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Lipid composition of the mantle and digestive gland of *Octopus vulgaris* juveniles (Cuvier, 1797) exposed to prolonged starvation

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Abstract Lipid composition of the mantle and digestive gland of *Octopus vulgaris* that were not fed for 27 days were determined. Every 3 days, three octopuses were killed and samples of the mantle and the digestive gland (DG) were taken, in order to determine total lipids as well as lipid classes and fatty acids. Composition in total lipids (TL) for the mantle was similar until day 21, then decreased and remained similar until the end of the experiment. Composition in total lipids for the DG decreased significantly after 3 days, then remained similar until day 21, and then decreased until the end of the experiment. As for the lipid classes, in the DG the main components were triglycerides and sterol esters. Sterol esters suffered strong reductions after 10 days of starvation, while triglycerides remained similar until day 21 and then decreased until the end of the experiment. Cholesterol decreased gradually throughout the experimental period. For polar lipids, phosphatidylcholine and phosphatidylethanolamine increased during the first 3 days and then decreased throughout the experiment. In the mantle, the only neutral classes that decrease were triacylglycerols and sterol esters, while no polar lipid classes decreased in this organ. It was noticeable the decrease in almost all fatty acids in the DG after 3 days of starvation, while in the mantle there were no differences in fatty acid concentrations during the experiment.

Keywords Fatty acids · Lipids · Lipid classes · Digestive gland · Mantle · *Octopus vulgaris* · Starvation

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Introduction

Octopus vulgaris is one of the most promising species for aquaculture diversification. It presents high growth rates (Aguado-Giménez and García-García 2003; Iglesias et al. 2006, 2007) and market price (FAO 2008), associated with a great demand throughout many regions of the world. The common octopus has a planktonic larval stage termed as paralarva (Young and Harman 1988) which lasts for 33–40 days when reared at a mean temperature of 24.7°C (Itami et al. 1963) prior to the benthic juvenile stage. The fattening of *O. vulgaris* is currently done in Galicia, North Spain (Iglesias et al. 1997), but with low profitability due to the dependence on juveniles captured from the wild and the absence of an adequate artificial diet (Domingues et al. 2009). These are two of the major bottlenecks for the commercial aquaculture of this species, which are enhanced by the high mortality in the paralarvae stage of the octopus culture cycle (Iglesias et al. 2007) and the impossibility of obtaining benthic juveniles on a commercial scale in captivity (Cerezo-Valverde et al. 2008).

Research on cephalopod nutrition started in the early 80s (Boucaud-Camou and Boucher-Rodoni 1983; Boucaud-Camou et al. 1985; DeRusha et al. 1989). One of the first studied species was cuttlefish (*Sepia officinalis*). Dry or moist pellets (Castro 1990; Lee et al. 1991; Castro et al. 1993; Castro and Lee 1994) or surimi, a fish myofibrillar protein concentrate (Castro et al. 1993; Castro and Lee 1994; Domingues 1999; Domingues et al. 2005), was initially used with poor results (Castro et al. 1993; Castro and Lee 1994; Domingues et al. 2005, 2006). In the past 3 years, a greater effort has been made to develop artificial diets in several cephalopods, especially in *O. vulgaris* (Cerezo-Valverde et al. 2008; Quintana et al. 2008) and *Octopus maya* (Domingues et al. 2007; Rosas et al. 2007).

Compared to fish, there is little knowledge on energetic physiology of cephalopods, such as food-storage reserves during starvation periods. Tait (1986) reported that the mantle and the digestive gland of *O. vulgaris* were the organs that suffered higher variations in biochemical composition after long-term starvation. Early research indicated that several cephalopod species use small amounts of lipids and/or carbohydrates during starvation (Mommsen and Hochachka 1981; O'Dor et al. 1984; Boucher-Rodoni and Mangold 1988; Segawa and Hanlon 1988; Boucher-Rodoni 1989) and have predominant amino acid metabolism (Ballantyne et al. 1981; Lee 1994). Nevertheless, the highest concentrations of lipid in cephalopods are consistently found in the digestive gland (Boucaud-Camou and Yim 1980; Mangold 1983; Moltschaniwskyj and Johnston 2006), which is an organ actively involved in lipid digestion and storage (Rosa et al. 2005). There is evidence that this organ is used for short-term lipid storage (Boucher-Rodoni et al. 1987; Fluckiger et al. 2008), and it is considered to be a good indicator of nutritional status (García et al. 2009). Furthermore, the importance of lipids in cephalopod metabolism has been demonstrated, both in early stages of development (Navarro and Villanueva 2000, 2003; Villanueva et al. 2002) or juveniles and adults (Domingues et al. 2003; Almansa et al. 2006; Ferreira et al. 2009; García et al. 2009).

Because of this, mantle and DG total lipid composition, lipid classes and fatty acids (FA) of up to 27-day starving octopuses were determined during this study in order to determine how they are kept, transformed and used by the animal in both organs under starvation conditions and determine the ability to use them as nutritional reserves. This study could provide information about essential lipid classes and FA for *O. vulgaris* in order to understand their importance for octopus metabolism and nutrition and help to develop efficient prepared diets for the fattening of this species.

Materials and methods

Capture, experimental design and sampling protocol

Octopuses were captured using artisanal bottom trawl nets in coastal waters of Huelva (South Spain) and brought to our research facility (Center IFAPA Agua del Pino, Cartaya, Spain). Animals were acclimated during 2 weeks in a flow-through system composed of 9 concrete tanks, with 4500 l of water each (3 m × 1 m, water depth of 1.5 m), and fed frozen squid (*Loligo gahi*) with a daily ration of 5% of body weight per day (BW d^{-1}) (wet weight of food/wet weight of the animal) provided twice a day at 09:00 h and 15:00 h.

After this period, a total of 30 immature octopuses (mean weight ± SD = 1618.3 ± 175.5 g) were randomly taken from the acclimation tanks and individually placed in a flow-through seawater system composed of 30 cylindrical tanks of 140 l each, and water flow of 30 l h^{-1}. No significant differences ($P > 0.05$) were found in initial total weights for the 30 octopuses. Water temperature was of 20 ± 1°C, salinity varied between 36 ± 1 ppt and pH was of 7.9 ± 0.1. Water flow was adjusted in all tanks to maintain oxygen concentration close to saturation levels. The natural photoperiod in South Spain was used for the experiment (14:10 h).

Every 3 days, three octopuses were killed. The first three octopuses to be killed were used as the control treatment since they were fed with squid before the start of the experiment and they were never starved. This was considered to be day 0 of the experiment. No food was provided to the rest of octopuses during the next 27 days.

Total weight (gram wet weight, g WW) and DG weight (g WW) were recorded for every animal in every sampling period. Octopuses were weighed alive in order to calculate initial and final total weight. In every sampling period, DG weight was recorded after octopuses were killed, after being anesthetized on ice for 2 min. Data obtained were used to calculate:

- Weight loss (%) = Final Weight × 100 × Initial Weight^{-1}
- Digestive Gland Index, DGI (%) = DG Weight × 100 × Total Weight^{-1}

Weight loss and DGI were recorded for every sampling period.

Three samples (2 g each) of the mantle and the DG of each animal were taken in order to determine total lipid, lipid classes and FA. Samples were collected and mixed with chloroform/methanol (2:1 volume/volume; v/v) and immediately placed on dry ice and stored at −80°C until lipid analysis.

Finally, moisture content was determined from 500 mg samples of the mantle and the DG using the method of Horwitz (1980).

Biochemical determinations: total lipids, lipid classes and fatty acids

Total lipid was extracted with chloroform/methanol (2:1 v/v) containing 0.01% of butylated hydroxytoluene (BHT) as antioxidant (Christie 1982). The organic solvent was evaporated under a stream of nitrogen and the lipid content determined gravimetrically.

Lipid classes were separated by one-dimensional double development high-performance thin-layer chromatography (HPTLC) using methyl acetate/isopropanol/chloroform/methanol/0.25% (weight/volume; w/v) KCl (25:25:25:10:9 by volume), as the polar solvent system and hexane/diethyl ether/glacial acetic acid (80:20:2 by volume), as the neutral solvent system. Lipid classes were quantified by charring with a copper acetate reagent

followed by calibrated scanning densitometry using a CAMAG TLC Scanner 3 dual wavelength flying spot scanner (Olsen and Henderson 1989).

TL extracts were subjected to acid-catalyzed transmethylation for 16 h at 50°C, using 1 ml of toluene and 2 ml of 1% sulfuric acid (v/v) in methanol. The resulting fatty acid methyl esters (FAME) were purified by thin-layer chromatography (TLC) and visualized with iodine in chloroform/methanol (2:1 v/v) 98% (v/v) containing 0.01% BHT (Christie 1982). Prior to transmethylation, nonadecanoic acid (19:0) was added to TL as internal standard. FAME were separated and quantified by using a Shimadzu GC-2010 gas chromatograph (GC) equipped with a flame ionization detector (250°C) and a fused silica capillary column RTX-WAXTM (10 m × 0.1 mm I.D.). Helium was used as carrier gas, and the oven initial temperature was 150°C, followed by an increase at a rate of 90°C min^{-1} to a final temperature of 250°C for 3 min. Individual FAME were identified by reference to authentic standards and to a well-characterized fish oil (FAME Mix C4–C24 and Menhaden Oil, SUPELCO, USA).

Total lipid content, neutral lipids (NL), polar lipids (PL) and lipid classes from the mantle and the DG of starving *O. vulgaris* for 27 days were calculated in % dry weight (% DW^{-1}). Fatty acids of total lipid from the mantle and DG of starving octopuses for 27 days was calculated in micrograms of fatty acid per milligram of tissue, mantle or DG and dry weight (μg FA mg DW^{-1}).

BHT, potassium chloride, potassium bicarbonate and iodine were supplied by Sigma Chemical Co. (St. Louis, MO). TLC (20 × 20 cm × 0.25 mm), and plates were purchased from Macherey–Nagel (Düren, Germany). Flat-bottom chamber for 10 × 10 cm plates (with stainless steel lid) and HPTLC (10 × 10 cm × 0.2 mm) plates, pre-coated with silica gel (without fluorescent indicator), were purchased from Merck (Darmstadt, Germany). All organic solvents for GC used were of reagent grade and were purchased from Panreac (Barcelona, Spain).

Statistics

Results are presented as means of triplicates ± SD. All data were checked for normal distribution with the one-sample Kolmogorov–Smirnoff test as well as for homogeneity of the variances with the Levene's test and, when necessary, arcsin transformation was performed. To all data expressed as percentage, arcsin transformation (Fowler et al. 2002) was applied directly. Changes on DGI through days of starvation were analyzed using partial correlation technique. When a normal distribution and/or homogeneity of the variances were not achieved, data were subjected to a Kruskall–Wallis nonparametric one-way ANOVA test based on rank transformation (Zar 1999). In all statistical tests used, $P < 0.05$ was considered statistically different. Differences in lipid composition of the mantle and DG between the sampling days were analyzed with one-way ANOVA's. When differences were found, a Tukey multiple comparison test was performed.

Also, polinomic grade 3 regressions ($y = a + bx + cx^2 + dx^3$; Zar 1999) were used to study total weight loss, variations in water content of both mantle and DG, and the DGI.

Results

A proportional reduction in total weight was recorded of *O. vulgaris* according to starvation time. At the end of the experiment and 27-day starvation *O. vulgaris* lost 35% of total weight ($P < 0.05$; Fig. 1).

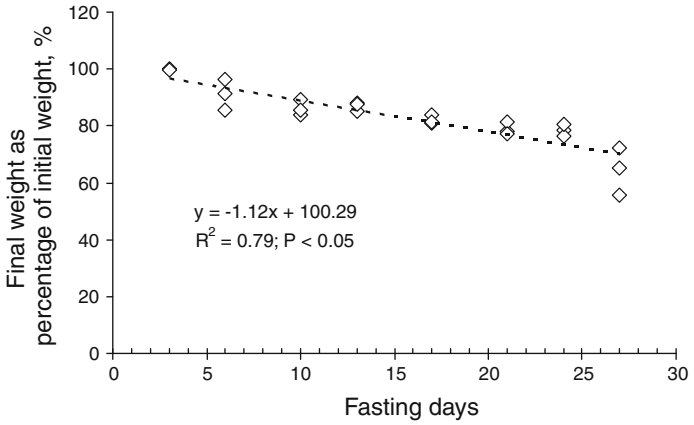

Fig. 1 Changes in living weight of *O. vulgaris* along starvation time (days). Values of final weight as a percentage of initial weight (%)

At the end of the experiment, mortality was of 10%, and the three octopuses died after 16, 20 and 23 days of starvation.

Table 1 shows weight of octopus and DG in gram wet weight (g WW), weight loss of octopus and DG in percentage (%), and DGI (%) during the experiment. After 27 days of starvation, octopuses had lost over 35% of their total weight, and 85% of the DG weight. The DGI showed significant changes after 3 days of starvation (Table 1), decreasing consistently during the 27 days of the starvation period.

Moisture of octopus mantle was similar (~80% WW) throughout the experiment (Table 2; Fig. 2), while moisture of the DG (Table 3) increased from the start (57.7% WW), being higher ($P < 0.05$) after 27 days without eating (76.3% WW). A polinomic grade 3 model of that increment was obtained both for the muscle and the DG moisture changes according to fasting condition (Fig. 3). Muscle and DG moisture changed suddenly at the beginning of the experiment; afterwards, a period of moisture stability was observed between days 10 to 17. After that period, fasting condition provoked a reduction in muscle moisture proportion, while an increment in DG moisture proportion was observed (Fig. 3).

TL from the mantle was different ($P < 0.05$) after 21 days of starvation and decreased from 5.45% DW at the start to 3.45% DW ($P < 0.05$) at the end of the experiment (Table 2). Total neutral lipids (TNL) in the mantle decreased gradually and were consistently significantly lower ($P < 0.05$) after 17 days of starvation. Total polar lipids (TPL) in the mantle only decreased after 21 days of starvation, but after 27 days they were not significantly lower ($P > 0.05$) than the control (Table 2). No lipid classes were significantly ($P > 0.05$) lower in the mantle after 27 days of starvation, compared to the control (Table 2).

TL from the DG decreased consistently from 36.5% DW to 9.4% DW, after 27 days of starvation but was only significantly different ($P < 0.05$) at 27 days of starvation (Table 3). TNL from the DG were only lower compared to the control after 27 days, while TPL were similar ($P > 0.05$) throughout the experiment, with the exception at the third day of starvation, where an important increase in TPL and all lipid classes occurred (Table 3). For the NL, only triacylglycerols (TG) and sterol esters (SE) were lower after 27 days compared to the control (Table 3). It is interesting to note that when not considering the

Table 1 Total weight, DG weight (g), weight loss (%), and digestive gland index (DGI, %) of starving *O. vulgaris* for 27 days

Starvation (d)	0	3	6	10	13	17	21	24	27
Octopus									
W (g)	1386.7 ± 41.8 ab	1664.0 ± 36.8 a	1568.7 ± 104.9 ab	1469.0 ± 276.3 ab	1266.7 ± 116.1 ab	1256.3 ± 221.0 ab	1176.0 ± 88.6 c	1242.7 ± 124.4 ab	1062.0 ± 177.5 d
WL (%)		0.2 ± 0.3	8.8 ± 5.4	13.8 ± 2.8	13.1 ± 1.7	18.0 ± 1.5	21.3 ± 2.2	21.5 ± 2.2	35.7 ± 8.3
DG									
W (g)	104.3 ± 21.6 a	68.6 ± 24.8 b	48.2 ± 6.0 bc	47.4 ± 11.6 bc	38.8 ± 6.6 bc	27.1 ± 13.6 c	20.4 ± 6.3 c	21.2 ± 3.7 c	14.9 ± 3.9 c
WL (%)		34.2 ± 23.7	53.7 ± 5.7	54.6 ± 11.1	62.8 ± 6.3	74.0 ± 13.0	80.4 ± 6.0	79.7 ± 3.5	85.8 ± 3.7
DGI (%)	7.7 ± 2.2 a	4.1 ± 1.1 b	3.1 ± 0.3 bc	3.2 ± 0.2 bc	3.1 ± 0.4 bc	2.1 ± 0.7 bc	1.7 ± 0.6 bc	1.7 ± 0.4 bc	1.4 ± 0.2 c

Data as mean of three octopus ± SD. Letters indicate significant differences ($P < 0.05$)

W weight, *WL* weight loss, *DG* digestive gland, *DGI* digestive gland index

Table 2 Moisture, total lipids, neutral lipids, polar lipids and lipid classes from the mantle of starving *O. vulgaris* for 27 days

Moisture (%)	80.07 ± 0.87	82.51 ± 2.08	82.45 ± 0.92	82.31 ± 0.97	81.84 ± 0.43	81.11 ± 0.55	82.53 ± 0.11	81.04 ± 1.69	80.07 ± 0.85
Starvation (d)	0	3	6	10	13	17	21	24	27
LC mantle (%DW)									
SM	0.04 ± 0.03	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.00	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.00	0.04 ± 0.02	0.05 ± 0.01
PC	0.87 ± 0.30 ab	0.86 ± 0.19 ab	1.05 ± 0.19 a	1.12 ± 0.21 a	0.77 ± 0.10 ab	0.89 ± 0.18 ab	0.62 ± 0.12 ab	0.53 ± 0.11 b	0.70 ± 0.04 ab
PS	0.42 ± 0.18	0.39 ± 0.09	0.42 ± 0.13	0.43 ± 0.06	0.37 ± 0.02	0.47 ± 0.09	0.33 ± 0.10	0.25 ± 0.05	0.37 ± 0.04
PI	0.20 ± 0.12 ab	0.13 ± 0.04 ab	0.17 ± 0.04 ab	0.22 ± 0.06 a	0.11 ± 0.01 ab	0.13 ± 0.06 ab	0.09 ± 0.04 ab	0.05 ± 0.01 b	0.10 ± 0.03 ab
PE	0.78 ± 0.24 ab	0.69 ± 0.15 ab	0.78 ± 0.16 ab	0.83 ± 0.12 a	0.68 ± 0.08 ab	0.78 ± 0.15 ab	0.52 ± 0.12 ab	0.43 ± 0.09 b	0.56 ± 0.04 ab
DAG	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CHO	0.79 ± 0.02 ab	0.85 ± 0.17 ab	0.93 ± 0.15 ab	0.97 ± 0.14 a	0.97 ± 0.15 a	0.98 ± 0.22 a	0.64 ± 0.10 ab	0.55 ± 0.10 b	0.72 ± 0.11 ab
FFA	0.03 ± 0.03 ab	0.04 ± 0.01 ab	0.03 ± 0.02 ab	0.01 ± 0.01 b	0.05 ± 0.02 ab	0.06 ± 0.02 a	0.01 ± 0.01 b	0.00 ± 0.01 b	0.02 ± 0.02 ab
TG	0.90 ± 0.25	0.66 ± 0.63	0.50 ± 0.48	0.32 ± 0.26	0.74 ± 0.35	0.33 ± 0.27	0.28 ± 0.11	0.15 ± 0.11	0.15 ± 0.12
SE	1.21 ± 0.34 a	0.60 ± 0.42 ab	0.65 ± 0.10 ab	**0.40 ± 0.12** b	**0.43 ± 0.19** b	**0.37 ± 0.05** b	**0.41 ± 0.03** b	**0.41 ± 0.07** b	0.56 ± 0.20 ab
UK	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.03	0.01 ± 0.01	0.00 ± 0.00	0.03 ± 0.05	0.06 ± 0.02	0.04 ± 0.01	0.08 ± 0.01
TL	5.24 ± 0.71 a	4.26 ± 1.47 ab	4.59 ± 0.72 ab	4.36 ± 0.31 ab	4.17 ± 0.86 ab	4.10 ± 1.01 ab	**2.99 ± 0.50** b	**2.46 ± 0.26** b	**3.31 ± 0.53** b
TNL	2.93 ± 0.15 a	2.14 ± 1.18 ab	2.11 ± 0.56 ab	**1.70 ± 0.16** bc	2.19 ± 0.69 ab	**1.74 ± 0.55** bc	**1.34 ± 0.23** bc	**1.11 ± 0.13** c	**1.48 ± 0.36** bc
TPL	2.30 ± 0.86 ab	2.11 ± 0.49 ab	2.47 ± 0.53 ab	2.66 ± 0.45 a	1.98 ± 0.20 abc	2.33 ± 0.47 ab	1.59 ± 0.36 bc	**1.31 ± 0.28** c	1.76 ± 0.16 abc

Data as mean of three octopus ± SD. Letters indicate significant differences ($P < 0.05$)

LC lipid classes, *SM* sphingomyelin, *PC* phosphatidylcholine, *PS* phosphatidylserine, *PI* phosphatidylinositol, *PE* phosphatidylethanolamine, *DAG* diacylglycerol, *CHO* cholesterol, *FFA* free fatty acids, *TG* triacylglycerols, *SE* sterol esters, *UK* unknown, *TL* total lipids, *TNL* neutral lipids, *TPL* polar lipids

Values in bold indicate significant differences

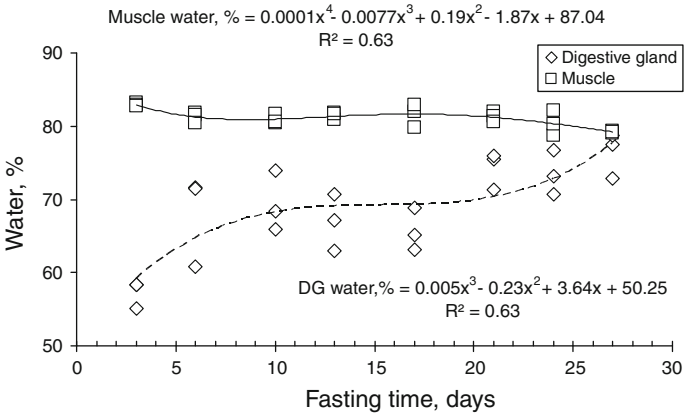

Fig. 2 Body water changes (y) of digestive gland and muscle of starving *O. vulgaris* for 27 days

first sampling period, TG maintained its proportion at the beginning, while after the 21st day it dropped. On the contrary, there was a drop on SE from the start (Table 3).

Generally, the FA in the mantle remained stable throughout the 27 days of starvation (Table 4). Composition was only lower for the 17th day of starvation for pentadecanoic acid (15:0), palmitoleic acid (16:1 *n*-7), docosapentaenoic acid (22:5 *n*-3) and docosahexanoic acid (DHA, 22:6 *n*-3), PUFA, and *n*-3 and *n*-3 HUFA. The arachidonic acid (ARA, 20:4 *n*-6), eicosapentanoic acid (EPA, 20:5 *n*-3) and DHA concentrations, as well as the DHA/EPA ratio remained unchanged throughout the experiment (Table 4).

For the DG, the majority of FA remained stable until 17 days of starvation (Table 5). After that, concentrations of almost all FA decreased or remained stable. Saturates, monoenes and PUFA decreased consistently after 17 days. Only two FA (22:1 *n*-9 and 22:5 *n*-3) decreased significantly ($P < 0.05$) after only 3 days of starvation (Table 5). The ARA concentration remained unchanged throughout the experiment, while DHA decreased gradually but was only significantly different ($P < 0.05$) at 27 days of starvation, while EPA concentrations decreased consistently after 17 days of starvation. The *n*-3 HUFA, *n*-9 and DHA/ARA ratio decreased gradually but were only significantly different ($P < 0.05$) at 21 days of starvation. The DHA/EPA ratio remained unchanged during the 27 days (Table 5).

Discussion

Major changes happened in the DG where the weight loss reached 85% at 27 days of starvation, being significant ($P < 0.05$) even only after 3 days (34%). Starvation period of 27 days provoked a loss of body mass between 28 to 45% of the initial weight, suggesting that animals were using muscle for their metabolic needs.

The DGI decreased consistently during the 27 days of the starvation period (from 7.7 to 1.4%) and showed significant changes even at the first sampling period, only after 3 days of starvation (from 7.7 to 4.1%) (Table 1). Castro et al. (1992) also reported a decrease in the DGI from 7.5% to 4.1% after 53 days of starvation in *S. officinalis*, although this decline was not as pronounced as the one obtained here. Moreover, the weight of the DG was also considerably reduced and the moisture content increased.

Table 3 Moisture, total lipids, neutral lipids, polar lipids and lipid classes from the digestive gland of starving *O. vulgaris* for 27 days

Moisture (%)	57.66 ± 2.29 a	59.82 ± 2.18 a	66.87 ± 9.11 ab	69.01 ± 4.01 ab	66.50 ± 0.93 ac	65.93 ± 2.45 ac	78.60 ± 3.09 b	74.47 ± 3.31 bc	76.30 ± 1.80 bc
Starvation (d)	0	3	6	10	13	17	21	24	27
LC DG (%DW)									
SM	0.03 ± 0.06	0.48 ± 0.09	0.00 ± 0.00	0.02 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.05	0.00 ± 0.00
PC	1.57 ± 0.26 b	**5.06 ± 1.45** a	1.75 ± 0.27 b	1.64 ± 0.46 b	1.45 ± 0.13 b	1.62 ± 0.22 b	1.74 ± 0.98 b	1.35 ± 0.21b	1.07 ± 0.07 b
PS	0.58 ± 0.06 c	**1.14 ± 0.36** a	0.88 ± 0.25 abc	**0.98 ± 0.32** ab	0.61 ± 0.09 bc	0.64 ± 0.15 bc	**1.07 ± 0.38** a	0.82 ± 0.07 abc	0.72 ± 0.11 abc
PI	0.22 ± 0.03	1.50 ± 0.37	0.33 ± 0.07	0.33 ± 0.09	0.22 ± 0.04	0.26 ± 0.05	0.34 ± 0.14	0.29 ± 0.03	0.22 ± 0.02
PE	1.21 ± 0.17 b	**3.33 ± 0.79** a	1.65 ± 0.32 b	1.53 ± 0.34 b	1.17 ± 0.16 b	1.22 ± 0.21 b	1.62 ± 0.78 b	1.28 ± 0.27b	0.90 ± 0.07 b
DAG	0.49 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CHO	3.04 ± 0.61	3.83 ± 1.05	4.17 ± 1.09	3.44 ± 1.02	5.31 ± 0.31	5.34 ± 0.26	3.56 ± 3.12	3.29 ± 1.64	1.41 ± 0.16
FFA	0.52 ± 0.24 bc	0.42 ± 0.09c	0.55 ± 0.15 bc	0.57 ± 0.24 bc	0.82 ± 0.09 ab	**1.02 ± 0.27** a	0.31 ± 0.26 c	0.29 ± 0.14 c	0.25 ± 0.13 c
TG	14.65 ± 3.46 a	**6.81 ± 1.97** bc	11.62 ± 4.18 ab	10.77 ± 4.23 ab	15.89 ± 2.15 a	16.59 ± 3.10 a	**6.21 ± 7.40** bc	**6.09 ± 3.59** bc	**2.54 ± 1.32** c
SE	13.13 ± 2.73 a	**6.30 ± 1.29** c	**7.15 ± 3.77** bc	**5.65 ± 2.04** cd	11.64 ± 0.98 ab	**7.49 ± 1.87** bc	**4.62 ± 4.65** cd	**3.05 ± 1.67** cd	**1.45 ± 0.55** d
UK	0.44 ± 0.12	0.64 ± 0.07	0.02 ± 0.04	0.32 ± 0.56	0.99 ± 0.03	1.63 ± 0.65	1.16 ± 1.17	0.62 ± 0.65	0.28 ± 0.12
TL	35.88 ± 7.64 a	29.61 ± 4.55 ab	28.13 ± 8.43 ab	25.27 ± 8.35 ab	38.15 ± 1.76 a	35.97 ± 1.05 a	20.77 ± 18.69 ab	17.17 ± 7.85 ab	**8.91 ± 2.11** b
TNL	31.83 ± 7.11 a	17.36 ± 1.52 ab	23.48 ± 9.11 ab	20.43 ± 7.33 ab	33.66 ± 2.04 a	30.44 ± 1.84 a	14.70 ± 15.25 ab	12.72 ± 7.04 ab	**5.73 ± 2.03** b
TPL	3.61 ± 0.54 b	**11.60 ± 3.11** a	4.63 ± 0.93 b	4.52 ± 1.22 b	3.50 ± 0.37 b	3.89 ± 0.63 b	4.91 ± 2.37 b	3.84 ± 0.63 b	2.98 ± 0.22 b

Data as mean of three octopus ± SD. Letters indicate significant differences ($P < 0.05$)

LC lipid classes, *SM* sphingomyelin, *PC* phosphatidylcholine, *PS* phosphatidylserine, *PI* phosphatidylinositol, *PE* phosphatidylethanolamine, *DAG* diacylglycerol, *CHO* cholesterol, *FFA* free fatty acids, *TG* triacylglycerols, *SE* sterol esters, *UK* unknown, *TL* total lipids, *TNL* neutral lipids, *TPL* polar lipids

Values in bold indicate significant differences

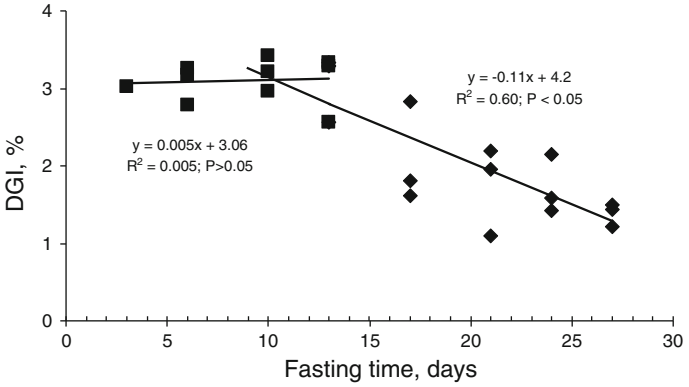

Fig. 3 Digestive gland index changes (y = DGI,%) through the starvation time (x) of *O. vulgaris*

Lipid composition of cephalopods in the mantle is close to 2% (Lee 1994). Both polar and neutral lipid fraction seemed to be equal in mantle content throughout the fasting ($\approx$50% each) (Almansa et al. 2006). Several studies (Sinanoglou and Miniadis-Meimaroglou 1998, 2000; Navarro and Villanueva 2000; Almansa et al. 2006; Ferreira et al. 2009) have shown that mantle of cephalopods is rich in phospholipids, especially phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cholesterol (CHO). Data from the present study confirm this profile for *O. vulgaris*. However, it is interesting to note the high content of mantle SE at the beginning of the experiment. In general, the mantle lipid composition remained stable, and only SE content showed differences ($P < 0.05$) after 10 days of starvation. This reduction in SE could be related to their use as energy source from the mantle of *O. vulgaris*.

The most abundant fatty acids in the mantle of *O. vulgaris* in this study were palmitic acid (16:0), DHA (22:6 *n*-3) and EPA (20:5 *n*-3), similarly to FA composition of the DG. The same fact is reported for mantle of *S. officinalis* by Ferreira et al. (2009) and for the mantle of many other cephalopod species by other authors (Sinanoglou and Miniadis-Meimaroglou 1998; Miliou et al. 2006; Zlatanos et al. 2006).

No important differences in FA and/or FA series were found in the mantle after 27 days of starvation. In this sense, it is interesting to notice that DHA/EPA ratios for this experiment ($\sim$1.5), even after 27 days of starvation, are close to the ones reported for the mantle of *O. vulgaris* by Miliou et al. (2006), between 1.5 and 1.9, Zlatanos et al. (2006) of 1.5, and by Sinanoglou and Miniadis-Meimaroglou (1998) of 1.5. Even when *O. vulgaris* (García et al. 2009) and *S. officinalis* (Ferreira et al. 2009) were in poor nutritional conditions, due to the use of inadequate diets, the ratio DHA/EPA in the mantle was maintained.

In general terms, mantle of cephalopods is characterized by no significant variations, despite the lipid composition of the diet, food ratios or maturation cycle (Moreno et al. 1998; Sinanoglou and Miniadis-Meimaroglou 1998; Moltschaniwskyj and Jackson 2000; Almansa et al. 2006; Ferreira et al. 2009).

DG of cephalopods has high lipid content and lipase activity (Moltschaniwskyj and Johnston 2006) and plays an important role in lipid digestion and metabolism, enzyme secretion, digestion and absorption (Semmens et al. 1995). There is evidence that the DG could be used for short-term lipid storage in some cephalopods (Castro et al. 1992; Fluckiger et al. 2008).

Table 4 Fatty acids of total lipid (μg FA mg DW^{-1}) from the mantle of starving octopuses for 27 days

Starvation (d)	0	3	6	10	13	17	21	24	27
M composition									
14:0	0.29 ± 0.12	0.35 ± 0.12	0.33 ± 0.14	0.29 ± 0.03	0.33 ± 0.07	0.14 ± 0.06	0.19 ± 0.04	0.17 ± 0.04	0.14 ± 0.01
15:0	0.07 ± 0.02 ab	0.10 ± 0.05 ab	0.12 ± 0.02 a	0.10 ± 0.02 ab	0.08 ± 0.01 ab	**0.04 ± 0.02 b**	**0.06 ± 0.01 b**	0.06 ± 0.02 ab	**0.05 ± 0.01 b**
15:1	0.05 ± 0.00	0.07 ± 0.08	0.12 ± 0.03	0.09 ± 0.03	0.09 ± 0.02	0.03 ± 0.03	0.07 ± 0.02	0.07 ± 0.00	0.06 ± 0.02
16:0	3.97 ± 1.70	4.20 ± 0.86	4.37 ± 0.67	4.24 ± 0.37	3.86 ± 0.10	2.20 ± 0.64	2.82 ± 0.66	2.54 ± 0.25	2.59 ± 0.27
16:1 *n*-7	0.18 ± 0.05 ab	0.30 ± 0.20 ab	0.37 ± 0.10 a	0.27 ± 0.03 ab	0.25 ± 0.02 ab	**0.10 ± 0.09 b**	0.17 ± 0.01 ab	0.18 ± 0.04 ab	0.14 ± 0.05 ab
17:0	0.18 ± 0.05	0.18 ± 0.07	0.26 ± 0.06	0.22 ± 0.02	0.18 ± 0.03	0.13 ± 0.04	0.16 ± 0.02	0.16 ± 0.03	0.16 ± 0.04
16:4 *n*-1	0.55 ± 0.14	0.57 ± 0.10	0.60 ± 0.10	0.69 ± 0.08	0.54 ± 0.03	0.39 ± 0.18	0.55 ± 0.14	0.49 ± 0.10	0.56 ± 0.07
18:0	1.18 ± 0.37	0.79 ± 0.15	0.99 ± 0.15	0.96 ± 0.04	0.86 ± 0.05	0.61 ± 0.26	0.84 ± 0.20	0.74 ± 0.15	0.91 ± 0.23
18:1 *n*-9	0.44 ± 0.17	0.41 ± 0.18	0.34 ± 0.11	0.32 ± 0.03	0.32 ± 0.04	0.17 ± 0.08	0.23 ± 0.05	0.21 ± 0.06	0.20 ± 0.02
18:1 *n*-7	0.28 ± 0.10	0.25 ± 0.07	0.30 ± 0.03	0.31 ± 0.15	0.25 ± 0.02	0.12 ± 0.05	0.15 ± 0.03	0.19 ± 0.11	0.19 ± 0.06
18:1 *n*-5	0.07 ± 0.03	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
18:2 *n*-6	0.07 ± 0.02	0.17 ± 0.19	0.19 ± 0.09	0.23 ± 0.15	0.16 ± 0.04	0.08 ± 0.07	0.10 ± 0.03	0.09 ± 0.01	0.10 ± 0.05
20:1 *n*-9	0.56 ± 0.20	0.53 ± 0.16	0.56 ± 0.10	0.53 ± 0.03	0.51 ± 0.04	0.29 ± 0.14	0.38 ± 0.12	0.37 ± 0.09	0.38 ± 0.04
20:2 *n*-6	0.06 ± 0.02	0.05 ± 0.01	0.08 ± 0.02	0.17 ± 0.18	0.08 ± 0.01	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.05 ± 0.03
20:4 *n*-6	0.41 ± 0.06	0.29 ± 0.14	0.35 ± 0.08	0.37 ± 0.08	0.30 ± 0.07	0.17 ± 0.09	0.27 ± 0.04	0.32 ± 0.10	0.30 ± 0.06
20:5 *n*-3	2.37 ± 0.41	2.43 ± 0.49	2.88 ± 0.54	2.73 ± 0.77	2.70 ± 0.43	1.38 ± 0.58	1.68 ± 0.40	1.73 ± 0.23	1.72 ± 0.25
22:1 *n*-9	0.25 ± 0.11	0.18 ± 0.05	0.21 ± 0.02	0.24 ± 0.03	0.22 ± 0.02	0.13 ± 0.06	0.18 ± 0.06	0.18 ± 0.03	0.21 ± 0.01
22:5 *n*-3	0.13 ± 0.02 ab	0.11 ± 0.03 ab	0.18 ± 0.02 a	0.15 ± 0.03 ab	0.11 ± 0.02 ab	**0.07 ± 0.04 b**	0.11 ± 0.02 ab	0.11 ± 0.01 ab	0.11 ± 0.03 ab
22:6 *n*-3	3.22 ± 0.55 ab	3.47 ± 1.02 ab	4.53 ± 1.19 a	3.72 ± 0.90 ab	3.86 ± 0.69 ab	**1.79 ± 0.83 b**	2.31 ± 0.52 ab	2.73 ± 0.64 ab	2.57 ± 0.31 ab
UK	0.56 ± 0.06	1.17 ± 1.02	1.66 ± 0.59	1.34 ± 0.28	0.90 ± 0.23	0.46 ± 0.47	0.84 ± 0.08	0.85 ± 0.28	0.70 ± 0.23
Totals									
Saturates	5.70 ± 2.27	5.63 ± 1.17	6.09 ± 0.78	5.82 ± 0.45	5.32 ± 0.10	3.13 ± 0.99	4.07 ± 0.92	3.69 ± 0.47	3.87 ± 0.54
Monoenes	1.92 ± 0.70	2.00 ± 0.74	2.16 ± 0.23	2.03 ± 0.33	1.85 ± 0.05	0.96 ± 0.50	1.35 ± 0.27	1.34 ± 0.25	1.32 ± 0.11
PUFA	7.19 ± 1.18 a	7.42 ± 1.79 ab	9.09 ± 1.69 a	8.66 ± 2.43 a	8.04 ± 1.13 a	**4.11 ± 1.76 b**	5.37 ± 1.20 ab	5.70 ± 1.06 ab	5.61 ± 0.80 ab

Table 4 continued

Starvation (d)	0	3	6	10	13	17	21	24	27
n-3	5.91 ± 1.04 ab	6.19 ± 1.57 ab	7.77 ± 1.74 a	6.96 ± 1.92 ab	6.83 ± 1.11 ab	**3.37 ± 1.44 b**	4.22 ± 0.98 ab	4.65 ± 0.88 ab	4.54 ± 0.64 ab
n-6	0.63 ± 0.03	0.56 ± 0.17	0.62 ± 0.05	0.82 ± 0.47	0.57 ± 0.07	0.30 ± 0.15	0.46 ± 0.09	0.47 ± 0.15	0.46 ± 0.11
n-9	1.24 ± 0.48	1.12 ± 0.37	1.12 ± 0.22	1.09 ± 0.07	1.05 ± 0.07	0.59 ± 0.28	0.79 ± 0.22	0.76 ± 0.18	0.79 ± 0.06
n-3 HUFA	5.78 ± 0.99 ab	6.05 ± 1.53 ab	7.60 ± 1.72 a	6.72 ± 1.77 ab	6.68 ± 1.12 ab	**3.28 ± 1.39 b**	4.14 ± 0.93 ab	4.57 ± 0.87 ab	4.41 ± 0.58 ab
n-3/*n*-6	9.66 ± 1.75	11.31 ± 2.31	12.61 ± 3.52	9.41 ± 2.73	12.04 ± 1.28	11.90 ± 2.46	9.10 ± 1.33	10.10 ± 1.60	10.14 ± 1.30
DHA/ EPA	1.38 ± 0.03	1.41 ± 0.14	1.56 ± 0.18	1.38 ± 0.17	1.43 ± 0.09	1.28 ± 0.09	1.38 ± 0.04	1.57 ± 0.18	1.50 ± 0.04
EPA/ ARA	5.94 ± 1.65	9.15 ± 2.28	8.40 ± 0.63	7.34 ± 1.13	9.34 ± 2.19	8.99 ± 1.84	6.12 ± 0.69	5.68 ± 1.18	5.79 ± 0.91
DHA/ ARA	8.21 ± 2.05 **a**	**12.68 ± 2.13 bc**	**13.04 ± 0.62 b**	10.03 ± 0.27 ab	**13.24 ± 2.21 b**	11.40 ± 1.81 ab	8.43 ± 0.80 ac	8.86 ± 1.78 ab	8.70 ± 1.56 ab
Mon/*n*-3 H	0.33 ± 0.06	0.33 ± 0.11	0.30 ± 0.08	0.31 ± 0.06	0.28 ± 0.06	0.28 ± 0.08	0.33 ± 0.02	0.30 ± 0.06	0.30 ± 0.02
Mon/ PUFA	0.27 ± 0.05	0.27 ± 0.08	0.24 ± 0.05	0.24 ± 0.05	0.23 ± 0.04	0.23 ± 0.06	0.25 ± 0.02	0.24 ± 0.04	0.24 ± 0.02
Mon/Sat	0.35 ± 0.02	0.35 ± 0.09	0.36 ± 0.06	0.35 ± 0.03	0.35 ± 0.01	0.29 ± 0.09	0.33 ± 0.01	0.36 ± 0.03	0.34 ± 0.04
18:1*n*-9/ *n*-3HUFA	0.08 ± 0.01	0.07 ± 0.03	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.05 ± 0.01

Data as mean of three octopus ± SD. Letters indicate significant differences ($P < 0.05$), and in bold are concentrations that are different from the control

M mantle, *PUFA* polyunsaturated fatty acid, *HUFA* highly unsaturated fatty acid, *DHA* docosahexanoic acid (22:6 *n*-3), *EPA* eicosapentanoic acid (20:5 *n*-3), *ARA* arachidonic acid (22:4 *n*-6), *Mon* monounsaturated fatty acid, *Sat* saturated fatty acid

Table 5 Fatty acids (μgFA mg DW^{-1}) of digestive glands of starving octopuses for 27 days

Starvation (d)	0	3	6	10	13	17	21	24	27
DG composition									
14:0	9.08 ± 1.80 a	6.11 ± 1.68 ab	5.08 ± 2.86 ab	**3.80 ± 1.76 b**	**3.44 ± 2.16 b**	5.29 ± 1.34 ab	**2.52 ± 1.24 b**	**1.91 ± 1.05 b**	**0.65 ± 0.42 b**
15:0	1.35 ± 0.26 a	0.55 ± 0.49 ab	0.66 ± 0.31 ab	0.68 ± 0.47 ab	0.69 ± 0.37 ab	0.99 ± 0.17 ab	0.60 ± 0.43 ab	0.44 ± 0.29 ab	**0.10 ± 0.07 b**
15:1	0.73 ± 0.06	0.86 ± 1.19	0.27 ± 0.05	0.57 ± 0.45	0.67 ± 0.20	1.04 ± 0.73	0.65 ± 0.57	0.51 ± 0.51	0.05 ± 0.03
16:0	48.70 ± 8.32 a	40.80 ± 9.45 ab	34.47 ± 15.24 ab	25.42 ± 4.78 abc	**20.56 ± 13.85 bc**	29.67 ± 9.43 abc	**17.95 ± 5.83 bc**	**11.91 ± 4.71 c**	**5.74 ± 1.63 c**
16:1 *n*-7	4.78 ± 1.28	2.44 ± 0.86	1.55 ± 1.31	1.78 ± 1.64	1.87 ± 0.70	2.71 ± 1.07	1.66 ± 1.15	1.14 ± 0.88	0.26 ± 0.13
17:0	1.37 ± 0.18 ab	1.79 ± 0.29 a	1.19 ± 0.78 ab	1.02 ± 0.34 ab	0.85 ± 0.42 ab	1.36 ± 0.20 ab	1.06 ± 0.46 ab	**0.59 ± 0.33 b**	**0.37 ± 0.02 b**
16:4 *n*-1	1.38 ± 0.16 ab	0.64 ± 0.38 a	1.27 ± 0.43 ab	1.41 ± 0.21 ab	1.13 ± 0.48 ab	1.17 ± 0.26 ab	**2.17 ± 0.71 b**	1.38 ± 0.18 ab	0.99 ± 0.19 ab
18:0	9.21 ± 1.26 a	6.68 ± 1.49 ab	6.89 ± 0.74 ab	5.88 ± 0.29 ac	**4.31 ± 2.77 bc**	6.77 ± 0.98 ab	6.03 ± 2.38 ac	4.24 ± 0.86 ac	**2.33 ± 0.10 c**
18:1 *n*-9	9.45 ± 1.55 a	5.94 ± 1.47 a	4.10 ± 2.80 ab	3.17 ± 2.02 ac	2.73 ± 1.69 ac	3.87 ± 0.43 ab	**2.01 ± 1.21 bc**	**1.35 ± 0.78 bc**	**0.43 ± 0.29 c**
18:1 *n*-7	5.56 ± 1.02 a	4.12 ± 0.98 ab	3.12 ± 1.70 ab	**2.58 ± 1.52 b**	**2.01 ± 1.01 b**	2.71 ± 0.48 ab	**1.38 ± 0.67 b**	**1.09 ± 0.38 b**	**0.30 ± 0.13 b**
18:1 *n*-5	1.08 ± 0.19 a	0.75 ± 0.17 ab	0.76 ± 0.24 ab	**0.49 ± 0.07 bc**	**0.40 ± 0.32 bc**	0.67 ± 0.16 ab	**0.38 ± 0.15 bc**	**0.29 ± 0.12 bc**	**0.12 ± 0.05 c**
18:2 *n*-6	1.09 ± 0.24	0.73 ± 0.35	0.54 ± 0.41	0.72 ± 0.85	0.66 ± 0.05	0.86 ± 0.55	0.60 ± 0.54	0.56 ± 0.32	0.04 ± 0.04
20:1 *n*-9	11.71 ± 1.37 a	7.17 ± 1.89 ab	5.34 ± 2.58 ab	**2.93 ± 2.61 bc**	4.45 ± 3.17 ac	5.26 ± 0.83 ab	**2.50 ± 1.67 bc**	**1.70 ± 1.17 bc**	**0.57 ± 0.23 c**
20:2 *n*-6	1.30 ± 0.30	0.69 ± 0.14	0.71 ± 0.17	0.55 ± 0.61	0.59 ± 0.19	0.72 ± 0.14	0.37 ± 0.16	0.38 ± 0.12	0.07 ± 0.03
20:4 *n*-6	3.46 ± 0.83	2.04 ± 0.37	2.80 ± 0.54	3.15 ± 0.60	2.12 ± 1.38	4.46 ± 1.17	4.19 ± 2.00	3.61 ± 0.97	1.86 ± 0.37
20:5 *n*-3	34.19 ± 7.36 a	22.33 ± 4.42 ab	21.45 ± 5.89 ab	18.82 ± 6.16 ac	**15.33 ± 9.26 bc**	25.58 ± 5.73 ab	**11.88 ± 4.88 bc**	**9.38 ± 4.73 bc**	**3.23 ± 1.42 c**
22:1 *n*-9	2.23 ± 0.39 a	**1.10 ± 0.29 bc**	**1.13 ± 0.34 bc**	**0.93 ± 0.21 bc**	**0.95 ± 0.59 bc**	1.29 ± 0.13 ab	**0.78 ± 0.46 bc**	**0.52 ± 0.21 bc**	**0.19 ± 0.04 c**
22:5 *n*-3	1.47 ± 0.26 a	**0.59 ± 0.20 bc**	0.80 ± 0.18 ab	**0.73 ± 0.23 bc**	**0.55 ± 0.27 bc**	**0.77 ± 0.08 b**	**0.35 ± 0.09 cd**	**0.27 ± 0.09 cd**	**0.10 ± 0.01 d**
22:6 *n*-3	67.67 ± 16.17 a	43.19 ± 6.23 a	43.02 ± 8.68 a	46.28 ± 12.69 a	31.99 ± 19.00 ab	59.09 ± 15.90 a	29.94 ± 14.83 ab	22.93 ± 12.37 ab	**7.96 ± 4.92 b**
UK	6.41 ± 1.23	6.58 ± 3.57	3.76 ± 2.73	4.66 ± 3.05	5.07 ± 1.75	8.94 ± 4.86	5.96 ± 4.46	4.08 ± 4.17	1.26 ± 0.25
Totals									
Saturates	69.93 ± 11.87 a	55.94 ± 12.77 ab	48.36 ± 19.70 ab	37.92 ± 6.68 ac	**29.85 ± 19.56 bc**	44.11 ± 11.52 ac	**28.23 ± 10.37 bc**	**19.12 ± 7.12 bc**	**9.19 ± 2.20 c**
Monoenes	37.58 ± 6.01 a	23.36 ± 5.64 ab	17.13 ± 8.71 ab	13.21 ± 8.93 ac	13.97 ± 8.13 ab	19.03 ± 2.60 ab	**10.40 ± 6.61 bc**	**7.17 ± 4.06 bc**	**2.09 ± 0.86 c**
PUFA	118.64 ± 27.31 a	75.05 ± 12.69 ab	74.70 ± 17.47 ab	75.51 ± 20.52 ab	**54.73 ± 32.71 bc**	96.45 ± 22.26 ab	**52.00 ± 24.45 bc**	**40.32 ± 19.16 bc**	**15.42 ± 6.94 c**
n-3	108.13 ± 24.99 a	68.40 ± 11.50 ab	66.76 ± 14.91 ab	67.56 ± 19.05 ab	**48.87 ± 29.44 b**	87.05 ± 21.38 ab	**42.99 ± 20.31 b**	**33.28 ± 17.46 b**	**11.49 ± 6.33 c**

Table 5 continued

Starvation (d)	0	3	6	10	13	17	21	24	27
n-6	6.61 ± 1.62	4.50 ± 0.97	5.03 ± 1.92	5.17 ± 1.62	3.61 ± 1.86	6.53 ± 0.91	5.25 ± 2.62	4.69 ± 1.44	2.26 ± 0.50
n-9	23.38 ± 3.26 a	14.21 ± 3.64 ab	10.57 ± 5.48 ab	7.03 ± 4.71 ac	8.13 ± 5.45 ac	10.41 ± 1.06 ab	**5.29 ± 3.34 bc**	**3.57 ± 2.12 bc**	**1.19 ± 0.49 c**
n-3 HUFA	104.46 ± 23.91 a	66.44 ± 10.94 ab	65.55 ± 14.41 ab	66.27 ± 18.38 ab	**48.00 ± 28.65 bc**	85.74 ± 21.26 ab	**42.34 ± 19.90 bc**	**32.70 ± 17.19 bc**	**11.35 ± 6.30 c**
n-3/*n*-6	16.39 ± 0.48 a	15.32 ± 0.83 a	13.81 ± 2.24 ab	13.26 ± 2.73 ab	13.01 ± 1.67 ab	13.39 ± 2.90 ab	**8.28 ± 0.29 c**	**6.83 ± 2.17 bc**	**4.86 ± 1.66 c**
DHA/EPA	1.97 ± 0.06	1.95 ± 0.13	2.03 ± 0.15	2.50 ± 0.46	2.10 ± 0.03	2.31 ± 0.25	2.45 ± 0.26	2.46 ± 0.27	2.34 ± 0.42
EPA/ARA	9.93 ± 0.34 a	10.96 ± 1.45 a	7.79 ± 2.23 a	5.88 ± 0.84 a	7.49 ± 0.64 a	5.91 ± 1.70 a	**2.91 ± 0.25 b**	**2.51 ± 0.91 bc**	**1.70 ± 0.45 c**
DHA/ARA	19.59 ± 0.10 ab	**21.34 ± 2.81 b**	15.61 ± 3.34 ab	14.57 ± 2.16 a	15.75 ± 1.61 ab	13.40 ± 2.54 a	**7.09 ± 0.18 c**	**6.07 ± 1.98 c**	**4.09 ± 1.78 c**
Mon/*n*-3 H	0.36 ± 0.03	0.35 ± 0.03	0.25 ± 0.07	0.19 ± 0.09	0.29 ± 0.01	0.23 ± 0.08	0.23 ± 0.05	0.21 ± 0.04	0.20 ± 0.06
Mon/PUFA	0.32 ± 0.02 a	0.31 ± 0.02 a	0.22 ± 0.06 ab	**0.17 ± 0.08 b**	0.26 ± 0.01 ab	0.21 ± 0.06 ab	**0.19 ± 0.04 b**	**0.17 ± 0.04 b**	**0.14 ± 0.03 b**
Mon/Sat	0.54 ± 0.01	0.42 ± 0.03	0.35 ± 0.03	0.33 ± 0.16	0.49 ± 0.06	0.46 ± 0.16	0.34 ± 0.12	0.35 ± 0.10	0.23 ± 0.06
18:1*n*-9/*n*-3HUFA	0.09 ± 0.01 a	0.09 ± 0.01 a	0.06 ± 0.03 ab	**0.05 ± 0.02 b**	0.06 ± 0.00 ab	**0.05 ± 0.01 b**	**0.04 ± 0.01 b**	**0.04 ± 0.01 b**	**0.04 ± 0.01 b**

Data as mean of three octopus ± SD. Letters indicate significant differences ($P < 0.05$), and in bold are concentrations that are different from the control

DG digestive gland, *PUFA* polyunsaturated fatty acid, *HUFA* highly unsaturated fatty acid, *DHA* docosahexanoic acid (22:6 *n*-3), *EPA* eicosapentanoic acid (20:5 *n*-3), *ARA* arachidonic acid (22:4 *n*-6), *Mon* monounsaturated fatty acid, *Sat* saturated fatty acid

Several studies (Fluckiger et al. 2008; Ferreira et al. 2009) have shown that the European cuttlefish DG is rich in PL (especially PC and PE according to Ferreira et al. (2009)) and NL, especially TG and SE (and in CHO by Ferreira et al. (2009)). The present work confirms that *O. vulgaris* DG displays a similar NL and PL pattern (Table 3). Also, in underfed conditions for *S. officinalis* (Ferreira et al. 2009) or starvation (present work), the levels of NL, specifically TG and SE, decreased significantly ($P < 0.05$).

Total lipids and lipid classes, excepting SE, showed no significant variations in the DG during the first 10 days, and composition of LC in % of total lipids was also maintained during this period. All this suggests that there was a regulated initial stage of lipid mobilization during the first 10 days of starvation, and compensatory mechanisms could be acting to maintain the physiological functions of the digestive gland, reflecting the adaptation of *O. vulgaris* to tolerate short-term starvation periods. After this period of time, lipids in the DG were more actively used.

The lipid decrease detected in this study was due to a decrease in NL fractions, mainly TG and SE. This suggests that SE and TG could be involved in lipid mobilization for energy purposes. It is known that TG may have a dual application: a metabolic, where it can be used as source for ATP production through oxidation, and/or structural, where it can be a source of FA for PL biosynthesis (Sargent et al. 1995). In this sense, it is interesting to note that all PL classes increased significantly after 3 days of starvation in the DG of *O. vulgaris* and TG decreased significantly during the same period. This peak could be associated to a possible biosynthesis of polar lipids from FA resultant from the energetic metabolism of TG during the early stages of starvation or to the genetic variability associated to living organism. Castro et al. (1992) also observed a strong variation in lipid classes from cuttlefish starved for 2 days and indicates as one of the possible explanations for this fact the high degree of variability among individual cephalopods (Clarke et al. 1989). The existence of synthesis or transport of lipids from other tissues other than the mantle and subsequent deposition in the gland during the early stages of starvation has been reported for cephalopods (Heras and Pollero 1989, 1990).

On the other hand, PL concentrations in the DG remained unchanged throughout the remaining 27 days of starvation. The more stable characteristic of polar lipids, since they are structural parts of membranes, could explain their unchanged concentrations in the DG throughout the 27 days of starvation.

The most abundant fatty acids in the DG of *O. vulgaris* in this study were DHA (22:6 *n*-3), palmitic acid (16:0) and EPA (20:5 *n*-3). These were also the three FA that were mostly mobilized during the starvation period and should be considered the most important FA in the DG of *O. vulgaris*. The same fact is reported for *O. vulgaris* (García et al. 2009) and *S. officinalis* (Ferreira et al. 2009).

Monoenes are a common energy substrate in marine species (Sargent et al. 1995). During this study, monoenes were used during starvation by octopuses, and a preferential use of saturates and monoenes (mainly) was detected after 21 days of starvation ($P < 0.05$). This can be expected because of the bigger energetic yield obtained through oxidation of monoenes and saturated fractions, the fact that many PUFA are essential and because of the higher levels of PUFA in the structural lipids. As in the present experiment, the DG of cuttlefish fed artificial diets (and in poor nutritional condition) showed lower contents of monoenes, saturates and PUFA, compared to natural diets (Ferreira et al. 2009). This was also reported for the squid *Loligo vulgaris* and *O. vulgaris* (Zlatanos et al. 2006), and the use of monoenes from the DG during long-term starvation periods in *S. officinalis* were also reported by Castro et al. (1992).

Like in other marine species, fatty acids of the *n*-6 series are minor components of TL composition, in contrast with those of the *n*-3 series (Sargent et al. 1995). However, it is noteworthy that no significant differences in levels of ARA in DG were detected in the present study, although differences were detected in other essential fatty acids as EPA and DHA; hence, the gradual decrease in the EPA/ARA ratio and DHA/ARA ratio observed. This could be due either to the maintenance of this FA or a possible ARA biosynthesis. In this sense, results on the fatty acid profile of *O vulgaris* paralarvae (Navarro and Villanueva 2000; Miliou et al. 2006) showed that ARA, 18:2 *n*-6 and other *n*-6 intermediate metabolites present surprizingly high values for a marine species. From these and other findings in other marine molluscs (Uki et al. 1986; Dunstan et al. 1996; Durazo-Beltrán et al. 2003), it is suggested that 20:4 *n*-6 might not be essential since ability for enzymatic bioconversion of C18 precursors to 20:4 *n*-6 could be present in this species. Moreover, Almansa et al. (2006) and Miliou et al. (2006) suggested a *n*-6 HUFA metabolism in cephalopods.

ARA is the primary precursor of eicosanoids in mammals and fish, competing with EPA in this role and is released from membrane phospholipids in response to several stimuli during "arachidonic acid cascade" (Miliou et al. 2006). Eicosanoids have a wide range of physiological actions, for example, in blood clotting, the immune response, the inflammatory response, cardiovascular tone, renal function, neural function and reproduction (Tocher 2003). Therefore, the scarce differences observed in ARA content during the present study could be associated with the important metabolic functions that this fatty acid has, and/or with the maintenance of cell membrane structure and function, as has been suggested by Miliou et al. (2006). All this suggests that ARA is an important fatty acid to be considered in *O. vulgaris* physiology.

No differences were detected in DHA/EPA ratio in the DG during the 27 days of fasting, suggesting that there was no preferential use in the DG of either during fasting or that a balance was tried to be maintained while using both. Similarly, Navarro and Villanueva (2003) reported the decrease in PUFA and particularly DHA in *O. vulgaris* paralarvae fed inadequate diets. On the contrary, Ferreira et al. (2009) reported different ratios in the DG of *S. officinalis*, which were much lower (<0.5), and in this case with significant differences between animals fed crustaceans (between 0.2 and 0.5) and artificial diets, which promote negative growth and poor condition (between 0.9 and 1.0). This author also reported higher ratios when feeding sardine (1.2). Fluckiger et al. (2008) reported lower ratios DHA/EPA in the DG of *S. officinalis*, compared to the present study, when feeding crustaceans (between 0.3 and 0.6) and higher ratios when feeding fish (between 1.5 and 1.8). Castro et al. (1992) also reported lower ratios DHA/EPA in the DG of this cephalopod species (<0.4). These findings may indicate that different routes of utilization of EPA and DHA between these two cephalopod species indicating that dietary requirements and diet formulation may require different approaches for both species.

DHA plays a multifunctional role in cell membrane physiology (Almansa et al. 2003; Horrocks and Farooqui 2004). On the other hand, EPA is one of the most important eicosanoids precursors, which are implicated in numerous physiological processes (Sargent et al. 1995). The importance of EPA and DHA for cephalopod juvenile nutrition, in this case *S. officinalis*, was also reported by Perrin et al. (2004).

In conclusion, mantle lipid stability should be beneficial form the consumer point of view. Also, results indicate that the DG could be used as an indicator of the nutritional status of the animal. In this case, levels of SE could be used to determine situations of short-term starvation or underfeeding, while low levels of TG and SE could indicate large periods of starvation or inadequate feeding. Although levels of ARA are low in the DG,

this appears to be an important FA in *O. vulgaris* lipid metabolism. Results also suggest a possible different metabolic route of the use of DHA and EPA in the DG between cephalopods, as *O. vulgaris* studied here, and *S. officinalis*. Similar Studies regarding protein and amino acid utilization in the mantle and DG during starvation (due to their important in cephalopod metabolism) should also be conducted, to better comprehend cephalopod general metabolism.

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