J. Algal Biomass Utln. 2013, 4 (2): 67–73
ISSN: 2229- 6905

Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © 2009 PHYCOSPECTRUM - ISSN: 2229 – 6905

Influence of micro algae in enrichment of *Artemia salina* for aquaculture feed enhancement

S. Lakshmanasenthil[1], T. Vinothkumar[1*], D. Geetharamani[2], T. Maruthupandi[3]

[1*] CMS College of Science and Commerce, Chinnavedampati (po), Coimbatore, Tamil Nadu, India. dr.tvinothkumar@gmail.com

[2] Dr. N.G.P. College of Arts and Science, Coimbatore, Tamil Nadu, India

3 CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India

Abstract

Artemia salina was enriched with different micro algae (*Chlorella vulgaris, Nannochloropsis granulata* and *Spirulina maxima*) with commercially available media A1 DHA SELCO. After enrichment process, proximate analysis was carried out by Lowry's method (protein), Anthrone method (carbohydrate) and phenol-Chloroform method (lipid). Results were compared with FAO nutrient standards for algae and *C. vulgaris* and *S. maxima* enriched *Artemia* showed prominent results. *Chlorella vulgaris* and *N. granulata* enriched *Artemia* showed high lipid which is the nutrient content of both algae. *Spirulina maxima* enriched *Artemia* showed the high protein which is the nutrient content of *S. maxima*. *Nannochloropsis granulata* and *C. vulgaris* enriched *Artemia* illustrated that good result in both protein and lipid. Concentration of the *Artemia* biomass was directly proportional with hours of enrichment. From this study, we can use algae (*C. vulgaris, N. granulata* and *S. maxima*) as an enrichment media instead of commercial A1 DHA SELCO media which give same nutrition profile to *Artemia*.

Keywords: nutrients, algae, FAO, enrichment, DHA

Introduction

Aquaculture, the farming of aquatic organisms contributed 40% to the total food source in the year 2000. It is expected to increase in the years 2020–2025. In aquaculture practice, brine shrimp (*Artemia*) nauplii are considered as the most widely used live diets for fish larvae (Sorgeloos et al. 2001). Over 85% of all marine animals' culture utilizes *Artemia* as a partial or sole diet (Hoff and Snell 2008). They have been used as a vector for the delivery of die-rent materials, such as nutrients (Espinosa and Allam 2006) and probiotics (Teresita et al. 2005).

The enrichment processes for *Artemia* nauplii significantly elevate all the fatty acids found in the nauplii, the most notable is the elevations of C20:5n-3 (EPA) and C22:6n-3 (DHA). Both have been implicated to be essential for larval growth and development in a number of fish species (Sorgeloos et al. 2001).

The essential highly unsaturated fatty acids (HUFAs) such as eicosapentaenoate (EPA) and docosa hexaenoate (DHA) are significantly higher in *Artemia* nauplii. The varying amounts of EPA and DHA reflect the differences in the amount of enrichment media used as well as quantitative and qualitative differences in the sources of these fatty acids (e.g., fish oil or algae) that make up the commercially prepared enrichment media. The enrichment process is time dependent. As the fatty acids and proteins are taken up by the *Artemia*, their fatty acid profiles and protein concentrations change according to the duration of the enrichment period. The length of the enrichment process should also be considered when preparing *Artemia* nauplii as a food for the larvae of ornamental fish (Tamaru et al. 2004).

Microalgae strains are recognized as an excellent source of proteins, carbohydrates, lipids, and vitamins, to be used as food and feed additives (Rocha et al. 2003). *Nanochloropsis* sp. and *Chlorella* sp. are well known source of EPA, an important polyunsaturated fatty acid (Hanhua and Kunsan 2003; Vishwanath et al. 2007). It is a well-known fact that *Spirulina* sp. is responsible for protein (Babadzhanov et al. 2004).

The aim of this study is (i) to enhance the protein and lipid content of *Artemia*, which is obligatory for fishes by the enrichment practice (ii) to reduce the cost by using micro algae as a natural enrichment medium (iii) to compare the natural enrichment with commercial medium A1 DHA SELCO for efficiency. The natural enrichment process is expected to the nutritient level of *Artemia* without any toxicity of chemical enrichment method.

Materials and Methods

Culture Collection

Microalgae (*C. vulgaris*, *N. granulata* and *S. maxima*) were obtained from the Dr. N.G.P. College of Arts and Science, Coimbatore, Tamil Nadu, India. Stock cultures of these strains were maintained in 25% Conway medium (Walne 1970) at 28±2 °C, 24 hrs dark/light cycle, with white fluorescent light at an intensity of 40 µmol photon $m^{-2}s^{-1}$ under continuous aeration. Batch cultures were scaled up to 20-30L and maintained until reaching exponential growth.

Composition of A1 DHA SELCO

A1 DHA SELCO media is a product of Artemia International LLC, USA [Moisture (30%); Crude ash (1%); Crude lipids (67%); Phosphorus (0.2%), Vitamin A (1,500,000 IU/kg); Vitamin D3 (150,000 IU/kg); Vitamin E (3600 mg/kg); Vitamin C (800 mg/kg) and Antioxidants (ethoxyquin) (http://www.artemia-international.com/default.asp?contentID=582#selco)].

Procedure for production and collection of *Artemia*

Artemia production was followed by the modified method of Sorgeloos et al. 2001. One gram of cyst of *Artemia salina* was taken in sea water (salainity 35 ppt). The dry cyst was placed in water to be hydrated. The free swimming nauplius, called as an instar 1, hatched out after 20 to 24 hrs. Sea water was maintained at 28–30 ˚C and at pH 8. The contents were subjected to sufficient aeration to keep the cysts in suspension. Air was provided through the open end of a half inch PVC pipe placed close to the bottom. A strong optimum illumination of 2000 lux was provided by placing two neon tubes (2X58w) just above the tank rianalm..

Enrichment and storage of *Artemia*

Fifty to hundred grams of A1 DHA SELCO was taken in 1L of water and 1 L of different microalgae emulsified by mixing vigorously for 3 mins. Freely hatched *Artemia salina* were transferred into varying concentration of A1 DHA SELCO (0.2, 0.4, 0.6 and 0.8 ml/2l) and enriched with *C. vulgaris*, *N. granulata* and *S. maxima* (250, 500, 750, 1000 ml/2L). After enrichment, samples were collected at different intervals (6, 12, 18, and 24hrs) and stored at at 4 to 10 °C in cold seawater to prevent the nutrient value of *Artemia.* A control was maintained without adding any enrichment media.

Proximate Analysis of *Artemia*

Stored *Artemia* were taken and dried to estimate the protein lipids and carbohydrates. Stored *Artemia* were taken and homogenized by morter and pestle. From that, 1 g of sampl was taken for each estimation. The estimation of protein was done by Lowry's method (Lowry et al. 1951). The estimation of carbohydrates was done by Anthrone method (Yemm and Willis 1954). The estimation of lipid was done by Phenol and Chloroform method (Morton et al. 1991).

Statistical Analysis

The collected data were tested thrice. Mean and SD values were done by SPSS (v16) software for windows.

Results

The concentration of crude protein, lipid and carbohydrates from *Artemia* enriched with different algae (*C. vulgaris*, *N. granulata* and *S. maxima*) are shown in Table 1. Though, four different concentrations of algae used, 1L concentration showed very good results among the all. Protein, lipid and carbohydrate concentration of each algae is compared. The protein concentration of *Artemia* enriched with *S. maxima* (spirulina) was found to the (0.55 ± 0.6, 0.77 ± 0.3, 0.86 ± 0.6 and 1.8 ± 0.7mg/g) (Table 1). Among the three algae used for enrichment, *S. maxima* exhibit significant enrichment result followed by *C. vulgaris* and *N. granulata*.

In Table 1, it is shown that the lipid content of *Artemia* enriched *C. vulgaris* and *N. granulata* is significantly higher than the enrichment with *Spirulina maxima.* However, *Artemia* enriched with *N. granulata* (2.08±0.42 (mg/g)) for 24 hrs showed the highest lipid concentration than the other enrichment process. The algae used for enrichment contain very low quantity of carbohydrate. It is reflected in the result of the recent study (Table 2). The enrichment process is time dependent. *Artemia*

enriched with algae showed bit increase in carbohydrate concentration when compared to enrichment by A1 DHA (Table 2). As fatty acids and protein are taken up by *Artemia*, their fatty acid profiles and protein concentrations change according to the duration of the enrichment. It is found that the length of an enrichment process should also be considered when preparing *Artemia* nauplii as food for the larvae of ornamental fish.

Table 1 Biochemical composition of *A. salina* enriched by natural media (algae)

Natural enrichment media (1L/2L)	Control	*Chlorella vulgaris*	*Nannochloropsis granulate*	*Spirulina maxima*
Hours	Concentration of protein (mg/g)			
6	0.06±0.02	0.12±0.09	0.16±0.02	0.55±0.60
12	0.065±0.01	0.19±0.05	0.30±0.03	0.77±0.30
18	0.07±0.03	0.39±0.03	0.39±0.03	0.86±0.82
24	0.067±0.1	0.55±0.15	0.59±0.05	1.80±0.70
Hours	Concentration of lipid (mg/g)			
6	0.06±0.01	0.38±0.02	0.38±0.17	0.12±0.02
12	0.06±0.04	0.42±0.06	0.52±0.05	0.19±0.06
18	0.063±0.03	0.85±0.20	1.20±0.09	0.35±0.01
24	0.07±0.01	0.95±0.18	2.08±0.42	0.45±0.10
Hours	Concentration of carbohydrate (mg/g)			
6	0.03±0.05	0.05±0.01	0.18±0.01	0.11±0.01
12	0.12±0.08	0.08±0.01	0.18±0.06	0.20±0.02
18	0.16±0.05	0.18±0.08	0.27±0.03	0.27±0.08
24	0.17±0.09	0.25±0.12	0.38±0.07	0.37±0.02

The concentration of crude protein, lipid and carbohydrate from *Artemia* enriched with A1 DHA SELCO media are shown in Table 2. From the results, it is found that the concentration and enrichment duration played a vital role in a process of enrichment. All the 4 concentrations (0.2, 0.4, 0.8 and 1.0 ml /L) were compared with control. Twenty four hour and 1.0 ml/L enriched *Artemia* showed the best one.

The results obtained from this study was compared with the FAO micro algae nutrient standards (Table 3). From this study, lipid and protein content of *C. vulgaris* and *S. maxima* enriched *Artemia salina* showed prominent result which reflects the nutrient content of those two algae (Table 3).

Table 2 Biochemical composition of *A. Salina* enriched by commercial media (A1 DHA)

Concentration of enrichment media (ml /L)	C	0.2	0.4	0.6	0.8	1.0
Hours	Concentration of protein (mg/g)					
6	0.07±0.01	0.18±0.04	0.26±0.05	0.19±0.03	0.22±0.02	0.11±0.05
12	0.072±0.01	0.28±0.01	0.35±0.01	0.38±0.01	0.39±0.01	0.29±0.07
18	0.08±0.01	0.43±0.03	0.43±0.09	0.49±0.02	0.58±0.03	0.55±0.06
24	0.09±0.02	0.65±0.05	0.64±0.04	0.72±0.07	0.88±0.20	0.98±0.01
Hours	Concentration of lipid (mg/g)					
6	0.07±0.01	0.10±0.01	0.12±0.01	0.16±0.08	0.30±0.02	0.30±0.01
12	0.07±0.05	0.28±0.02	0.29±0.05	0.25±0.03	0.38±0.07	0.49±0.02
18	0.08±0.03	0.43±0.09	0.48±0.02	0.37±0.04	0.59±0.01	0.58±0.01
24	0.09±0.01	0.52±0.10	0.57±0.01	0.54±0.01	0.71±0.04	0.79±0.01
Hours	Concentration of carbohydrate (mg/g)					
6	0.07±0.01	0.15±0.01	0.19±0.02	0.16±0.07	0.21±0.01	0.30±0.04
12	0.08±0.01	0.23±0.05	0.30±0.01	0.27±0.06	0.33±0.03	0.39±0.06
18	0.08±0.03	0.44±0.02	0.42±0.01	0.38±0.02	0.44±0.01	0.49±0.03
24	0.08±0.02	0.49±0.06	0.50±0.02	0.59±0.01	0.62±0.02	0.78±0.01

Table 3 Chemical Composition of Algae Expressed on A Dry Matter Basis (%) (FAO 1997)

Name of the strain	Protein	Carbohydrates	Lipids	Nucleic acid
Scenedesmus obliquus	50-56	10-17	12-14	3-6
Scenedesmus quadricauda	47	-	1.9	-
Scenedesmus dimorphus	8-18	21-52	16-40	-
Chlamydomonas rheinhardii	48	17	21	-
Chlorella vulgaris	51-58	12-17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	-
Spirogyra sp.	6-20	33-64	11-21	-
Dunaliella bioculata	49	4	8	-
Dunaliella salina	57	32	6	-
Euglena gracilis	39-61	14-18	14-20	-
Prymnesium parvum	28-45	25-33	22-38	1-2
Tetraselmis maculata	52	15	3	-
Porphyridium cruentum	28-39	40-57	9-14	-
Spirulina platensis	46-63	8-14	4--9	2-5
Spirulina maxima	60-71	13-16	6-7	3-4.5
Synechococcus sp.	63	15	11	5
Anabaena cylindrica	43-56	25-30	4-7	-

Discussion

Microalgae are utilised in aquaculture as live feed for bivalve larvae and spat (Tokusoglu and Unal 2003), early larvae of crustaceans and marine fish (Zou et al. 2000). Our results demonstrate that there is variation in the growth rate as well as in the EPA and DHA content of *Artemia* enriched with different algal species. Marine algae are the major producers of x3-PUFAs

(EPA and DHA), the fresh water algae predominantly have saturated or monosaturated fatty acids. When algae are used as food in the marine chain, it is important to know the lipid and fatty acid composition of the actual algal species used in order to know the nutrient requirement.

The rearing of the majority of marine fish larvae requires the use of live food. The rotifer *Brachionus plicatilis* and the anostracan *Artemia* are the two organisms mostextensively used for this purpose. Brine shrimps are typically filter-feeders that consume organic detritus, microscopic algae and bacteria. Blooms of microscopic algae are favorite habitats of *Artemia*. Large populations of *Artemia* develop when they feed on the algae and heterotrophic bacteria that are produced by these blooms. Brine shrimp populations exhibited significant growth when the cultures are fed with algae, rice bran (Lavens and Sorgeloos 2000), soybean meal or whey powder.

Lipids are major sources of metabolic energy throughout the embryonic developmental stages in fish (Naceur et al. 2012). The amount of lipids as well as the lipid classes catabolized vary among species and other sources of energy, such as carbohydrates and proteins, are also utilized. The amount of lipids in eggs generally correlates with the time interval between spawning and egg hatching (Naceur et al. 2012). Several studies have shown that the qualitative and quantitative lipid content of the feed as well as the feeding regime during gonadogenesis have a major impact on spawning and egg quality (Naceur et al. 2012). Similar results was reported by Lakshmanasenthil et al., (2012) in the same field of study and he was reported that the nutrient content of *N. salina*, *C. salina* and *S. subsalsa* were transferred to the enriched *Artemia*.

Artemia are utilized in aquaculture as live feeds for the larval and early juvenile stages of fish and shell fishes. A1 DHA SELCO medium used for enhancing the nutritional value of *Artemia*, is not economically feasible. In the present study, the maximum protein content of 0.33±0.07 to 1.05 mg/g (Table 1) was observed upon enrichment with *S. maxima* (spirulina). These results reflected the nutrient content of spirulina. *Spirulina maxima* contains about 60% (51–71%) protein (Babadzhanov et al. 2004). It is a complete protein containing essential amino acids, though with reduced amounts of methionine, cysteine and lysine when compared to the proteins of meat, eggs and milk. However, It is superior to typical plant protein, such as that from legumes (Babadzhanov et al. 2004). Overall, while spirulina is often marketed as an excellent source of protein, per gram spirulina is approximately 30 times more expensive than protein.

Replacement of A1 DHA SELCO media by using micro algae as a natural enrichment media in *Artemia* culture system possesses lot of advantages. Micro algae are enriched with nutrient (as sole component or as food additive to basic nutrients) essential for coloring the flesh of the fish and for other biological activities. They are primarily photoautotrophic and few species are heterotrophic in nature. Unlike terrestrial plants, which require fertile land or irrigation, microalgae can grow in a wide range of habitats (Tamaru et al. 2004).

In the present study, the maximum lipid content was obtained with *C. vulgaris and N. granulata* 0.97±0.72 and 1.31±0.61 respectively at 24 hrs enrichment period. In a early study, *Chlorella sp.* has been reported to yield EPA at 39.9% of total lipids (Zou et al. 2000). *Chlorella sp.* is considered as a protein source also (Zou et al. 2000). *C. vulgaris* considered as a promising algae for industrial applications because of its ability to accumulate high levels of polyunsaturated fatty acids. It is mainly used as an energy-rich food source for fish larvae and rotifers (Lavens and Sorgeloos 2000). Moreover, fatty acid play a major role in growth of many marine organisms and most of the microalgal species have moderate to high percentages (7–34%) of EPA. Prymnesiophytes (*Pavlova* sp. and *Isochrysis* sp.) and Cryptomonads are relatively rich in DHA (0.2–11%) while eustigmatophytes, *Nannochloropsis* and diatoms have the highest percentages of amino acid (0–4%) (Walne 1970). The present study reports that enrichment process is time depend, the maximum protein, carbohydrate and lipid content was obtained at 24hrs, it is suggested that more than 24 hrs of enrichment is suitable for harvesting the *Artemia*.

The levels of soluble carbohydrates were found to be higher in nauplii than in adults and lower in all life stages. Carbohydrates is not a significant factor for *Artemia* at all stages of its life. The nutrient levels were significantly reduced in 24 hrs post hatch and unfed adult *Artemia*. It is therefore, essential that *Artemia* be utilized as quickly as possible in order to avoid nutrient degradation.

Conclusion

In this study, the proximate composition analyses of crude protein, lipid and carbohydrate showed a linear increase in concentration in the *Artemia* biomass. The increase was directly proportional to the hours of enrichment and the concentration of enrichment media. The results showed the nutrients such as protein, carbohydrate and lipid of *C. vulgaris, N. granulata* and *S. maxima* significantly enhanced the above said nutrient concentration in *Artemia* by the enrichment process. As *C. vulgaris* and *N. granulata* are high in lipid content by the enriched *Artemia* also contained high lipids. *Spirulina maxima* enriched *Artemia*

contains high protein. But *N. granulata* showed the good result in protein also. A1 DHA SELCO also showed very good but the expense is very high compare to algae. From this study, we can use algae (*C. vulgaris, N. granulata* and *S. maxima*) as an enrichment media instead of commercial A1 DHA SELCO media, which give the same nutrition level.

Acknowledgement

The authors are thankful to the authorities of CMS college of Science and Commerce and Dr. N.G.P. College of Arts and Science for their facilities.

References

Babadzhanov AS, Abdusamatova N, Yusupova FM, Faizullaeva N, Mezhlumyan LG, Malikova M (2004) Chemical composition of spirulina platensis cultivation in Uzbekistan. Chem Nat Comp 40 (3): 276-279.

Espinosa EP, Allam P (2006) Comparative Growth and Survival of Juvenile Hard Clams, Mercenaria mercenaria, Fed Commercially Available Diets. Zoo Biology 25:513–525.

FAO (1997) Renewable biological systems for alternative sustainable energy production. FAO Agricultural Services Bulletin. Chapter 6. Version 128.

Hanhua H, Kunsan G (2003) Optimization of growth and fatty acid composition of a unicellular marine picoplankton, Nannochloropsis sp., with enriched carbon sources. Biotechnol Lett 25: 421-425.

Hoff FH, Snell TW (2008) Plankton Culture Manual. Florida Aquafarms, Inc., Dade City, Florida. 186 pp.

Lakshmanasenthil, S., Marudhupandi, T., Ajithkumar, T.T., Nandhinidevi, K., Balasubramanian, T (2012) Exigent of micro algae for the enrichment of *Artemia salina*. Journal of aquaculture feed science and nutrition 4(2): 34-38.

Lavens P, Sorgeloos P (2000) The history, present, status and prospects of the availability of *Artemia* cysts for aquaculture. Aquaculture 181: 397-403.

Lowry OH, Rosenbrough N, Fair AC, Randall RJ (1951) Protein measurements with folin phenol reagent. J Biol Chem 193:265-275.

Morton JM, Kirkpatricka RL, Smith P (1991) Comments on estimating total body lipids

from measures of lean mass. Condor 93: 463-465.

Naceur HB, Jenhani FBR, Romdhane MS (2012) Biometrical study of Artemia spp. (Anostraca: Artemiidae) cysts harvested from different salt lakes in Tunisia. North-Western Journal of Zoology 8 (2): 308-314.

Rocha MS, Garcia EC, Henriques HF (2003) Growth aspects of the marine microalga Nannochloropsis gaditana. Biomol Eng 20: 237-242.

Sorgeloos P, Dhert P, Candreva P (2001) Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture 200: 147–159.

Tamaru CS, Pang L, Ako H (2004) Ornamental fish feeds - Part 1. Effect of different maturation diets on the spawning of armored catfish, *Corydoras aeneus*. Int Aquafeed 7(4):33-35.

Teresita DNJ, Montiel M, Leticia G, Canché R (2005) Biomass production and nutritional value of Artemia sp. (Anostraca: Artemiidae) in Campeche, México Rev Biol Trop (Int. J. Trop. Biol. ISSN-0034-7744) 53 (3-4): 447-454.

Tokusoglu O, Ünal MK (2003) Biomass Nutrient Profiles of Three Microalgae: Spirulina platensis, Chlorella vulgaris, and Isochrisis galbana. Journal of Food Science 68 (4).
Vishwanath P, Torsten K, Elisabeth O, Gjermund V, Hans RG (2007) Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacult Int 15:1–9.

Walne PR (1970) Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria and Mytilis. Fish Invest 26: 162

Watanabe T, Arakawa T, Kitajima T, Fujita S (1984) Effect of nutritional quality of broodstock diets on reproduction of red seabream. Nippon Suisan Gakkaishi 59, 1023-1028.

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57: 508–514.

Zou N, Zhang C, Cohen ZVI, Amos RI (2000) Production of cell mass and eicosapentaenoic acid (EPA) in ultrahigh cell density cultures of Nannochloropsis sp. (Eustigmatophyceae). Eur. J. Phycol. 35: 127±133.