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Original Article

Efficacy of fish oil- and linseed oil-enriched *Artemia* nauplii on growth performance and stress resistance of tiger barb larvae (*Puntius tetrazona*)

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Abstract: Fish oil is the important fat source in fish nutrition. High demand for fish oil and low global supply arise a need of alternative oils in fish culture. Plant oils are a good candidate in this case. The aim of the present study was to compare the efficacy of fish oil and linseed oil on growth and stress resistance of tiger barb (*Puntius tetrazona*) larvae. Artemia nauplii were enriched by 2.5, 5 and 7.5 of each oil. The enriched nauplii were offered to larvae for 14 d. thereafter, fish were fed non-enriched nauplii for another 14 d. At the end of the trial, larvae were subjected to osmotic stress and their survival was recorded. There was no significant difference in final weight, SGR and weight gain among the treatments at day 14. However, oil type and oil levels significantly affected these parameters after 28 d. Fish of 2.5-LO and 5-FO groups showed the best and worst performance, respectively. There was no significant difference in survival rate among the treatments, after 14 and 28 d; however, oil type significantly affected survival of the larvae after osmotic stress. Survival of larvae fed on linseed oil-enriched nauplii was significantly higher than that of those fed on fish oil-enriched nauplii. Linseed oil showed significantly better results in growth performance and stress resistance compared to fish oil. It is concluded that linseed oil is more suitable than fish oil for Artemia enrichment to feed tiger barb larvae. The potential reasons for the better performance of larvae fed on linseed oil-enriched Artemia were discussed.

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Introduction

Lipids are the main energy sources for fish, especially at early life stage. Docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6) are the important fatty acids in fish nutrition (Sargent et al., 2002). These fatty acids support suitable growth and survival of fish as well as required energy for reproduction (Sargent et al., 1999).

Live foods, particularly Artemia, are used in freshwater and saltwater aquaculture. *Artemia* nauplii contains 50-60 % proteins (all amino acids at the sufficient level) and 5-20 % lipid (rich in long chain fatty acids), which is of particular importance in fish nutrition (Sorgeloos et al., 1986). Artemia is capable of transferring different materials such as vitamins, amino acids and drugs to fish larvae. Essential fatty acids, particularly EPA and DHA, are necessary for growth, survival and resistance to diseases in fish larvae. Feeding on enriched *Artemia* nauplii improves in non-specific immune mechanisms, resistance to diseases and environmental stresses (Gapasin et al., 1998; Lim et al., 2002). On the other hand, there is diffidence among the biochemical composition of different *Artemia* strains, which this difference can be minimized by enrichment (Leger et al., 1986).

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Fish oil has been extensively used in fish nutrition; because it contains sufficient level of long chain unsaturated fatty acids (EPA and DHA). However, global fish oil supply is not sufficient for aquaculture, because of decrease in marine catchment (Wassef et al., 2007). Accordingly, alternative oils are needed for fish nutrition. Plant oils are a good candidate for fish nutrition, which can be used sustainably. One of these oils is linseed oil. Linseed oil contains high levels of linolenic and linolenic acid that are precursor EPA, DHA and ARA. Freshwater fish can utilize these precursors (Sargent et al., 2002), so that, linseed oil seems to be a good alternative for fish oil in freshwater fish nutrition.

Tiger barb (*Puntius tetrazona*) is a freshwater species, which naturally found in Sumatra, Brunei, Thailand and Malaysia (Welcomme, 1988). This species is native to southwest Asia, however, was reintroduced to the habitats from which they have been eliminated (Ng and Tan, 1997). In Iran, this species is of particular interest due to its beauty and low price. Tiger barb needs live food in early life stages, such as many fish species. It is not cleared that whether fish oil and linseed oil leave different effect on growth performance and stress resistance of tiger barb. Thus, the aim of this study was to compare the efficacy of fish oil- and linseed oil-enriched *Artemia* nauplii on growth performance and stress resistance of tiger barb larvae.

Materials and methods

Cysts of *Artemia franciscana* were used for this study. To hatch the cysts, 1 g of cysts were dispersed in 1 L water in a conical jar supplied with aeration from bottom. Water salinity and temperature was 33 ppt and 28 ºC, respectively (Dhont and Van Stappen, 2003). Hatching occurred 18 h after incubation. Hatching rate was determined by counting three 0.1-ml-samples of the jar water.

To prepare oils emulsions, 0.5 g lecithin was mixed with 100 ml distilled water in a blender for 5 min. 100 ml of this emulsion was used for each concentration (2.5, 5 and 7.5 %) of either fish oil or linseed oil. To enrich the nauplii, 2 ml of each concentration was added to 1 L water containing 200000 nauplii for 6 h. After 6 h, inoculation was repeated and nauplii was enriched for another 6 h. Then the nauplii were transferred to the tanks of larvae.

Tiger barb larvae (2.3±0.09 mg) were purchased from a commercial hatchery. 2160 larvae were randomly stocked in 18 glass aquariums supplied with continuous aeration. To acclimatize the larvae, they were fed non-enriched Artemia for a couple of days. Then aquariums were divided into 6 groups with three replicates: 2.5 % fish oil (2.5-FO), 5% fish oil (5-FO), 7.5% fish oil (7.5-FO), 2.5 % linseed oil (2.5-LO), 5% linseed oil (5-LO) and 7.5% linseed oil (7.5-LO). Fish were fed *ad libitum*, 4 times a day, for a 14-d period. Thereafter, all treatments were fed by non-enriched *Artemia* nauplii for another 14-d period. Growth performance was recorded at the end of each 14-d period. Water temperature, pH and dissolved oxygen were 28±0.7 ºC, 7.2±0.12 and 6.4±0.34 ppm, during the feeding trials.

At the end of the experiment, fish were subjected to osmotic stress (exposure to 13 ppt water) and survival of each treatment was recorded.

Data were examined for normality. Data were subjected to two way ANOVA to find the effect of oil type and oil concentration on tested parameters. Duncan test was used to delineate significant difference among the treatments. Data were presented as mean ± SD.

Results

Growth performance of different treatments is presented in Table 1. There were no significant difference in final weight, SGR and weight gain among the treatments at day 14. However, oil type and oil levels significantly affected these parameters after 28 d. Generally, fish of 2.5-LO and 5-FO groups showed the best and worst performance, respectively (Table 1).

There was no significant difference in survival rate among the treatments, after 14 and 28 d; however, oil type significantly affected survival of the larvae

Table 1. Growth performance of tiger barb larvae fed on *Artemia* nauplii enriched with either fish oil or linseed oil, over 14 and 28 days. Different letters in each column show significant difference. n = 3.

	14-d			28-d		
	Final weight	SGR	Weight gain	Final weight	SGR	Weight gain
2.5-LO	24.1±0.7	16.5±0.5	914±91	145.1±5.3 c	14.7±0.3 c	6126±525 c
5-LO	27.6±0.4	17.0±0.3	985±51	130.1±2.3 b	14.4±0.1 b	5524±248 b
7.5-LO	23.7±0.8	16.4±0.4	895±81	132.4±1.1 b	14.3±0.2 b	5454±323 b
2.5-FO	24.1±0.8	16.7±0.3	932±50	123.9±5.8 ab	14.1±0.1 b	5105±269 b
5-FO	24.2±0.7	16.8±0.4	948±58	115.8±2.7 a	13.6±0.2 a	4439±181 a
7.5-FO	24.7±0.9	16.7±0.8	936±90	124.0±4.0 ab	14.2±0.1 b	5134±115 b
P value						
Oil type	0.40	0.82	0.86	0.0001>	0.0001>	0.0001>
Oil level	0.19	0.61	0.59	0.02	0.009	0.01
Type × Level	0.13	0.79	0.74	0.21	0.07	0.1

Table 2. Survival of tiger barb larvae fed on *Artemia* nauplii enriched with either fish oil or linseed oil, over 14 and 28 days. Different letters in each column show significant difference. n = 3.

	14 d	28 d (Before stress)	28 d (After stress)
2.5-LO	91.6±0.7	94.4±2.1	75.5±3.8 b
5-LO	90.5±1.7	93.6±0.6	70.0±6.6 b
7.5-LO	91.4±1.3	93.6±0.9	80.6±6.6 b
2.5-FO	93.3±3.1	94.1±4.3	51.1±7.6 a
5-FO	93.3±1.3	95.0±1.6	62.2±10.1 a
7.5-FO	94.1±0.9	95.1±2.1	51.1±7.6 a
P value			
Oil type	0.11	0.22	0.0001>
Oil level	0.81	0.83	0.21
Type × Level	0.83	0.56	0.002

after osmotic stress (Table 2). Survival of larvae fed on linseed oil-enriched nauplii was significantly higher than that of those fed on fish oil-enriched nauplii (Table 2).

Figure 1 shows efficacy of fish oil and linseed oil to improve growth performance and stress resistance of tiger barb larvae. Accordingly, linseed oil showed significantly better results in all cases compared to fish oil.

Discussion

Fish oil has been the first choice oil for fish diet formulation, for a long time. However, great demand and tiny supply of fish oil caused it not to be a sustainable oil source for aquaculture. Thus, alternative oils, such as plant oils, are needed to ensure sustainable fish production.

Although there are many studies focused on the use of plant oils as the alternative for fish oil in the fish feeds (Alexis, 1997; Lupatsch et al., 1997; Oliva-Teles, 2000; Izquierdo et al., 2003; Montero et al., 2003; Wassef et al., 2007), few study can be found on the effect of linseed oil on turbot (*Psetta maxima*) and Atlantic salmon (*Salmo salar*) nutrition and performance (Regost et al., 2003; Menoyo et al., 2007). Replacement of fish oil by linseed oil resulted decrease in growth performance turbot, which is not in agreement with the present study. It is because that turbot is a marine species, which needs EPA and DHA (Sargent et al., 2002) that are abundantly found in fish oil not linseed oil. However, Menoyo et al. (2007) showed that linseed oil could be an alternative for fish oil in the Atlantic salmon diet, without detrimental effects, which is in agreement

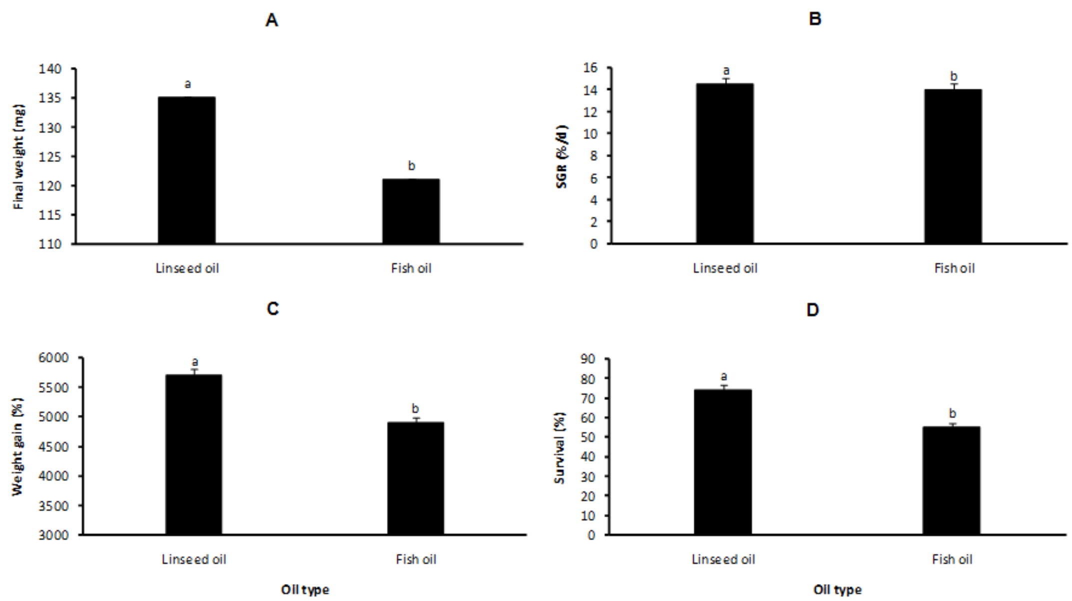

Figure 1. Comparison of the effect of fish oil and linseed oil on final weight (A), SGR (B), weight gain (C) and stress resistance (D) of tiger barb larvae. n = 9.

with the present results. Atlantic salmon, like tiger barb, is a freshwater species that needs linolenic and linolenic acids which are richly found in linseed oil. This explains the better performance of larvae fed on linseed oil-enriched nauplii compared to those fed on fish oil-enriched nauplii.

There was no difference in growth and survival among the treatments, after 14 d. Previous study showed there was no significant difference in survival and growth performance of dolphin (*Coryphaena hippurus*) larvae fed by rotifer enriched by squid (rich in DHA and EPA) and plant oils (Ostrowski and Divakaran, 1990). However, in the present study, larvae showed significantly different performance at day 28. This showed that feeding on enriched nauplii could affect the larvae performance in the future, when they did not feed on enriched nauplii. The underlying mechanism of this phenomenon is not clear and needs further research.

Linseed oil led to better resistance to stress compared to fish oil, in the present study. It is believed to be related to better nutritional status of larvae fed on linseed oil-enriched nauplii compared to those fed on fish oil-enriched nauplii. It is demonstrated that freshwater species need both n-3 and n-6 fatty acids (Sargent et al., 2002), which are richly available in linseed oil. On the other hand, fish oil is more unsaturated than linseed oil, and in turn, is more under the risk of lipid oxidation (Jalali et al., 2010). Since no antioxidant was used in the present study, lower survival of larvae fed on fish oil-enriched nauplii could be related to more oxidative stress compared to those fed on linseed oil-enriched nauplii. Similar results were reported by Jalali et al. (2010).

In conclusion results showed that linseed oil is more beneficial than fish oil for tiger barb larvae. Using linseed oil in Artemia enrichment could enhance growth and stress resistance of tiger barb. These effects seem to be related to high concentration of linolenic and linolenic acid concentration in linseed oil that are necessary for freshwater species.

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