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Dietary Prebiotic Immunogen Supplementation in Reproductive Performance of Platy (*Xiphophorus maculatus*)

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Abstract: This study was conducted to evaluate the effect of dietary prebiotic immunogen on the reproductive performance of a livebearing ornamental species, platy (*Xiphophorus maculatus*). Fish were fed under four regimes, 0% (A), 0.5% (B), 1% (C) and 1.5% (D), immunogen mixed diet for a period of 26 weeks. Results indicated that Gonadosomatic Index (GSI), fry production, relative fecundity and fry length were significantly ($p<0.01$) higher in group D as compared to the control (group A) and other experimental groups. The percentage of deformed fry was not significantly affected by the various dietary treatments ($p<0.05$). Moreover, fry survival (%) was found lowest in control group. With the observed results, we are able to conclude that female platy fish broodstocks benefit from inclusion of prebiotic immunogen in diet during their reproductive stages.

Key words: Prebiotic, immunogen, *Xiphophorus maculatus*, reproductive performance, fry production, Iran

INTRODUCTION

The culture of ornamental fish is one of the most economic and profitable areas of fish farming activities. The annual international exports of ornamental fish in 2002 were about US $200 million in value (Vannuccini, 2004; Ghosh *et al*., 2007). Poeciliid species demonstrate viviparous strategy with female storing transferred sperms within the ovary followed by internal egg fertilization and hatching of youngs (Chong *et al*., 2004). Platies (*Xiphophorus maculatus*) are a popular freshwater ornamental species mainly produced in various tropical countries.

They are easy to breed and keep. In addition, they accept all kinds of food (Ling *et al*., 2006; Ghosh *et al*., 2007). 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). However, research on optimization of diets to improve the reproductive performance is still in its infancy. As defined by Gibson and Roberfroid (1995), 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. In addition, prebiotics may affect growth, feed utilization and body composition (Grisdale-Helland *et al*., 2008). The 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). Examples of prebiotics include mannanoligosaccharides (White *et al*., 2002), oligofructose and inulin (Teitelbaum and Walker, 2002), oligofructose and fructooligosaccharides (Grisdale-Helland *et al*., 2008). Li and Gatlin III (2004) reported the increase in survival and resistance to *Streptococcus iniae* in hybrid striped bass when fed a diet supplemented with Grobiotic™-AE (a commercial prebiotic consisting of a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products).

Staykov *et al*. (2007) demonstrated that dietary supplementation with 2 g kg^{-1} Mannanoligosaccharide (MOS) improved the growth, feed efficiency and survival of rainbow trout as compared with those fed the basal diet. They also showed that the non-specific immune system was positively affected when the diet was supplemented with MOS. Generally, immunogen is a commercial prebiotic mixture of β-glucans and mannanoligosaccharides. The use of prebiotics for

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enhancing growth parameters and in improving disease resistance ability has been well documented in fish (Darwish *et al*., 2002; Mahious *et al*., 2006; Refstie *et al*., 2006; Grisdale-Helland *et al*., 2008) but research on the effect of feeding prebiotics on the reproductive performance of fish are lacking. Therefore, the principal objective of this study was to evaluate the effect of dietary immunogen levels on reproductive performance of platy (*Xiphophorus maculatus*).

MATERIALS AND METHODS

Prebiotic: A commercial prebiotic immunogen was obtained from the International Commerce Corporation USA, INC.

Test diets: The ingredients and proximate compositions of the basal and experimental diets are given in Table 1. The experimental diets were prepared by incorporating immunogen to the feeds in the concentration of 0.5% (B), 1% (C) and 1.5% (D). Control diet (A) was also prepared using the same composition of ingredients except the prebiotic immunogen. To prepare the diets, first, ingredients were blended thoroughly with additional water to make a paste of each diet. The paste were then cold extruded and cut into pellets to obtained 1 mm pellets. The diets were air-dried and stored at -2°C (Sardar *et al*., 2007) in air tight containers until fed.

Fish: One month old juveniles of platies (*Xiphophorus maculatus*) 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 two 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.

Experimental design and feeding diet: 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 ten 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 (temp., 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			
Compositions	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, 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. Contained (g kg^{-1} mix): $MgSO_4.2\ H_20$, 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

Proximate analysis 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).

Studies on the 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.

Statistics: All data obtained from experiments 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. Significance was also set at 5% level.

RESULTS AND DISCUSSION

The results of the Gonadosomatic Index (GSI) levels are shown in Fig. 1. The gonadosomatic index in *X. maculatus* increased with an increase in the concentration of prebiotic supplementation in feed. The fish of the experimental group D recorded the highest GSI (7.06±0.043%) followed by C (6.91±0.012%), B (6.90±0.003%) and A (6.65±0.011%). Although, results reveal a general trend of increased relative fecundity (Fig. 2) and total fry production per female (Fig. 3) with increasing levels of dietary probiotic, the differences among group A (control), B and C were not significant ($p>0.05$).

Fish of the experimental group D exhibited the highest value of relative fecundity and fry production per female. The experimental group D, recorded the maximum weight (2.46±0.033) and length (6.56±0.026) of fry. The lowest length of fry was observed in group B and the minimum weight of fry was recorded in control group A. Moreover, there were no significant differences in the weight and length of fry among the experimental groups A-C (Table 2).

The results of the present study showed that supplementation of feed with immunogen had no significant ($p>0.05$) impact on the percentage of deformed fry of *X. maculatus* within the treated groups and control group (Table 2). The fish of the experimental group D exhibited the highest value (55.64±0.73) of fry survival

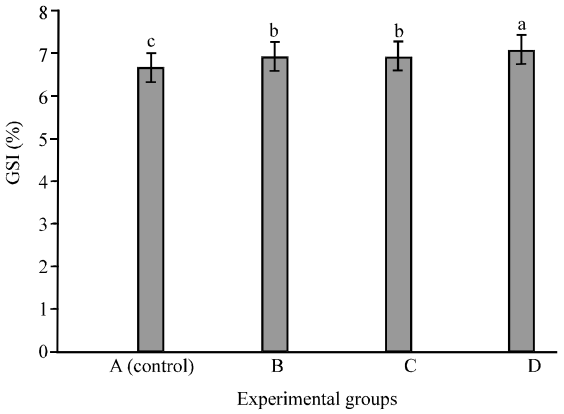

Fig. 1: Gonadosomatic index (%) of different experimental groups of *Xiphophorus maculatus*; data are expressed as mean±SE; values receiving same superscript are statistically not significant ($p>0.05$)

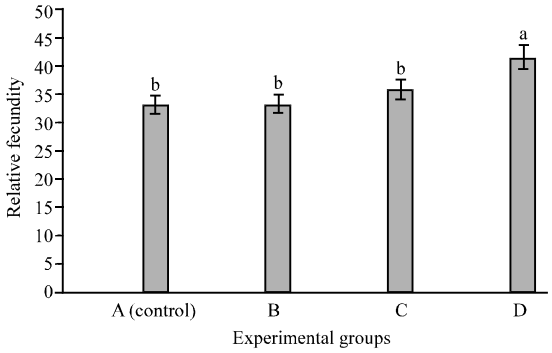

Fig. 2: Relative fecundity of different experimental groups of *Xiphophorus maculatus*; means with the same letters are not significantly different ($p>0.05$); data are expressed as mean±SE

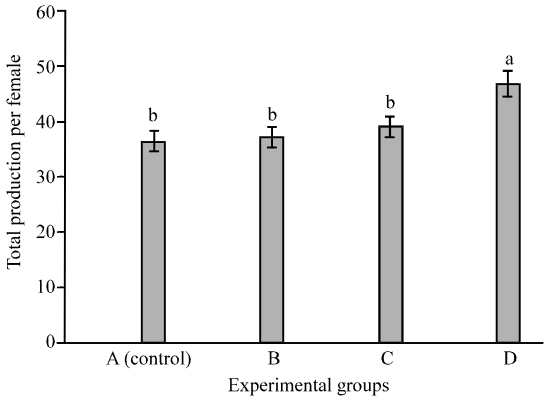

Fig. 3: Total fry production per female of different experimental groups of *Xiphophorus maculatus*; means with the same letters are not significantly different ($p>0.05$); data are expressed as mean±SE

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

Experimental groups	A (control)	B	C	D
Fry length (mm)	6.32±0.005^{b}	6.29±0.012^{b}	6.34±0.012^{b}	6.56±0.026^{a}
Fry weight (mg)	2.23±0.033^{b}	2.36±0.066^{ab}	2.33±0.033^{ab}	2.46±0.033^{a}
Deformed fry (%)	3.97±0.654^{a}	4.89±1.211^{a}	4.15±0.770^{a}	2.37±1.190^{a}

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

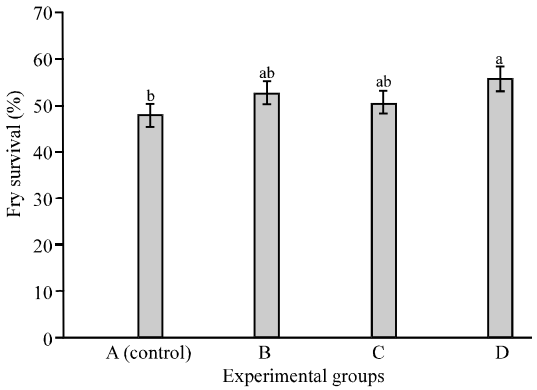

Fig. 4: Fry survival (%) of different experimental groups of *Xiphophorus maculatus*; data are expressed as mean±SE; values receiving same superscript are statistically not significant (p>0.05)

which was significantly different (p<0.05) from the lowest value (47.77±1.18) exhibited by fish of the experimental group A (control group) (Fig. 4).

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 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 present study demonstrated that dietary supplementation of prebiotic immunogen favorably influenced the gonadosomatic index, fry production, relative fecundity and fry survival. 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, D generally showed the best performance in the experiment.

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