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Effects of Yeast Probiotic (Thepax) Enrichment on Biochemical Parameters of *A. urmiana* Nauplii

[1]Zeynabsadat Fazeli, [2]Ghobad Azari-Takami and [3]Seyyed Amirmohsen Fazeli
[1]Department of Aquaculture, Faculty of Agricultural Sciences,
Islamic Azad University, Science and Research Unit, Tehran, Iran
[2]Department of Animal Breeding and Nutrition,
Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3]Department of Aquaculture, Islamic Azad University, Azadshahr Unit, Azadshar, Iran

Abstract: Nauplii *Artemia urmiana* was enriched with Thepax - a commercial product containing yeast cells of *Saccharomyces cerevisiae*. The Thepax-induced changes in biochemical parameters such as crude protein, fat, fiber, ash and energy were determined in the nauplii enriched at different times (6, 12 and 18 h) after hatching. To identify these changes, each group of nauplii (6, 12 and 18 h post-hatching) was enriched with 10^7 colony forming units (cfu mL^{-1}) for a 24 h period. Enrichment with Thepax increased the ash content but decreased crude fat, protein and energy contents of *Artemia urmiana* nauplii ($p<0.05$). Thepax had no effects on crude fiber ($p>0.05$). As shown by the results of this study, it seems that probiotic enrichment with Thapax is favorable to improve the mineral (ash) content of *Artemia urmiana* nauplii. However, supplemented yeast probiotic with lipid emulsion and proteolytic enzymes is suggested for the nauplii enrichment.

Key words: Enrichment, biochemical parameters, yeast probiotic, *Artemia urmiana*

INTRODUCTION

Artemia is one of the most important live feeds for commercial production of fish and shellfish larvae. This organism, in turn, can be fed on a wide variety of feeds since it is a continuous nonselective and particle filter feeder. In result, the nauplii have been considered as a live feed and a multipurpose vector.

Many studies have been performed to improve the nauplii feature as a feed and or vector. Probiotic enrichment of *Artemia* nauplii by *Saccharomyces* sp. have been used for the both aims. For example Gomez-Gill *et al.* (1998) and Patra and Mohamed (2003) attempted to improved vector function of the nauplii by probiotic *Saccharomyces* sp.

Some studies have shown compositional changes of the nauplii body following enrichment by *Saccharomyces cerevisiae*. Lim *et al.* (2005) showed that use of the yeast single cell protein can increase lipid content of the enriched *Artemia franciscana* nauplii.

Thepax (Manufactured by Doxol Co., Italy) is a manipulated product of *S. cerevisiae* yeast cells with controlled reproduction and supplemented by amino acids, minerals and vitamins. This supplementary ingredients have positive effects on growth and provide a favorable intestinal flora e.g., Lactobacilli.

Data on yeast probiotic enrichment of *Artemia urmiana* nauplii found in Uromie Lake of Iran are insufficient. Fazeli and Azari-Takami (2006) have found the best time and concentration of Thepax for probiotic enrichment of *Artemia urmiana* nauplii. Some locally published data have been provided about enriching effect of a bacterial Probiotic on vector function of *Artemia urmiana* nauplii. This project was conducted to study the thepax-induced changes in chemical composition of the nauplii enriched at different times after hatching.

MATERIALS AND METHODS

Artemia cyst hatching: *Artemia urmiana* cysts were decapsulated using a chemical process according to Gomer-Gill *et al.* (1998). The decapsulated cysts were hatched in a sealed flask with 1000 mL of autoclaved saline water (3/0 g L^{-1} salinity). The cysts were stocked at a density of 2 g L^{-1} and incubated at 28±1°C under pH = 7.

The hatching container was vigorously aerated to keep the cysts in suspension and exposed to light. After 24 h, the newly hatched nauplii were collected aseptically in a 120 μm-pores sieve and washed thoroughly with sterile distilled water.

Corresponding Author: Z. Fazeli, Department of Aquaculture, Faculty of Agricultural Sciences, Islamic Azad University, Science and Research Unit, Tehran, Iran Tel: +98(173) 3222995

Enrichment treatment: To specify the effect of Thepax on biochemical compositions of *A. urmiana* nauplii, 1 g Thepax was added to each conical-cylindrical container containing 6 L of sterilized 30 ppt salt water. In result, solutions of 10^7 cfu mL^{-1} *Saccharomyces cerevisiae* were prepared within each container. 6, 12, 18 h post-hatched nauplii were stocked at rate of 2 g L^{-1} to the solutions. Three separate containers were considered for each nauplii group. The treatment period with Thepax was 24 h for each nauplii enrichment group. There was also a Thepax-free control group for each of the three nauplii groups. At the end of each enrichment treatment, blowing process was interrupted and the nauplii were collected by siphoning. This time corresponded to 30, 36 and 42 h post-hatching, respectively for 6, 12 and 18 h post-hatching treatments.

Laboratory analyses: After measuring dry matter, duplicate samples (>0.5 g) were weighted and incinerated in a Thermolyne muffle at 550°C overnight and total ash was calculated. Crude protein was measured using a microkejdal method with catalyst. Energy content was determined by bomb calorimeter (Isoperibol calorimeter, 6200, Parr Co.). Crude fat was determined by extraction with petroleum using AOAC methodology (Ellis, 1984; AOAC, 1996). Crude fiber was determined according to Van Soest (1994).

Statistical analysis: Treatments were compared by one-way Analysis of Variance (ANOVA). In case of in - homogeneity, comparisons of means were made using Duncan's multiple range test at 5% level of significance using SPSS (version 13.0). The significance level was set at $p<0.05$.

RESULTS

Chemical composition of *A. urmiana* Nauplii: Compared with control nauplii, the amount of protein was less in 30 and 36 h post-hatching enriched nauplii ($p<0.05$) (Fig. 1). However, there was no significant difference in crude protein of enriched and control 42 h post-hatched nauplii ($p>0.05$). In both enriched and control nauplii, the more mature were the nauplii, the higher was its crude protein content. However, there were no significant differences among 30, 36 and 42 h post-hatched nauplii ($p>0.05$).

The comparison of crude fat content of enriched and control nauplii suggest a reducing effect of Thepax on the crude fat. As shown in Fig. 2, the crude fat content of enriched 30 and 42 h post-hatched nauplii was less than the controls' ($p<0.05$). However, the enriched 36-h nauplii have shown the highest crude fat content with a significant difference with control counterparts ($p<0.05$).

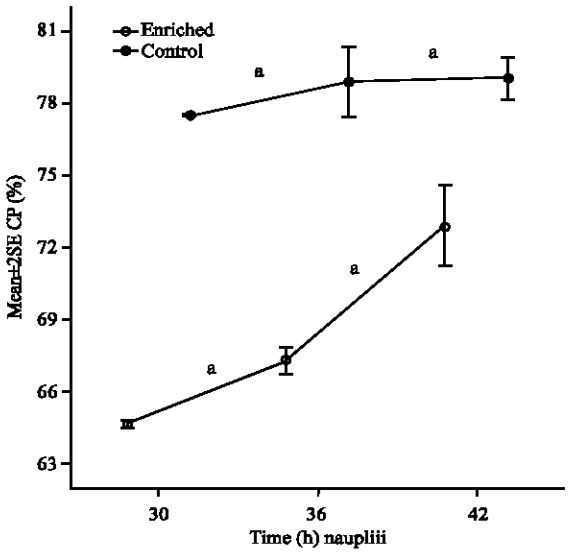

Fig. 1: The Crude Protein (CP) of enriched and control *Artemia* nauplii with different post-hatching treatment periods. The vertical axis shows the percentage of crude protein within the nauplii and the horizontal axis shows ages of the nauplii after a 24 h treatment. (a) Significant difference was seen between different post-hatching treatment periods ($p<0.05$). (b) No significant difference ($p>0.05$)

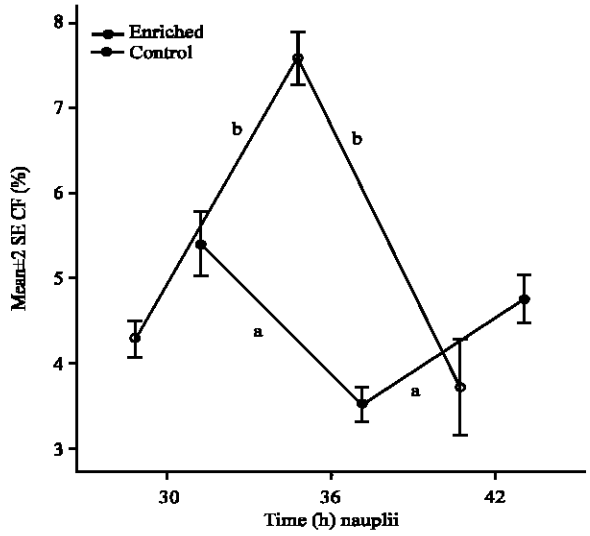

Fig. 2: The Crude Fat (CF) of enriched and control *Artemia* nauplii with different post-hatching treatment periods. The vertical axis shows the percentage of crude fat within the nauplii and the horizontal axis shows ages of the nauplii after a 24 h treatment. (a) Significant difference was seen between different post-hatching treatment periods ($p<0.05$). (b) No significant difference ($p>0.05$)

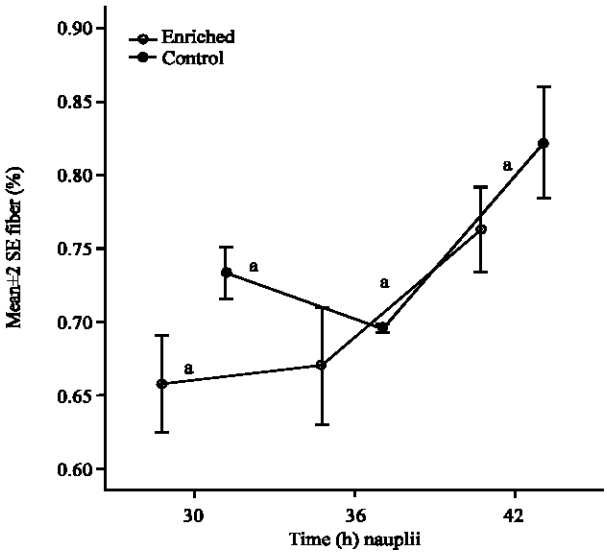

Fig. 3: The crude fiber of enriched and control *Artemia* nauplii with different post-hatching treatment periods. The vertical axis shows the percentage of crude fiber within the nauplii and the horizontal axis shows ages of the nauplii after a 24 h treatment. (a) Significant difference was seen between different post-hatching treatment periods ($p<0.05$) and (b) No significant difference ($p>0.05$)

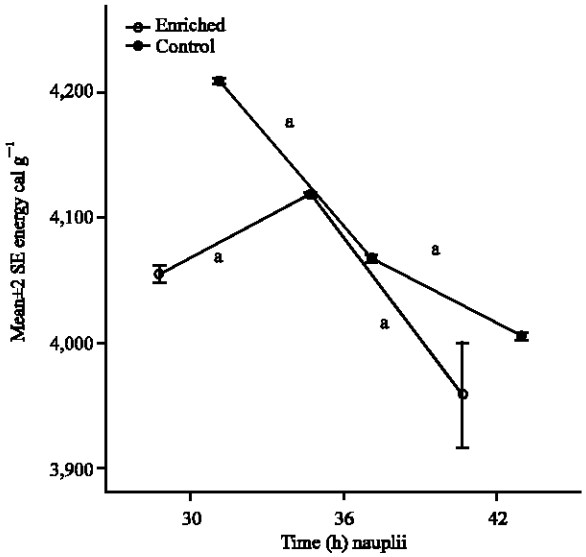

Fig. 4: The crude energy of enriched and control *Artemia* nauplii with different post-hatching treatment periods. The vertical axis shows the crude energy (cal g^{-1}) within the nauplii and the horizontal axis shows ages of the nauplii after a 24 h treatment. (a) Significant difference was seen between different post-hatching treatment periods ($p<0.05$) and (b) No significant difference ($p>0.05$)

Within enriched groups, there were no significant differences among crude fat contents of 30, 36 and 42 h post-hatched nauplii ($p>0.05$). In contrast, significant differences were seen among fat content of control nauplii where highest between 30 and 36 h post-hatched nauplii ($p<0.05$).

No significant difference in crude fiber content between enriched and control nauplii were found ($p>0.05$) (Fig. 3). Within enriched and control nauplii, there were significant differences among crude fiber contents of 30, 36 and 42 h post-hatched nauplii ($p<0.05$). In enriched nauplii, the more mature were the nauplii at the onset of enrichment, the higher was its crude fiber content.

The energy of enriched nauplii in all different times was significantly less than the control nauplii ($p< 0.05$). Within enriched and control nauplii, there were significant differences among crude energy contents of 30, 36 and 42 h post-hatched nauplii ($p<0.05$). In the case of the enriched nauplii, the highest energy content was for 36 h nauplii. In control nauplii, the more mature were the nauplii, the less was its crude energy content (Fig. 4).

The ash contents of the enriched nauplii was significantly higher than the control groups ($p<0.05$).

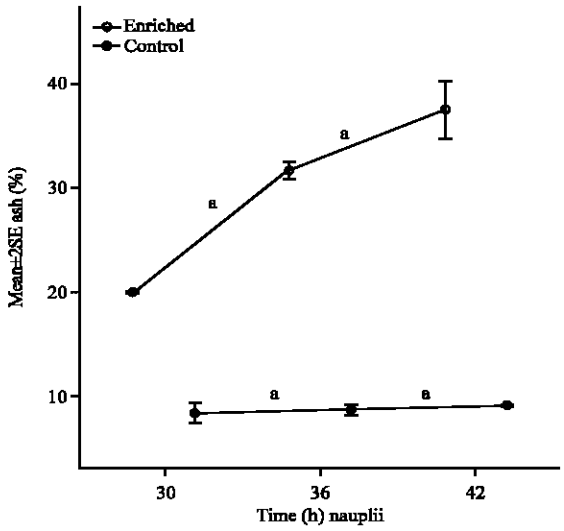

Fig. 5: The ash content of enriched and control *Artemia* nauplii with different post-hatching treatment periods. The vertical axis shows the percentage of ash within the nauplii and the horizontal axis shows ages of the nauplii after a 24 h treatment. (a) Significant difference was seen between different post-hatching treatment periods ($p<0.05$) and (b) No significant difference ($p>0.05$)

Within enriched and control nauplii, there were significant differences among crude ash contents of 30, 36 and 42 h post-hatched nauplii ($p<0.05$) (Fig. 5).

DISCUSSION

According to present results, decreases in crude protein, crude lipid and energy contents were observed in the enriched nauplii compared to control nauplii. In addition, the results suggest no Thepax-induced changes in crude fiber but significant increases in the ash content of enriched nauplii.

Tovar *et al.* (2002) showed a decrease in secretion of amylase and tripsin following treatment of sea bass fish by *Saccharomyces cerevisiae*. In the case of *Artemia nauplii*, this finding can suggest that the cells of *Saccharomyces cerevisiae* might be indigestible within the nauplii body. If so, no adequate protein can be taken by the enriching nauplii and no significant changes in nauplii protein profile would be seen. A possible strategy to overcome this problem, the removal treatment of yeast cell wall can be performed by enzymatic digestion before enrichment as suggested by Peter *et al.* (1990). In addition, we suggest a concurrent supplementation of the probiotic yeasts with proteolytic enzymes.

Torja *et al.* (2003) found a low content of free fatty acids in *Saccharomyces cerevisiae* and suggest a concurrent use of lipid emulsion during yeast probiotic enrichment of *Artemia* nauplii. This finding in conjunction with short time of enrichment can explain the lower fat content of enriched nauplii in comparison to control nauplii.

In contrast, Lim *et al.* (2005) found significant increase in crude lipids by enriching *Artemia franciscana* nauplii with wild *Saccharomyces cerevisiae*, compared with the newly hatched nauplii ($p\leq0.05$). Manqué *et al.* (2004) suggested that feeding or enrichment *Artemia nauplii* with *Saccharomyces cerevisiae* as well as *Dunaliella tertiolecta* can provide the highly unsaturated fatty acid (HUFA n = 3).

In addition, Barclay and Zeller (1996) suggested that the yeasts supplemented with lipid emulsions as well as bacteria and micro capsulated compounds can be an alternative for commercial material such as Selco to fortify the unsaturated fatty acid profile of aquatic animals. In fact, the lipid supplemented yeasts have high concentrations of unsaturated fatty acids including Doxahexanoic Acid (DHA) (22:6n-3).

As shown in this study, Rudneva and Shaida (2005), Ortega *et al.* (1999), Vanhaeck *et al.* (1983) and Boulton and Huggins (1978) also showed that the more mature were the nauplii, the less was its crude fat content. Similar findings were seen for the energy by these authors. To explain the decrement of energy during nauplii maturation, they referred to decrease of fat content and higher biological activity of the mature nauplii in comparison to younger nauplii.

The ash content of the Thepax is high. The higher ash content of the enriched nauplii can be explained by this concept.

Greco *et al.* (2002) showed that ash content increase following yeast enrichment was limited to adult *Artemia salina* var. San Franciso, which was attributed to the ability of the adult animals in swallowing the yeasts.

CONCLUSIONS

As shown by the results of this study, it seems that probiotic enrichment with Thapax is favorable to improve the mineral (ash) content of *Artemia urmiana* nauplii. However, supplemented yeast probiotic with lipid emulsion and proteolytic enzymes is suggested for the nauplii enrichment. In addition, some new studies can be helpful to understand changes of vitamins and other nutritional elements of *Artemia urmiana* nauplii following the yeast enrichment.

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