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Effect of Dietary Supplementation of Vitamin C on the Reproductive Performance of Female Livebearing Ornamental Fish

Javadi Mousavi Mahsa and Jafari Vali Allah
Department of Fishery Sciences,
University of Agricultural Sciences and Natural Resources of Gorgan, Golestan, Iran

Abstract: This study was conducted to evaluate the effect of dietary vitamin C (Ascorbic Acid, AA) on reproductive performance of a freshwater ornamental species, the platy (*Xiphophorus maculatus*). Vitamin C was incorporated in fish feed at 6 different concentrations of 0.0 (A), 0.1 (B), 0.2 (C), 0.4 (D), 0.8 (E) and 1.6 (F) g kg^{-1} of dry diet with 5% of their body weight daily in two split doses for a period of 20 weeks. Results indicated that Gonadosomatic Index (GSI), fry production and relative fecundity were significantly higher in group F as compared to the control group (A) and other experimental groups ($p<0.05$). There were no significant differences in the weight and length of fry among the dietary treatments ($p>0.05$). The results also showed that the percentage of deformed fry was significantly lower in experimental groups (except the group B) compared to the control group ($p<0.05$). Moreover, the highest and lowest fry survival (%) were observed in group F and A, respectively. Collectively, this study showed that female broodstocks benefit from inclusion of vitamin C in diet during their reproductive stages.

Key words: *Xiphophorus maculatus*, reproductive performance, vitamin C, gonadosomatic index, nutrition, Iran

INTRODUCTION

The ornamental fish sector is one of the most economic and profitable areas of fish farming activities. The platies (*Xiphophorus maculatus*) are a very popular group of ornamental fish species due to the existence of a variety of body colors. They are also easy to breed and keep. In addition, they accept all kinds of food (Ling *et al*., 2006; Ghosh *et al*., 2007). Poeciiid 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).

Proper nutrition has long been recognized as a critical factor in improving 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 performances is still in its infancy. There is a positive correlation between the supply of nutrients during vitellogenesis in the broodstock diet and reproductive related factors such as a better oocyte development and maturation, higher rate of vitellogenesis, larger egg size and better embryonic development (Milton and Arthington, 1983; Seghal and Toor, 1991; Blom and Dabrowski, 1995; Ling *et al*., 2006; Ghosh *et al*., 2007). Vitamin C (Ascorbic Acid, AA) plays a key role for normal physiological functions in fish (Lim and Lovell, 1978). Since most teleosts are unable to synthesize ascorbic acid due to the lake of L-gulonolactone oxidase, an exogenous source of vitamin C is required in fish diets. The existence of a need for a dietary supply of ascorbic acid for growth and development has evidenced in a number of fish (NRC, 1993). Inadequate supply of dietary vitamin C usually resulted in a number of deficiency signs such as reduction in growth, skeletal deformities, impaired collagen formation and haemorrhaging (Halver *et al*., 1969; Gouillou-Coustans *et al*., 1998). Therefore, the objective of thepresent experiment was to investigate the effect of dietary vitamin C levels on various reproductive aspects of platy (*Xiphophorus maculatus*) (Table 1).

MATERIALS AND METHODS

Preparation of test diets: The experimental diets were prepared by incorporating vitamin C (L-ascorbic acid) to the feeds in the concentration of 0.1 (B), 0.2 (C), 0.4 (D),

Corresponding Author: Javadi Mousavi Mahsa, Department of Fishery Sciences,
University of Agricultural Sciences and Natural Resources of Gorgan, Golestan, Iran

Table 1: Composition of basal diet supplemented with vitamin C

Composition	Diet A (control)	B	C	D	E	F
Ingredients (g kg^{-1})						
Fish meal[a]	500.00	500.00	500.00	500.00	500.00	500.00
Whole wheat meal	420.00	420.00	420.00	420.00	420.00	420.00
Soybean oil	40.00	40.00	40.00	40.00	40.00	40.00
Vitamin premix without vitamin C[b]	20.00	20.00	20.00	20.00	20.00	20.00
Mineral premix[c]	20.00	20.00	20.00	20.00	20.00	20.00
Vitamin C[d]	0.00	0.10	0.20	0.40	0.80	1.60
Proximate composition[e]						
Crude protein	38.90	38.81	38.65	38.84	38.54	38.12
Crude lipid	11.30	11.37	11.41	11.45	11.60	11.10
Ash	10.51	10.61	10.45	10.35	10.70	10.21
Moisture	9.40	9.20	9.40	9.10	9.50	9.30
Gross energy (MJ kg^{-1})	20.40	20.30	20.50	20.20	19.90	19.70

[a]Fish meal: Pars kelika Co., Mirood, Iran [b]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. [c]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. [d]Supplemented as ascorbyl polyphosphate. Sigma Aldrich Company, Poole, Dorset, UK. [e]Expressed as percent dry matter unless indicated otherwise

also prepared using the same composition of ingredients except vitamin C. The ingredients were grinded, milled, weighted, mixed and pelleted with meat mincer through a 0.8 mm die. The diet were air-dried and stored at -2°C (Sardar *et al*., 2007) in tight containers until use.

Experimental animals: About 1 month old juveniles of livebearing ornamental fish *Xiphophorus maculatus* were purchased from a commercial fish farm at Tonokaban, Mazandaran, Iran. They were kept in 200 L plastic containers with recirculated and aerated water for 2 month until they reached sexual maturity. All fish were fed with control feed at 5% of their body weight daily in two split doses. Throughout this period, males separated from females at earliest sign of sexual differentiation to avoid reproduction activities. The sex of fish was based on male secondary sexual characters and determined by external examination specially using anal fin as a marker. Female poecilidae can retain active sperm in crysts in their ovaries and oviducts 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 female were used for this study.

Experimental design: Virgin females aged 20 weeks (0.62-0.65 g) were used for experiment. Four replicate tanks (60 L) stocked with 10 females were used for each diet. Group A received the basal diet and acted as control. Group B-F were fed diet containing 0.1, 0.2, 0.4, 0.8 and 1.6 g vitamin C kg^{-1} dry diet, respectively. During the experimental period, fish were fed with feed at 5% of their body weight daily in 2 split doses. Virgin males aged 3 months were also kept separately in a glass aquarium (200 L) and fed frozen bloodworms twice daily. During the experimental period, three males were randomly selected and introduced to each experimental tank at an interval of 30 days. These male were left with the females for 5 days before returning them to holding glass aquaria. During feeding, males were separated from females using plastic sheet. Moreover, bundles of tied-nylon strings were placed into each experimental tank as shelter for new free swimming fry to avoid cannibalism by parental fish.

Proximate analysis of diet: Analysis of dry matter (by oven drying at 105°C for 24 h), crud protein (Kjeldahl apparatus, nitrogen×6.25) crude lipid (extraction with petroleum ether by Soxhlet apparatus) 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:

$$\text{Relative fecundity} = \frac{\text{Total fry production at throughout experimental period}}{\text{Mean weight of female (g)}}$$

Total fry production per female is equal total fry harvested throughout experimental period per number of female

$$\text{Gonadosomatic index (\%)} = \frac{\text{(Ovary weight)}}{\text{(Body weight)}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{(Total live fry (No.) after t)}}{\text{Total fry production (No.) throughout experimental period)}} \times 100$$

where, t is the days of experiment.

Statistical analysis: Data were analyzed using one-way Analysis of Variance (ANOVA) and significant difference among treatment means were compared using Duncan's test. Significance was 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 vitamin C supplementation in feed. The fish of the experimental group F recorded the highest GSI (7.16±0.023%), followed by E (6.75±0.020%), D (6.70±0.122%), C (6.65±0.064%), B (6.35±0.095%) and A (6.30±0.080%). Similarly, the highest relative fecundity was observed in group F followed by group E and D whereas the control group (A) showed the lowest relative fecundity (Fig. 2). The results of the mean total fry production per female are shown in Fig. 3. Total fry production was lowest for the control group (A) while group F showed the highest level. In addition, there was no significant difference ($p>0.05$) between group A (control) and B. There were no significant differences ($p>0.05$) in the average weight and length of fry among the different experimental groups (Table 2). Similarly, no significant differences ($p>0.05$) were observed in the final weight and length of female broodstock (Table 2). The percentage of deformed fry was found to be significantly lower ($p<0.05$) in fish fed diets containing vitamin C (except group B) and significantly highest ($p<0.05$) in fish of the experimental group (A) fed the control diet (Fig. 4). The fish of the experimental group F exhibited the highest value (57.76±0.815) of fry survival which was significantly different ($p<0.05$) from the lowest value (47.53±0.371) exhibited by fish of the experimental group A (control group) (Fig. 5).

In fishes, it has been established that vitamin C plays an important role in fish reproduction (Blom and

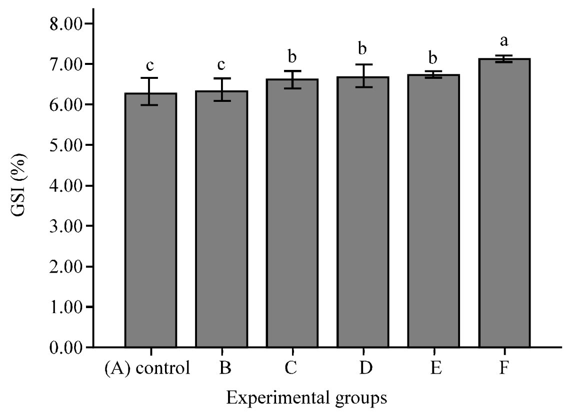

Fig. 1: Gonadosomatic index (%) of different experimental groups of *Xiphophorus maculatus*. Means with the same letters are not significantly different ($p>0.05$). Data are expressed as mean±SE

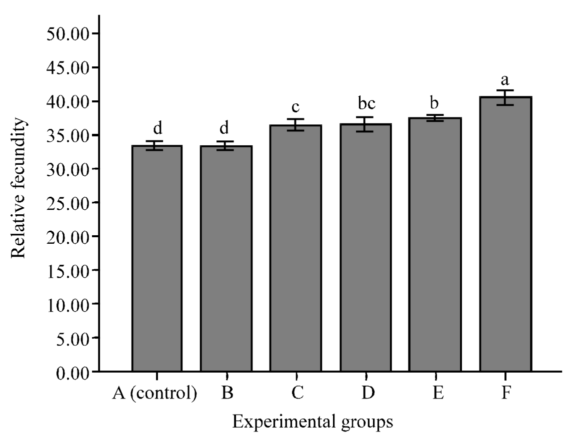

Fig. 2: Relative fecundity of different experimental groups of *Xiphophorus maculatus*. Means with the same letters are not significantly different ($p>0.05$). Data are expressed as mean±SE

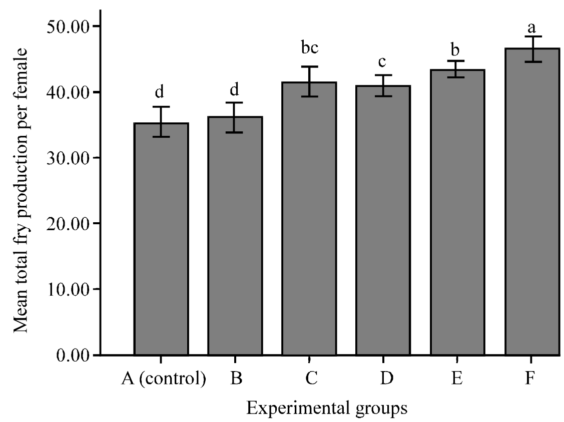

Fig. 3: Total fry production per female of different experimental groups of *Xiphophorus maculatus*. Means with the same letters are not significantly different ($p>0.05$). Data are expressed as mean±SE

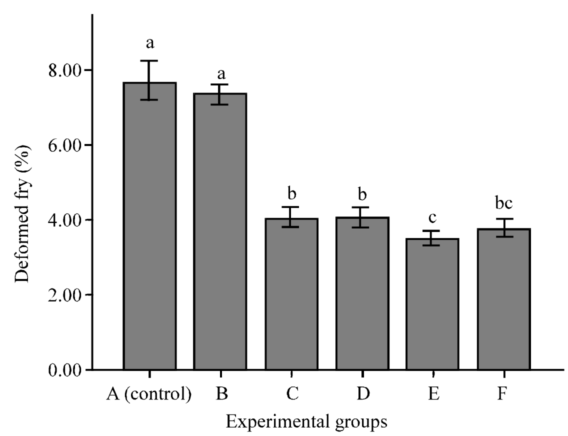

Fig. 4: The percentage of deformed fry of different experimental groups of *Xiphophorus maculatus*. Means with the same letters are not significantly different ($p>0.05$). Data are expressed as mean±SE

Table 2: Average weight and length of different experimental groups of *Xiphophorus maculatus*

Experimental groups	A (control)	B	C	D	E	F
Initial weight (g)*	0.592±0.006^a	0.597±0.009^a	0.600±0.011^a	0.602±0.009^a	0.605±0.008^a	0.607±0.011^a
Initial length (mm)*	30.25±0.8800^a	30.0±1.35000^a	30.25±0.8500^a	30.75±0.4700^a	30.50±0.6400^a	31.00±0.4000^a
Final weight (g)*	1.127±0.004^a	1.122±0.007^a	1.125±0.008^a	1.125±0.002^a	1.127±0.004^a	1.127±0.007^a
Final length (mm)*	39.00±0.7000^a	39.50±1.1900^a	38.75±0.4700^a	38.00±1.5800^a	40.0±0.91000^a	39.00±1.2200^a
Fry length (mm)	6.52±0.0200^a	6.53±0.0220^a	6.53±0.0290^a	6.52±0.0540^a	6.52±1.0150^a	6.53±0.0320^a
Fry weight (mg)	2.42±0.0850^a	2.37±0.0470^a	2.42±0.0850^a	2.37±0.0470^a	2.45±0.0640^a	2.45±0.0280

^{a*}Average per female; Means with the same letters in each row are not significantly different (p>0.05). Data are expressed as mean±SE

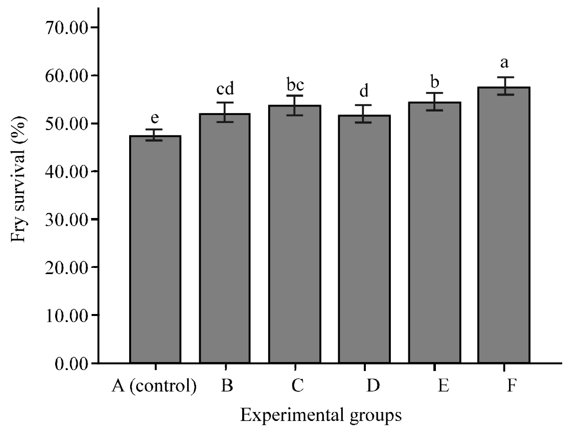

Fig. 5: Fry survival (%) of different experimental groups of *Xiphophorus maculatus*. Means with the same letters are not significantly different (p>0.05). Data are expressed as mean±SE

Dabrowski, 1995; Emata *et al*., 2000; Dabrowski and Ciereszko, 2001). Vitamin C was known to increase reproduction performance such as ovarian growth in common carp (Watanabe and Takashima, 1977), higher percentage of normal eggs and fecundity in gilthead seabream (Izquierdo *et al*., 2001). Diets deficient in AA resulted in high mortality of eyestalk-ablated *Marsupenaeus japonicus* females (Alava *et al*., 1993a) retarded the ovarian development of *M. japonicus* (Alava *et al*., 1993b) and decreased the hatching rate of *F. indicus* eggs (Cahu *et al*., 1995). The results of this study showed that vitamin C supplemented diets improved the gonadosomatic index, relative fecundity, fry production and fry survival (%). Moreover, the percentage of deformed fry was decreased. These revealed that vitamin C incorporated diets helped to increase the reproductive performance of the experimental fish. These observations are in agreement with the finding of Dabrowski (2001) who reported that vitamin C is necessary for successful reproduction in rainbow trout, apparently in protecting oxidation sensitive genetic material in gametes and probably has the same function in warm-water fish. Even though the mechanism for AA's effects on reproductive performance of fish is not clear, studies with rainbow trout (Liu *et al*., 1997) indicated that the mechanism could be based on the oxidant function of AA.

CONCLUSION

It can be conducted that vitamin C we used in this study could increased the reproduction performance of *X. maculatus*. Moreover, further studies are needed to determine the mechanisms responsible for the improvement of reproductive performance of fish.

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