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Effects of supplemented diets by levamisole and *Echinacea purpurea* extract on growth and reproductive parameters in angelfish (*Pterophyllum scalare*)

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Abstract. The aim of this study was evaluation the effects of supplemented diets by levamisole and *Echinacea purpurea* (Moench, 1794) extract on growth and reproductive parameters in angelfish, *Pterophyllum scalare* (Lictenstein, 1823). 54 Uniform angelfish (average weight 3.8±0.29 g) were selected. The fishes were fed by three diets which included commercial extruder diet (control), commercial extruder diet supplemented by 0.25 ppt levamisole (T_1) and commercial extruder diet supplemented by 0.25 ppt *Echinacea purpurea* extract (T_2). The experiment was conducted for 3 months. The weight gain in T_2 was significantly higher than other groups ($p<0.05$). There was no significant difference ($p>0.05$) in fecundity and hatching rate among groups but the best survival of yolk-sac absorption period was found in group fed supplemented diet by *Echinacea* extract ($p<0.05$). This study indicates that growth performance can be improved by dietary *Echinacea* extract supplementation and larval survival can be promoted by it.

Key Words: supplemented diets, levamisole, *Echinacea purpurea*, growth, reproductive parameters, angelfish (*Pterophyllum scalare*).

چکیده. هدف از این مطالعه ارزیابی اثرات جیره های تکمیل شده توسط لوامیزول و عصاره گیاه اکیناسه روی پارامترهای رشد و تکثیر ماهی آنجل بود. 30 عدد ماهی آنجل یک شکل (میانگین وزن 3.8±0.29 گرم) انتخاب شده بودند. سه تیمار طراحی شد شامل گروه شاهد که به آن جیره بالانس شده داده می شد و دو گروه دیگر جیره هایی مشابه با گروه شاهد که توسط لوامیزول و عصاره گیاه اکیناسه به میزان 0.25 ppt برحسب وزن خشک بدن تکمیل شده بود دریافت می کردند (T_1 و T_2). آزمایش به مدت 3 ماه به طول انجامید. وزن بدست آمده در گروه تغذیه شده با جیره تکمیل شده با اکیناسه به طور معناداری بالاتر از سایر گروه ها بود ($p<0.05$). اختلاف معناداری بین هماوری و میزان تفریخ در بین گروه ها مشاهده نشد($p>0.05$) اما بهترین میزان بقا در دوره جذب کیسه زرده در گروه تغذیه شده با جیره تکمیل شده توسط عصاره اکیناسه مشاهده شد ($p<0.05$). این مطالعه نشان می دهد که کارایی رشد می توان توسط جیره های تکمیل شده با عصاره اکیناسه بهبود یابد و بقای لاروی توسط آن می تواند افزایش یابد.

کلمات کلیدی: جیره های تکمیل شده، لوامیزول، اکیناسه، رشد، پارامترهای تولیدمثلی، ماهی آنجل

Introduction. Ornamental fish farming is an important primary industry (Lim & Wong 1997). Angel fish is one of the most popular freshwater fish species in the aquarium trade industry (García-Ulloa & Gómez-Romero 2005). Due to its body coloration, shape and economical value (Luna-Figueroa 2003) the angel fish represents one of the most important ornamental cichlid species. *Pterophyllum scalare* (Lictenstein, 1823) is, without question, the most popular and generally more available member of the entire family Cichlidae. Both the silver and a myriad of artificially selected color and finnage varieties are commercially produced. These Cichlids make a magnificent solo display, but there is no practical reason for excluding other fish from their aquarium.

In recent years, the concern about bacterial resistance to antibiotics in livestock industry has led to legislation minimizing/eliminating the use of such compounds. The use of the immunostimulants in aquaculture is becoming popular, enhancing the activity of the non-specific defense mechanisms and increasing disease resistance (Raa 1996).

The medicinal use of Echinacea can be traced back to the American Indians, who regarded *Echinacea* as among the most favourable remedies to treat wounds, snake bites, headache and the common cold (Moerman 1998). In the middle of the 20th century, *Echinacea purpurea* (Moench, 1794) has been introduced as a medicinal plant to

Europe, where it has been used against infections and for stimulation of the immune response. Echinacea seems to activate the macrophages and other immunological functions in laboratory animals and humans and there is considerable evidence of the role played by the polysaccharidic fraction in the immunostimulatory effect of echinacea preparations. The polysaccharides (heteroglycans), isolated from *E. purpurea*, have been particularly investigated in their capacity to activate macrophages and other components of the immune system in mice, rats and humans (Bauer 1999).

Antibiotics, drugs and chemicals have been used for treating fish disease caused by environmental stress and other factors for years (Stoskopf 1993). However, these are often effective for only a short time and may accumulate in the environment. In the past, the immunological approach to preventing fish disease has been by vaccination against specific pathogens, where vaccines were used for treating a particular disease (Siwicki et al 1994). Immunostimulants comprise a group of biological and synthetic compounds that enhance the nonspecific defense mechanisms of animals (Siwicki et al 1994). Levamisole is a levo-isomer of tetramisole (Findlay & Munday 2000). Previous studies have suggested that levamisole treatment leads to an enhanced state of resistance to various kinds of infections.

The present study was conducted to determine the effects of supplemented diets by levamisole and *Echinacea purpurea* on growth and reproductive parameters in *Pterophyllum scalare*.

Material and Method. For this study, 54 uniform prematured angel fish were randomly selected and 6 fish stocked into each aquarium with tree replications per treatment. Angel fish, obtained from Institute of Ornamental Fish Hatchery in Babol (Iran), were transferred to the place of experiment and acclimated for 2 weeks. Fish were fed to satiation twice per day. The feeding trials were conducted in 9 (80×30×40 cm) glass aquaria. Gentle aeration was provided by air stones. During the experiment, the water quality parameters were monitored during the trial and average value for temperature, dissolved oxygen, hydrogen ion concentration (pH) and salinity were 26±2 ºC, 5.7-7.7 mg L^{-1}, 6.9-7.8 units and 0.1 mg L^{-1} respectively. Light/dark cycle of 12:12 h was maintained during the feeding trial.

Fresh *Echinacea* parts were washed with deionized water, dried at 37° C and ground well. Dried plant powders were then soaked in 70% ethanol (1:1 ratio) individually for 48 h (Eloff 1998; Punitha et al 2008). The slurry was then filtered with Wathman No. 1 filter paper and centrifuged for 5 min at 5000 rpm. The filtrate obtained from ethanol was evaporated to dryness at 40° C in a rotary evaporator (IKA® HB10 basic, China) and the water extract was then freeze-dried by using a freeze drier system (Operon: FDB-5503, Korea). Finally, the dried extract was stored at 4° C until use (Arabshahi-Delouee & Urooj 2007).

We used three diets which included commercial extruder diet (control), commercial extruder diet supplemented by 0.25 ppt levamisole (T_1) and commercial extruder diet supplemented by 0.25 ppt *Echinacea purpurea* extract (T_2). Nutrient compositions of experimental diets are given Table 1. The experiment was conducted for 12 weeks with angelfish.

Table 1

Nutrient composition of experimental diet (%)

Diet	Commercial extruder diet
Crude protein	48
Crude fat	12
Crude fiber	3
Ash	12
Vitamin	3
Moisture	12
Filler	10

Reproductive parameters were investigated for one pair of each replication. Feeding was continued during the spawning period. Spawning, hatching, survival, yolk-sac absorption period and distance between two consecutive spawning for each pair of fish were investigated. The following variables were calculated:

Weight Gain (WG) = final fish weight – initial fish weight
Per day growth (g) = Mean weight gain (g)/Number of days (Tacon 1990)
Specific growth rate (SGR) = (ln W_t – ln W_0) ×100 t^{-1} (Hevroy et al 2005)
W_t and W_0 were final and initial fish weights (g), respectively and t is time (days) between ln W_t and ln W_0.
Daily growth rate (DGR) = {100× (Final weight (g)-Initial weight (g)/ (days×Initial weight (g)); (De Silva & Anderson 1995)

The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) (using SPSS 16.0 programme) to test for effects of dietary treatments. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test. For each comparison, statistically significant differences were determined by setting the aggregate type I error at 5% ($P<0.05$).

Results. Growth performance showed a significant difference ($p<0.05$) among treatments. The fishes which were fed supplemented diet by *Echinacea* extract show the best Final mean weight, Mean weight gain, Per day growth, Specific growth ratio (SGR) and Daily growth rate (DGR) (see Table 2).

Table 2

Growth parameters of the experimental diets

Parameters	Control	T_1	T_2
Initial mean weight (g)	3.79±0.04 [a]	3.80±0.04 [a]	3.80±0.02 [a]
Final mean weight (g)	7.28±0.23 [b]	7.14±0.17 [b]	7.79±0.18 [a]
Mean weight gain (g)	3.48±0.19 [b]	3.34±0.13 [b]	3.99±0.17 [a]
Per day growth (g)	0.06±0.02 [a]	0.06±0.04 [a]	0.07±0.04 [a]
Specific growth ratio (SGR)	0.65±0.09 [b]	0.63±0.03 [b]	0.72±0.06 [a]
Daily growth rate (DGR)	1.53±0.23 [b]	1.46±0.18 [b]	1.75±0.19 [a]

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

Reproductive parameters were investigated for each group in two consecutive spawning (see Tables 3-4). There were no significant differences ($p>0.05$) among groups in fecundity and hatching (%), however they were better in T_2. Also larval survival in yolk-sac absorption period (%) was higher in the group fed with *Echinacea* extract supplemented diet ($p<0.05$).

Table 3

Reproductive parameters in the experimental groups (first time)

Parameters	Control	T_1	T_2
Fecundity	430.67±65.25 [a]	419.67±86.69 [a]	479.67±26.84 [a]
Hatching (%)	78.16±8.22 [a]	75.49±11.98 [a]	85.92±3.74 [a]
Larval survival (%)	60.48±1.81 [b]	60.26±3.88 [b]	77.74±8.25 [a]

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

Results of second spawning was the same as first time and there were no difference between them.

Table 4

Reproductive parameters in experimental groups (second time)

Parameters	Control	T_1	T_2
Fecundity	398.67±20.03 [a]	459.33±63.17 [a]	544.67±107.51 [a]
Hatching (%)	82.74±14.44 [a]	82.58±8.84 [a]	83.65±10.96 [a]
Larval survival (%)	59.02±5.39 [b]	63.80±5.46 [b]	78.97±4.43 [a]

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

Period between two consecutive spawning was measured. It was shorter in T_2 group than two other groups.

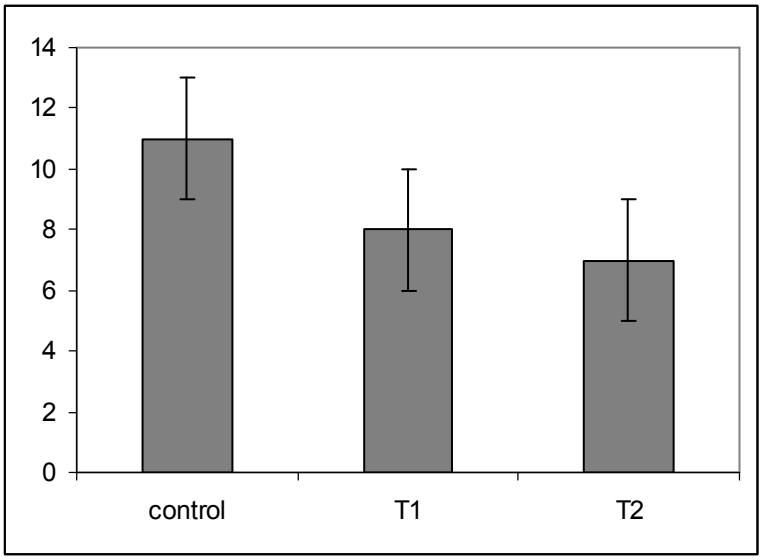

Figure 1. Period between two consecutive spawning in experimental groups.

Discussion. The world trend to improve food security and to use natural products will drive the chemically synthesized antibiotics and growth promoters out of use. The search for alternative, natural solutions has begun in order to substitute their use (Csep et al 2010). In order to replace antibiotics, the attention was focused also on plants known to have benefic effects on human health (aromatic plants, medicinal plants, spices and plants extracts). Although the majority of plants are known for centuries and are used in the traditional medicine, their way of action is not fully understood, the existing data being at most empirical. Phytoadditives are fodder additives obtained from medicinal plants or plants extract. They are being used on a wide range, by humans as well as by animals, including fish (Gabor et al 2010).

The *Echinacea* supplemented diet improved the feed performance which agreed with the results of Maass et al (2005) who reported that the *Echinacea botanicals* administered as feed additive improved feed conversion. This observation is also in agreement with the finding of Salah et al (2008).

Maqsood et al (2009) reported that dietary levamisole supplementation (250 mg levamisole kg^{-1} of diet) significantly enhances the growth parameters of *Cyprinus carpio* when compared with the fish fed on control diet. Similar findings were reported by Misra et al (2005) and Gopalakannan & Venkatesan (2006) while in the present study there was no significant difference in growth performance between T_2 group when compared with the fish fed on control diet.

It is well accepted in the literature that broodstock nutrition has significant impact on reproductive performance (Izquierdo et al 2001; Babe & Labbe 2010). In this study improving in larval survival in yolk-sac absorption period (%) in T_2 can be due to nutrients in diets that mixed with *Echinacea*. Moreover, reducing of time between two

consecutive spawning in T_2 can be via better providing in essential nutritions for gametogenesis.

Conclusions. The present study demonstrates that use of *Echinacea purpurea* extract in the angelfish diet has positive effects on the growth performance and reproductive parameters.

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