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Manipulation of fatty acids in the estuarine clam *Meretrix casta* (Gmelin, 1791) by supplementation with the microalgal diet, *Isochrysis galbana*

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ABSTRACT

The present study evaluated the changes in fatty acid profile of the estuarine clam *Meretrix casta*, an important food organism used in the larval rearing of scyllarid lobsters, after supplementation with the microalgal species *Isochrysis galbana*. The uptake and assimilation of lipids from the microalgal feed were verified by gas chromatographic analysis of fatty acids in the clam tissues after eight days of feeding with *I. galbana*. Increase in concentrations of polyunsaturated fatty acids (PUFA), in particular docosahexaenoic acid (DHA, C22:6n3) and C18:2n-6, was observed in clams supplemented with *I. galbana*. Changes in monounsaturated fatty acid (MUFA) composition were less marked and related to the increasing proportions of C18:1, after supplementation. Feeding with *I. galbana* also induced a decrease in the proportion of saturated fatty acids, which was related to decrease in proportions of both C16:0 and C18:0. Although the fatty acid composition showed significant differences, the gross lipid content of the clam tissues did not seem to be excessively influenced by the algal feeding. Tissues from clams supplemented with *I. galbana* are being evaluated as feed for sand lobster larval trials.

Keywords: Clam, Fatty acid, *Isochrysis galbana, Meretrix casta*, Microalgae, Supplementation

Introduction

Larval nutrition is one of the major bottlenecks for the culture of many species of crustaceans. Bivalve molluscs such as the estuarine clam *Meretrix casta* are the major food organisms used in the larval rearing of several crustaceans, including the *Thenus* spp. of scyllarid lobsters. In general, bivalves are rich sources of n-3 fatty acids such as C20:5n-3 (eicosapentaenoic acid) and C22:6n-3 (docosahexaenoic acid). Due to the fact that marine molluscs cannot synthesise essential fatty acids *de novo*, algal lipids form the main source of these fatty acids. However, the nutritional quality, especially the lipid composition of adult bivalves does not remain constant and is greatly influenced by seasonal changes. Seasonal variations in fatty acid composition have been reported for several marine bivalve molluscs, including clams and mussels (Beninger and Stephan, 1985; Zlatanos, 2008). These variations are closely linked to the availability and composition of their natural diet comprising of phytoplankton. Phytoplankton availability is, in turn, dependent on environmental parameters such as water temperature, salinity, wave action *etc*. Earlier reports indicate that the nutritional quality and growth rates of bivalves could be improved by supplemental feeding with microalgal diets rich in highly unsaturated fatty acids (HUFAs) (Chu and Greaves, 1991; Caers *et al.*, 1998).

In the present study, the effect of supplemental feeding with microalgae on the lipid profile of clams was examined. The microalgal species *Isochrysis galbana* was chosen as the supplementary diet for this experiment because it is relatively easy to produce in large quantities and possesses a high content of polyunsaturated fatty acids (PUFA). The objective of this study was to assess whether the nutritional quality of clams could be augmented and standardised by this process, in order to compensate the seasonal variations of naturally occurring algae in seawater.

Materials and methods

Adult estuarine clam *M. casta* in the size range of 30-32 mm collected from the wild were acclimated at 25 °C in ambient seawater (30 ppt salinity) prior to the start of the experiment. After the acclimation period, clams were randomly divided into two groups of 200 animals each and placed in 500 l circular HDPE tanks continuously

supplied with filtered seawater under constant aeration. The temperature was maintained at 25 °C.

Preliminary trials were conducted to study the effect of various cell concentrations of *I. galbana* on ingestion rates of the clam, in order to determine the concentration range suitable for use in the supplementation experiments. The optimum microalgal concentration for clams in the size range of 30-32 mm was found to be around 2 x 10^5 cells ml^{-1} $animal^{-1}$, because the best filtration rates at which near total feed utilisation could be achieved was obtained at this concentration. Hence, it was decided to use this concentration range for the supplementation experiments.

The microalgae were grown in 3 l batch cultures under controlled conditions of temperature (25 °C), pH (7.5-8.0) and salinity (35 ppt), with continuous illumination. Filtered and sterilised seawater enriched with Walne's medium was used for culture. The algae were harvested in the exponential phase and cell concentrations of the cultures were counted using a haemocytometer. Clams held in the experimental tank were fed with *I. galbana* added to the seawater at regular intervals to keep the microalgal concentration constant, while the control group was fed only with phytoplankton contained in seawater. The duration of the experiment was 8 days.

Animals were sampled at the end of eight days of dietary conditioning. Total dry weight biomass, tissue and hepatopancreas (HP) weights from clams fed with *I. galbana* and from control clams were recorded. The overall lipid profile and fatty acid composition of tissues from control and the experimental clams were investigated. Total lipid extracts were subjected to direct transesterification using 10% acetyl chloride in methanol. The resulting fatty acid methyl esters (FAME) were analysed by gas chromatography (GC), using Agilent 6890 series gas chromatograph (Agilent Technologies, USA), equipped with a fused silica capillary column (30 mm x 0.25 mm i.d.) and a flame ionisation detector (FID). The oven temperature programme involved an initial increase from 150 °C to 180 °C at 10 °C min^{-1}, a hold at 180 °C for 7 min, followed by another increase to 215 °C at 5 °C min^{-1} and a final hold at 215 °C for 15 min. Helium was used as the carrier gas. The component fatty acids were identified by a comparison of retention times with that of known fatty acid standards and results are reported as percentages of the total identified fatty acids.

Results and discussion

The fatty acid profile of *M. casta* from the wild (control) showed a considerable contribution of saturated fatty acids (SFA) in tissues (54.2%), while polyunsaturated fatty acids (PUFA) were less abundant (23.6%) (Fig. 1). It is well known that bivalves have only very limited capability to synthesise PUFAs (Chu and Graeves, 1991). The major SFA proportion was primarily due to the high percentage of short-chain fatty acids, palmitic (C16:0) and stearic (C18:0) acids. These results are similar to previously reported data in other bivalve species (Prato *et al.*, 2010). The monounsaturated fatty acids (MUFA) amounted to 22.1%, with oleic acid (C18:1) being the predominant fatty acid. The main PUFAs detected in clam tissue were 20:5n3 (7.8%) and 22:6n3 (10.5%) (Table 1).

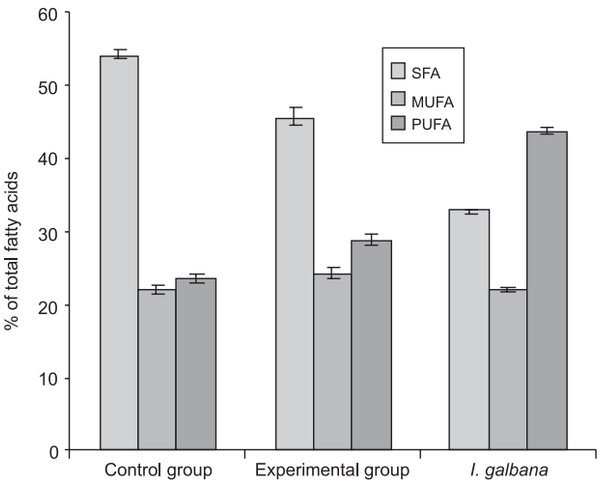

Fig. 1. Percentage composition (% of total fatty acids ± SD) of fatty acid
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

Table 1. Composition of major fatty acids in *Meretrix casta* supplemented with *Isochrysis galbana*

Fatty acids[a]	Control group (%)	Supplemented group (%)
14:0	1.08 (0.1)	2.1 (0.3)
16:0	23.3 (2.0)	18.7 (1.2)
17:0	4.4 (0.6)	4.1 (0.5)
18:0	25.4 (1.1)	20.7 (1.0)
16:1n-7	3.7 (0.3)	3.2 (0.2)
18:1n-9	18.4 (0.9)	21.0 (1.2)
18:2n-6	2.1 (0.7)	2.5 (0.4)
18:3n-3	3.2 (0.3)	4.3 (0.5)
20:5n-3	7.8 (1.0)	7.0 (0.5)
22:6n-3	10.5 (1.0)	15.1 (1.8)
Total SFA	54.2	45.6
Total MUFA	22.1	24.2
Total PUFA	23.6	28.9

Results are expressed as percentage of total fatty acids

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. n=3 (SD in parentheses)

The salient result obtained after eight days of feeding with microalgal diet (*I. galbana*) was an increase in

concentration of PUFA (Fig. 1), in particular 22:6n3 (10.5 to15.1%). PUFAs have been shown to be of specific importance for nutrition of crustacean larvae and the quantity of 22:6n3 (DHA) has been shown to affect larval survival (Caers *et al.*, 1999). *Isochrysis* species of microalgae are considered a rich source of these lipids. Overall, the pattern of fatty acids observed in this study for experimental clams fed on *I. galbana* reflects the profile reported by other workers (Caers *et al.*, 1998) for the same microalgal species (Table 2).

Table 2. Fatty acid composition (% of total fatty acids) of *Isochrysis galbana*

Fatty acids[a]	%
14:0	20.8 (0.2)
16:0	11.9 (0.1)
16:1n-7	4.5 (0.1)
18:1n-9	15.7 (0.3)
18:1n-7	1.5 (0.1)
18:2n-6	6.3 (0.2)
18:3n-3	9.1 (0.2)
18:4n-3	9.8 (0.1)
18:5n-3	5.4 (0.7)
20:5n-3	1.0 (0.1)
22:6n-3	12.1 (0.1)
Total SFA	32.7
Total MUFA	22.0
Total PUFA	43.7

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. n=3 (SD in parentheses).

[a]Minor components (present at levels below 1%) were not included in the table.

Supplementation with *I. galbana* induced a decrease in the proportion of SFA (Fig. 1), which was related to a decrease in the proportions of both C16:0 and C18:0 from 23.3% to 18.7% and 25.4% to 20.7%, respectively (Table 1). Saturation of fatty acids has been shown to increase as particulate organic matter is oxidised in the water column, especially during conditions of low nutrient availability, high levels of detritus and limited phytoplankton growth (Parrish *et al*., 2005). Changes in MUFA concentration and composition were less marked, and were related to the increasing proportions of C18:1 (from 18.4% to 21%), after supplementation.

In general, the fatty acid composition of the algae was reflected in that of the experimental animals to some extent; for example, the high levels of 18:1n9, 18:3n3 and 22:6n3 in the algal diet resulted in higher levels of these fatty acids in the experimental clams. Previous studies have also illustrated that the fatty acid composition of the diet affected the fatty acid profile of bivalves (Beninger and Stephan, 1985; Cears *et al.*, 1998). However, the levels of C14:0 and 18:4n3 which were high in the algal diet, were not clearly expressed in the fatty acid composition of the experimental animals. Although the fatty acid composition showed differences, the overall lipid content of clams fed with microalgae remained relatively unchanged (control clam 2.6%; microalgae-fed clam 2.8%). This result is in agreement with that obtained for clams fed with *Skeletonema costatum* (Baud *et al.*, 1990; Piveteau *et al.*, 1999). This could be due to the fact that lipid content increases in bivalves only during gametogenesis (Piveteau *et al.*, 1999).

Tissues from clams supplemented with *I. galbana* are being evaluated as feed for sand lobster larval trials. Future investigations will include conditioning of clam with other phytoplankton species such as *Skeletonema* and *Nannochloropsis* and comparing their effect on quality of scyllarid larvae.

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