EFFECTS OF A MONO-ALGAL DIET AND STARVATION ON THE LIPID CLASSES OF THE DIGESTIVE GLAND OF THE SCALLOP *FLEXOPECTEN GLABER*

Khaoula TELAHIGUE[a*], Imen RABEH[a], Tarek HAJJI[a,b], Nabila GHAZALI[a], Wièm MASMOUDI[a,c], Mohamed Salah ROMDHANE[d] & M'hamed EL CAFSI[a]

[a]*:Université Tunis EL Manar, Faculté des Sciences de Tunis, Département de Biologie, Unité de recherche de Physiologie et d'écophysiologie des organismes aquatiques, Université Tunis El Manar, 2092 Tunis, Tunisie.*
[b]*: Institut supérieur de Biotechnologie de Sidi Thabet, Biotechpole Sidi Thabet 2020 Ariana, Tunisie*
[c]*: Institut supérieur de pêche et d'aquaculture de Bizerte, 7080 Rimel Bizerte, Tunisie.*
[d]*: Institut National Agronomique de Tunisie, 43 Av. Charles Nicolle 1082 Tunis, Tunisie.*

(Reçu le 18 Janvier 2013, accepté le 09 Mars 2013)

ABSTRACT: The effects of a mono-algal diet and the starvation trials on the content of the lipid classes and the fatty acid composition of the digestive gland of the scallop *Flexopecten glaber* were assessed in 2010. Results have shown that starvation induces the mobilization of triacylglycerol into phospholipids in the digestive gland of the scallop *F. glaber*. Also, food deprivation induced the depletion of energetic fatty acids (14:0, 16:0, 20:5n-3) and the selective retention of essential and structural-type fatty acids (20:4n-6, 22:6n-3, 22:2). A significant increase of the non methylene interrupted dienoic fatty acid (22:2) was noted in the triacylglycerol of specimens exposed to mono-specific-diet based on the microalga *Isochrysis galbana* clone Tahitian (T-Iso). This would be explained by the substitution to the 20:5n-3 through *de novo* synthesis of the 22:2.

Key words: Digestive gland, *Isochrysis galbana*, fatty acid, food deprivation, Pectinids.

RESUME : Les effets d'un régime alimentaire mono-spécifique ainsi que la privation alimentaire sur les catégories lipidiques et leur composition en acides gras ont été étudiés au niveau de la glande digestive du pétoncle glabre *Flexopecten glaber* en 2010. Les résultats ont montré que la privation alimentaire provoque la mobilisation des triacylglycérols vers les phospholipides dans la glande digestive du pétoncle glabre. En outre, la privation alimentaire induit l'épuisement des acides gras énergétiques (14:0, 16:0, 20:5n-3) et la rétention sélective des acides gras essentiels et structuraux (20:4 n-6, 22:6 n-3, 22:2). Une augmentation significative de l'acide gras non interrompu par un méthylène (22:2) a été notée dans le triacylglycérol des spécimens exposés à un régime trophique mono-spécifique basé sur la microalgue *Isochrysis galbana* Tahiti clone (T-Iso). Cela est vraisemblablement due à un phénomène de substitution du 20:5n-3 par la synthèse *de novo* du 22:2.

Mots clés : Glande digestive, *Isochrysis galbana*, acides gras, privation alimentaire, Pectinidés.

INTRODUCTION:

Bivalve molluscs living in coastal environments are adapted to resist to relatively long periods of nutritive stress [1]. Under a period of high energy demands or stressful conditions, bivalves' survival depends on their metabolic reserves [2, 3]. Amongst metabolites, lipids are strongly involved in nutritional and physiological processes by providing an efficient source of energy and essential fatty acids [4]. According to many authors, the digestive gland represents the primary lipids storage organ in scallops [5 - 7]. Under physiological stress or during the process of gametogenesis, a transfer of metabolites from the digestive gland to the gonad and other tissues was described in other scallop species [3, 6].

Numerous studies have reported the effects of diet variations on the lipid content and fatty acids composition of scallops [8 - 13]. Nevertheless, few authors have investigated the effects of diet variations on the composition of fatty acids in the digestive gland [14 - 16]. To the authors'

* Corresponding author, e-mail: k_telahigue@yahoo.fr

knowledge, no studies have focused on the starvation impact on lipids content of the digestive gland of scallops.

The present work was carried out in order to point out the fatty acid dynamics in lipid classes of the *F. glaber* digestive gland fed on monoalgal diet. It also permits us to assess the relative importance of different fatty acids and the strategy adopted by *F. glaber* to modulate its endogenous lipidic reserves to overcome the starvation trial.

MATERIAL AND METHODS:

Experimental design and animal sampling

A total of 46 wild adult *Flexopecten glaber* individuals of mean shell height 43±3 mm were collected by scuba diving from the Bizerte lagoon in the North East of Tunisia in the spring season (May 2010). Six specimens were sacrificed in the laboratory and considered as initial condition or "T0" (time zero) group.

The other 40 specimens were divided into two equal groups and placed in two tanks. Each tank contained 70 L of filtered and UV-treated seawater maintained at constant temperature (15°C) and salinity (35psu). A photoperiod of 12-h light/12-h dark was sustained over the duration of the experiment.

During 3 weeks, the first lot was exclusively fed on monoalgal diet based on the microalgae *Isochrysis galbana* clone Tahitian (T-Iso). A feeding rate of 2.10^9 cells $scallop^{-1}day^{-1}$ was adopted. This is comparable with levels considered in previous studies on Pacific lion's paw scallop *Nodipecten subnodosus* [17] and the doughboy scallop *Mimachlamys asperrima* [18]. The second group was maintained unfed. A gentile aeration was applied in both tanks to ensure an adequate supply of oxygen and to maintain algal cells in suspension during the experimentation in the nourished lot. Water was filtrated on a daily basis over the night and changed twice per week.

Total lipid extraction for fatty acid analysis

After a 21 days period of acclimation, scallops were sacrificed using liquid nitrogen. The digestive gland was removed, weighed and frozen at -30°C. Lipids were extracted according to the method of Folch [19] with the solvent mixture chloroform-methanol (2:1, v/v) containing 0.01% buthylated hydroxy toluene (BHT) as an antioxidant. After solvent evaporation with nitrogen, lipids were transferred with solvent mixture into pre-weighed vial. The solvent mixture was over-evaporated under nitrogen and the extract was dried in a vacuum dessiccator. Lipids were re-dissolved in the solvent mixture and concentrated at 10mg/ml.

Lipid classes' separation

Lipid classes were separated using thin-layer chromatography (TLC) with one dimensional double development as described by Olsen and Henderson [20]. Doses of 500 µl of lipid extracts were separated on plates (20x20 cm, silica gel 60, Merck, Germany) using hexane: diethyl ether: glacial acetic acid (80: 20: 2, v/v) as developing solvent for neutral lipids and methyl acetate: isopropanol: chloroform: methanol: 0.25 % KCl (25: 25: 25: 10: 9, v/v) as developing solvent for polar fraction. Lipid classes were visualized under UV light after spraying with 0.1% 2'-7' dichloro-fluorescein in absolute methanol.

Analysis of fatty acids

After evaporation to dryness, lipid extracts and fractions were trans-esterified according to Cecchi method [21]. Methyl nonadecanoate 19:0 (Sigma), which didn't exist in our samples, was added as internal standard. Separation of FAMEs was carried out on a HP 6890 gas chromatograph with a split/splitless injector equipped with a flame ionization detector at 275°C, and a 30 m HP Innowax capillary column with an internal diameter of 250 µm and a film thickness of 0.25 µm. Injector temperature was held at 250°C. The oven was programmed to rise from 50 to 180°C at a rate of 4°C /min, from 180 to 220°C at 1.33°C/min and to stabilize at 220°C for 7 min. Nitrogen was the carrier gas. Identification of FAMEs was based on the comparison of their retention times with those of a mixture of methyl esters (SUPELCO PUFA-3). Fatty acid peaks were integrated and analyzed using HP chemstation software.

Statistical analysis

To assess significant differences between means, data was analyzed using the software Statistica Version 6.0 according to the One Way Analysis of Variance method (ANOVA). In this respect, Duncan test was applied and differences were considered significant when $p<0.05$.

RESULTS:

Content of lipid classes

Figure 1 and 2 represent the concentrations (mg/g wet weight^{-1}) of different lipid classes in the digestive gland of *F. glaber* under different diet conditions. The concentration of each lipid class was calculated from the fatty acid content of each fraction.

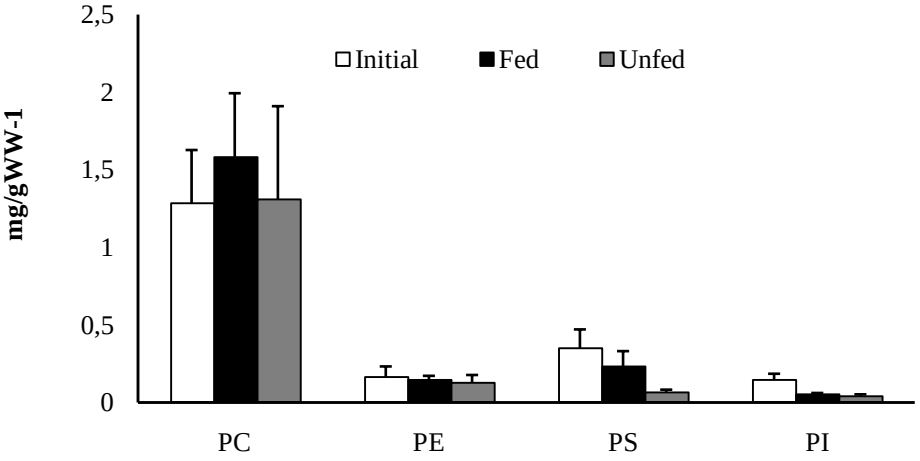

Figure 1: Effects of the mono-specific-algal diet and the starvation on the polar lipid classes' content of the digestive gland (expressed in mg/g of wet weight) of *Flexopecten glaber* (collected from the Bizerte lagoon in May 2010). PC (phosphatidylcholine), PE (phosphatidylethanolamine), PS (phosphatidylserine), PI (phosphatidylinositol).

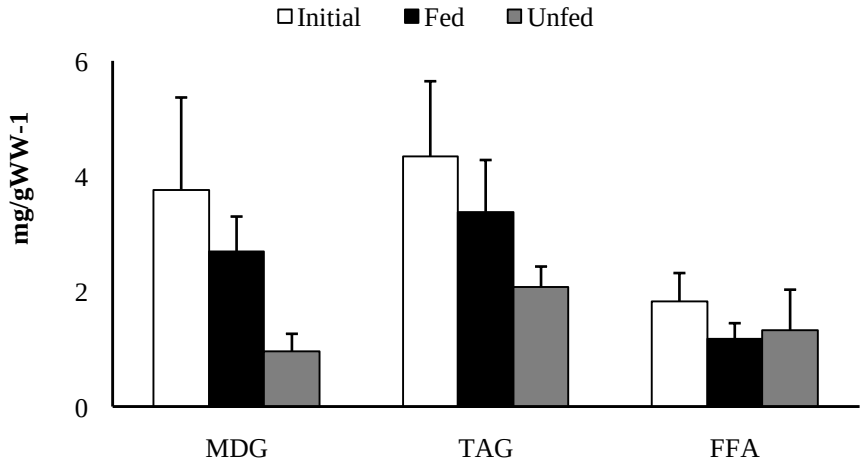

Figure 2: Effects of the mono-specific-algal diet and the starvation on the neutral lipid classes' content of the digestive gland (expressed in mg/g of wet weight) of *Flexopecten glaber* (collected from the Bizerte lagoon in May 2010). MDG (mono-diacylglycerols), TAG (triacylglycerol), FFA (free fatty acids).

Results have shown that phosphatidylcholine (PC) constituted the principal polar lipid fraction in the digestive gland of *F. glaber* at different diet conditions (Fig. 1). However, in the neutral fraction (Fig. 2), triacylglycerol (TAG) was the predominant lipid class. After 3 weeks of starvation, phosphatidylserine (PS) and phosphatidylinositol (PI) fractions remained at very low concentrations compared to the initial condition ($p<0.05$). Nevertheless, The PC and phosphatidylethanolamine (PE) fractions showed insignificant variations ($p>0.05$).

In the neutral fraction, the decreases of mono-diacylglycerols (MDG) and TAG concentrations were more important in the starved scallops than in those maintained under mono-specific-algal diet.

Results reported in Table 1 showed that the major lipid compound of the digestive gland of *F. glaber* was the neutral fraction with percentages exceeding 70% in the different diet conditions. Under monoalgal diet and food deprivation, neutral lipids decreased to 78.4 and 74.1% against an increase of polar lipids to 21.6 and 25.9%, respectively ($p<0.05$). The decrease in the neutral lipids fraction led to a decrease in the nonpolar/polar lipids ratio and the triacylglycerol/ phospholipids' ratios.

Table 1: Percentages of neutral and polar lipids in the digestive gland of *Flexopecten glaber* (collected from the Bizerte lagoon in May 2010) at initial, under monoalgal diet and starvation conditions. NL: Neutral lipids, PL: Polar lipids

	Initial	Monoalgal diet	Starved
Total neutral lipids	83.8±2.7	78.4±1.6	74.1±0.8
Total polar lipids	16.2±0.5	21.6±1.2	25.9±1.0
NL: PL ratio	5.2±0.7	3.6±1.1	2.9±1.4
TAG: Phospholipids ratio	2.3±0.8	1.7±0.5	1.4±0.3

Dietary fatty acid composition

The fatty acid profile of the microalgae *Isochrysis galbana* that was fed to scallops is given in Table 2. The diet exhibited a predominance of 18:0; 18:1; 18:2*n*-6, 18:3*n*-3; 18:4*n*- 3 and 22:6*n*-3. Total polyunsaturated fatty acids (PUFAs) accounted for 31.9% of total fatty acids and those from the *n*-3 series were 4 folds more abundant than those from the (*n-6*) series.

Table 2: Fatty acid composition of the microalgae *Isochrysis galbana* (T-Iso) brought in May 2010 from the National Institute of Sea Sciences and Technologies, Monastir center (Tunisia). Data are expressed as percentages of total fatty acids. SAFA: sum of saturated fatty acids, MUFA: sum of monounsaturated fatty acids, PUFA: sum of polyunsaturated fatty acids.

Fatty acid	Fatty acid in diet (% of total fatty acids, *n*=3)	
	Mean	S.D
14:0	6.83	2.40
15:0	1.12	0.53
16:0	21.18	4.90
17:0	0.76	0.10
18:0	27.66	10.49
20:0	0.25	0.07
22:0	0.11	0.02
∑SAFA	57.91	5.76
14:1	0.06	0.02
15:1	0.03	0.01
16:1	3.32	1.13
18:1	6.09	1.88
20:1*n*-9	0.42	0.13
20:1*n*-7	0.26	0.15
∑MUFA	10.18	2.94
16:2	0.20	0.02
16:3	0.27	0.08
16:4	0.32	0.10
18:2*n*-6	5.90	1.84
20:2*n*-6	0.16	0.15
20:3*n*-6	0.10	0.01
20:4*n*-6	0.15	0.06
18:3*n*-3	9.44	2.81
18:4*n*-3	7.32	2.19
20:3*n*-3	0.27	0.08

20:4*n*-3	1.26	0.35
20:5*n*-3	1.37	0.47
22:3*n*-3	0.86	0.33
22:5*n*-3	0.24	0.07
22:6*n*-3	4.05	0.92
∑PUFA	31.91	4.77
n-6	6.31	1.37
n-3	24.81	3.66
n-3/n-6	3.93	1.06
22:6/20:5	2.95	0.55

Fatty acids composition of lipid classes

TAG fatty acids composition in the digestive gland of the *F. glaber* exposed to different nutritional condition are reported in table 3.

TAG fraction of the control specimens (initial) is dominated by the saturated fatty acids (SAFAs) group, which represents 50% of the total fatty acids. Among this group, the major fatty acids are the 14:0, the 16:0 (16.9 and 19.7 %, respectively) and, at lesser level, the 18:0 (9.3%). The main unsaturated fatty acids, represented in terms of percentage, were as follows: monounsaturated (MUFA) 16:1n-7 (9.5%) and 20:1*n*-9 (5%); polyunsaturated 20:5*n*-3 (6.1%) and 22:6*n*-3 (9.8%). The most abundant (*n*-6) fatty acids were the 18:2*n*-6 (1.6%) and the 20:4*n*-6 (2.1%).

After 3 weeks of exposure to a monoalgal diet, the TAG fraction showed a decrease in the ∑SAFA and ∑MUFA against an increase in the ∑PUFA ($p<0.05$). These results were a consequence of the decrease in the 14:0 and the 16:0 percentages in the SAFA group and 16:1*n*-7 and 20:1*n*-9 in MUFAs. In this group, we have recorded an increase in the 16:1*n*-9.

In the PUFA group, the mono-specific-algal diet induced an increase of several polyunsaturated fatty acids: 16:3; 22:2; 18:3*n*-3; 20:4*n*-3; 22:6*n*-3 and 20:4*n*-6. However, we noted a diminution in the amount of 20:5*n*-3($p<0.05$).

Under fasting, the most severe declines occurred in the 14:0; 16:1*n*-7; 20:5*n*-3 and 22:6*n*-3. Nevertheless, starvation has led to a significant increase ($p<0.05$) in the 18:0 (from 9.3 to 32.8%) and the 18:2*n*-6 (from 1.6 to 5.6%). A decrease in *n*-3 PUFAs was noticed with a simultaneous increase in the *n*-6 ones. Therefore, leading to a decline in the *n*-3:*n*-6 ratio($p<0.05$).

Table 3: Fatty acid composition of the triacylglycerol fraction in the digestive gland of *Flexopecten glaber* (collected from the Bizerte lagoon in May 2010, n=6) under different diet conditions. Data are expressed as mean percentages of total fatty acids. SAFA: sum of saturated fatty acids, MUFA: sum of monounsaturated fatty acids, PUFA: sum of polyunsaturated fatty acids.

Fatty acid	Initial mean±SD	Monoalgal diet mean±SD	Starved mean±SD
14:0	16.9±0.1	4.2±0.9	3.3±1.2
15:0	0.5±0.0	1.8±0.5	1.2±0.2
16:0	19.7±3.8	10.8±1.7	16.3±2.8
17:0	2.0±0.7	1.5±0.2	5.1±1.1
18:0	9.3±1.7	11.4±2.0	32.8±4.0
20:0	1.4±0.3	1.0±0.2	0.3±0.0
22:0	0.1±0.0	0.2±0.0	0.2±0.0
∑SAFA	49.8±6.6	30.8±4.9	59.1±5.3
16:1*n*-9	0.4±0.0	5.7±1.2	3.5±1.2
16:1*n*-7	9.5±0.9	0.2±0.0	1.5±0.5

18:1*n*-9	3.0±0.5	3.2±0.9	3.7±1.2
18:1*n*-7	1.9±0.2	2.0±0.0	3.0±0.7
20:1*n*-9	5.0±1.3	2.2±0.5	4.1±0.9
20:1*n*-7	1.5±0.7	2.1±0.2	1.2±0.3
∑MUFA	21.4±2.9	15.4±1.7	16.9±2.2
16:2	0.4±0.0	1.2±0.3	1.1±0.1
16:3	1.6±0.4	11.2±0.9	0.7±0.1
16:4	0.7±0.0	0.4±0.0	1.4±0.2
21:5	0.3±0.0	0.7±0.1	0.4±0.0
22:2	0.8±0.1	6.0±1.3	0.4±0.1
18:3*n*-3	1.0±0.2	4.5±1.9	0.7±0.1
18:4*n*-3	0.0±0.0	0.4±0.1	1.2±0.0
20:3*n*-3	0.8±0.2	0.2±0.0	1.0±0.4
20:4*n*-3	0.5±0.1	5.1±1.9	4.6±1.5
20:5*n*-3	6.1±1.8	1.6±0.6	0.8±0.1
22:3*n*-3	0.7±0.3	2.1±0.7	1.1±0.2
22:5*n*-3	0.9±0.4	0.5±0.0	0.8±0.0
22:6*n*-3	9.8±1.6	12.8±2.9	1.4±0.3
18:2*n*-6	1.6±0.5	1.0±0.1	**5.6±1.9**
20:2*n*-6	0.6±0.0	1.4±0.4	0.9±0.3
20:3*n*-6	0.7±0.1	0.6±0.1	0.6±0.0
20:4*n*-6	2.1±0.4	3.8±0.6	1.4±0.1
∑PUFA	28.8±3.4	53.7±5.8	24.0±3.1
∑*n*-3	19.9±3.8	27.4±3.9	11.6±1.2
∑*n*-6	5.0±1.3	6.8±1.3	8.5±2.9
n-3/*n*-6	4±0.5	4.0±1.9	1.4±0.2
22 :6/20 :5	1.6±0.3	8.1±2.1	1.8±0.5

The composition of the phospholipids' fatty acid in the digestive gland of the control specimens (Table 4) was similar to that of the TAG fraction. Nevertheless, the following differences are noticed: The phospholipid fraction contained more PUFA (40.9%) but less saturated and monounsaturated fatty acids (43.2 and 15.9% respectively); the major saturated fatty acid is the C16:0. Monoalgal diet and starvation led to a slight decrease of the 16:0 against a significant increase of the 18:0 and consequently an elevation of the ∑SAFA.

Among the MUFA group, starvation induced an elevation of the *n*-9 series fatty acids (mainly the 18:1*n*-9 and 20:1*n*-9). Under mono-specific-diet, the 16:1*n*-9 percentage showed an increase, nevertheless the 20:1*n*-7 percentage decreased sharply ($p<0.05$).

In PUFA group of nourished scallops, the percentages of the 18:4*n*-3, 22:2, 20:4n-3 and 20:2*n*-6 increased while the 18:3*n*-3, 20:5*n*-3, 22:6*n*-3 and 20:4*n*-6 decreased ($p<0.05$). After 3 weeks of food deprivation, we recorded an increase in the percentages of the following fatty acids: 22:2, 20:4*n*-3, 22:6*n*-3, 20:2*n*-6 and 20:4*n*-6 ($p<0.05$). On the other hand, a strong decline in the proportions of the 18:3*n*-3 (from 11.8 to 1.1%) and the 20:5*n*-3 (from 12.5 to 1.9%) was recorded ($p<0.05$). Consequently, the decline in the ∑PUFA and the *n*-3:*n*-6 ratios were more apparent in the polar fractions of starved scallops than in the fed specimens.

Table 4: Fatty acid composition of the phosphatidylcholine fraction in digestive gland of *Flexopecten glaber* (collected from the Bizerte lagoon in May 2010, n=6) under different diet conditions. Data are expressed as mean percentages of total fatty acids. SAFA: sum of saturated fatty acids, MUFA: sum of monounsaturated fatty acids, PUFA: sum of polyunsaturated fatty acids.

Fatty acids	Initial n=6	Monoalgal diet n=6	Starved n=6
14:0	6.8±1.7	10.0±0.9	3.2±0.4
15:0	0.1±0.0	0.6±0.1	0.4±0.1
16:0	27.2±3.5	22.3±1.6	22.0±1.8
17:0	1.5±0.4	1.8±0.4	2.7±0.9
18:0	5.8±1.3	14.2±1.5	17.5±1.9
20:0	1.7±0.4	0.5±0.1	0.3±0.0
22:0	0.1±0.0	0.5±0.0	0.2±0.0
∑SAFA	43.2±5.7	49.9±5.2	46.3±5.5
16:1*n*-9	0.2±0.0	5.7±1.9	0.5±0.1
16:1*n*-7	5.9±1.8	0.2±0.0	3.7±0.3
18:1*n*-9	3.5±1.1	2.8±0.5	8.4±2.1
18:1*n*-7	3.8±0.8	2.1±0.2	3.2±0.9
20:1*n*-9	0.9±0.1	1.6±0.4	3.6±1.1
20:1*n*-7	1.7±0.6	1.4±0.1	0.4±0.0
∑MUFA	15.9±3.2	13.7±1.4	19.9±2.8
16:2	0.4±0.0	0.2±0.0	0.6±0.1
16:3	0.6±0.1	1.6±0.5	1.5±0.2
16:4	0.3±0.0	0.5±0.1	0.4±0.0
21:5	0.8±0.1	0.7±0.2	0.4±0.1
22:2	1.0±0.2	2.2±0.7	2.8±0.6
18:3*n*-3	11.8±0.9	9.4±1.1	1.1±0.4
18:4*n*-3	0.1±0.0	1.0±0.1	0.5±0.0
20:3*n*-3	0.2±0.0	0.4±0.0	0.1±0.0
20:4*n*-3	0.1±0.0	3.2±0.8	6.4±1.8
20:5*n*-3	12.5±1.2	5.9±1.9	1.9±0.8
22:3*n*-3	0.9±0.1	1.0±0.2	1.2±0.5
22:5*n*-3	0.9±0.3	0.6±0.1	1.0±0.3
22:6*n*-3	7.5±1.8	5.1±1.8	8.8±1.9
18:2*n*-6	1.6±0.5	1.4±0.4	1.6±0.8
20:2*n*-6	0.5±0.1	1.4±0.1	1.1±0.4
20:3*n*-6	0.3±0.0	0.7±0.1	1.3±0.2
20:4*n*-6	1.6±0.3	0.9±0.2	2.9±0.8
∑PUFA	40.9±4.8	36.3±3.3	33.8±1.9
∑*n*-3	33.9±2.9	26.7±1.9	21.1±2.8
∑*n*-6	4.0±1.2	4.5±0.6	6.9±1.5
n-3/*n*-6	8.6±3.1	6.0±1.9	3.1±0.9
22 :6/20 :5	0.6±0.1	0.9±0.1	4.5±1.4

DISCUSSION:

Content of lipid classes

As revealed in many marine invertebrates including bivalves, TAG was the quantitatively most important lipid class in the digestive gland of *F. glaber*. This result is in accordance with the

findings of Napolitano and Ackman [5] in *Placopecten magellanicus;* Pazos[7] in *Pecten maximus* and Palacios [22] in *Nodipecten subnodosus.* The high amount of this neutral fraction is related to the digestive gland role as a storage organ. In fact, according to Giese [23] lipids are primarily stored as triacylglycerol (TAG) droplets in the digestive glands of marine bivalves.

The high content of triacylglycerols, found in wild *F. glaber* digestive gland, is most likely correlated with the food availability in the studied area. In fact, Dridi [24] recorded a peak of chlorophyll "a" in the lagoon of Bizerte during the spring season, which corresponds to our sampling period. Other authors have also attributed the high of triacylglycerol in the digestive gland of *P. maximus* and *P. magellanicus* to the high levels of chlorophyll "a" [16, 5].

During fasting, *F. glaber* showed decreasing levels of TAG and MDG, compared to those registered in control specimens. This strategy was adopted in order to sustain the phospholipid content at normal level in spite of the food deprivation. This finding was confirmed by the NL:PL ratio variations. In fact, the lower NL:PL and triacylglycerol:phospholipids ratios, suggest an increase in the mobilization of triacylglycerols into phospholipids. Same results were recorded in adult oyster *Crassostrea virginica* and in clams spat *Tapes philippinarum* [25, 26].This result is supported by the findings of Gallager [27]. These authors outlined a preferential catabolism of triacylglycerol in *Crassostrea virginica* and *Mercenaria mercenaria* larvae's under starvation.

In this study, after three weeks of food deprivation, the high percentage of lipid reserve, compared to the polar one, suggests that the other metabolites (carbohydrates and/or protein) were selectively metabolized at the beginning of the experiment. In bivalves, the time-course for mobilization of different substrates (proteins, lipids and carbohydrates) to produce energy during fasting depends on many parameters such as: species, development stage, organ and initial biochemical composition. Numerous authors have confirmed the bivalve preferences for carbohydrates compared to lipids as energetic substrates in dietary emergency [28, 29, 30].

Composition of the dietary fatty acids

The flagellate *I. galbana* has already been found to be an important component of scallop diets [31] and widely used in mariculture due to its high content of long chain polyunsaturated fatty acids (PUFAs) [32].

Lipid profile of the microalgae *I. galbana,* used in this study, showed that medium chain (C18) and the long chain (C22) fatty acids represented more than 60% of the total fatty acids. Among the PUFA group, the DHA (22:6*n*-3) constituted the major compound. These results are in agreement with those previously reported by Parrish [33].

Composition of the lipid classes fatty acids

The composition of the fatty acids of marine organisms is a combination of assimilated and de *novo* produced fatty acids [34]. Napolitano and Ackman [5] reported that fatty acid composition of the digestive gland of scallops is quite influenced by the composition of the fatty acids of the phytoplankton. In the present study, the high concentrations of 14:0, 16:1*n*-7, 20:5*n*-3 and 22:6*n*-3 in the digestive gland of the control specimen suggests that their planktonic diet is a combination of diatoms and dinoflagellates as already reported in other studies [35,36]. However, the DHA:EPA amount ranging between 0.6 and 1.6 could be explained by a dominance of dinoflagellates in their diet as suggested by Taylor and Savage [37].

When fed on mono-specific-algal diet, the digestive gland of *F. glaber* revealed high proportion of the 22:6*n*-3 (mainly in the TAG fraction). This high amount of DHA is probably derived from the microalgae diet based on *I. galbana*. This result is confirmed by Waldock and Holland [4] who demonstrated the limited or total incapability of bivalves to synthesize this fatty acid. The same

result was recorded for the 18:3*n*-3. The important amount of this fatty acid (mainly in the polar fraction of fed scallops) is derived from the diet which exhibits high amount of the 18:3*n*-3.
In the same condition, the presence of EPA in the PC and ARA in the TAG fractions, in relatively high proportions (this is despite their low percentages in the diet) is resulting from the endogenous lipid reserves of the scallop. This could be explained by the fact that bivalves have a limited capacity for de novo synthesis of long-chain PUFA ([38, 15]. Besides, Caers [39] revealed that high amounts of 20:5*n*-3 and 22:6*n*-3 in *Crassostrea gigas* fed on the microalgae *Dunaliella tertiolecta* is assumed to result from endogenous reserves that were built up prior to the initiation of the experiment.
The 22:2 non methylene interrupted dienoic fatty acid (22:2NMID), previously recorded in Pectinids [40, 7, 9] was detected with high amount in the TAG fraction (6%) of the *F. glaber* digestive gland. This result is opposite to those recorded in several Pectinids species in which the highest amount of NMID were recorded in the polar fraction such in *Nodipecten subnodosus* [41], *Pecten maximus* [7] and *Argopecten purpuratus* [9].
Although absent in the diet, the notable amount of 22 NMID confirms the capabilities of this species to *novo* biosynthesize PUFA NMID fatty acids. This capacity to biosynthesize NMIDs has been demonstrated in molluscs from 16:1*n*-7 and 18:1*n*-9 through elongations and Δ5 desaturation [42]. In this study, the *novo* biosynthesis of the 22:2 fatty acids coincided with the drastic decrease of their precursor 16:1*n*-7 in the TAG and PC fractions. According to Klingensmith [43], the NMID may act as a substitute for essential fatty acids such as 20:4*n*-3, 20:5*n*-3, and 22:6*n*-3. In this study, the noticed increase in 22:2 coincided with low levels of 20:5*n*-3. Thus, the 22:2 NMID seems to substitute the EPA which is poorly contributing to the diet.
In both lipid fractions (TAG and PC) of scallop *F. glaber*, 3 weeks fasting period induced a decrease of the energetic saturated fatty acids (14:0 and 16:0) against an increase of the 18:0 characterized by structural-type function. These results reflected the fatty acid reserves depletion and the selective retention of structural-type fatty acids. Those findings revealed the strategy adopted by *F. glaber* to resist the period of diet shortage. This phenomenon of selective accumulation was also recorded for the 18:1*n*-7, 18:1*n*-9 and the 20:1*n*-9 MUFA in TAG and PC of starved scallops. This accumulation could be related to their precursor role in the biosynthesis of NMID as suggested by Zhukova [42]. The selective accumulation of 18:1 and 20:1 MUFA was also recorded in the starved clam *Tapes philippinarum* spat [9]. Specific *n*-7 MUFA retention was observed in starved juvenile scallop *Placopecten magellanicus* [44].
The NMID are selectively retained in polar lipid class when *F. glaber* is starving. This would be related to the involvement of the NMID in peculiar membrane properties and their implication in the control and the repair of structural and functional inadequacies due to the long chain PUFA shortage [45].
During the fasting trial, the combined effects of the increasing 22:6*n*-3 and the decreasing 20:5*n*-3 proportions, in the polar fraction, caused a drastic change in the 22:6*n*-3/20:5*n*-3 ratio from 0.6 to 4.5. This result confirmed the preferential incorporation of 22:6*n*-3 compared to 20:5*n*-3, as recorded by other authors in starved juveniles of *Placopecten magellanicus* [44]. The maintenance of high level of DHA was attributed to its major role in the maintenance of the structural and functional integrity of cell membranes, as reported by Delaunay [38].
In this study, the increasing level of the ARA recorded in the polar fraction (table 4) of unfed *F. glaber* agrees with other studies in which a selective retention of the 20:4*n*-6 was reported in starved bivalves [44, 9, 29]. This would mean that for starving F. glaber, the 20:4n-6 increased level would be due to selective retention. However, the release of ARA could result from the phospholipids hydrolyzes under the Phospholipases A2 (PLA 2) action [46].

Studying the phospholipase activities in scallop juveniles *Pecten maximus*, Hoehne-Reitan [47] have reported that the PLA 2 activity was significantly higher in starved juveniles compared to the fed scallops.

The increasing amount of the arachidonic acid in starved *F. glaber* was coupled with the precursor's role of this fatty acid in the eicosanoids synthesis and consequently with improvement of the immune function. In fact, the starvation stress compromises immunological activities in bivalves as recorded in the scallop *Chlamys farreri* [48] and the oyster *Saccostrea glomerata* [49]. Moreover, Hurtado [50] have associated the eicosanoid metabolites release with immune response in the oyster *Crassostrea corteziensis*.

CONCLUSION:

As physiological adjustment, starved *F. glaber* mobilize their endogenous lipid reserve to provide energy in order to sustain their basic metabolism necessary for survival. At the fatty acid level, the strategy adopted by this bivalve to survive the starvation trial consisted of the selective retention of essential and structural-type fatty acids as DHA, AA and NMID (mainly in the PC fraction).

EPA deficiency induced the *de novo* biosynthesis of the NMID by *F. glaber* fed on *I. galbana*. This was especially noticed in the TAG fraction as a substitute to the 20:5n-3 and in order to maintain high level of the PUFAs group.

Acknowledgements

The authors wish to express gratitude to Mr Raouf Besbes from the "Institut National des Sciences et Technologies de la Mer de Monastir, Tunisie" for his helpful assistance in providing the microalgal that were crucial for this experiment. Similar thanks go to the anonymous reviewer for improving the final version.

REFERENCES:

[1] I. Laing, ***J. Exp. Mar. Biol. Ecol.*, 1993,** 173, 111.
[2] C. Berthelin, K. Kellner, M. Mathieu, ***Comp. Biochem. Physiol. (B)*, 2000**, 125, 359.
[3] C. J. Lodeiros, J. J. Rengel, H. E. Guderley, O. Nusetti, J. H. Himmelman, ***Aquaculture,* 2001,** 199, 63.
[4] J. Waldock, D. L. Holland, ***Lipids,* 1984,** 19, 332.
[5] G. E. Napolitano, R. G. Ackman, ***Comp. Biochem. Physiol. (B)*, 1992**, 103, 645.
[6] G. Le Pennec , M. Le Pennec , G. Beninger, ***J. Mar. Biol. Assoc. U.K.*, 2001,** 81, 663.
[7] A. J. Pazos, J. L. Sanchez, G. Roman, M. L. Perez-Paralle, M. Abad, ***Comp. Biochem. Physiol. (B)*, 2003a,** 134, 367.
[8] P. Soudant, J. Moal, Y. Marty, J. F. Samain, ***J. Exp. Mar. Biol. Ecol.*, 1996**, 205, 149.
[9] M. Caers, P. Coutteau, K. Cure, V. Morales, G. Gajardo, P. Sorgeloos, ***Comp. Biochem. Physiol. (B)*, 1999**, 123, 97.
[10] N. Nevejan, I. Saez, G. Gajardo, P. Sorgeloos, ***Aquaculture,* 2003,** 217, 613.
[11] A. Farias, J. G. Bell, I. Uriarte, J. R. Sargent, ***Aquaculture,* 2003**, 228**,** 289.
[12] F. Pernet, V. M. Bricelj, C. C. Parrish, ***J. Exp. Mar. Biol. Ecol.*, 2005,** 327, 115.
[13] R. Tremblay, S. Cartier, P. Miner, F. Pernet, C. Quéré, J. Moal, M. L. Muzellec, M. Mazuret, J. F. Samain, ***Aquaculture,* 2007**, 262, 410.
[14] G. E. Napolitano, M. A. Silva, R. G. Ackman, ***Mar. Biol.*, 1993**, 117, 647.
[15] M. Caers, P. Coutteau, P. Sorgeloos, G. Gajardo, ***Aquaculture*, 2003,** 217, 437.
[16] A. J. Pazos, A. Silva, V. Vazquez, M. L. Perez-Paralle, G. Roman, J. L. Sanchez, M. Abad, ***Comp. Biochem. Physiol. (B)*, 2003b,** 134, 435.
[17] L. M. Roldan-Carrillo, A. N. Maeda-Martinez, A. Masso-Rojas, M. T. Sicard-Gonzalez, ***J. shellfish res.*, 2005,** 24, 353.
[18] W. A. O'connor, M. P. Heasman, S. J. O'connor, ***Aquacult. Res.*, 2000,** 31, 627.
[19] J. Folch, M. Lees, G. H. Sloan Stanley, ***J. Biol. Chem.*, 1957,** 226, 497.
[20] R. E. Olsen, R. J. Henderson, ***J. Exp. Mar. Biol. Ecol.*, 1989**, 129, 189.
[21] G. Cecchi, S. Basini, C. Castano, ***Revue française des corps gras,* 1985**, 32, 163.
[22] E. Palacios, I. S. Racotta, O. Arjona, Y. Marty, J. R. Le Coz, J. Moal, J. F. Samain, ***Aquaculture,* 2007,** 266, 266.
[23] A. C. Giese, ***Physiol. Rev.*, 1966,** 46, 244.
[24] S. Dridi, M. S. Romdhane, M. El Cafsi, ***Aquaculture,* 2007**, 263, 238.
[25] M. L. Swift, D. White, M. B. Ghassemieh, ***Lipids,* 1980**, 15, 129.

[26] M. Caers, P. Coutteau, P. Sorgeloos, *Aquaculture*, **2000,** 186, 157.
[27] S. M. Gallager, R. Mann, G. C. Sasaki, *Aquaculture*, **1986,** 56, 81.
[28] T. H. Carefoot, P.Y. Qian, B. E. Taylor, T. West, J. Osborne, *Aquaculture*, **1993,** 118, 315.
[29] M. Pirini, M. P. Manuzzi, A. Pagliarani, F. Trombetti, A. R. Borgatti, V. Ventrella, *Comp. Biochem. Physiol. (B)*, **2007,** 147, 616.
[30] W. Liu, Q. Li, F. Gao, L. Kong, *Fisheries Sci*., **2010,** 76, 737.
[31] C. A. Gillis, P. E. Dabinett, *Bull. Aquac. Assoc. Can.*, **1989**, 89, 77.
[32] S. W. Jeffrey, M. R. Brown, J. K. Volkman, The Haptophyte Algae, pp. 287-302, in Haptophyte as feedstocks in mariculture, J. C. Green, B. S. C. Leadbeater editors, Clarendon Press, Oxford, **1994.**
[33] C. C. Parrish, J. S. Wells, Z. Yang, P. Dabinett, *Mar. Biol.*, **1998,** 133, 461.
[34] C. E. Allen, P. A. Tyler, C. L. Van Dover, *J. Mar. Biol. Assoc. U.K.*, **2001**, 81, 817.
[35] G. E. Napolitano, R. G. Ackman, M. W. N. Ratnayake, *J. World Aquacult. Soc.*, **1990,** 21, 122.
[36] G. E. Napolitano, R. J. Pollero, A. N. Gayoso, B. A. MacDonald, R. J. Thompson, *Biochem. Syst. Ecol.*, **1997**, 25, 739.
[37] A. G. Taylor, C. Savage, *Aquaculture*, 2006, 261, 430.
[38] F. Delaunay, Y. Marty, J. Moal, J. F. Samain, *J. Exp. Mar. Biol. Ecol*., **1993**, 173, 163.
[39] M. Caers, S. D. Utting, P. Coutteau, P. F. Millican, P. Sorgeloos, *Mar. Biol.*, **2002,** 140, 1157.
[40] E. Kraffe, P. Soudant, Y. Marty, *Lipids*, 2006, 41, 491.
[41] E. Palacios, I. S. Racotta, E. Kraffe, Y. Marty, J. Moal, J. F. Samain, *Aquaculture*, **2005,** 250, 270.
[42] N. V. Zhukova, *Biochim. Biophys. Acta*, **1986,** 878, 131.
[43] J. S. Klingensmith, *Lipids*, **1982,** 17, 976.
[44] P. Coutteau, J. D. Castell, R. G. Ackman, P. Sorgeloos, *J.* shellfish *res.*, **1996**, 15, 259.
[45] E. Kraffe, P. Soudant, Y. Marty, *Lipids*, **2004,** 39, 59.
[46] E. A. Denis, *J. Biol. Chem.*, **1994,** 269, 13057.
[47] K. Hoehne-Reitan, S. N. Økland, K. I. Reitan, *Aquacult. Nutr*., **2007,** 13, 45.
[48] B. Xu, M. Chen, H. Yang, S. Zhao, *J. Shellfish Res*., **2008,** 27, 1195.
[49] D. Butt, S. Aladaileh, W. A. O'Connor, D. A. Raftos, *Aquaculture*, **2007**, 264, 82.
[50] M. A. Hurtado, M. Reza, A. M. Lbarra, M. Wille, P. Sorgeloos, P. Soudant, E. Palacios. *Aquaculture*, **2009,** 294, 300.