Nutritional Value of a Heterotrichous Ciliate, *Fabrea salina* with Emphasis on Its Fatty Acid Profile

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ABSTRACT : *Fabrea salina* is a hypersaline ciliate having importance as a live food source for juvenile stages of aquatic animals including smaller invertebrates. The analysis of this ciliate for proximate and biochemical composition was carried out. The moisture, protein, fat, carbohydrate and ash content of *F. salina* from natural sources were 86.66±0.380, 56.66±0.494%, 36.66±0.614%, 1±0.073% and 4±0.182%, respectively. Gas chromatographic analysis (percent area below the curve) revealed that the presence of oleic acid was higher over other fatty acids in both natural and cultured *F. salina*. The absolute content of oleic acid was higher in natural (18.91% area) than in the cultured (10.74% area) *F. salina*. Linoleic and linolenic acids were also among major fatty acids with the percentage area of 16.29 and 14.58, respectively. The number of fatty acids in cultured *Fabrea* was less as compared to the natural ones and the oleic acid was followed by palmitic acid, palmitoleic acid, linoleic acid and stearic acid. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 7 : 995-999)***

Key Words : Ciliate, *Fabrea salina*, Proximate Composition, Fatty Acid Profile

INTRODUCTION

The commercial production of fish and shellfish species increases every year due to the advancement in culture techniques which necessitates the identification of new sources of food organisms and their mass cultivation. Very few studies have been carried out on the nutritional quality of live feeds used in hatcheries for seed production of aquaculture species. Indeed zooplankton are a valuable source of protein, amino acids, various kinds of lipids, fatty acids, minerals and enzymes to the juvenile predators of fish and shellfish species (Watanabe et al., 1983; Millamena et al., 1990). Fujita (1979) has reported the species specific live feed for different early developmental stages of some fish species. Lipid profile has been studied extensively in poultry too (Pal et al., 2000; Sinky et al., 2003).

Ciliates are most specialized protozoa and are found in a variety of habitats. They form an important component of estuarine and coastal marine ecosystems as both consumers of bacteria and prey for metazoan grazers (Stoecker and Cappuzzu, 1990; Sanders and Wickham, 1993). The biochemical composition of ciliates is of prime importance in understanding the nutritional benefits for the growth and reproduction that metazoans acquire by consuming these animals. *Fabrea salina* is a much suitable candidate species as a live food for mariculture purposes. Natural propagation of *Fabrea salina* has been reported from several diverse environments such as salt marshes, hypersaline lakes and solar salterns (Ellis, 1937; Post et al., 1983; Yufera, 1985; Pandey and Yeragi, 1998). The average cell size of *Fabreasalina* is 250-350 μm, making it appropriate as larval feed. Sometimes it also grows to 600 μm size (Pandey, 2001). The biochemical composition of fresh water ciliates *viz. Tetrahymena* or *Paramecium* (Wood, 1988), are well documented while very less information is available in marine ciliates. The present study was carried out to determine the nutritional quality of *Fabrea salina* in terms of its biochemical composition with special reference to fatty acid profile in naturally available as well as cultured *Fabrea*.

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MATERIALS AND METHODS

Collection of sample

Fabrea salina was harvested from the high salinity reservoirs of Mira Road salt pan of Mumbai coast during the month of May, the peak of salt production period when this ciliate is found in greater abundance. A plankton net made up of bolting silk having 40 μm mesh size was used for the purpose. The sample was concentrated and made ready for biochemical studies. The ciliate was also cultured in the institute at 65‰ salinity and at 28±1°C temperature, by using egg-custard at 10 mg/l as food source. The various ingredients of egg-custard which are shown in the Table 1 were mixed thoroughly and cooked in a water bath for 20 minutes to make it in semi-solid form. Before feeding, the custard was passed through a bolting silk cloth of 20 μm mesh size to make ingestible particle size.

Proximate analysis

The *Fabrea salina* was analysed as per AOAC (1990) for protein (micro Kjeldahl method), fat (by ether extraction), moisture (by weight difference after drying for 24 h at 105°C) and the ash content (using muffle furnace).

Table 1. Ingredients and their quantity used in making egg-custard

Ingredients	Quantity
Hen's egg	1 (no.)
Milk powder	15.0 g
Corn flour (finely ground)	10.0 g
Prawn flour (finely ground)	1.5 g
Agar-agar	2.0 g
Yeast	1.0 g
Vitamin mix	1.0 g
Cod-liver oil	1 ml
Fresh water	60 ml

Fat esterification and gas chromatographic analysis

Fatty acid methyl ester (FAME) of the extracted fat was prepared by reacting with methanolic sodium hydroxide, boron trifluoride, heptane and sodium chloride by the Method 969.33 of AOAC (1990). An aliquot of the upper heptane solution was removed and dried with anhydrous Na_2SO_4. The dried matter was diluted to a concentration of 7% for gas chromatography, using the model Chemito 8610 with diethylene glycol succinate (DEGS) column. The temperature was adjusted at 60°C for 4 minutes, then raised to 200°C at a rate of 5°C per minute. Injector and Flame Ionization Detector (FID) temperatures were 280 and 300°C, respectively. The temperature of oven was maintained at 220°C. The sample size was 0.2 µl. Identification of fatty acids was carried out on the basis of retention times of the standard mixture of fatty acids.

RESULTS

Proximate composition

The analysis revealed that *Fabrea salina* contained 56.66±0.494% protein, 36.66±0.614% fat and 1±0.073% carbohydrate. The ash content was 4.0±0.182% while moisture content was recorded as 86.66±0.380%.

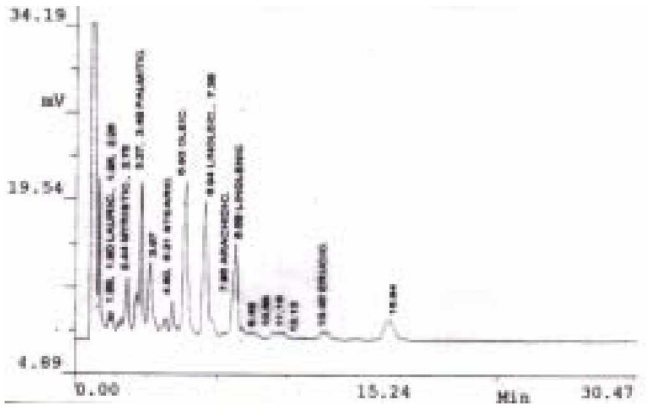

Figure 1. Fatty acid profile of naturally available *Fabrea salina*.

Fatty acid profile

During culture, a maximum density of 82 *Fabrea salina*/ml was obtained on 6th day and thereafter the population declined gradually to 44/ml on 7th day. The results of analysis of fatty acid profile of naturally available *Fabrea salina* and that of cultured on egg-custard are shown in the Table 2 and 3, respectively while the chromatogram for the same is given in Figure 1 and 2. Oleic acid (18:1n-9) with percentage area of 18.91 was found to be the most abundant fatty acid in *Fabrea salina* harvested from the natural environment. This is followed, in decreasing order of their abundance as linoleic acid, linolenic acid, palmitic acid, caprylic acid, stearic acid, lauric acid, marystic acid,

Table 2. Fatty acids in naturally available *Fabrea salina*

Fatty acid	Descriptive name	Systematic name	Area (%)	Retention time (minutes)
Saturated				
8:0	Caprylic	Octanoic	3.89	1.24
12:0	Lauric	Dodecanoic	0.79	1.80
14:0	Marystic	Tetradecanoic	0.79	2.44
16:0	Palmitic	Hexadecanoic	11.29	3.49
18:0	Stearic	Octadecanoic	3.28	5.21
20:0	Arachidic	Eicosanoic	0.19	7.95
Unsaturated				
18:1n-9	Oleic	9-Octadecenoic	18.91	5.92
18:2n-6	Linoleic	9,12-Octadecadienoic	16.29	6.94
18:3n-3	Linolenic	9,12,15-Octadecatrienoic	14.58	8.59
22:1n	Erucic	13-Docosenoic	0.13	13.45

Table 3. Fatty acids in cultured *Fabrea salina*

Fatty acid	Descriptive name	Systematic name	Area (%)	Retention time (minutes)
Saturated				
16:0	Palmitic	Hexadecanoic	6.78	2.62
18:0	Stearic	Octadecanoic	2.04	4.04
Unsaturated				
16:1n-7	Palmitoleic	9-Hexadecenoic	4.04	2.98
18:1n-9	Oleic	9-Octadecenoic	10.74	4.57
18:2n-6	Linoleic	9,12-Octadecadienoic	2.50	5.46

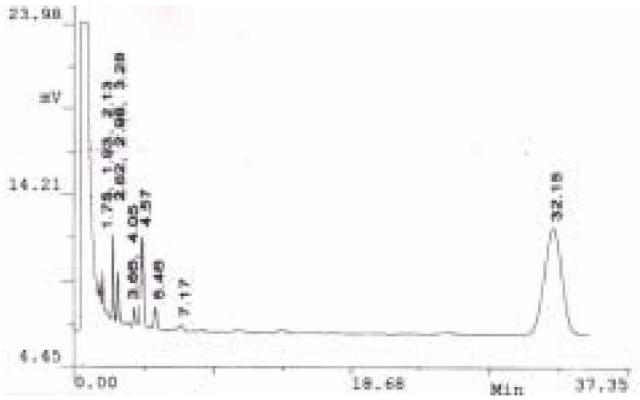

Figure 2. Fatty acid profile of cultured *Fabrea salina*.

arachidic acid and erucic acid. In addition to oleic acid, another but least abundant monounsaturated fatty acid (MUFA) recorded was erucic acid (22:1n) with an area of 0.13% and the retention time of 13.45 minutes.

Fabrea salina contains a good amount of two polyunsaturated fatty acids (PUFA). These are linoleic acid (18:2n-6) and linolenic acid (18:3n-3) having the area of 16.29 and 14.58%, respectively. Out of six saturated fatty acids, palmitic acid (16:0) was the most abundant with an area of 11.29% while the least abundant was arachidic acid (20:0) with 0.19% area.

The number of fatty acids in cultured *Fabrea salina* was noted to be less than that in naturally available animals. Nevertheless, the majority of these fatty acids are unsaturated ones. The oleic acid (10.74%) was the most abundant one, followed in decreasing order by palmitic acid, palmitoleic acid, linoleic acid and stearic acid. Palmitoleic acid (16:1n-7) which is a MUFA, has an area and retention time of 4.04% and 2.98 minutes, respectively.

DISCUSSION

Most of the fishes and crustaceans depend on zooplankton at some stage of their life cycle and some thrive even exclusively on these minute animals throughout their life. Therefore, success in culturing planktivorous fry relies principally upon the zooplankton, their nutrient composition and density (Fernando, 1994).

The higher protein content (56.66±0.494%) in *Fabrea salina* indicates that macronutrient requirements can be satisfied for its most predators and higher lipid content allows it to withstand extreme environmental conditions such as hypersalinity and higher temperature prevailing in its natural habitat. The biochemical composition of natural zooplankton can vary seasonally (Khan and Qayyum, 1971) and also it can be affected by the level of nutrients in water (Vijverberg and Frank, 1976). The microalga *Dunaliella* is the principal diet for *Fabrea salina* during the periods of its great abundance in summer months (Pandey, 2001). Its capability to survive in hypersaline environments is associated with its unique ability to produce very high concentrations of intracellular glycerol. It is the only eukaryotic microorganism known to thrive in highly saline water bodies such as the Dead Sea in Israel (Volcani, 1944) and the Great Salt Lake, Utah (Post, 1977). *Fabrea salina* is not equipped to synthesize carbohydrates and hence contains very less quantity of these biomolecules. Moreover, carbohydrate and protein are interconvertible and when energy requirements for the growth and reproduction are satisfied, these moities are converted into fat. Once fat is formed, it is only stored or oxidized to release energy but not normally reconverted into proteins or carbohydrates.

It has been established that certain long-chain polyunsaturated fatty acids (PUFA) are essential to many organisms. Considerable research has been focused on the availability and the role of PUFA in larval rearing in hatcheries (Watanabe et al., 1983) and in the natural environment (Sergent and Whittle, 1981). However, there is remarkably little experimental evidence supporting the superiority of diets containing more PUFA for herbivores. In contrast to plant fatty acids, the fatty acids of animal origin are usually simple, straight chained and may contain up to six double bonds. One remarkable feature of animal fat is their limited ability to form characteristic PUFA. Therefore, to a large extent they depend on external sources to satisfy their PUFA requirements. The fatty acid composition may change according to the seasonal succession of phytoplankton species (Jeffries, 1970). Harvey et al. (1997) have observed the rapid incorporation of dietary fatty acids by bacterivorous ciliates *Pleuronema* sp. and *Fabrea salina* grown on algal diet. The bacterial fatty acids, including branched, odd chain length and hydroxy-fatty acids were readily incorporated by *Pleuronema* sp., with the distribution of fatty acids in this ciliate showing strong resemblance to its prey. The presence of bacterial fatty acids in *Fabrea* which was grown on *Isochrysis galbana* was due to either reduced turnover of fatty acids for *Fabrea* as compared to *Pleuronema* or ingestion of bacteria in addition to the alga provided. In the present study, the presence of palmitoleic acid in cultured *Fabrea* and its absence in the natural one reveals the incorporation of this MUFA from egg-custard, the diet used to grow the ciliate. Further the variation in the number and amount of constituent fatty acids in these two sources is also due to the availability of food of varied quality in these systems. *Fabrea salina* is known to grow well on bacteria, yeasts and marine algae and shows differential growth responses to varying diet (Repak, 1986).

The presence of saturated fatty acids 14:0, 16:0 and 18:0 in the natural sample of *Fabrea salina* in the present study has also been recorded by Harvey et al. (1997) who grew this ciliate on *Isochrysis galbana*. Similarly, as in the

present study, they also observed oleic acid in smaller amount. Further in contrast to the observations of Harvey et al. (1997) which reveals the total lack of PUFA in *Fabrea salina* (feeding on *I. galbana* in which PUFA generally accounts for 22% of total fatty acids). two PUFAs. linoleic acid and linolenic acid in natural sample and one. linoleic acid in the cultured sample have been recorded. In several ways the essential fatty acids of *Fabrea* are of great significance to the organisms at higher trophic levels in food chain. For example, EFAs in high concentration along with lipids constitute the structural elements of the cells and tissues.

In the microalga *Dunaliella*, about 30-50% of the cellular material is lipids (Javor. 1989). The prevalence of C_{16} and C_{18} saturated and unsaturated fatty acids. with the dominance of $C_{16:0}$ is almost similar to the distribution of these fatty acids in *Fabrea salina* in the present study. This alga synthesizes trace amounts of C_{20} and C_{22}. C_{17} and highly branched ($C_{20:6}$) fatty acids.

Ciliates are important grazers of phytoplankton (Capriulo and Carpenter, 1983) and bacteria (Sherr et al., 1986) and thus provide a link to channel particulate and dissolved organic matter to metazoans (Azam et al.. 1983). In estuarine environment. bacterivorous ciliates are often as important as flagellates (Sherr et al., 1987) and provide the most direct connection between bacteria and copepods. As reviewed by Pierce and Turner (1992), the existence of a trophic link between copepods and ciliates has been well established and the presence of bacterial fatty acids in ciliates may prove useful as tracers of ciliate and bacterial ingestion by metazoans. *Acartia tonsa*. the common estuarine copepod. is shown to feed upon small bacterivorous ciliates (Sherr et al.. 1987; Ederington et al., 1995). Recent studies with *Acartia tonsa* feeding on *Pleuronema* sp. have shown that bacterial fatty acids and tetrahymenol can be directly transferred from bacteria to copepods. with ciliates as the intermediary (Ederington et al., 1995).

The biochemical analysis of *Fabrea salina* shows its potentiality as live food organism for mariculture purposes to rear the young stages of finfish and shellfish species. Its ability to form dormant cysts during unfavourable environmental conditions and hatching of these cysts under controlled conditions increase its practical application in larviculture practices. It has been successfully cultured on preliminary as well as mass scale by offering different kinds of food source such as dry yeast. *Dunaliella* and egg-custard (Pandey and Yeragi. 2001). Although young stages of fish and shellfish can also be grown on specially formulated diets richer in protein content than that of live foods. the importance of later is always superior in this respect (Pandey and Yeragi. 2000). Moreover. there is immense scope to manipulate its nutritional quality as per the larval needs by means of food offered to it. In fact essential nutrients. pigments *etc.* may be manipulated and bioencapsulated into *Fabrea* for prophylactics and therapeutic purposes to cope with nutritional deficiencies and diseases in fish and crustacean larvae.

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