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Evaluation of the Biochemical Composition of Four Marine Algae and Its Nutritional Value for Brine Shrimp.

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Abstract: *Microalgae are utilized in aquaculture as a live feed for the crustaceans, ablone, zooplanktons, etc. The present study was aimed to examine the nutritional status of Artemia sp. nauplii enriched with four different algal sources namely Chaetoceros calcitrans, Skeletonema coastaum, Duniella salina & D. bardawil and also the amount of beta-carotene assimilated when enriched with the two green algae. Artemia sp. nauplii enriched with D. salina showed high amounts of protein and carbohydrate, whereas Artemia sp. fed with Chaetoceros calcitrans showed high amounts of lipid. The protein profile of Artemia sp. enriched with different algal sources did not show prominent differences in the polypeptide bands. However, high amount of beta-carotene was assimilated in Artemia sp. nauplii when enriched with D. salina. Hence this study showed that the microalgae D. salina can be used as a potential feed to improve the nutritional status of Artemia sp. nauplii.*
Keywords: *Microalgae, nutritional status, Artemia sp., beta carotene.*

I. Introduction

Aquaculture is becoming a major system for augmenting animal production, to supplement the stagnating marine fish production and to compensate the growing uncertainties of marine resource sustainability (Sankar and Ramachandran, 2002). Hence developing new technology, new breeds and newly domesticated breeds of fishes and live feeds offer a great hope for the future with a promise for blue revolution in the century to match the green revolution (Bernado, 2003). The first feeding of any cultivable organism is the most 'critical phase' of their life when they need the right type of nourishment for their survival and growth. Live diets include three groups of organisms namely phytoplankton such as microalgae (2 - 20 µm) and zooplankton such as rotifers (50 - 200 µm) and brine shrimp, *Artemia* sp. (200 - 300µm) (Annon, 2000). *Artemia* sp. is extremely important as a standard live feed for over 85% of marine species (Kinne, 1977). *Artemia* sp. is a biologically uncontaminated readily available and acceptable larval feed (Takami, 1993; Reddy & Thakur, 1998), possessing several features such as: small size, easy ingestion (Lèger et al., 1986), high nutritional value, unchanging food requirement from nauplii to adult (Helfrich, 1973) and high tolerance to various culture environments (Lèger *et al*., 1987). Moreover they also can be used as biovehicles by which the nutritional components can be administered to the fish and shrimp larvae. This phenomenon is known as bioencapsulation of live food organisms (Tamaru *et al*., 2000). Microalgae which are at the base of the food chain represent the third largest aquacultured crop in the world (Hanisak, 1998 & Annon 2000). In nature, most fishes and shrimps feed on varied types of natural phytoplanktons and zooplanktons. Farming of marine animals, including both finfish and invertebrates – chiefly crustaceans (shrimps) and mollusks requires microalgae as feed at some point in the life cycle (Jeffrey *et al*., 1994). However microalgae are also used widely to improve the nutritional content of zooplankton live feeds by allowing the zooplankton to fill their digestive systems with microalgae before subsequently being fed to the fish or shrimp larvae. In this "conditioning" strategy the zooplanktons serve as bags of appropriate size that partially digest the algae and stimulate components to the larvae. The green algae *Dunaliella*, produces abundant β-carotene (Wikifors, 2000) (an accessory light harvesting pigment) (Glazer, 1983). Carotenoids contribute to the health and reproduction in fishes, as well as their pigmentation. It is well documented that crustaceans are unable to biosynthesize carotenoids and therefore, the carotenoids are included in the feed and fed to fishes and shrimps through *Artemia* sp. nauplii. The aim of this present study is to explore the nutritional evaluati of *Artemia* sp. nauplii enriched with different algal sources.

II. Materials And Methods

The algal cultures of the diatoms: *Chaetoceros calcitrans* and *Skeletonema coastaum* and the green algae: *Dunalliella salina* and *D. bardawil* were obtained from the Algal Culture Collection at the Center for Advanced Studies in Botany, University of Madras. The diatoms were maintained in F/2 medium (Guillard and Ryther, 1962) and the green algae were maintained in De Walne's medium.

Hatching of Artemia cysts

The cysts of *Artemia* sp. obtained from the Department of Zoology, University of Madras, were used for this investigation. *Artemia* cysts were hatched as per the standard procedure outlined by Sorgeloss *et al*., 1986. The hatched nauplii from the decapsulated cysts (more than 90 % Instar – I) were siphoned out and used for the enrichment experiments.

Enrichment of Artemia nauplii

The 24 h old 600 nauplii in 1L of seawater were fed separately with the microalgae *viz*; *Cheatoceros calcitrans* (22nd day), *Skeletonema coastatum* (20th day), *Dunaliella salina* (15th day) and *D. bardawil* (21st day) (obtained from the Centre for Advanced studies in Botany, University of Madras) at different cell concentrations viz; 50 - 70 cells/mL, 30 - 60 cells/mL, 30 - 80 cells/mL, 40 – 90 cells/mL, 20 - 80 cells/mL and 50 - 100 cells/mL respectively. The experiment was conducted for a period of 24 h. Every 3 h interval the animals were observed under microscope and recorded their gut region. The collected nauplii are washed in fresh water and their nutritional status was evaluated.

Biochemical Analysis

Fifty mg fresh weight of the *Artemia* nauplii enriched with different algal sources were taken and estimated for different biochemical constituents. The total protein was quantified following the method of Bradford, 1976. The total carbohydrate was estimated as per the method of Dubois *et al.*, 1956.The total lipid was determined using the method of Jordifolch lees, 1956. SDS-PAGE was carried out using the modified method of Laemmli (1970). The *Artemia* sp. nauplii enriched with the four different microalgae were studied for its carotenoid content by following the method of Schwartz and Patroni-Killam (1985). The amount of carotenoids extracted were scanned under UV visible spectrophotometer at 450 nm and further confirmed by TLC. All the values were given on wet weight basis, with three replications. The values were analyzed using oneway ANOVA by the Agres statistical software package.

III. Results and Discussion

In the present study, four different microalgae were used as feed to live *Artemia* sp. The protein, lipid and carbohydrate content of four different algal sources were estimated. Among the four algal sources used to enrich *Artemia* sp., *Dunaliella salina* showed high amount of protein content (69 µg/ml)followed by *D. bardawil*, *Chaetoceros calcitrans* and *Skeletonema coastatum* . as shown in figure (1). *Dunaliella salina* enriched nauplii showed high content of carbohydrate (189 µg/ml) followed by *Chaetoceros calcitrans*, *Skeletonema coastatum* and *D. bardawil* (Fig. 3). However high lipid content was recorded in the *Artemia* nauplii enriched with *Chaetoceros calcitrans*(Fig. 2). The above results are in similar to the findings of D'souza (1999) who reported that Penaid larvae fed with *Chaetoceros* sp. showed high protein and lipid content. The protein profile using the SDS-PAGE of *Artemia* sp. enriched with different algal sources did not show prominent differences in the polypeptide bands as well as when compared the control (Fig 6). The beta- carotene extracted from *Artemia* sp. nauplii enriched with *D. salina* (1.66 µg/ml) as shown in figure 4 and *D. bardawil* (0.94 µg/ml) showed a peak at 450 nm . The extracted beta-carotene was subjected to TLC showed an Rf value (0.85) similar to the authentic beta-carotene (Fig. 5). Our present findings are in congruent with Boonyaratpalin et al. (2001), who reported that the *Artemia* nauplii enriched with *D. salina* contained beta-carotene, this nauplii when fed to the shrimps imparted colouration. The *Artemia* sp. enriched with beta-carotene via the algal source serves as bioencapsulator where it can be transferred up the food chain as many crustaceans are unable to synthesize carotenoids *de novo* (Goodwin, 1984) and moreover the microencapsulated live diets enhanced the growth and survival of shrimps and fishes (Pedroza, *et al*. 2004). Carotenoids not only impart coloration but also improve tolerance to stress conditions and the immunity of aquatic animals (Hunter, 2000, Supamattaya *et al.*, 2005). It has been shown that *Penaeus monodon* showed enhanced resistance to white spot syndrome viral infection when fed with diet enriched with *D. salina* (Madhumathi & Rengasamy, 2011), which is rich source of carotenoids.

Therefore a good selection of algal species is a prerequisite to support the aquaculture industry inorder to improve nutritional quality, healthy growth and hatchery efficiency. Hence it could be concluded from the present study that among the four microalgae tested *D. salina* could be used as a potential live feed to improve the nutritional status of *Artemia* sp. nauplii.

Figure 1:

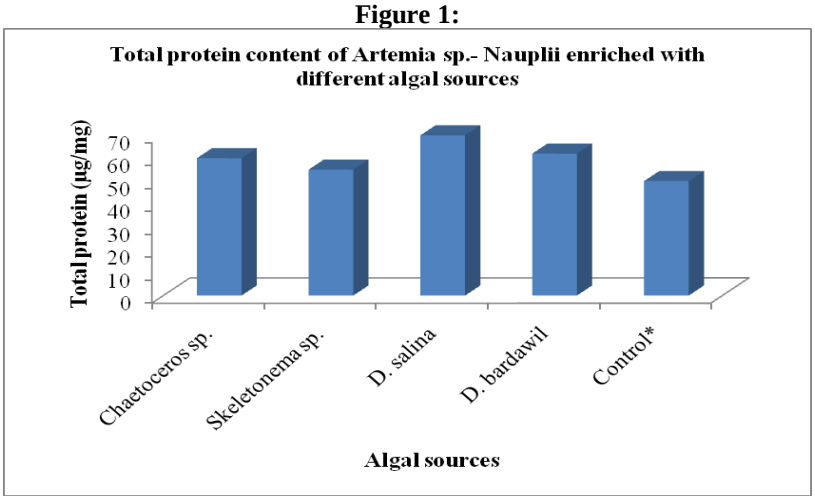


Figure 2:

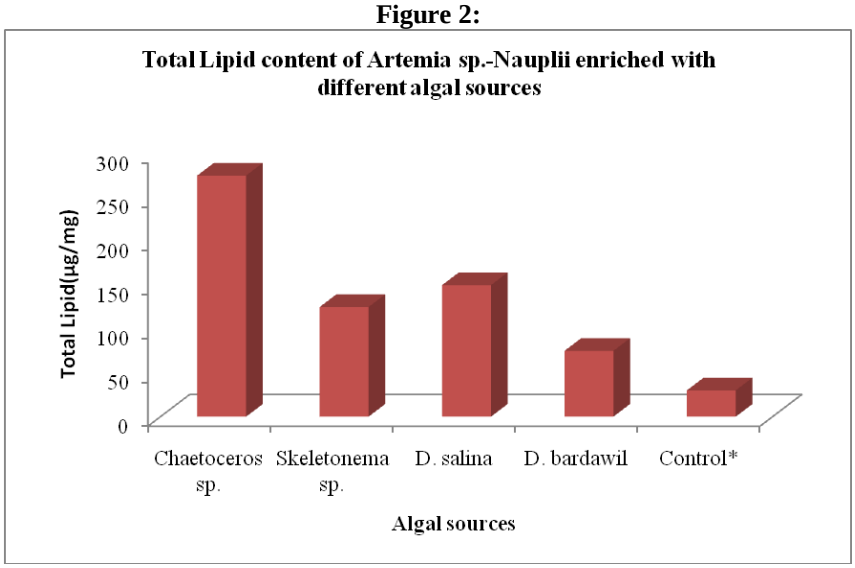
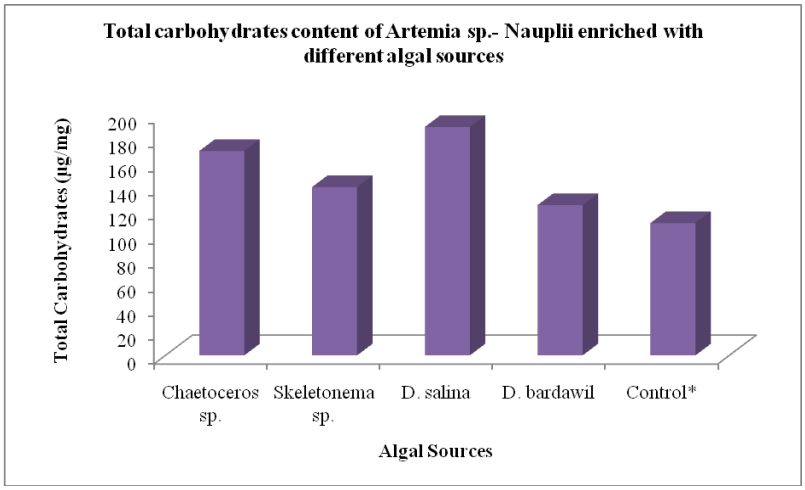


Figure 3:



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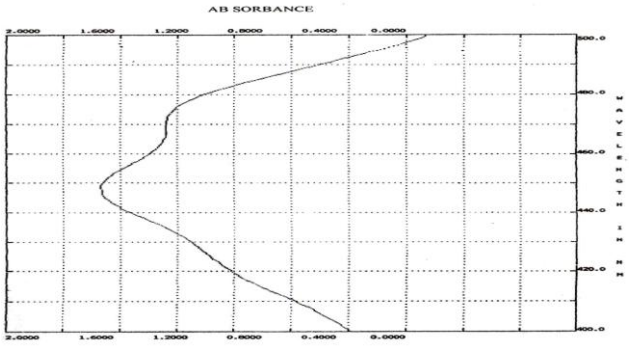
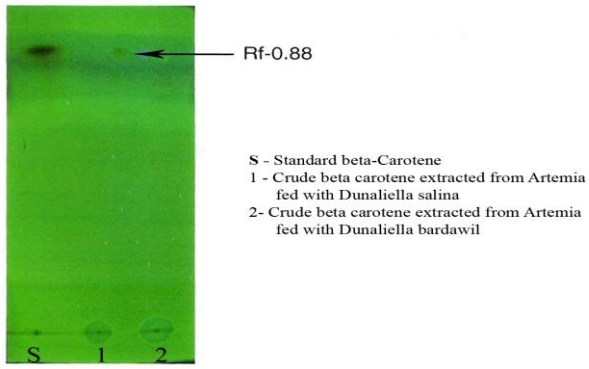


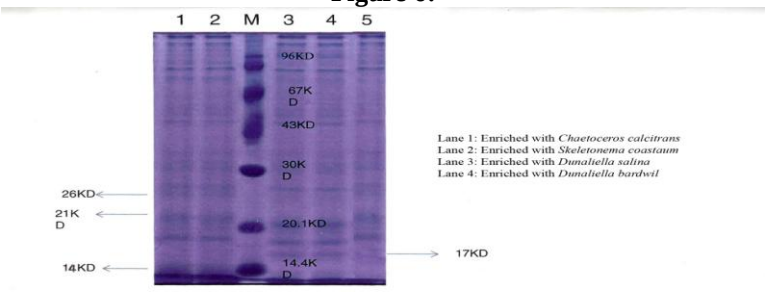
Quantitative analysis of crude extract of beta-carotene from Artemia sp. enriched with Dunaliella salina

Figure 5:



Qualitative analysis of crude extract of beta-carotene from Artemia sp. enriched with green algae (Thin layer chromatography under UV Light)

Figure 6:



Protein profile of Artemia sp. enriched with different algal sources

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