J Krishi Vigyan 2023, 11 (Supple) : 15-20 DOI : 10.5958/2349-4433.2023.00077.6

Biometric and Fatty Acid Profile of the Brine Shrimp *Artemia franciscana* Enriched with Marine Microalgal Species belonging to Prymnesiophytes and Eustigmatophytes

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ABSTRACT

Naturalized *Artemia franciscana* strains were collected from the Kelambakam hypersaline habitats along the Southeastern coast of India. Naturally occurring microalgae *Nannochloropsis oculata*, *Dicrateria inornata, Pavlova viridis,* and *Isochrysis galabana* has been used as Poly Unsaturated Fatty acid (PUFA) enrichment diet for *Artemia* nauplii. *Artemia* was enriched for different time intervals (0, 1, 3, 5, 7, 9 h) to find the optimum enrichment duration for the biometrical characters of the *Artemia* nauplii and to compare their suitability as fatty acid enrichment source. The length and width of *Artemia* nauplii enriched with microalgae exhibited a marginal increase up to 7 h of enrichment followed by a significant increase after 9h. Lipid contents of the nauplii enriched with *N. oculata* and *I. galabana* were high (26.20 and 26.25, respectively) at three hours of enrichment and observed a significant decrease at nine hours of enrichment. Total PUFA content of the *Artemia* nauplii enriched by *I. galabana, P. viridis*, and *D. inornata* was increased at seven hours of enrichment and on further enrichment (9 h), PUFA content was found to be significantly reduced. Maximum DHA was recorded in *Artemia* nauplii enriched with *I. galabana* (3.69% at 7 h), and it was found to be significantly higher than nauplii enriched with other microalgae. The microalgae-induced naupliar enrichment concerning essential PUFAs like DHA and EPA does not require more than 7 h enrichment while maintaining the naupliar size at their minimum for use in larval feeding.

Key Words: Artemia franciscana, Biometry, Dicrateria inornata, Isochrysis galabana, Nannochloropsis oculata, Pavlova viridis Enrichment,Polyunsaturated fatty acids.

INTRODUCTION

The brine shrimp *Artemia* nauplii (Crustacea: Branchiopoda: Anostraca) is popularly used as a live feed and, more precisely, as a carrier of nutritional elements for finfish and crustacean larval rearing (Vikas *et al*, 2012; Ronnestad *et al*, 2003). *Artemia* is a highly sought-after live feed in aquaculture because of its texture, size, and storage ability. Though *Artemia* nauplii are extensively used as live food in larviculture, and were reported to lack certain essential nutritional elements required for the larvae. Among those, long-chain polyunsaturated fatty acids (LC-PUFAs) are worth mentioning (Sorgeloos *et al,* 1991) that have a significant role in determining the growth and survivability of finfish and shellfish larvae (Rainuzzo *et al*, 1997). The studies explain why research on enriching *Artemia* with PUFAs viz., EPA (20:5n3) and DHA (22:6n3) before their use as live prey has received considerable attention to increasing larval survival rates. Several commercial enrichment diets such as DHASelco, A1-Selco, and Protein Selco supplement the PUFA profiles of *Artemia* and other live feeds used in mariculture (Chakraborty *et al*, 2010; Tamaru *et al*, 1999; Biswas *et al*, 2006). However, these commercial enrichment formulations are highly expensive and susceptible to oxidation, giving way to forming harmful *trans* fatty acids and undesirable oxidation products.

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A significant source of long-chain PUFAs in aquatic animals is from the primary producers through the food web, mainly from various microalgae. Microalgae play an important role in aquaculture since they constitute the basis of the food chain, being the main diet for molluscs and used for the first-feeding of fish and crustacean larvae (Ferreira *et al*, 2008). They are renewable reservoirs of PUFAs, and, therefore, can be a potential enrichment diet of live feed and mariculture (Hu *et al*, 2008). PUFAs like microalgae are much more stable than commercially available enrichment emulsion formulations. It is significant that the levels and ratios of 22:6n3: 20:5n3: 20:4n6 in live microalgal cells more closely resemble natural larval diets, and the probabilities of natural protection of PUFAs by natural antioxidants in microalgae are advantageous. The longer n3 and n6 fatty acids (>18 carbon atoms) dominate the composition of marine microalgae species and can be transferred to higher organisms via live feeds, such as *Artemia* (Chakraborty *et al,* 2010). Multivariate applications for fatty acid analysis were started in the last part of the 1980s (Ulvund *et al,* 1988). The most common multivariate method is principal components analysis (PCA). In this study, the efficiency of marine microalgae *viz.*, *Nannochloropsis oculata*, *Dicrateria inornata, Pavlova viridis,* and *Isochrysis galabana* on the PUFAs content of the *Artemia* nauplii have been validated. Simultaneously, the effects of enrichment duration on the biometrical characters of the nauplii in 0, 1, 3, 5, 7, and 9 h time intervals have also been studied. The diet-induced changes in the fatty acid composition of enriched *Artemia* concerning the initial fatty acid content of the nauplii and possible metabolic changes of the fatty acids for various durations have been reported. The enrichment experiment›s main objective was to find the candidate microalgae as live food enrichment diets and the optimum duration to harvest *Artemia* nauplii with minimum size to use in larviculture.

MATERIALS AND METHODS

Preparation of stock culture of microalgae for enrichment

The experimental microalgae selected in this study belonged to the family prymnesiophytes (*viz*., *P. viridis* and *I. galabana*) and eustigmatophytes (*viz*., *N. oculata* and *D. inornata*), and the stock of the microalgae culture required for the study was taken from the marine microalgae culture facility of CMFRI, Cochin.

Enrichment procedure for the Artemia nauplii with the microalgae

Artemia cysts were collected from the hypersaline habitats of Kelambakam, Tamil Nadu (12047› N 800 13› E). The samples were brought to the wet laboratory of Central Marine Fisheries Research Institute (CMFRI), suitably cleaned, and processed by bipartite floatation technique with brine and freshwater as detailed earlier (Sorgeloos *et al,* 1983), and stored under refrigeration until further use. Decapsulation and hatching o*f Artemia* cysts (strain designation CKF) were performed following established procedures with suitable modifications (Sorgeloos, 1986). Metanauplii-1 was harvested from the hatching container using a sieve (120µm), rinsed thoroughly with filtered sea water (35ppt), and transferred to 3 l enrichment containers at a final density of 100 nauplii ml-1 at room temperature (28 ± 1oC). The four different enrichment diets, viz. *N. oculata*, *D. inornata, P. viridis,* and *I. galabana* were prepared and enriched (50 X 104±25 cells mL-1). This concentration was found to be sufficient to feed the *Artemia* during the 9 h enrichment duration, as proved by the residual microalgal cells in *Artemia* culture tanks after even 9 h. Samples of *Artemia* were harvested in triplicate at six different intervals (0, 1, 3, 5, 7, and 9 h) during the enrichment period with bolting silk scoop mesh (200 µm). Randomly collected nauplii were fixed with Lugol's iodine for biometrical measurements. The samples were thoroughly rinsed with double distilled water and preserved at -200C until further use for biochemical estimation.

Biometrical measurement of nauplii at different time intervals

The maximum length and width of Artemia nauplii fixed with Lugol's iodine were determined using a light microscope (Leica, USA) with the overhead camera (DIIGIEYE) 330/210 (Image Analyzer Microscope) and software (Dewinter Biowizard).

Estimation of total lipids and fatty acids

Lipid content in the *Artemia* nauplii and microalgae (350 mg wet weight) was estimated following the method reported by Bligh and Dyer (1959) with suitable modifications. The esterified fatty acid content of the microalgal species and the enriched *Artemia* nauplii were analyzed by gas-liquid chromatography with an FID detector using fatty acid methyl ester standard (Supelco FAME 37 standard).

Statistical analyses

Statistical evaluation was conducted with SPSS program 13.0 (SPSS Inc, Chicago, USA). Descriptive statistics were calculated for all the studied traits. Analyses were carried out in triplicate (n=3), and the means of all parameters were examined for significance by analysis of variance (ANOVA). Pearson correlation coefficient between length and width of the *Artemia* nauplii at different time intervals was calculated. A significance level of 95% ($p< 0.05$) was used throughout. Principal component analysis (PCA) is often used to reduce the dimensionality of data profiles containing intercorrelated variables.

RESULTS AND DISCUSSION

Nutritional profile

Artemia nauplii are a candidate and most sought-after live feed for larval cultures that prefer soft textured prey items to meet their feed intake demands. Aquatic finfish and shellfish larvae have little ability to synthesize the long-chain PUFAs from shorter carbon chain precursors using the desaturase and elongase enzymes, so all of the essential PUFAs have to be supplied in the diet. However, *Artemia* nauplii are considered to be an incomplete live feed for marine larvae because of their paucity of essential polyunsaturated fatty acids (PUFAs), *viz.*, n3 PUFAs, eicosapentaenoic acid (EPA, 20:5n3), docosahexaenoic acid (DHA, 22:6n3), and n6 PUFA, *viz.*, arachidonic acid (A.A., 20:4n6) (Watanabe *et al*, 1994). Therefore, it is essential to enrich *Artemia* nauplii with n3 and n6 PUFAs, before using them as a live feed. These PUFAs low in *Artemia* and nauplii are essential for finfish and crustacean larvae and must be incorporated by external means in this live feed. There are reports of using commercially available formulations viz., DHA Selco, Protein Selco, A1 Selco, Etc. to enrich live feed like rotifer and *Artemia* nauplii. Due to the slighter shelf-life of these commercial formulations, there is growing interest in marine microalgae that are reported to contain considerably high contents of PUFAs to enhance the essential n3 and n6 PUFAs content in the *Artemia* nauplii (Chakraborty *et al*, 2007; Refsgaard *et al*, 1998). Several microalgae species are reported to possess higher contents of PUFAs and are significant contributors to the marine food web as a renewable source (Hartvigsen *et al*, 2000). Earlier, it was reported that microalgae belonging to prymnesiophytes (e.g., *Pavlova* sp. and *Isochrysis* sp.) and cryptomonads are relatively rich in DHA (0.2-11% TFA), whereas eustigmatophytes (*Nannochloropsis* spp.) and diatoms (*Chaetoceros* spp.) have higher percentages of EPA and A.A. (Lavens *et al,* 1991). The advantage of microalgae in enriching live feed is that they are significant contributors to the marine food chain as a renewable source, and no undesirable products are expected

The present study signifies the importance of microalgae as an enrichment diet for enhancing the nutritional profile of *Artemia* nauplii. It also brings out the protocol for optimum enrichment concerning naupliar size and the nutritional profile. It was established that microalgae belonging to prymnesiophytes (*Pavalova* sp and *Isochrysis* sp) were rich in DHA, whereas eustigmatophytes

(*Nannochloropsis* sp and *D. inornata*) have higher percentages of EPA (Chakraborty *et al,* 2007; Lavens *et al,* 1991; Renaud *et al,* 1994). Therefore, the microalgal enrichment diets (*N. oculata, P. viridis, D. inornata,* and *I. galabana*) were used in this study to improve the essential fatty acid composition of *Artemia* nauplii. This study has established that enrichment with selected microalgae can be effectively implemented to improve the nutritional contents of live feed, particularly *Artemia* nauplii, before being fed to marine larvae. Naupliiar size was reported to be one of the major limiting factors in determining the superiority of *Artemia* strain. *Artemia* nauplii enriched with microalgae for 0, 1, 3, 5, 7, and 9 h exhibited a marginal increase in length and width up to 5-7 h and then significantly increased at final enrichment duration (9 h). 5-7 h is considered the threshold enrichment time to maintain the optimum nutritional balance of *Artemia* nauplii, particularly fatty acids, while keeping the growth rate at its minimum. Therefore, longer enrichment (more than 7 h) is not advocated because of the rapid growth rate of the live feed after 7 h, which diminishes larval feed ingestability. High feed ingestion rates were observed during the 5-7 h enrichment period, which generally coincided with the size of live prey (*Artemia* nauplii), corresponding to 19-20% of the length of the predator larvae. An earlier study suggested the most favorable relationship of prey size to predator length as 0.2, thus signifying the importance of small prey size in larviculture (Barros *et al,* 2003).

The fatty acid composition of enriched *Artemia* nauplii varied as a function of microalgal dietary treatment and enrichment time. Diet-induced changes in the polyunsaturated fatty acid composition of enriched *Artemia* for various durations (1-9 h) revealed that *D. inornata* yielded the best performance (33.9% PUFA), followed by *P. viridis* (31% PUFA) and *I. galabana* (27.6%) during the enrichment period. During the initial phase of the enrichment, nauplii lipid concentration was high, indicating the accumulation of lipids in naupliar cells. Reitan (1997) reported a continuous increase in lipid contents in live feed rotifer (*Branchionus plicatilis*) enriched with microalgae and their ability to modify dietary fatty acids (Navarro *et al,* 1999). The role of PUFAs in aquaculture nutrition has been extensively investigated during the past two decades, particularly for live feeds (Sargent *et al,* 1999; Deering *et al,* 1997). The long chain PUFAs *viz.,* EPA (20:5n3) and A.A. (20:4n6) were reported to be involved in the production and modulation of eicosanoids (Brown, 1994), whereas DHA (22:6n3) was reported to maintain structural and functional integrity in larval cell membranes including neural function (Chakraborty *et al,* 2007). Though PUFAs are essential in larval development, they have a limited ability to synthesize the PUFAs *viz.*, 20:5n3 and 22:6n3 in the required quantity to meet their demand. This demands supplementing these essential fatty acids to larvae through live feeds like *Artemia* nauplii (Sargent *et al,* 2002).

Fatty acid profiling of the four microalgal species shortlisted for the enrichment of *Artemia* nauplii showed considerable differences in their total SFA, PUFA, and MUFA content. Total PUFA content was highest in *I. galabana* (43.31%), followed by *D. inornata* (39.01%), revealing their superiority over the other experimental microalgae. Though DHA was found to be highest (9.75%) in *I. galabana*, EPA content was found to be lower (4.11%) than recorded in *N. oculata* (9.69%) and *P. viridis* (9.54%). The differences in the essential fatty acid content of the four microalgae used for the enrichment study have been reflected in the fatty acid profile of the enriched *Artemia* nauplii. Accordingly, *Artemia* nauplii enriched with *I. galabana* exhibited a significantly higher DHA/EPA ratio (0.9) at 7 h of enrichment than in the other three species. A key aspect of fatty acid dynamics in *Artemia* and other zooplankton is whether they modify dietary fatty acids and, if so, to what extent these modifications take place (Ruiz *et al,* 2008). It was apparent from the present study that the contents of essential fatty acids, *viz.,* EPA, and

DHA in *Artemia* nauplii exhibited an incremental trend with the enrichment progress up to 5-7 h. Significantly, DHA, considered an essential fatty acid for larval nutrition, was found to be highest in *Artemia* nauplii enriched with *I. galabana* for 7 h (3.69%). P. viridis was the next best microalgae to enrich *Artemia* nauplii due to the high DHA (2.72%) content of the live feed after 5 h of enrichment. Subsequently, *Artemia* nauplii enriched with this microalga exhibited a significantly higher DHA/EPA ratio (0.65) at 5 h of enrichment, second best after *I. galabana.* The The results revealed higher PUFA content in *Artemia* nauplii enriched with *P. viridis, I. galabana, N. oculata,* and *D. inornata* than those reported earlier (Tamaru *et al,* 1999), where commercial enrichment media were used to enrich live feed. The content of EPA in *Artemia* nauplii enriched with *N. oculata* (5h-7.81%) and *I. galabana* (7h-7.58%) was found to be higher than recorded using commercial formulations *viz.,* Menhaden oil (250 ppm, 1.02%), DHA Selco (300 ppm, 1.81%), Selco (300 ppm, 0.59%), DHA MicroFeast L-10a (250 ppm, 0.82%), and Algamac-2000 (200 ppm, 0.68%) (Tamaru *et al,* 1999). The content of DHA in *Artemia* nauplii enriched with *I. galabana* (7h-3.69%) too was found to be higher than recorded in commercial formulations *viz*., Menhaden oil (0.5%), DHA Selco (2.33%), Selco (0.25%), DHA MicroFeast L-10a (0.16%), and Algamac-2000 (0.16%) (Tamaru *et al,* 1999) at identical concentrations. As detailed earlier, the present study revealed the superiority of microalgae as a live feed enrichment source over commercially available emulsions. The principal compound analysis (PCA) of 7 h enriched *Artemia* nauplii and the factor loading plot revealed the negative correlation ∑between MUFA with the ∑C18 PUFA, PUFA, ∑n3, ∑n6, 18:4n3, and ∑PUFA/∑SFA. The close relation of the 18:4n3 and ∑n3 revealed the impact of 18:4n3 in determining the total n3 fatty acid content. The results revealed the advantage of *I.galabana* over others as a suitable enrichment diet for enhancing the nutritional profile of *Artemia* nauplii.

CONCLUSION

The present study is significant in identifying *Isochrysis galabana* as the candidate microalgae that can offer an excellent nutritional package to the live feed *Artemia* nauplii through enrichment of PUFAs, which are reported to be essential for mariculture larvae. An optimized protocol to enrich *Artemia* for use as a live feed has been established in this study. The study also established the optimum enrichment duration of *Artemia* nauplii as 7 h to acquire a balanced fatty acid profile in the said live feed required for larviculture while maintaining the nauplii at a suitable size for feeding the mariculture larvae. Results from the present study validate the potential use of renewable sources like microalgae *I. galabana* as an enrichment diet to improve the nutritional quality of *Artemia* nauplii compared with importing and using more expensive enrichment techniques for use as potential live feed for larviculture.

ACKNOWLEDGMENTS

The authors are thankful to the Director, CMFRI, Cochin, for providing the necessary facilities to carry out the work.

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Received on 2/8/2023 *Accepted on 12/11/2023*