

**Growth, survival and stress resistance of tiger barb  
(*Puntius tetrazona*) larvae fed on linseed oil-enriched  
*Artemia franciscana* nauplii**

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**Abstract**

Effect of feeding on linseed oil (LO)-enriched *Artemia* was investigated on growth, survival and stress resistance of tiger barb (*Puntius tetrazona*) larvae. Larvae were fed by LO-enriched (2.5, 5 and 7.5 %) as well as non-enriched *Artemia* nauplii for 14 d, followed by 14 d feeding on non-enriched *Artemia*. Fish fed on enriched nauplii showed significant increase in growth performance at both 14<sup>th</sup> and 28<sup>th</sup> d. Also, fish fed on enriched *Artemia*, especially those fed on 7.5 % LO, showed greater resistance in response to osmotic and hypoxia stress at 28<sup>th</sup> d. The results suggest that LO enriched *Artemia* nauplii are capable to promote growth and stress response in tiger barb larvae.

**Keywords:** Catfish, Fatty acid, Liver, Fatty tissue

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## Introduction

Lipids are an important source of energy and essential fatty acids (EFA) in aquaculture. The importance of long-chain highly unsaturated fatty acids (HUFA), such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6) in fish nutrition is well established (Sargent *et al.*, 2002). These fatty acids are required for fish optimal growth, development and reproduction (Sargent *et al.*, 1999). Despite efforts to develop artificial alternatives (Leger *et al.*, 1986; Jones *et al.*, 1993) brine shrimp (*Artemia* sp.) nauplii remain an important live food for fish larvae. *Artemia* sp. nauplii consists of 50-60% protein (all main amino acids in enough content and of 20-50% long – chain fatty acids in much content) that is important in nutrition of aquatic creatures (Sorgeloos *et al.*, 1986).

Due to compulsory nutrition, there is the ability of transferring different materials in different amounts to fish by *Artemia* sp. nauplius. *Artemia* sp. is important in fish culture due to high nutrient value and ease of access. Its various forms are applied at different stages of growth as a primary food. Its usability as a carrier of vitamins, pigments, chemicals, vaccines and hormones has made it a crucial means of enriched food source in aquaculture (Sorgeloos *et al.*, 1986). Biochemical composition is different in various *Artemia* types and this difference could be decreased by various nutrient enrichment (Leger *et al.*, 1986).

*Artemia* sp. is deficient in the case of some EFA, especially DHA (Figueiredo *et al.*, 2009). Considering the importance of EFA in early life stages of fish, it is necessary to enrich *Artemia* nauplii with EFA. Linseed oil is an ideal source of linolenic acid (18:3n-3) (Matthews *et al.*, 2000; Rahimi *et al.*, 2009). Although it does not contain high amounts of ARA, it contains relatively high amount of linolenic acid (18:2n-6 *Cis*) (Matthews *et al.*, 2000) which could be converted to ARA (Sargent *et al.*, 2002). Therefore *Artemia* sp. nauplii enrichment with LO is beneficial for optimizing EFA levels of nauplii.

Tiger barb, (*Puntius tetrazona*) is naturally found in Sumatra, Borneo, Thailand and Malaysia (Welcomme, 1988). However, it was introduced to the other parts of the world mainly as a popular ornamental fish species. On the other hand it is artificially bred and reared for its natural population conservation, sale to fish hobbyists, as well as reintroducing to habitats in which they have been eliminated (Ng and Tan, 1997). Like many other species, early life of tiger barb is dependent on live food, especially *Artemia* sp. nauplii. Thus the aim of the present study was to determine the effect of LO-enriched *Artemia franciscana* nauplii on growth performance, survival and resistance to stress in tiger barb larvae.

## Materials and methods

### *A. nauplii and enrichment protocol*

Hatching technique was based on Dhont and Van Stappen (2003). Briefly, cysts of *A. franciscana* were artificially hatched by adding 1 g cysts to 1 liter water (33 g/l). Temperature and light intensity was maintained at 28°C and 2000 lux, respectively. Continuous aeration was provided to ensure dissolved oxygen up to 5 mg/l as well as for suitable turbulence for cysts. Hatching rate was determined by counting and averaging the nauplii of three samples (0.1 ml) under stereoscopic loupe (Olympus, SZX7, Japan).

LO emulsion was prepared by mixing of 0.5 g lecithin with 100 ml distilled water, by an electric blender for 5 min. To this mixture, 2.5, 5 and 7.5 g LO was added and mixed for 1.5 min (Clawson and Lovell 1992). Three batches of 200000 nauplii were added to three conical-bottom cylinders (1 L water). Two milliliters of LO emulsions (2.5, 5 and 7.5%) were inoculated to the cylinders. After 6 hours, inoculation was repeated as the first one. Six hours after the second inoculation nauplii were added to the corresponding fish aquariums.

#### *Larvae and Growth trial*

Tiger barb larvae were obtained from artificial propagation. A total of 1440 larvae were used for the growth trial. The larvae were distributed into 12 glass aquariums. They were fed on *Artemia* nauplii for 2 days as acclimation period. Then, the aquariums were assigned into 4 groups; one control and three enriched groups. The control group was fed on non-enriched *Artemia*, while the enriched groups were fed (until satiation, four times a day) on one of the 2.5, 5 and 7.5 % LO-

enriched *Artemia* for 14 days. After 14 days, all groups were fed (until satiation) on non-enriched *Artemia*, for another 14-days period. The larvae weight and length were measured at the beginning of the experiment as well as 14 and 28 days thereafter. Water temperature, pH and dissolved oxygen were maintained at  $28 \pm 0.7$  °C,  $7.2 \pm 0.12$  and  $6.4 \pm 0.34$  mg/l.

#### *Stress resistance*

After 28 days, all groups were exposed to osmotic and hypoxia stress. For osmotic stress, the fish were exposed to 6, 10 and 12 g/l salinity, in a 24-hours-interval basis. Three tanks were assigned for each treatment, each containing 20 fish. Survival was recorded every 24 hours, before exposure to the next salinity. For hypoxia challenge, aeration was ceased for dissolved oxygen to be dropped down to 4 mg/l. Fish remained under this condition for 4 days, when survival was recorded. Three tanks were assigned for each treatment, each containing 20 fish.

#### *Statistical analyses*

Normality of data was confirmed by Shapiro-Wilk test. Data were subjected to one-way ANOVA and Duncan test to detect significant difference. All analyses were performed using the statistical software SPSS version 16.  $P < 0.05$  was considered significantly different. Data are presented as treatment mean  $\pm$  SD.

## **Results**

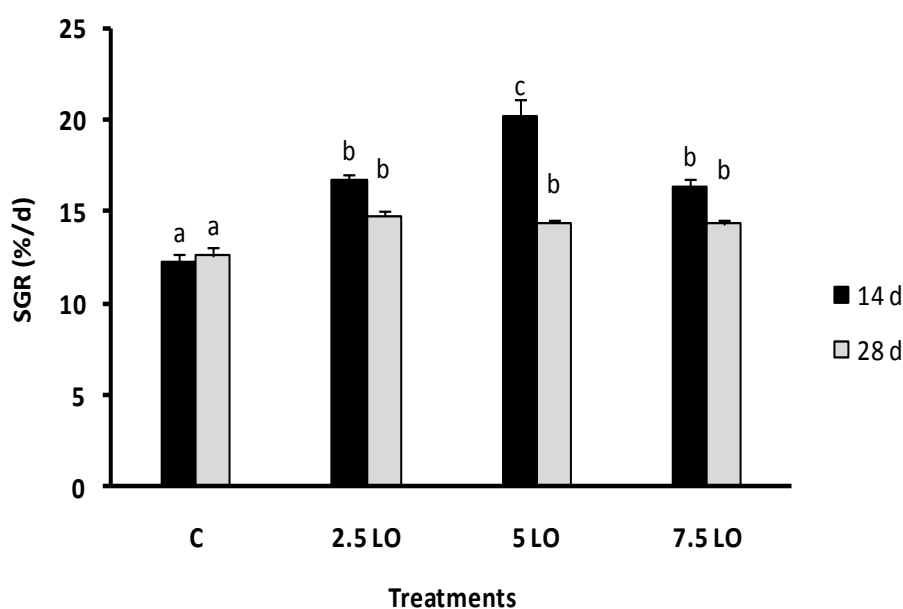
#### *Growth performance*

The fish fed on enriched *Artemia* showed significant increase in weight, length, weight gain (WG) and specific growth rate (SGR), at both 14<sup>th</sup> and 28<sup>th</sup> day (Table 1;

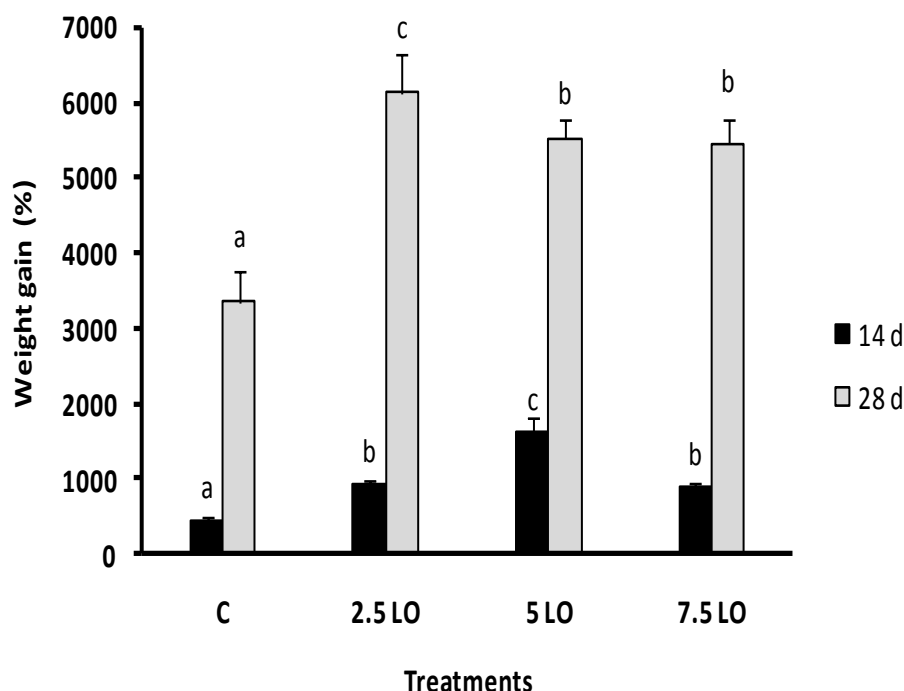
Fig. 1 and 2). There was no significant change in total mortality among the treatments, during 14 and 28 days rearing periods (Table 2).

**Table 1: Growth performance of the tiger barb fed on enriched and non-enriched *Artemia* over 14 and 28 days. Different letters in each row show significant difference (Duncan test).  $n = 3$ .**

|                          | Control           | 2.5 LO            | 5 LO              | 7.5 LO            |
|--------------------------|-------------------|-------------------|-------------------|-------------------|
| Initial                  |                   |                   |                   |                   |
| Weight (mg)              | $2.3 \pm 0.2$     | $2.3 \pm 0.1$     | $2.3 \pm 0.1$     | $2.4 \pm 0.1$     |
| Total length (mm)        | $6.15 \pm 0.02$   | $6.19 \pm 0.01$   | $6.14 \pm 0.02$   | $6.14 \pm 0.02$   |
| 14 days                  |                   |                   |                   |                   |
| Weight (mg)              | $17.8 \pm 0.3^a$  | $24.1 \pm 0.6^b$  | $39.6 \pm 0.4^c$  | $23.6 \pm 0.7^b$  |
| Total length (mm)        | $10.07 \pm 0.1^a$ | $11.63 \pm 0.2^b$ | $12.21 \pm 0.3^c$ | $11.49 \pm 0.2^b$ |
| Cumulative mortality (%) | $9.16 \pm 2.2$    | $6.67 \pm 3.0$    | $6.39 \pm 1.9$    | $5.83 \pm 0.8$    |
| 28 days                  |                   |                   |                   |                   |
| Weight (mg)              | $110 \pm 7.9^a$   | $145 \pm 5.3^c$   | $130 \pm 2.2^b$   | $132 \pm 9.8^b$   |
| Total length (mm)        | $14.2 \pm 0.4^a$  | $15.7 \pm 0.2^b$  | $16.0 \pm 0.2^b$  | $17.6 \pm 0.4^c$  |
| Cumulative mortality (%) | $15.8 \pm 3.6$    | $12.5 \pm 7.1$    | $11.4 \pm 3.5$    | $10.3 \pm 2.9$    |



**Figure 1: SGR of the tiger barb fed on enriched (2.5 LO, 5 LO and 7.5 LO) and non-enriched (C) *Artemia* over 14 and 28 days. Different letters above the bars show significant difference at 14<sup>th</sup> or 28<sup>th</sup> day, separately (Duncan test).**



**Figure 2:** Weight gain of the tiger barb fed on enriched (2.5 LO, 5 LO and 7.5 LO) and non-enriched (C) *Artemia* over 14 and 28 days. Different letters above the bars show significant difference at 14<sup>th</sup> or 28<sup>th</sup> days, separately (Duncan test).

#### *Stress resistance*

The control group showed significantly impaired survival compared with the enriched groups, following both osmotic and hypoxia stresses (Fig. 3 and 4). There were no significant differences in stress resistance among the enriched groups. Survival of the control group exposed to 10 and 13 g/l saltwater reached zero, while

the enriched groups showed near 80 and 50 % survival, respectively (Fig. 3). The fish fed on 7.5 LO-enriched nauplii showed significantly higher survival rate compared with the other groups, following hypoxia (Fig. 4). The control group showed the lowest survival rate compared with the other groups following hypoxia (Fig. 4).

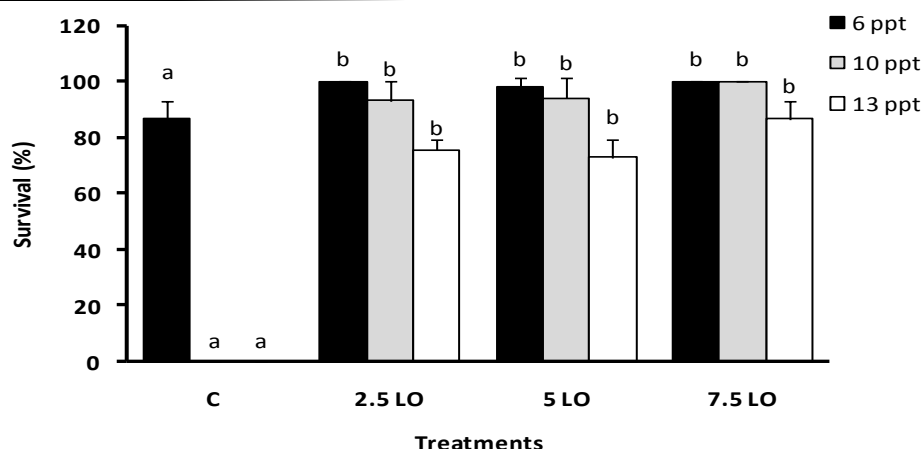


Figure 3: Osmotic stress resistance of the tiger barb following 28 days feeding on enriched (2.5 LO, 5 LO and 7.5 LO) and non-enriched (C) *Artemia*. Different letters above the bars show significant difference for each salinity, separately (Duncan test).

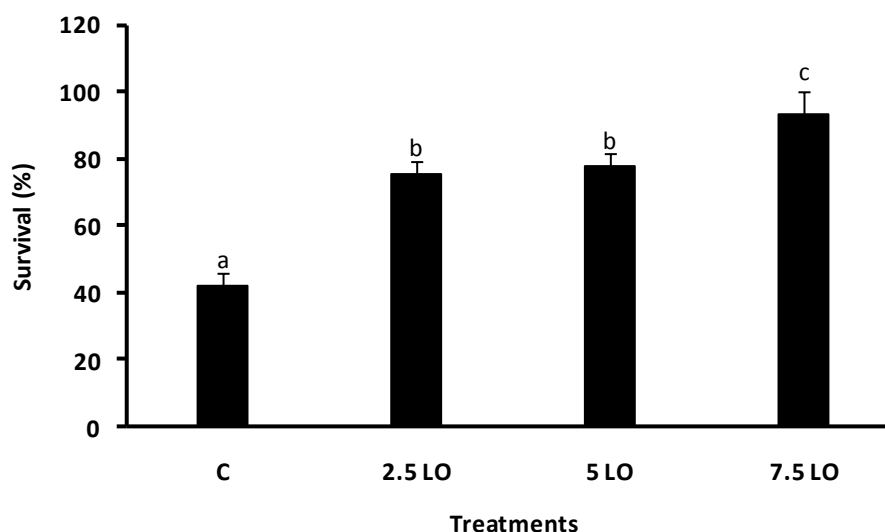


Figure 4: Hypoxia stress resistance of the tiger barb following 28 days feeding on enriched (2.5 LO, 5 LO and 7.5 LO) and non-enriched (C) *Artemia*. Different letters above the bars show significant difference (Duncan test).

## Discussion

*Artemia* is often an inferior food source for larvae compared with wild zooplankton. However, the capability of *Artemia* to produce any amount of biomass over a

short time, in contrast to zooplankton, and the possibility of enrichment with essential nutrients ensures its widespread use in larviculture (Støttrup and McEvoy, 2008). Tiger barb showed a better performance

when fed by LO-enriched *Artemia* compared with non-enriched *Artemia*. Previous studies on marine fish larvae such as turbot (*Scophthalmus maximus*) (Gatesoupe and Le Milinaire, 1985), Japanese flounder (*Paralichthys olivaceus*), red sea bream (*Pagrus major*) (Izquierdo *et al.*, 1989), gilthead sea bream (*Sparus aurata*) (Koven *et al.*, 1993; Rainuzzo *et al.*, 1997), *Limanda ferruginea* (Copeman *et al.*, 2002) and freshwater fish larvae such as Beluga (*Huso huso*) (Jalali *et al.*, 2008) showed the advantage of n-3 HUFA on growth performance. Linseed oil contains about 50 and 15 %, of linoleic and linolenic acid, respectively (Rahimi *et al.*, 2009). Linolenic acid is precursor of EPA and DHA and linoleic acid is precursor of ARA (Sargent *et al.*, 2002). Thus higher growth performance of the larvae fed on LO-enriched nauplii might be due to the endogenous production of EPA, DHA and ARA.

In this study, the positive effects of feeding LO-enriched *Artemia* for 14 days were kept by the fish over the next 14 days (during which they fed on non-enriched *Artemia*). It showed that the fast growth ability of tiger barb larvae, when fed on enriched live food can be maintained even if feeding continues with non-enriched live food. Jalali *et al.* (2008) reported a similar observation in Beluga larvae fed on fish oil-enriched *Artemia* followed by non-enriched *Daphnia* sp.

Tiger barb fed on enriched *Artemia* also demonstrated a better survival rate than those fed on non-enriched *Artemia* during osmotic and hypoxia challenges. It

is believed that eicosanoids' production via their precursor, linolenic acid, was responsible for such stress resistance in the fish fed on LO-enriched nauplii. Eicosanoid has an important role in stressful conditions (Sargent *et al.*, 2002). Eicosanoid production is a normal physiological process, with excess eicosanoid production often occurring in pathological conditions (Sargent *et al.*, 2002). On the other hand, it is possible that higher stress resistance in the tiger barb fed on enriched *Artemia* was related to endogenous production of DHA (from linolenic acid) compared to those fed on non-enriched *Artemia*. In fact, DHA is important in rapid development of visual and neural tissues in larval stages as reported for *Coryphaena hippurus* (Sargent *et al.*, 2002). Moreover, larvae fed on higher DHA gained bigger body size, which might be related to their better stress resistance, as reported in *C. hippurus* (Kraul *et al.*, 1993). Likewise, Jalali *et al.* (2008) reported higher survival rate, and larger body size in Beluga (*H. huso*) larvae fed on HUFA enriched *Artemia* compared to those fed on non-enriched *Artemia*.

It is concluded that tiger barb larvae fed on LO-enriched *Artemia*, over 14 days, showed better growth performance, which lasted until 28 days. They also demonstrated better stress resistance compared to those fed on non-enriched *Artemia*, which could be attributed to the higher EPA intake or the higher body size, as a result of higher DHA intake.

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