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Effect of Dietary Supplementation with Probiotic on Reproductive Performance of Female Livebearing Ornamental Fish

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Abstract: A study to determine the effect of varying dietary probiotic levels on the reproductive performance of a freshwater ornamental species, the swordtail (*Xiphophorus helleri*) was carried out. The commercial probiotic, Primalac were mixed thoroughly with the artificial feeds at concentration of 0.0% (A), 0.04% (B), 0.09% (C) and 0.14% (D) and fed to healthy fish for a period of 26 weeks. Results showed that supplementation of feed with probiotic increased significantly ($p<0.05$) the Gonadosomatic Index (GSI), fecundity and fry production of female broodstock. Fry production, fecundity and fry survival were significantly higher in group B as compared to the group A (control) ($p<0.05$). The highest significant Gonadosomatic Index (GSI) and fry weight were observed in group B ($p<0.01$). The fish of experimental groups B and A recorded the lowest and highest deformed fry (%), respectively. Based on these data, it is concluded that female swordtail broodstocks benefit from inclusion of primalac in diet during their reproductive stages. Further study in needed into the mechanism (s) of action for probiotics such as Primalac and their application in aquaculture.

Key words: Probiotic, primalac, *Xiphophorus helleri*, relative fecundity, gonadosomatic index, Iran

INTRODUCTION

The ornamental fish sector is one of the most economic and profitable areas of fish farming activities. The swordtails (*Xiphophorus helleri*) are a very popular group of ornamental fish species due to the existence of a variety of body colors and fin patterns. Moreover, they are easy to breed (Ling *et al*., 2006). The swordtails are viviparous breeders with female storing transferred sperms within the ovaries for internal egg fertilization followed by hatching of eggs and a gestation period of approximately 27 days before release of free swimming embryos (Siciliano, 1972; Ling *et al*., 2006). In aquaculture nutrition, lipid, protein, fatty acids, vitamins E and C and carotenoids influencing various reproduction processes such as fertilization, larval development and fecundity (Izquierdo *et al*., 2001).

There are many studies reporting nutrient mixture, hormones, antibiotics, chemotherapeutants and herbal products are used as nutrient supplements for broodstock 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. As the alternative to repair these deficiencies, application of probiotics in aquaculture is new and developing venture which needs further research in fish. Probiotic is a preparation of or a product containing viable, defined microorganisms in sufficient numbers which alter the microflora (by implantation or colonization) in a compartment of host and by that extract beneficial health in this host (Schrezenmeir and Vrese, 2001).

Several mechanisms have been explained as the modest of action for probiotic bacteria. The competitive exclusion based on the removal of the pathogen by the beneficial population is believed to plays an important role in this process (Gatesoupe, 1999; Ghosh *et al*., 2008). Many researchers also reported that the enhancement of animal growth can be attributed to the nutritional benefits of probiotic bacteria such as production of important digestive enzymes, availability of minerals and trace elements and vitamin production (Helerer *et al*., 1998; Ghosh *et al*., 2008). Although, the use of probiotics in growth performance and in improving disease resistance ability has been well studied (Bogut *et al*., 1998; Robertson *et al*., 2000; Rengipipat *et al*., 2000; Ghosh *et al*., 2007, 2008), research on the effect of feeding probiotics on various reproductive performance of fish is

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elusive and only poorly been studied yet. The current study was therefore, designed to investigate the effects of dietary Primalac (a commercial probiotic) levels on reproductive performance of swordtail.

MATERIALS AND METHODS

Probiotic bacteria strain: A commercial probiotic Primalac a mixture of equal proportions of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium thermophilum* was obtained from the Nikandishan Farjad Commerce Corporation, Tehran, Iran.

Preparation of control and probiotic feed: The ingredients and proximate compositions of the basal and experimental diets are shown in Table 1. The experimental diets were prepared by incorporating Primalac to the feeds in the concentration of 0.04% (B), 0.09% (C), 0.14% (D) of the diets. Control diet (A) was also prepared using the same composition of ingredients except Primalac. To prepare the diets, first ingredients were blended thoroughly with additional water and 1% binder to make a paste of each diet. The pastes were then cold extruded and cut into pellets. The diets were air-dried and stored at -2°C (Sardar *et al*., 2007) in tight containers until use.

Table 1: Formulation (dry weight %) and chemical composition of the experimental diets

Chemical composition	Diet			
	A (control)	B	C	D
Ingredients (%)				
Fish meal[a]	40.00	40.00	40.00	40.00
Whole wheat meal	10.00	10.00	10.00	10.00
Barley meal	10.00	10.00	10.00	10.00
Soybean meal	14.00	14.00	14.00	14.00
Corn meal	10.00	10.00	10.00	10.00
Fish oil[b]	5.00	5.00	5.00	5.00
Sunflower oil	3.00	3.00	3.00	3.00
Soybean oil	3.00	3.00	3.00	3.00
Lecitine[c]	2.00	2.00	2.00	2.00
Vitamin premix[d]	1.00	1.00	1.00	1.00
Mineral premix[e]	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	0.75	0.75	0.75	0.75
Chromic oxide[f]	0.25	0.25	0.25	0.25
Probiotic primalac[g]	0.00	0.04	0.10	0.14
Proximate chemical composition[h]				
Crude protein	34.90	34.81	34.65	34.84
Crude lipid	16.30	16.37	16.41	16.45
Ash	10.41	10.71	10.45	10.25
Moisture	8.40	8.20	8.40	8.10
Gross energy (kcal g^{-1})	5.45	5.44	5.44	5.45

[a]Fish meal: Pars kelika Co., Mirood, Iran; [b]Herring oil: [c]Aquagran, Riceland (USA); [d]Vitamin premix contained the following vitamins (each kg^{-1} diet): Vitamin A, 10 000 IU; Vitamin D_3, 2000 IU; Vitamin E, 100 mg; Vitamin K, 20 mg; Vitamin B_1, 400 mg; Vitamin B_2, 40 mg; Vitamin B_6, 20 mg; Vitamin B_{12}, 0.04 mg; Biotin, 0.2 mg; Choline chloride, 1200 mg; Folic acid, 10 mg; Inositol, 200 mg; Niacin, 200 mg; Pantothenic calcium, 100 mg; [e]Contained (g kg^{-1} mix): $MgSO_4$, $2H_2O$, 127.5; KCl, 50.0; NaCl, 60.0; $CaHPO_4$, $2H_2O$, 727.8; $FeSO_4$ $7H_2O$, 25.0; $ZnSO_4$ $7H_2O$, 5.5; CuSO, $5H_2O$, 0.785; $MnSO_4$, $4H_2O$, 2.54; $CoSO_4$, $4H_2O$, 0.478; $Ca(IO_3)_2$, $6H_2O$, 0.295; $CrCl_3$, $6H_2O$, 0.128; [f]Sigma Aldrich Company, Poole, Dorset, UK; [g]Nikandishan Farjad Commerce Corporation, Tehran, Iran; [h]Expressed as percent dry matter unless indicated otherwise

Experimental animals: About 1 month old juveniles of swordtails (*Xiphophorus helleri*) were purchased from a commercial fish farm at Gorgan, Golestan, Iran. The processes of raising, conditioning and selection swordtails for feeding trial were according to Ghosh *et al*. (2007).

Feeding and experimental design: Virgin females aged 4 month (average weight 0.60-0.61 g and length 3.2-3.3 mm) were used for experiment. Fish were divided randomly into 4 groups (A-D). About 4 replicate tanks (60 L) were used for evaluation of each diet with a total of 10 females selected and stocked in each tank. Group A received the basal diet and acted as control. Group B-D were fed with probiotic Primalac at 0.4, 0.9 and 1.4 g kg^{-1} of feed, respectively. The fish were fed with feed at 5% of their body weight daily in two split doses throughout the experimental period at 09:00 and 17:00 h. The feeding trial lasted for 26 weeks. Virgin males aged 4 months were kept separately in a large tank (250 L) and fed frozen bloodworms twice daily.

During the experimental period, 3 males were randomly selected and introduced into each of the 4 different groups at an interval of 30 days. These males were left with the females for 5 days before returning them to the holding tank. The wastes and fecal matter were siphoned out on every 3rd day. During feeding, males were separated from females using plastic sheet, bundles of tied-nylon strings were placed into each experimental tank as shelter for new free-swimming fry to avoid cannibalism by parental fish. The water quality parameters were monitored every day and maintained at optimal level by regular water exchange (temperature, 24.3±1.5°C; dissolved oxygen, 7.1±0.52 mg L^{-1}; salinity, 0.43±0.07 ppt; pH, 7.65±0.21 units; ammonia-nitrogen<0.18).

Proximate composition of diet: Analysis of dry matter (by oven drying at 105°C for 24 h), crude lipid (extraction with petroleum ether by Soxhlet apparatus), crud protein (kjeldahl apparatus, nitrogen×6.25) and ash (incineration in a muffle furnace at 600°C for 4 h) were performed for feed (AOAC, 2000).

Determination of reproductive parameters: Reproductive performances were calculated as follows:

- Relative fecundity = Total fry production at throughout experimental period/mean weight of female (g)
- Total fry production per female = Total fry harvested throughout experimental period per number of female

- Gonadosomatic index (%) = (Ovary weight/body weight)×100
- Survival (%) = (Total live fry (No.) after t/total fry production (No.) throughout experimental period)× 100 where t is the days of experiment

Data and statistical analysis: In order to determine significant differences, results were analyzed by a 1 way Analysis of Variance (ANOVA) using the SAS (2002-2003) package. Differences among means were determined and compared by LSD's test. Differences were also considered significant when $p<0.05$.

RESULTS AND DISCUSSION

Total fry production per female was highest for the group B while there was no significant difference ($p>0.01$) among A (control), C and D (Fig. 1). The highest relative fecundity was observed in group B followed by group C

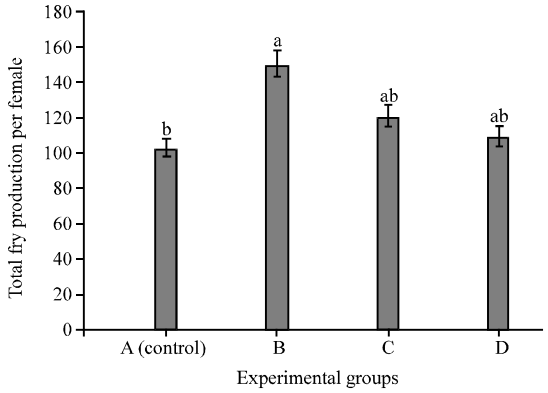

Fig. 1: Total fry production per female of different experimental groups of *Xiphophorus helleri*. Means with the same letters are not significantly different ($p>0.05$). Data are expressed as mean±SE

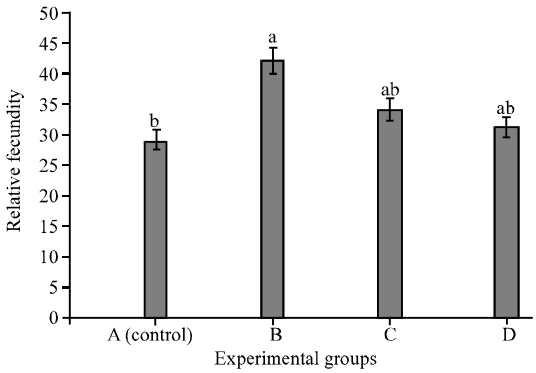

Fig. 2: Relative fecundity of different experimental groups of *Xiphophorus helleri*. Means with the same letters are not significantly different ($p>0.05$). Data are expressed as mean±SE

relative fecundity (Fig. 2). The Gonadosomatic Index (GSI) and D whereas the control group (A) showed the lowest of fish fed the probiotic feeds (group B-D) were significantly higher ($p<0.01$) than fish fed the control feed (group A). Moreover, the fish of the experimental group B exhibited the highest value (11.09±0.05%) of GSI (Fig. 3). The results of the weight and length of fry are shown in Table 2. The fish of the experimental group B recorded the highest average length of fry and the fish of the experimental group D recorded the lowest. The average length of fry in the experimental groups A and B were found to differ significantly ($p<0.01$) from that in the experimental group C and D. The average weight of fry was found highest in the experimental group B and lowest in group D and A. In addition, no significant difference was observed in average weight of fry between the experimental group D and A (control group).

The percentage of deformed fry were found to be significantly higher ($p<0.05$) in control (A) and lower in group B (Fig. 4). Although, the experimental group B recorded the highest survival rate of fry and the experimental group A (control group) the lowest, the differences among experimental groups A, C and D were not significant ($p>0.05$) (Fig. 5). The results showed that Primalac supplemented diets favorably influenced the GSI, relative fecundity, fry survival, total fry production per female, reduction in fry deformity and higher average weight and length of fry. There is a positive correlation between the presence of proteins and fatty acids in the

Table 2: Weight and length of fry in different experimental groups of *Xiphophorus helleri*

Experimental groups	A (control)	B	C	D
Fry length (mm)	7.43±0.011^{a}	7.44±0.005^{a}	7.31±0.017^{b}	7.32±0.028^{b}
Fry weight (mg)	5.33±0.005^{c}	5.67±0.023^{a}	5.41±0.011^{b}	5.31±0.011^{c}

Means with the same letters in each row are not significantly different ($p>0.05$); data are expressed as mean±SE

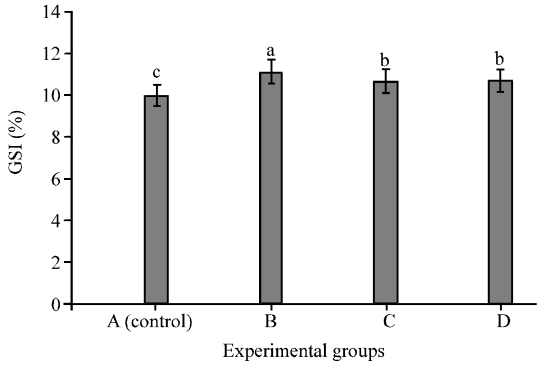

Fig. 3: Gonadosomatic index (%) of different experimental groups of *Xiphophorus helleri*; Data are expressed as mean±SE. Values receiving same superscript are statistically not significant ($p>0.05$)

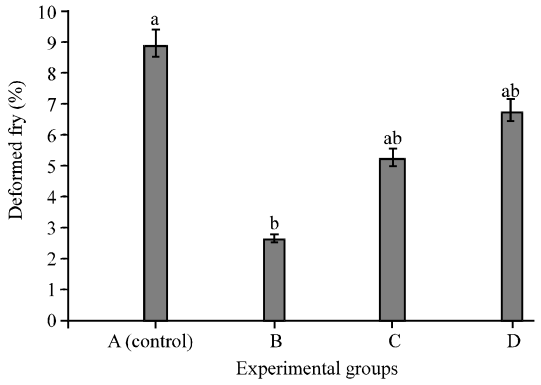

Fig. 4: Deformed fry (%) of different experimental groups of *Xiphophorus helleri*. Data are expressed as mean±SE. Values receiving same superscript are statistically not significant (p>0.05)

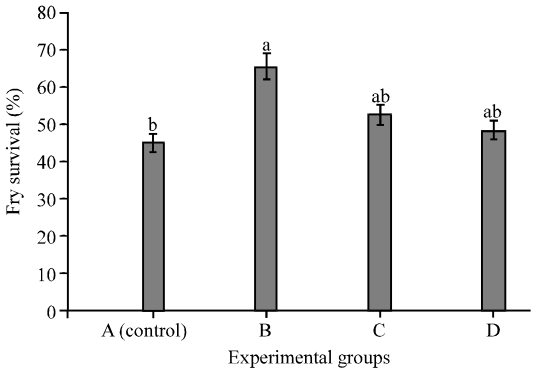

Fig. 5: Fry survival (%) of different experimental groups of *Xiphophorus helleri*. Data are expressed as mean±SE. Values receiving same superscript are statistically not significant (p>0.05)

broodstoock diet and reproductive-related factors such a better oocyte development and maturation, higher rate of vitellogenesis and larger egg size (Milton and Arthington, 1983; Shim *et al*., 1989; Seghal and Toor, 1991; Ghosh *et al*., 2007; Dahlgren, 1980; Ling *et al*., 2006). The results revealed that the probiotic incorporated diets helped to increase the reproductive performance of the experimental fish. This is in agreement with the report of Ghosh *et al*. (2007) that reproductive performance was enhanced in the probiotic feed fed fish.

These could be attributed to the balanced production of essential nutrients (particular essential fatty acids) by intestinal probiotic bacteria (Irianto and Austin, 2002; Ghosh *et al*., 2007). Several studies have shown the importance of balancing the composition of dietary unsaturated fatty acids such as arachidonic acid, docosahexaenoic acid and eicosapentaenoic acid in fish to ensure optimized broodstock reproductive performances and enhance larval quality (Ling *et al*., 2006). Moreover, essential fatty acids can also supply energy to sustain the spawning activities (Ling *et al*., 2006; Ghosh *et al*., 2007). Probiotic bacteria also affect the production of the vitamins, particularly B group vitamins (Golden and Gorbach, 1992; Ghosh *et al*., 2007). Hence, higher survival rate and lower deformed fry could be linked to the intestine probiotic bacteria which produce B group vitamins. Ghosh *et al*. (2007) reported that the synthesis of vitamin B_1 and B_{12} by the probiotic bacterial strain *Bacillus subtilis* could have accounted for the reduced numbers of dead and deformed fry in some species of livebearing ornamental fish fed diets containing *B. subtilis*. These observations are in agreement with the finding of Ketola *et al*. (1998) who reported that Thiamin (Vitamin B_1) can reduce the mortality of progeny in the Atlantic salmon.

CONCLUSION

In the study, the commertial probiotic (Primalac) researchers used in this study considerably could enhance the reproductive performance of *Xiphophorus maculatus*. In addition, among different groups, B generally showed the best performance in the experiment.

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