supplemented feeds during the experimental period (Table 1). The experimental feeds were given to the fishes twice a day and the control group was also maintained. The fish were fed with feed at 5% of their body weight daily into two split doses throughout the experimental period. The unutilized feed and fecal matter were collected before each morning and water was changed one in every two days.

Table 1. Proximate composition of control and different experimental diets.

Proximate composition	T_1	T_2	T_3	T_4	T_0
Moisture	34.84	34.60	34.23	35.18	34.10
Protein	5.18	5.34	5.30	4.81	4.16
Lipid	10.35	10.62	10.73	10.64	10.84
Ash	8.65	9.16	9.04	8.31	8.80

Values are represented as mean.

Parameters Analyzed

Different parameters of reproductive performances such as relative fecundity (Total fry production throughout experimental period / Mean weight of female in gram), gonado somatic index (Ovary weight /Body weight X 100), fry survival (Total live fry after time / Total fry production X100),dead fry, deformed fry, weight and length of fry and weight and length of adult. All data obtained from experiments were analyzed by one-way analysis of variance (ANOVA) to compare the significance between control and experimental diets. Significance was considered at 5% ($P<0.05$) level.

RESULTS AND DISCUSSION

The results of this study demonstrated that incorporation of probiotics in feed favorably influenced the reproductive performance of live bearer's *P. sphenops* in terms of high fecundity, high gonado somatic index, high fry survival, reduction in fry mortality and deformity and higher average weight and length of fries (Table 2).

Table 2. Reproductive performance of different experimental groups of *Poecilia sphenops.*

Experimental groups	T_1	T_2	T_3	T_4	T_0
Relantive fecundity	17.02±0.58	75.14±1.73	12.12±0.91	14.03± 0.84	7.04± 1.52
Fry survival	16.04 ±0.50	75.06 ±1.73	41.02 ±18.35	38.12 ±1.156	4.05± 0.37
Dead fry	1.59 ±1.88	-	-	1.52 ±1.92	1.68± 0.57
Deformed fry	0.23± 0.50	-	-	-	1.02 ±0.577
Fry weight(g)	1.05 ±0.10	1.32 ±0.10	0.84± 0.10	1.16 ±0.58	0.83± 0.04
Fry length(mm)	1.14 ±0.12	1.06 ±0.12	1.12 ±0.04	1.17 ±0.92	1.40 ±0.10
Weight of spawning females(g)	2.92 ±0.04	3.36± 0.10	3.20 ±0.10	3.0 ±0.08	3.42 ±0.10
Length of spawning females(mm)	4.28± 0.04	4.46 ±0.08	3.91 ±2.50	4.01 ±0.58	4.02 ±0.58
GSI (%)	6.88 ±0.01	12.12 ±0.01	9.67 ±0.02	8.13± 0.65	6.06 ±0.01

Values are represented as mean ± SE.

The results clearly showed that dietary probiotic supplementation had beneficial effects on relative fecundity of black molly, *P. sphenops*. Efficiency of probiotic incorporation on reproductive performance of *P. sphenops* resulted better than control (P<0.05). The different treatments of vibact were also differed significantly for relative fecundity, among the four different concentrations, 0.4% of vibact supplemented to *P. sphenops*, the greatest effect (75±1.732) was obtained during 60 days of experimental period. Reproduction is gated by the state of body energy reserve and is sensitive to different metabolic cues; the neuroendocrine mechanisms responsible for the tight coupling between energy homeostasis and fertility are represented by metabolic hormones and neuropeptides that integrate the hypothalamic center governing reproduction, controlling the expression and release of growth hormone releasing hormone (GHRH) (Fernandez, *et al.,* 2006; Castellano *et al.,* 2009; Zohar *et al.,* 2010).Thus, full activation of the hypothalamic pituitary gonadal axis at puberty and its proper functioning in adulthood critically depend on adequate body energy stores (Hill *et al.,* 2008). The identification of the adipose hormone, leptin, which signals the magnitude of energy stores to the hypothalamic centers governing reproduction (Goumenou *et al.,* 2003), represented an important step toward understanding the mechanisms controlling this interplay.

The dietary administration of an indigenous spore-forming bacillus probiont resulted in an effective probiont colonization and proliferation in the host digestive tract (Rengpipat *et al.,* 2000). Probiotic bacteria established in the gut enhance brood stock and larval nutrition by synthesizing essential nutrients (protein and essential fatty acids) and enzymes (amylase, protease and lipase) (Irianto and Austin, 2002). Probiotic bacteria in the fish intestine enhances host enzymes secretion by the superior maturation of fish intestinal secretary cells (Tovar *et al.,* 2002), which increases the digestive efficiency of the complex protein and lipids included in the diet, thus increasing the rate at which they can be assimilated by the host animal. Probiotic bacteria also produce B group vitamins (Goldin and Gorbach, 1992) and the production and supply of vitamin B complex and certain unknown stimulants could have played a key role in the elevated reproductive performance of the probiotic feed-fed fish. In this present study, total fry production was higher in probiotic supplemented groups than the control.

The average numbers of dead and deformed fry were found to vary significantly (P<0.05) in the *P. sphenops*, with the higher values being recorded in control feed-fed fish and the lower values in probiotis feed-fed fish. The average weight and length of released fry were also significantly (P<0.05) more in the probiotic feed-fed fish. The synthesis of B Complex vitamins, particularly thiamine (vitamin B1) and vitamin B_{12} by the probiont *Bacillus subtilis*, could have accounted for the reduced numbers of dead and deformed fry in the probiotic diet fed fish (Ghosh *et al.,* 2007). This may be the reason for the reduction of dead and deformed fries in the probiotic experimental group (0.2 to 0.8%) than the control fish.

The present investigation showed highest fry survival in the probiotic supplemented fishes and maximum values were recorded in 0.4% of probiotic incorporation during the experimental period. These findings were in agreement with the findings of Abasali and Mohamad (2011), who noticed a highest value of fry survival in platy, *Xiphophorus maculatus.* Supplementation of feed with probiotics significantly (P<0.05) increased the gonodo somatic index, fecundity and fry production of spawning females and length and weight of fry in all the treated groups. The number of dead and deformed fry were also significantly lower (P<0.05) in fish fed with the probiotic feeds. The use of higher concentration of the probiont in diet did not always lead to significantly improved reproductive performance of the spawners.

This The beneficial influence of vibact probiotic on reproductive performance was possibly due to alternation of the fish intestinal microflora and improving the beneficial mixed bacteria culture by probiotic ingredients, particularly *Streptococcus faecalis, Clostridium butyricum, Bacillus mesentericus* and *Lactic acid bacillus*. Higher survival rate and lower

*****Corresponding author e-mail: e-mail: venisekar1958@gmail.com

deformed fry could be linked to the intestine probiotic bacterial population which produces B complex vitamins. It is important to define the probiotic levels administrated to fish to avoid overdosing and under dosing with resultant study considered different probiont levels and concluded that a probiotic concentration of 0.4% was sufficient for enhanced reproductive performance, and that the use of a higher concentration of probiotic cells did not always yield significantly better results. The results showed that dietary probiotic supplementation improved the reproductive performance in terms of higher gonado somatic index, fecundity and fry survival of the *P. sphenops*. Collectively, this study showed that female livebearers benefit from inclusion of probiotics in diet during their reproductive stages.

CONCLUSIONS

It can be concluded that the commercial probiotic preparation Vibact used in this study can improve the reproductive performance of female black molly fish brood stocks during reproductive stages. Moreover, further studies are needed to determine the mechanisms responsible for the improvement of reproductive performance of fish. There is an ever-increasing commercial interest in the ornamental fish trade in India and all over the world; the application for probiotics in aquaculture will be beneficial especially for improving reproductive efficacy of the ornamental fishes. The field of probiotics intended for aquaculture organisms is now gaining much importance. There are many valuable probionts available in the markets. However, research on optimization of diets to improve the reproductive performance is still in its infancy.

CONFLICT OF INTERESTS

The author declares that there are no conflicts of interests associated with this article.

REFERENCES

Abasali, H. and Mohammad, S., 2011. Dietary probiotic immunogen supple-mentation in reproductive performance of platy (*Xiphophoru smaculatus*). *Vet. Res.*, 4(3): 66-70.

Abrenouch, A., Ali, A.A., Chebbaki, K., Akharbach, H. and Mohammed, S., 2010. Effect of diet (fatty acid and protein) content during spawning season on fertility, eggs and larvae quality of common progy (*Pagrus pagrus* Linnaeus 1758). *Agr. Biol. J. North America*., 135: 21-25.

AOAC, 1990. Official methods of analysis of AOAC, Vol.1, 15th edition, Association of official analytical chemists, Arlington, VA, USA.

Castellano, J.M., Roa, R.M., Luque, C., Dieguez, E., Aguilar, L., Pinilla and Tena–abolicSempere, D., 2009. Kiss-1/Kiss peptins and the metabolic control of reproduction: Physiologic roles and putative physio pathological implications, Peptides, 30: 57-66.

Chong A.S.C., Ishak, S.D., Osman, Z. and Hashim, R., 2004. Effect of dietary protein level on the reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquaculture*., 234: 381-392.

Dosta, M., Barrera, T.C., Francisco, J., Perrino, F., Reyes, L.M., Gutiérrez, H.H. and Suárez, S.C., 2012. Bacteria with probiotic capabilities isolated from the digestive tract of the ornamental fish *Pterophyllum scalare*. http://dx.doi.org/10.5772/45954.

Dernekbasi, S., Unal, H., Karayucel, I. and Aral, O. 2010. Effect of dietary supplementation of different rates of spirulina (*Spirulina platensis*) on growth and feed conversion in guppy (*Poeciliarecticulate* Peters, 1860). *J. Ani. Vet. Adv.*, 9(9): 1395-1399.

Dharmaraj, S. and Dhevendran, K., 2010. Evaluation of *Streptomyces* as a probiotic feed for the growth of ornamental fish *Xiphophorus helleri*. *Food Techn. Biotechn.*, 48(4): 497–504.

Fernandez, R., Martini, A.C., Navarro, J.M., Castello, C., Dieguez, E., Auliar, L., Pinnilla, P. and Tenasempere, M. 2006. Novel signals for the integration of energy balance and reproduction. *Mol. Cel. Endocrinol.*, 254: 127-132.

Ghosh, S., Sinha, A. and Sahu, C., 2007. Effect of probiotic on reproduction performance in female live bearing ornamental fish. *Aqu. Res.*, 38: 518-526.

Ghosh, S., Sinha, A. and Sahu, C., 2008. Dietary probiotic supplementation in growth and

health of live-bearing ornamental fishes. *Aqu.Nut.,* 14: 289-299.

Gioacchini, G., Maradonna, F., Lombardo, F., Bizzaro, D., Olivotto, I. and Carnevali, O., 2010. Increase of fecundity by probiotic administration in zebra fish (*Danio rerio*). *Res Reprod.,* 140: 953-959.

Goldin, B.R. and Gorbach, S.L., 1992. Probiotics for Humans, In: Probiotics.The Scientific Basis (Ed.by Fuller, R.), pp: 355-376.

Goumenou, A.G., Matalliotakis, I.M., Koumantakis, G.M. and Pandis, D.K., 2003. The role of leptin in fertility. *Eur. J. Obstet. Gynecol. Reprod. Biol.,* 106: 118-124.

Hernandez, L.H.H., Barrera, T.C., Mejia, J.C., 2010. Effects of the commercial probiotic *Lactobacillus casei* on the growth, protein content of skin mucus and stress resistance of juveniles of the Porthole livebearer *Poecilia gracilis* (Poecilidae). *Aquacult. Nutr.,* 16 (4): 407–411.

Hill, J.W., Elmquist, J.K. and Elias, S., 2008. Hypothalamic pathways linking energy balance and reproduction. *Am. J. Physiol. Endocrinol. Metab.,* 294(5): 827-832.

Irianto, A. and Austin, B., 2002. Probiotic in aquaculture. *J. Fish Dis.,* 25: 633-642.

Izquierdo, M.S., Fernandez-pelacios and Tacon, A.G.J., 2001. Effect of brood stock nutrition on reproductive performance of fish, *Aquacult.,* 197: 25-42.

Langer, S., Bakhtiyar, Y., Lakhnotra, R. 2011.Replacement of fish meal with locally available ingredients in diet composition of *Macrobrachium dayanum*. Afr. J. Agr. Res., 6(5): 1080-1084.

Lombardo, F., Gioacchini, O., Carnevali, O. 2011. Probiotic-based nutritional effects on killifish reproduction. *Fish.*Aquaculture J., 7: 27-33.

Mansa, M.J. and Allah, V.J. 2011. Effect of dietary supplementation of vitamin C on the reproductive performance of female live bearing ornamental fish. *J. Ani. Vet. Adv.,* 10(16): 2074-2078.

Martinez Cruz, P., Ana L., Ibanez, Oscar, A., Hermosillo, M. and Ramirez Saad, H.C., 2012. Use of probiotics in aquaculture. *ISRN Microbiology*, Article–13.

Nekoubin, H., Javaheri, S., Imanpour, R.M., 2012. Effect of symbiotic (*Biomin imbo*) on fecundity and reproductive factors of zebra fish (*Danio rerio*). *Wor. J. Fish Mar. Sci.,* 4(1): 65-67.

Rengipipat, S., Rukpratanpom, S., Piyatriratiti V.S. and P. Menasaveta. 2000. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiotic bacterium (*Bacillus* S11). *Aquacul.,* 191: 271-288.

Tovar D., Zambonino, J., Cahu G., Gatesoupe, E., Vazquez-juarez, R. and Lesel, R. 2002. Effect of yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Centrarchuslabrax*) larvae. *Aquacult*, 204: 113-123.

Zohar, Y., Mun-Oz-Cueto, J.A., Elizur. A. and Kah, O., 2010. Neuro endocrinology of reproduction in teleost fish. *Gen. Comp. Endocrinol.,*165: 438-455.