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Influence of Dietary Organic Acids Supplementation on Reproductive Performance of Freshwater Angelfish (*Pterophyllum scalare*)

Sara Shahidi, Maziar Yahyavi and Delaram Nokhbe Zare

Department of Fisheries, Islamic Azad University,
Bandarabbas Branch, Bandarabbas, Iran

Abstract: A 2-month feeding trial was carried out with angelfish (3.45±0.05 g) by application of four diets including commercial diet (control), commercial diet with 0.1% organic acids (T1), commercial diet with 0.2% organic acids (T2) and commercial diet with 0.4% organic acids (T3) to investigate the proper dose and study the effect of organic acids on reproductive parameters. Experiment was conducted in a completely randomized design and data analysis was done by Excel and SPSS software packages. A significant difference ($p<0.05$) was observed between T1 and control in absolute fecundity and gonadosomatic index, while there is no significant difference among these two groups with T2 and T3 ($p>0.05$). There is no remarkable difference in hatchability among experimental treatments. Larval survival until yolk-sac absorption was higher in T1 than other groups, significantly. To sum up, results indicate that it will be efficient to add 0.1% of organic acids supplement to the angelfish diet because of promotion in reproductive parameters and increasing in larval survival.

Key words: Angelfish · Organic Acids · Reproductive Parameters

INTRODUCTION

Angelfish is one of the most popular freshwater fish species in the aquarium trade industry [1,2]. Due to its body coloration, shape and economical value [3] the angelfish represents one of the most important ornamental cichlid species. *Pterophyllum scalare* (Schultze, 1823) is, without question, the most popular and generally more available member of the entire family Cichlidae [4].

Proper nutrition has long been recognized as a critical factor in promotion normal growth and sustaining health of fish [5]. Prepared diets not only provide the essential nutrients that are required for normal physiological function but also may serve as the medium by which fish receive other components that may affect their health and reproductive performance [6,7]. However, research on optimization of diets to improve the reproductive performance is still in its infancy.

As a group of chemicals, organic acids are considered to be any organic carboxylic acid, including fatty acids and amino acids, of the general structure R-COOH. Not all of these acids have effects on gut microflora. In fact, the organic acids associated with specific antimicrobial activity are short-chain acids (C1–C7) and are either simple monocarboxylic acids such as formic, acetic, propionic and butyric acids, or are carboxylic acids bearing an hydroxyl group (Usually on the á carbon) such as lactic, malic, tartaric and citric acids. Salts of some of these acids have also been shown to have performance benefits. Other acids, such as sorbic and fumaric acids have some antifungal activity and are short chain-carboxylic acids containing double bonds. Organic acids are weak acids and are only partly dissociated. Most organic acids with antimicrobial activity have a pKa, the pH at which the acid is half dissociated, between 3 and 5. A very important objective of dietary acidification is the inhibition of intestinal bacteria competing with the host for available nutrients

Corresponding Author: Sara Shahidi, Department of Fisheries, Islamic Azad University, Bandarabbas Branch, Bandarabbas, Iran.

and a reduction of possibly toxic bacterial metabolites, e.g. ammonia and amines, thus improving weight gain of the host animal. Furthermore, Commercial mixtures of organic acids are widely used to control pathogenic bacteria such as Salmonella species and *Escherichia coli* in feed ingredients and terrestrial livestock feeds [8].

The use of additives such as prebiotics for enhancing growth parameters and in improving disease resistance ability has been well documented in fish [9,10], but research on the effect of feed additives on the reproductive performance of fish are lacking. Therefore, the principal object of this study was to evaluate the effect of dietary organic acids supplementation on reproductive performance of angelfish (*Pterophyllum scalare*).

MATERIAL AND METHODS

Fish and Experimental Design: Healthy and uniform prematured angelfish (n=240) were obtained from Institute of Ornamental Fish Hatchery (Shiraz, Iran). They were acclimated to new conditions for 2 weeks in six glass aquaria and fed on the basal experimental diet without dietary organic acids. At the end of the acclimation period, fish with an average weight of approximately 4.93 ± 0.14 g were randomly selected and stocked in aquaria (Triplicate groups per dietary treatment according to four test diets) at a density of 20 fish per aquaria (80×40×35 cm). Gentle aeration was provided by air stones. Fish were reared at 27 ± 2 °C with a 12:12 h light/dark photoperiod for 8 weeks. Water pH was registered between 7.2 and 7.8 and dissolved oxygen between 6.5 and 7.5 mg L^{-1}. The experiment was carried out in triplicate.

Test Diets: In the present study, fishes were fed by commercial extruder diet (Biomar) to satiation. The analyzed proximate composition of the basal diet is shown in Table 1. The experimental diet was supplemented with Orgacids™ (Sunzen, Malaysia) to give 0, 1, 2 and 4 g of mixed OA kg^{-1} diet. This product contained formic acid, citric acid, malic acid, lactic acid, ortophosphoric acid and tartaric acid. The first treatment was considered as the control. Fish were fed by hand to apparent satiation (Visual observation of first feed refusal) two times per day (At h: 9 and 16).

Table 1: Composition of basal diet

composition	(%)
Protein	54
Lipid	15
Ash	10
Vitamin	2

Reproduction Measurement: Reproductive parameters were investigated for five pairs of each replication. Feeding was continued during the spawning period. Absolute fecundity, hatching rate, survival until yolk-sac absorption and gonadosomatic index for each pair of fish were investigated according to Kasiri *et al.* [4].

Statistical Analysis: Data are presented as means ± standard deviation (SD). The effect of dietary organic acids on reproductive parameters was analyzed by one-way analysis of variance (ANOVA). Multiple comparisons among means were made with Duncan's new multiple range tests and significance was set at $p < 0.05$. Survival and ratio data were arcsine-transformed prior to analysis. All the statistical analyses were performed using the software SPSS (Version 16, SPSS Inc., Chicago, IL, USA).

RESULTS

As shown in Figure 1, absolute fecundity of fish fed diet 1 g OA kg^{-1} (595±46.55) was greater than other groups. There were no significant differences (p>0.05) in fecundity amount among the control, T1 and T2. Absolute fecundity was found to be significantly (p<0.05) lower in fish fed OA at level of 4 g kg^{-1} diet than the other groups (510.75±47.28).

According to Figure 2, the same value was observed in hatching rate among experimental groups, approximately.

On the one hand, there were statistically significant differences in larval survival until yolk-sac absorption between fish fed diet 1 g OA kg^{-1} and other groups (Figure 3). On the other hand, there were no remarkable differences in larval survival among the other groups (Control, T2 and T3).

As far as figure 4 is considered, with respect to gonadosomatic index, no difference was detected among control, T1 and T2. In addition, no statistical difference was observed among control, T2 and T3. Gonadosomatic index in T1 was higher relative to other experimental groups.

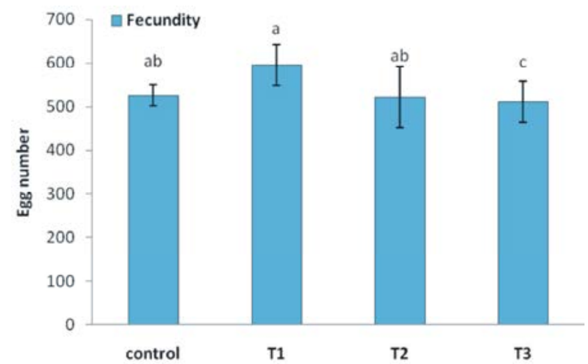

Fig. 1: Absolute fecundity in experimental groups.

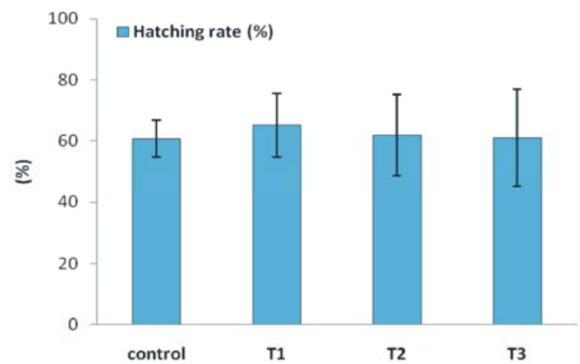

Fig. 2: Hatchability in experimental groups.

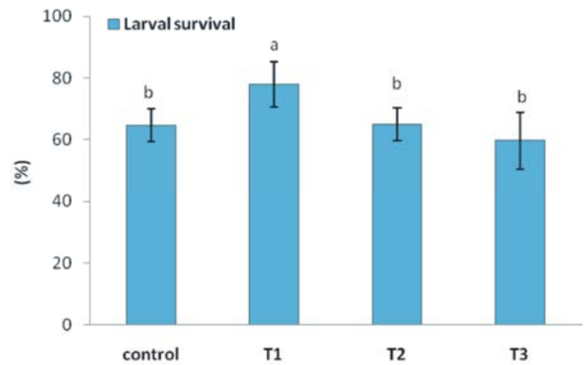

Fig. 3: Larval survival in experimental groups.

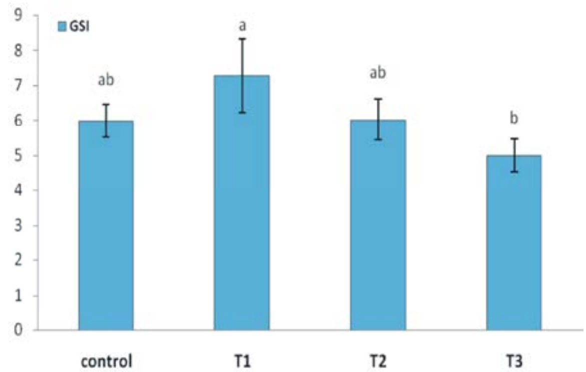

Fig. 4: Gonadosomatic index in experimental groups.

DISCUSSION

Results reveal that use of OA at the level of 0.1% promotes reproductive performance in angelfish breeders, since enhancement in absolute fecundity, gonadosomatic index and larval survival until yolk-sac absorption. OA presence in fish diet can act as a prebiotic and accumulate beneficial bacteria in the gut (Such as Bifidobacteria and Lactobacilli). Beneficial bacteria enhance nutrition by synthesizing essential nutrients (Fatty acids, proteins and vitamins) and enzymes (Protease, lipase and amylase) [11,12]. So this function can increase enzymes secretion which increases the digestive efficacy of the complex proteins and lipids included in the diet and improve food digestion and absorption by the host [13,14]. There is a positive correlation between the presence of proteins and fatty acids in the broodstock diet and reproductive-related factors such as better oocyte development and maturation, higher rate of vetillogenesis and larger egg size [15,16]. Our findings in this study are in agreement with reports of Ghosh *et al.* [13] and Hajibeglou and Sudagar [14] in female livebearing ornamental fish. They asserted that reproductive performance was enhanced in the fish fed supplemented diet with probiotic. This could be attributed to the balanced production of essential nutrients (In particular essential fatty acids) by intestinal beneficial bacteria. Several study have shown the importance of balancing the composition of dietary unsaturated fatty acids such as arachidonic acids, docosahexaenoic acid and eicosapentaenoic acid in fish to ensure optimized broodstock reproductive performance and enhance larval quality [17-18]. Moreover, essential fatty acids can also supply energy to sustain the spawning activities [13,19]. Accumulation of beneficial bacteria also affects the production of the vitamins, particularly B group vitamins [13,20]. Hence, in our study higher survival rate in T1 could be linked to the better performance of intestine bacteria which produce B group vitamins. Ghosh *et al.* [13] showed that the synthesis of vitamin B_1 and B_{12} by *Bacillus subtilis* could have accounted for reducing dead fry in four species of livebearing ornamental fish. This observation is in agreement with the finding of Ketola *et al.* [21] who pointed out that Thiamin (Vitamin B_1) can reduce the mortality of progeny in the Atlantic salmon. In addition, Khajepour *et al*. [22] observed that citric acid could improve some hematological and biochemical parameters in Beluga (*Huso huso*) which have direct relation with physiological statues and survival rate.

In conclusion, this research revealed that supplemented diet with 0.1% Orgacids™ improve reproductive performance of angelfish breeders. This trend can be related to promotion in physiological characters and modifying microflora in the gut.

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