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Comparison of Two Microalgal Diets. 1. Influence on the Biochemical and Fatty Acid Compositions of Raw Oysters (*Crassostrea gigas*)

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Oyster farming in France is a traditional activity. Each year, 149 000 tons of oysters are fattened before being sold. More and more oyster farmers supplement the diet of oysters by microalgae to optimize the fattening process and to improve both the growth and flesh quality of oysters. In the present study, oysters were supplemented by two microalgae: *Skeletonema costatum* and *Tahitian isochrysis* clone. The ash, protein, carbohydrate (including glycogen), and lipid contents were analyzed. The growth of oysters was improved when they were fattened with microalgae, leading to an increase in the condition index. The chemical composition of oysters was influenced by the chemical composition of the microalgae, especially the level of glycogen, which was significantly increased for oysters supplemented by microalgae. The fatty acid profile of oysters fattened by microalgae was positively correlated with the fatty acid profiles of *S. costatum* and *T. isochrysis*. These results show the effectiveness of supplementation by microalgae on the growth and on the biochemical composition (glycogen and fatty acids especially) of oysters.

KEYWORDS: ***Crassostrea gigas*** **oyster;** ***Skeletonema costatum*****;** ***Tahitian isochrysis*** **clone; biochemical composition; lipid composition; fatty acid profile**

INTRODUCTION

France is the greatest producer of oysters (*Crassostrea gigas*) in Europe (*1*). Most of the oysters produced are used by the French, who are the greatest consumers of seafood in Europe. The Pacific oyster *C. gigas* was introduced into Atlantic waters when a disease decimated the Portuguese oyster (*Crassostrea angulata*) in the 1970s. The Pacific oyster, which has been reared in Japan for centuries (*2*), is very sensitive to climatic variation. Indeed, although this species is well adapted to the French coast, it remains an exotic species (*3*) unused to the fall in temperature and the shortening of the photoperiod that occurs in autumn. Moreover, the quality of oysters is influenced by their food quality and quantity, according to the environmental conditions (*4*). The flesh quality of oysters is dependent on their biochemical content. To limit these fluctuations in quality, more and more oyster farmers carry out a fattening process. This consists of rearing microalgae in ponds and then feeding them to oysters in ponds. Thus, both the food quality and quantity as well as the seawater temperature and salinity can be controlled, all factors which can modify the biochemical composition of oysters.

Microalgae, including *Skeletonema costatum* and *Tahitian isochrysis* clone, are often used in aquaculture. Indeed, *S. costatum* has been used by Bougrier et al. (*5*), McCausland et al. (*6*), Méléder et al. (*7*), and Piveteau et al. (*8*) to study the growth or the biochemical composition of juvenile or adult oysters *C. gigas*. Concerning *T. isochrysis*, it has been used to feed oyster *C. gigas* spat (*6*, *9*). These two microalgae are used for their nutritional value and for their high fatty acid content, especially their high content of polyunsaturated fatty acids (*5*−*9*).

Very little work has been published on the biochemical composition of Pacific oysters (*2*, *3*, *8*). In contrast, there is much research concerning their fatty acid composition (*2*, *6*, *8*, *10*). To our knowledge, only McCausland et al. (*6*) have fed juvenile oysters with *S. costatum* and *T. isochrysis* to study their growth and their biochemical composition. Our study deals with the influence and the comparison of microalgae *S. costatum* and *T. isochrysis* on the growth of adult oysters, on their biochemical composition, on their lipid changes, and on their fatty acid profile.

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MATERIALS AND METHODS

Reagents. Butylated hydroxytoluene, nonadecanoic acid, trifluoroboride/methanol (14%), and glucose standard solution at 100 mg/dL were obtained from Sigma-Aldrich (Germany). Hexane, toluene, phenol, trichloroacetic acid, sodium hydroxide, anhydrous sodium carbonate, Folin−Ciocalteau reagent, and bovine albumin were purchased from Merck (Germany). Acetone, anhydrous sodium sulfate, sulfuric acid, copper sulfate, and sodium−potassium tartrate were purchased from Panreac (Spain). Chloroform was purchased from Cluzeau (France).

Oysters. Oysters *C. gigas* were obtained from the Bay of Bourgneuf in Vendée on the Atlantic coast of Western France. Oysters were collected outside the maturation period, from January to March 2001, at the adult stage. After collection, they were stored alive at 4 °C for 6 days.

Microalgae. Microalgae *S. costatum*, which is a diatom, was reared outside in an 80 000-L pond with underground saltwater, the composition of which was given by Baud (*11*). Natural light was used for photosynthesis. The temperature varied from 15 to 17 °C, depending on the climatic conditions, and the saltwater salinity was 33−34 g/L. The flagellate *T. isochrysis* was grown in a 300-L tank containing underground saltwater and vitamin B12, in a special room at 22 °C. Photosynthesis was assured by artificial light. The salinity of the saltwater was 33−34 g/L.

Oysters' Diet. The experiments took place in Bouin in the Bay of Bourgneuf. Three 600-L plastic tanks containing 400 oysters were supplied by seawater at 14 °C. In the first tank (oyster control group), oysters were fed only with natural phytoplankton contained in seawater containing about 2 μg/L of particulate organic matter (POM). In the second and third tanks, oysters were fed with seawater enriched with *S. costatum* (4.8 mg of POM/L) and *T. isochrysis* (5.1 mg of POM/L), respectively. The content of organic matter in the tank was measured by weighing a Whatman filter GF/C in which a known volume (V) of microalgae was filtered. The filter was rinsed with ammonium formate, dried at 56 °C for 24 h, and then weighed (P_1). The filter was then calcined at 450 °C for 1 h and weighed (P_2). The POM was calculated as follows: POM (mg/L) $= 1000 \times (P_1 - P_2)/V$. In terms of cells, 2×10^9 cells of *S. costatum* per oyster per day and 8×10^9 cells of *T. isochrysis* per oyster per day were given to oysters. The cells were counted with a scanner using a Malassez cell (0.0025 mm^2 area $\times$ 0.2 mm depth). Seawater salinity, temperature, and oxygen content were measured regularly. Seawater salinity was measured using a conductivity meter (WTW, USA). Oxygen content was determined using a dissolved oxygen meter (WTW). An oxygenation ramp allowed the oysters to be oxygenated, and the mixing of the seawater column permitted a good distribution of the microalgae in the tank. Seawater was renewed at a rate of 600 L h^{-1} tank^{-1}.

The experiment lasted 6 weeks. Oysters were sampled at time 0 and after 6 weeks of experimentation for all the analyses.

Condition Index. The growth of the oysters was evaluated through the determination of their condition index. The condition index was evaluated on 30 oysters by measuring the whole weight of oysters and their flesh weight. This index was measured in order to evaluate how the oyster used the internal cavity volume for the development of its tissues. The condition index is calculated as CI $= (W_f \times 100)/W_t$, where CI is the condition index, W_f is the flesh weight of the oyster, and W_t is the total weight of the oyster (AFNOR). The condition index can vary from 6 to 9, according to the quality of the oysters. In our study, oysters at the beginning of the experiment had a condition index of 6.7.

Biochemical Analyses. Thirty freeze-dried oysters were crushed to a fine powder. The protein content of 8 mg of oyster powder was measured in triplicate by the colorimetric method described by Lowry et al. (*12*). The carbohydrate and glycogen contents of 5 mg of oyster powder were determined in triplicate by the method of Dubois et al. (*13*). Lipids of oysters and of microalgae (*S. costatum* and *T. isochrysis*) were extracted by a mixture of chloroform/methanol (2:1, v/v) using the method of Folch et al. (*14*), and their content was measured by a gravimetric method. Thirty oysters were crushed, and six lipid extractions were made on 10 g of the crushed mixture. The lipid contents of 50 g of *S. costatum* and 20 g of *T. isochrysis* pastes were determined in triplicate. The microalgae pastes were obtained by centrifugation of the particles in suspension in the underground saltwater.

Table 1. Biochemical Composition of Oysters of the Control Groups and Those Fed *S. costatum* and *T. isochrysis* after 6 Weeks of Experimentation

	OCG-S0	OCG-S6	OS–S6	OI–S6
condition index[a]	6.7 (1.6) a	7.2 (1.1) a	9.4 (1.6) a	8.2 (1.6) a
ash[b] (% dry matter)	24.6 (2.3) a	29.6 (2.1) b	20.8 (0.9) c	25.9 (0.9) a
protein[b] (% dry matter)	49.0 (1.3) a	40.3 (1.9) b	36.4 (1.9) c	37.6 (2.2) bc
carbohydrate[b] (% dry matter)	15.0 (0.1) a	21.7 (4.1) b	32.2 (0.3) c	34.0 (3.8) c
glycogen[b] (% dry matter)	7.4 (0.1) a	9.7 (1.7) b	29.9 (0.1) c	23.2 (1.4) d
lipid[c] (% dry matter)	8.5 (1.1) a	7.9 (0.3) a	11.1 (0.8) b	9.9 (0.1) c

[a] OCG, oyster control group; OS, oysters supplemented by *S. costatum*; OI, oysters supplemented by *T. isochrysis*; S0, control point; S6, level after 6 weeks of experimentation. [b] Mean (tandard deviation), $n = 30$. [c] Mean (standard deviation), $n = 3$. [d] Mean (standard deviation), $n = 6$. Values in the same row followed by different letters are significantly different by ANOVA ($p < 0.05$).

Fatty Acid Analyses. The composition of fatty acids in oysters and in the two microalgae was determined by gas chromatography after their transmethylation by a mixture of trifluoroboride/methanol (14%), as described by Morrison and Smith (*15*). The fatty acid methyl esters (FAMEs) were analyzed by using a gas chromatograph (HP model 5890 series II chromatograph, Hewlett-Packard Co., Palo Alto, CA). One microliter of extract was injected in a split mode on an injector set at 250 °C. The FAMEs were separated on a capillary column (DB-23, 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μm thickness, J&W Scientific, Folsom, CA). The oven temperature was set at 150 °C for 3 min and then increased at a rate of 10 °C/min to 180 °C for 7 min, followed by a further increase at a rate of 5 °C/min to 215 °C, with a final time of 15 min. The helium carrier gas flow was 1 mL/min. The detector was a flame ionization detector set at 280 °C. Nonadecanoic acid (2 mg) was used to quantify the FAMEs. A commercial solution of standards (Sigma Chemical Co., Saint Louis, MO) was injected in order to identify FAMEs on the basis of their retention index. The identification of FAMEs was confirmed by using a gas chromatograph (HP 5890 II, Hewlett-Packard Co.) coupled with a mass spectrometer equipped with an electronic impact source (HP 5971 II mass-selective detector, Hewlett-Packard Co.). The temperatures of the oven and of the injector were the same as previously used. The MSD conditions were as follow: ionization energy, 70 eV; mass range, 33−300 u; scan rate, 2.0 scans/s; electron multiplier voltage, 200 V; interface temperature, 180 °C. The spectra of FAMEs were compared with those of an internal library at our laboratory and with a commercial one (NBS 75k).

Statistical Treatment. Data acquisition and statistical treatment (variance analysis ANOVA) were performed on Statgraph 4.0 software.

RESULTS AND DISCUSSION

Biochemical Composition. Table 1 shows the change in the biochemical composition of unfed oysters and oysters supplemented with microalgae. The oyster control group contained about 90% moisture. The main components of the oysters were protein (49.0% of dry matter) and ashes (24.6% of dry matter). They contained 8.5% lipids, and the rest was composed of carbohydrates including glycogen. The same oysters after 6 weeks at 14 °C in the tank showed a reduced protein content (from 49.0% to 40.3%) and an increase in carbohydrates (from 15.0% to 21.7%). The fluctuation in the biochemical composition was probably due to bad weather conditions, which led to a decrease in the water salinity (24 g/L instead of 33 g/L as usually found in this region). It may also have been due to a lack of quality of shellfish in the Bay of Bourgneuf since the oil pollution by the tanker "Erika" in December 1999. Laing and Earl (*16*) have already shown that the level of salinity can affect oysters, and particularly the metamorphosis of oyster

larvae; however, they do not specify in which sense. According to our experiment, the decrease of the seawater salinity resulted in mortality of oysters and their limited growth. Linehan et al. (*2*) have shown that seasonal variation has an important impact on the biochemical composition of oysters. In the same way, the climatic conditions also have a great importance.

The condition index was increased in oysters supplemented by microalgae. The condition index was 7.2 for the oyster control group versus 9.4 and 8.2 respectively for oysters supplemented by *S. costatum* and *T. isochrysis*, showing an improvement in the growth of oysters. The glycogen content increased, indicating a good availability and a good assimilation of microalgae in the habitat. Castell and Trider (*17*) have shown a glycogen content increase when oysters are fed both carbohydrates and lipids. Glycogen is the main energy source of oysters and plays an essential part in the reproductive cycle (*2*, *18*). The lipid content of oysters fed microalgae increased with the contribution of the lipids of microalgae. The increase in lipids in oysters supplemented by *S. costatum* was higher than that in oysters supplemented by *T. isochrysis*. This is in agreement with the lipid content of the microalgae, which is 6.7 and 4.3% of dry matter for *S. costatum* and *T. isochrysis*, respectively.

Fatty Acid Composition. Table 2 shows the fatty acid composition of microalgae *S. costatum* and *T. isochrysis* and the fatty acid composition of oysters of the control group and those supplemented by microalgae at the beginning and after 6 weeks of experimentation. A significant difference in the content of the fatty acids of the *n*−9, *n*−7, *n*−6, and *n*−3 series was observed between the oyster control group at the beginning of the experiment (week 0) and after 6 weeks of experimentation. As for the biochemical composition, the climatic variation changed the fatty acid composition. The fatty acid composition, however, followed the tendency found by Linehan et al. (*2*), McCausland et al. (*6*), and Piveteau et al. (*8*). The control group oysters were mainly composed of saturated fatty acids and polyunsaturated fatty acids. The main fatty acids in oysters were palmitic acid (C16:0), eicosapentaenoic acid (C20:5 *n*−3), and docosohexaenoic acid (C22:6 *n*−3). C20:5 *n*−3 and C22:6 *n*−3 have been found to be essential fatty acids in oysters (*19*). An enriched diet in these last two fatty acids increased the growth of bivalves (*20*).

The content of C16:0 in oysters was steady, even with the supplementation by the microalgae. The diatom *S. costatum* contains 27.2% of saturated fatty acids and the flagellate *T. isochrysis* 39.5%. No significant difference in saturated fatty acids content was observed between oysters fed microalgae and the oyster control group at week 6. Nevertheless, a statistical difference was observed between oysters fed the two microalgae. Saturated fatty acids were not accumulated in oysters.

A significant difference was observed for *n*−9 fatty acid content, the level of which was 2.7% for oysters supplemented by *S. costatum* and 7.3% for oysters supplemented by *T. isochrysis*. The difference was due to the presence of C18:1 *n*−9, high in oysters fattened with *T. isochrysis*, in agreement with the content of this fatty acid in the microalgae.

A significant difference ($p < 0.05$) in *n*−7 fatty acid content was observed between oysters supplemented by microalgae (6.2% for oysters supplemented by *S. costatum* and 2.8% for oysters supplemented by *T. isochrysis*). This is related to the content of *n*−7 fatty acid in the microalgae. Indeed, *S. costatum* contains 21.3% of *n*−7 fatty acid (compared to 5.8% for *T. isochrysis*). The *n*−7 fatty acid level increases due to the accumulation of the C16:1 *n*−7 of the microalgae, but it could also be due to bioconversion of C16:0 (largely present in the microalgae) to C16:1 *n*−7 (*21*).

Table 2. Evolution of Total Fatty Acid Composition of Control Group Oysters and Those Supplemented by *S. costatum* and *T. isochrysis* after 6 Weeks of Experimentation (in % of the Total Fatty Acid)[a]

fatty acid	microalgae: *S. costatum*	microalgae: *T. isochrysis*	experimentation time: OCG-S0	OS-S6	OCG-S6	OI-S6	statistical effect
11:0	0.0 (0.0)	0.1 (0.0)	0.0 a	0.1 b	0.1 b	0.1 b	*
12:0	1.5 (0.1)	1.9 (0.1)	0.0 a	0.1 b	0.0 a	0.0 a	**
13:0	nd	nd	0.0 a	0.1 b	0.1 b	0.2 c	**
14:0	14.1 (2.2)	24.9 (0.6)	0.3 a	1.7 b	5.2 c	9.7 d	***
15:0	0.5 (0.1)	0.6 (0.1)	1.2 a	0.5 b	0.3 c	0.4 b c	*
16:0	10.7 (1.7)	11.0 (0.3)	26.9 a	22.5 b c	21.2 c	24.0 b	**
17:0	nd	0.2 (0.1)	0.1 a	4.0 b	2.0 c	2.7 d	**
18:0	0.3 (0.1)	0.5 (0.1)	1.1 a	7.3 b	5.1 c	4.4 d	**
20:0	0.1 (0.0)	0.3 (0.1)	0.1	0.2	0.2	0.1	ns
∑SFA	**27.2 (3.8)**	**39.5 (0.8)**	**29.7 a**	**36.5 ab**	**34.2 a**	**41.6 b**	*
14:1 *n*–9	0.2 (0.0)	0.3 (0.1)	0.0 a	1.3 b	0.7 c	0.7 c	*
18:1 *n*–9	0.1 (0.0)	13.0 (0.4)	5.3 a	3.6 b	1.7 c	5.2 ab	*
20:1 *n*–9	nd	nd	7.9 a	1.2 b	0.3 c	1.3 b	*
∑*n*–9	**0.3 (0.0)**	**13.3 (0.4)**	**13.2 a**	**6.1 b**	**2.7 c**	**7.3 b**	**
16:1 *n*–7	21.2 (0.4)	5.8 (0.1)	0.2 a	2.1 b	6.1 c	2.7 b	*
17:1 *n*–7	0.1 (0.0)	nd	0.0 a	0.2 b	0.1 a	0.1 a	*
∑*n*–7	**21.3 (0.4)**	**5.8 (0.1)**	**0.2 a**	**2.3 b**	**6.2 c**	**2.8 d**	*
∑MUFA	**21.6 (0.4)**	**19.1 (0.5)**	**13.4 a**	**8.4 b**	**8.9 ab**	**10.1 a**	*
18:2 *n*–6	0.6 (0.1)	8.9 (0.1)	0.1 a	0.8 b	0.8 b	5.7 c	***
20:2 *n*–6	nd	nd	0.1 a	0.2 b	0.1 a	0.6 c	**
20:3 *n*–6	nd	nd	0.0 a	0.1 b	0.1 b	0.2 c	***
20:4 *n*–6	0.1 (0.1)	nd	0.1 a	5.6 b	1.8 c	2.7 d	**
22:2 *n*–6	nd	nd	0.0 a	0.0 a	0.0 a	0.1 b	***
∑*n*–6	**0.7 (0.1)**	**8.9 (0.1)**	**0.4 a**	**6.8 b**	**2.9 c**	**9.3 d**	***
16:2 *n*–4	4.1 (0.1)	0.8 (0.1)	0.0 a	0.0 a	1.3 b	0.0 a	***
16:3 *n*–4	5.0 (0.4)	0.1 (0.0)	0.1 a	0.1 a	1.0 b	0.0 a	***
∑*n*–4	**9.1 (0.6)**	**0.9 (0.1)**	**0.1 a**	**0.1 a**	**2.3 b**	**0.0 a**	***
18:3 *n*–3	0.1 (0.1)	6.9 (0.1)	0.1 a	2.2 b	1.5 c	5.6 d	*
18:4 *n*–3	3.4 (0.3)	15.1 (0.4)	2.6 a	1.9 b	1.6 b	1.1 c	*
20:3 *n*–3	nd	nd	0.2 a	0.1 a	0.1 a	0.4 b	***
20:5 *n*–3	23.5 (1.9)	0.8 (0.1)	28.9 a	22.1 b	32.4 c	10.6 d	**
22:6 *n*–3	4.0 (0.3)	8.6 (0.5)	24.4 a	20.5 b	13.7 c	19.7 b	**
∑*n*–3	**31.0 (2.6)**	**31.4 (1.0)**	**56.1 a**	**46.9 b**	**49.3 b**	**37.4 c**	*
16:4 *n*–1	10.6 (0.8)	0.1 (0.1)	0.2 a	0.1 a	1.4 b	0.1 a	***
∑*n*–1	**10.6 (0.8)**	**0.1 (0.1)**	**0.2 a**	**0.1 a**	**1.4 b**	**0.1 a**	***
∑PUFA	**51.4 (4.1)**	**41.4 (1.1)**	**56.9 a**	**53.8 ab**	**56.0 ab**	**46.8 b**	*

[a] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; OCG, oyster control group; OS, oysters supplemented by *S. costatum*; OI, oysters supplemented by *T. isochrysis*; S0, control point; S6, level after 6 weeks of experimentation; nd, not detected. Values in the same row followed by different letters are significantly different by ANOVA at 5% (*), 1% (**), and 1‰ (***). ns, not significant.

A significant difference was observed for *n*−6 fatty acid content in oysters supplemented by *S. costatum* and *T. isochrysis* (2.9% and 9.3%, respectively). This is in agreement with the content of *n*−6 fatty acid in microalgae: *T. isochrysis* contains 8.9% compared to 0.7% in *S. costatum*. It is interesting to note that the level of C18:2 *n*−6 increased in oysters supplemented by *T. isochrysis* due to its high content of this fatty acid (8.9% of total fatty acids). This fatty acid was slightly elongated to C20:2 *n*−6 (0.6% for oysters fed with *T. isochrysis* versus 0.2% for the oyster control group). However, there was no desaturation of 18:2 *n*−6 to C20:4 *n*−6 according to the decrease of this fatty acid in oysters fattened with *T. isochrysis*, thus confirming the results of Caers et al. (*22*). Moreover, the level of C20:4 *n*−6 for oysters fattened by *T. isochrysis* is low, in accordance with its low level in *T. isochrysis*. In addition, the C20:4 *n*−6 content for oysters fattened by *S. costatum* and *T. isochrysis*

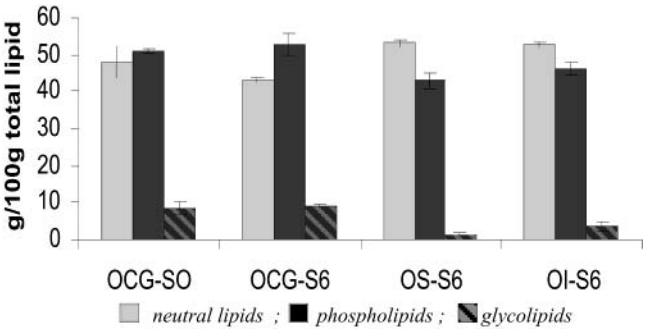

Figure 1. Neutral lipids and polar lipids of oysters (mean ± standard deviation, $n = 6$). OCG, oyster control group; OS, oysters supplemented by *S. costatum*; OI, oysters supplemented by *T. isochrysis*; S0, control point; S6, level after 6 weeks of experimentation.

was low compared to that of the oyster control group after 6 weeks of experimentation. Knauer and Southgate (*19*) have shown the retention of this fatty acid in unfed spat oysters, proving its essential character.

T. isochrysis contains C18:3 $n-3$ (6.9% versus 0.1% for the diatom), which led to an increase of its presence in oysters. C18:4 $n-3$ fatty acid content was found to be low in oysters fed the flagellate. This result is in agreement with the results of McCausland et al. (*6*), who showed that this fatty acid was not retained in the lipid content of juvenile oysters. In oysters fattened by *T. isochrysis*, the C20:5 $n-3$ content decreased and that of C22:6 $n-3$ tended to stay steady, thus showing that there was no elongation of the C18:3 $n-3$ (found in abundance in oysters) in C20:5 $n-3$ and C22:6 $n-3$. Moreover, oysters were not able to elongate C18:3 $n-3$ to C18:4 $n-3$. These results are in accordance with those of Langdon and Waldock (*20*). In relation to the level of C20:5 $n-3$ in the two microalgae, it increased significantly in oysters supplemented by *S. costatum* and decreased significantly in those fed *T. isochrysis*. In the same way, the content of C22:6 $n-3$ was steady in the oysters supplemented by *T. isochrysis*, whereas it was significantly lower in oysters fattened by *S. costatum*, due to the low amount of C22:6 $n-3$ in *S. costatum*. Oysters are able to incorporate these essential fatty acids in their lipids. The significant difference ($p < 0.05$) for $n-3$ fatty acid content in oysters supplemented by the two microalgae was essentially due to the large decrease in C20:5 $n-3$ in oysters fattened by *T. isochrysis* and its large increase in oysters fattened by *S. costatum*.

The content of PUFA in the oyster control group after 6 weeks of experimentation and in oysters supplemented by *S. costatum* was not significantly different. Nevertheless, the content of $n-6$, $n-4$, and $n-1$ fatty acids was significantly different between these two groups of oysters. The sum of $n-6$, $n-4$, $n-3$, and $n-1$ fatty acids resulted in an equal level of PUFA but a different fatty acid profile.

Figure 1 shows that the oyster control group at the beginning and after 6 weeks of experimentation had an equivalent distribution of neutral and polar lipids (phospholipids and glycolipids). The glycolipid level was low. The oyster control group contained more phospholipids than neutral lipids, as shown by Soudant et al. (*10*) in juvenile oysters. Oysters supplemented by microalgae had a neutral lipid level that increased to the detriment of polar lipids. The fatty acids of microalgae are accumulated in neutral lipids and to a lesser extent in phospholipids, according to the results of Piveteau et al. (*8*). Caers et al. (*22*) have shown that neutral lipids are used as an energy source and phospholipids as a reserve energy source.

Figure 2 shows the profile of C20:5 $n-3$ and C22:6 $n-3$ in neutral lipids and in phospholipids. The fatty acid profile of neutral lipids showed the same trends as for total lipids, unlike phospholipids, for which the differences were less marked, especially for the oyster control group. For oysters supplemented by *S. costatum*, C20:5 $n-3$ was accumulated in great amounts in neutral lipids. Oysters supplemented by *T. isochrysis* had a decreasing level of C20:5 $n-3$ both in neutral lipids and in polar lipids, and the C22:6 $n-3$ fatty acid content was steady both in neutral lipids and in phospholipids. The decrease of C22:6 $n-3$ in oysters supplemented by *S. costatum* suggests the poor ability of oysters to elongate and desaturate fatty acids such as C20:5 $n-3$, which are highly present in this microalgae. Waldock and Holland (*23*) have shown that juvenile oysters are able to elongate and desaturate long-chain fatty acids, but not in sufficient quantity to cause an accumulation.

Factors implicated in the different biochemical compositions of oysters fed *S. costatum* and *T. isochrysis* are the ability to be ingested, the ability to be digested, and the biochemical composition of the microalgae (*9*). This experiment shows the ability of oysters to assimilate both microalgae. The increase in the condition index of oysters fattened by microalgae reveals an increase in oysters' growth. The increase in the glycogen level indicates the ability of oysters to store carbohydrate in the form of glycogen as an energy reserve. These results also demonstrate the ability of oysters to accumulate supplied lipids and to modify their fatty acid profile according to that of the microalgae. The value $n-3/n-6$ is high for oysters supplemented by *S. costatum* (16.8 versus 6.9 for the oyster control group and 4.0 for oysters supplemented by *T. isochrysis*), showing its good nutritional supply.

Josephson (*24*) had shown the role of certain PUFAs, found in large amounts in oysters fed both microalgae, as aroma precursors. Our next study consists of correlating the aroma

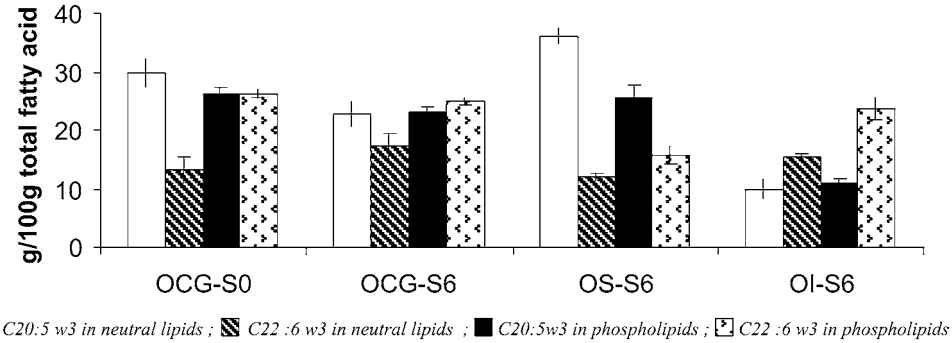

Figure 2. C20:5 *n*–3 and C22:6 *n*–3 levels in neutral lipids and in phospholipids (mean ± standard deviation, $n = 6$). OCG, oyster control group; OS, oysters supplemented by *S. costatum*; OI, oysters supplemented by *T. isochrysis*; S0, control point; S6, level after 6 weeks of experimentation.

profile of oysters fed *S. costatum* and *T. isochrysis* with their fatty acid profile and then highlighting the role of fatty acids as precursors of aroma compounds for adult oysters *C. gigas* (Pennarun et al., 2003).

ABBREVIATIONS USED

CI, condition index; FAME, fatty acid methyl ester; MUFA, monounsaturated fatty acid; POM, particulate organic matter; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

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