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# Effect of Prebiotic Immunogen on Reproductive Performance in Female Swordtail *Xiphophorus helleri*

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**Abstract:** To determine the effect of immunogen as a commercial prebiotic on the reproductive performance of a freshwater ornamental species, swordtail (*Xiphophorus helleri*), fish were fed diets containing four doses of immunogen viz. 0% (A), 0.5% (B), 1% (C) and 1.5% (D) for 26 weeks. Results showed that all experimental groups showed higher fry survival (%), relative fecundity and fry production compared to control (A) ($p<0.01$). Weight and length of fry were significantly higher in group C and D as compared to other groups ($p<0.01$). The Gonadosomatic Index (GSI) was found highest in group C and lowest in control group. The results also showed that the percentage of deformed fry was significantly higher in control group as compared to other groups. Collectively, this study showed that reproductive performance of female swordtail can be improved by dietary prebiotic immunogen supplementation. Further study is needed into the mechanism (s) of action for prebiotics such as immunogen and their application in aquaculture.

**Key words:** Prebiotic, immunogen, *Xiphophorus helleri*, reproductive performance, gonadosomatic index, Iran

## INTRODUCTION

The livebearing category of ornamental fish is the most popular of all ornamental fish due to the existence of a variety of body colors and fin patterns. Moreover, they are easy to breed and accept all kinds of feed (Ling *et al*., 2006; Ghosh *et al*., 2007).

Among livebearers, *Xiphophorus helleri* is one of the most important and familiar ones. Swordtails are viviparous breeders with females storing transferred sperms within the ovaries for internal egg fertilization followed by 27 days before release of free swimming embryos (Siciliano, 1972; Ling *et al*., 2006). Within aquaculture, broodstock nutrition is still poorly understood due to difficulties in conducting studies involving proper feeding and reproduction of broodstock (Chong *et al*., 2004).

Proper nutrition has long been recognized as a critical factor in promoting normal growth and sustaining health of fish. Prepared diets not only provide the essential nutrients that are required for normal physiological functioning but also may serve as the medium by which fish receive other components that may affect their health and reproductive performance (Gatlin, 2002; Li and Gatlin III, 2004). A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health (Gibson and Roberfroid, 1995). Many researchers reported that prebiotic bacteria may affect the growth, feed utilization, health status and body composition of the animals. It is believed that these results are inconclusive however and may be affected by the prebiotic chosen, species, dietary supplementation level and duration of use (Grisdale-Helland *et al*., 2008).

Staykov *et al*. (2007) reported that the growth, feed efficiency and survival of rainbow trout fed a diet supplemented with 2 g $kg^{-1}$ Mannanoligosaccharide (MOS) were significantly greater than that of the fish fed the basal diet. Li and Gatlin III (2004) demonstrated that dietary supplementation with Grobiotic™-AE (a commercial prebiotic) increased the survival and resistance to *Streptococcus iniae* of hybrid striped bass as compared to the basal diet.

The use of prebiotics for enhancing growth parameters and in improving disease resistance ability has been well documented in fish (Darwish *et al*., 2002; Li and Gatlin III, 2004; Mahious *et al*., 2006; Refstie *et al*., 2006; Grisdale-Helland *et al*., 2008) but research on the effect of feeding prebiotics on various reproductive aspects of fish are lacking. This study was therefore, taken up with the novel objective of supplementing immunogen (a

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commercial prebiotic mixture of β-glucans and mannanoligosaccharid) in the diet of swordtails to evaluate its effects on their reproductive performance.

## MATERIALS AND METHODS

**Experimental diets:** The ingredients and proximate compositions of the basal and experimental diets are shown in Table 1. Prebiotic immunogen (International Commerce Corporation USA, INC) was incorporated into three experimental diets, viz. 0.5% (B), 1% (C) and 1.5% (D). Control diet (A) was also prepared using the same composition of ingredients, except the immunogen. Initially, dry ingredients were mixed thoroughly and 1% binder was added. Sufficient water along with the oil ingredients were then added to make a paste of each diet. The paste was then cold extruded and pelletized using a hand pelletizer to obtain 1 mm pellets. Finally, the diets were air-dried and stored at -2°C (Sardar *et al*., 2007) in air tight containers until fed.

**Experimental animals:** One month old juveniles of swordtails (*Xiphophorus helleri*) were purchased from a commercial fish farm at Gorgan, Golestan, Iran. They were kept in 500 L plastic containers with recirculated and aerated water for 3 months until they reached sexual maturity. They were fed with basal experimental diet (Table 1) without supplementation of the Immunogen at 5% of their body weight daily in 2 split doses. Throughout this period, males were separated from females at earliest sign of sexual differentiation to avoid reproduction activities. Female poecilidae can retain active sperm in crypts in their ovaries and oviduct for a period of up to 8 months and become pregnant without another copulation (Dzikowski *et al*., 2001; Ghosh *et al*., 2007). Therefore, only virgin females were used for this study.

**Feeding and experimental design:** Virgin females aged 4 months (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). Four 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 prebiotic immunogen at 0.5, 1 and 1.5% 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 (Hikari®, Hayward, CA, USA) twice daily. During the experimental period, three 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°C±1.5; 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).

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

| | Diet | | | |
|---|---|---|---|---|
| Composition | 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 |
| Prebiotic immunogen[g] | 0.00 | 0.50 | 1.00 | 1.50 |
| **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): $MgSO_4.2H_20$, 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]International Commerce Corporation USA, INC. [h]Expressed as percent dry matter unless indicated otherwise

**Feed analysis:** 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

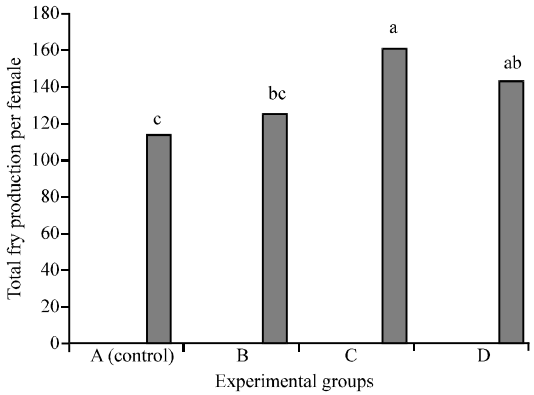


Fig. 1: Total fry production per female of different experimental groups of *Xiphophorus maculatus*. Means with the same letters are not significantly different (p>0.05). Data are expressed as mean±SE

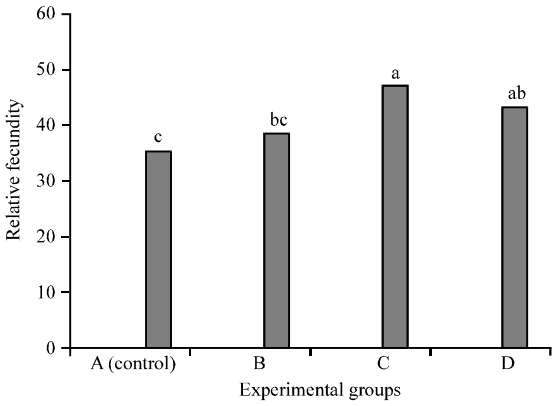


Fig. 2: Relative fecundity of different experimental groups of *Xiphophorus maculatus*. Means with the same letters are not significantly different (p>0.05). Data are expressed as mean±SE

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 day of experiment (Fig. 1).

**Statistical analysis:** In order to determine significant differences, results were analyzed by a one 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

The fish of the experimental group C exhibited the highest value (160±6.93) of total fry production per female which was significantly different (p<0.01) from the lowest value (114±3.46) exhibited by fish of the experimental group A (control group). Similarly, the highest relative fecundity was observed in group C followed by group D and B whereas the control group (A) showed the lowest relative fecundity. The fish fed with the prebiotic feed C (11.08±0.008), D (10.82±0.032) and B (10.23±0.11) exhibited significantly higher (p<0.01) values of Gonadosomatic Index (GSI) than fish fed the control feed (9.73±0.029) (Fig. 2 and 3).

The average weight and length of fry were significantly higher (p<0.01) in group C and D as compared to the control (group A) and group B (Table 2). The average weight and length of fry in the experimental groups C and D were found to differ significantly (p<0.01) from that in the experimental groups A (control) and B. The percentage of deformed fry was found to be significantly lower (p<0.01) in fish of the experimental groups (B-D) fed the prebiotic feeds and significantly higher (p<0.01) in fish of the experimental group (A) fed the control feed (Fig. 4). The percentage of fry survival differed significantly (p<0.01) between the groups with the highest value recorded in fish of the

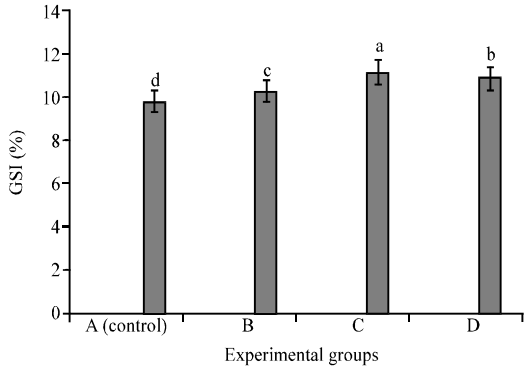


Fig. 3: Gonadosomatic index (%) of different experimental groups of *Xiphophorus maculatus*. Data are expressed as mean±SE. Values receiving same superscript are statistically not significant (p>0.05)

Table 2: The percentage of deformed fry and average weight and length of fry in different experimental groups of *Xiphophorus helleri*

| | Experimental groups | | | |
|---|---|---|---|---|
| Parameters | A (control) | B | C | D |
| Fry length (mm) | 7.01±0.028[b] | 7.03±0.029[b] | 7.18±0.018[a] | 7.17±0.010[a] |
| Fry weight (mg) | 5.18±0.011[b] | 5.17±0.020[b] | 5.32±0.005[a] | 5.32±0.003 |

[a]Means with the same letters in each row are not significantly different (p>0.05). Data are expressed as mean±SE

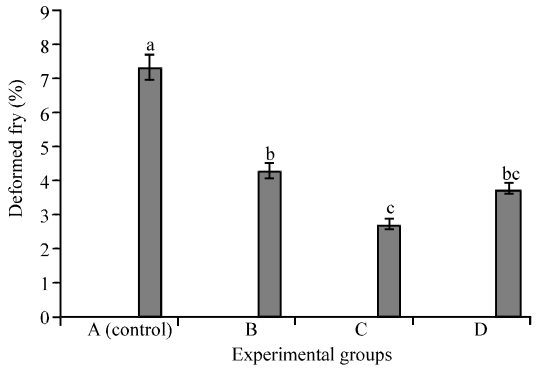


Fig. 4: Deformed fry (%) of different experimental groups of *Xiphophorus helleri*. Data are expressed as mean±SE. Values receiving same superscript are statistically not significant (p>0.05)

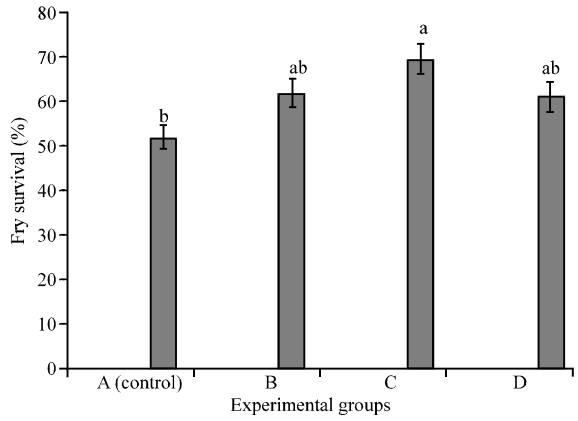


Fig. 5: Fry survival (%) of different experimental groups of *Xiphophorus maculatus*. Data are expressed as mean±SE. Values receiving same superscript are statistically not significant (p>0.05)

group C (69.95±2.91) and the lowest in fish of the control (group A) (52.13±1.72) (Fig. 5). Prebiotics mainly consist of oligosaccharides promoting beneficial bacterial growth within the gastrointestinal tract (Yazawa *et al*., 1978). Immunogen is a commercial prebiotic which mainly composed of β-Glocans (BG) and Mananoligosaccharid (MOS). β-glocan is used as immunostimulatory feed ingredients (Burrells *et al*., 2001), vaccine adjuvant (Rorstad *et al*., 1993) and also has anti-inflammatory effect (s) (Andersson *et al*., 2000).

MOS may function as a prebiotic, favourity growth of beneficial bacteria in the gut (such as Bifidobacteria and Lactobacilli) (Refstie *et al*., 2010). The beneficial influence of Immunogen on reproductive performance was possibly due to alteration of the fish intestinal microflora and improving the beneficial bacteria growth by immunogen ingredients, particularly mannanoligosaccharid. Beneficial bacteria as probiotic bacteria, enhance nutrition by synthesizing essential nutrients (fatty acids, proteins and vitamins) and enzymes (protease, amylase and lipase). The results of this study demonstrated that incorporated of prebiotic immunogen in feed favorably influenced the reproductive performance of *X. helleri* in terms of high GSI, high fry production, high relative fecundity, high fry survival, reduction in fry deformity, higher average weight and length of fry.

These could be attributed to the balanced production of essential nutrients (in 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 (Sargent, 1995; Mazorra *et al*., 2003; 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 four 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

The commercial prebiotic (immunogen), we used in this study, considerably could enhance the reproductive performance of *Xiphophorus maculatus*. In addition among different groups, C generally showed the best performance in the experiment.

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