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GROWTH, PARTIAL ENERGY BALANCE, MANTLE AND DIGESTIVE
GLAND LIPID COMPOSITION OF *Octopus vulgaris* (Cuvier, 1797) FED WITH
TWO ARTIFICIAL DIETS.

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Abstract

Effects of two binders (gelatine and alginate), were tested on growth, survival, partial energy balance and lipid composition of mantle and digestive gland of *Octopus vulgaris*. Octopus average weight was of 713.0 ± 127.9 g at the start of the experiment. The three diets tested (10 octopuses per diet) were: 1) CON, (*Loligo gahi*) as a control diet, 2) GEL, composed of squid paste (*L. gahi*) (30%), fish hydrolyse CPSP® (10%), and high quality fish meal (50%), agglutinated with 10% of gelatine and 3) ALG, composed of squid paste (*L. gahi*) (30%), fish hydrolyse CPSP® (10%), and high quality fish meal (50%), all agglutinated with 10% of alginate. Growth rates were 1.4 ± 0.2 , 0.2 ± 0.2 and -0.2 ± 0.2 % bw d⁻¹, for octopuses fed CON, GEL and ALG diets, respectively, and were higher when fed CON, compared to the two artificial diets. The GEL diet promoted higher growth and none of the artificial diets tested promoted significant growth. The digestive gland of octopuses had higher concentrations of fatty acids than the mantle. Fatty acid composition of the mantle was similar between CON and the two artificial diets. Digestive gland of animals fed CON had higher concentrations of fatty acids, than those fed the artificial diets.

Keywords: Binders, lipids, nitrogen excretion, octopus, oxygen consumption.

Introduction

Octopus vulgaris presents high growth rates (Aguado & Garcia-Garcia, 2003; Iglesias *et al.*, 2006; 2007), high market price (Garcia-Garcia *et al.*, 2004) and great demand throughout many regions of the world, which makes

1
2 it a potential species for aquaculture diversification. The fattening of this
3 species is presently done in Galicia, North Spain (Iglesias *et al.*, 1997), with
4 low profitability, due to the absence of an artificial diet, among other aspects
5 (Garcia-Garcia *et al.*, 2004). In fact, the two major bottlenecks for the
6 commercial aquaculture of this species are the high mortality in the paralarvae
7 stage (Iglesias *et al.*, 2006) and the inexistence of adequate artificial feeds
8 (Domingues *et al.*, 2005; 2006; 2007, Rosas *et al.*, 2007).

9
10 Research on artificial diets for cephalopods initiated in the early 90's
11 (Castro, 1991; Lee *et al.*, 1991; Domingues, 1999). Both moist or dry pellets
12 (Castro, 1991; Lee *et al.*, 1991; Castro *et al.*, 1993; Castro & Lee, 1994) or
13 surimi, a fish myofibrillar protein concentrate (Castro *et al.*, 1993; Castro &
14 Lee, 1994; Domingues, 1999; Domingues *et al.*, 2005), were initially used, with
15 poor results (Castro *et al.*, 1993; Castro & Lee, 1994; Domingues *et al.*, 2005;
16 2006). Recently, either *O. vulgaris* (Cerezo-Valverde *et al.*, 2008; Quintana *et*
17 *al.*, 2008) or the Yucatan octopus, *Octopus maya* (Domingues *et al.*, 2007;
18 Rosas *et al.*, 2007), have been used as research animals to understand the
19 metabolic requirements in order to develop artificial diets.

20
21 The use of binders as stabilizers is common practice in aquaculture. Corn
22 and wheat starch, arabic gum, polymers and cellulose, among others, are
23 used as binders (Yamamoto & Akiyama, 1995). Alginate concentration in diets
24 can alter faeces hardness and reduce pollution (Cho & Bureau, 2001; Brinker
25 *et al.*, 2005), while its concentration in diets also affects nutrient digestibility
26 (Morales *et al.*, 1993; Arzel *et al.*, 1995). A negative effect of this binder has
27 been reported for sea bass (*Dicentrarchus labrax*), when inclusions higher
28 than 15% were used (Gabaudan, 1987).

29
30 Cerezo-Valverde *et al.*, (2008) used gelatine and alginate as agglutinants
31 of bogue (*Boops boops*) and shrimp, to prepare diets for *O. vulgaris*, which
32 promoted negative or moderate growth. However, an artificial wet diet based
33 on crab muscle tissue agglutinated with gelatine was fed to *O. maya*,
34 delivering adequate growth and survival, compared to crab muscle tissue that
35 is commonly used as a control diet (Rosas *et al.*, 2007; Quintana & Rosas.,
36 2007); diets agglutinated with alginate promoted moderate growth for juveniles
37 of the same species (Aguila *et al.*, 2007).

Energetics can be defined as the quantification of the exchange and transformations of energy and matter between living organisms and their environment (Lucas, 1993). When applied in nutritional studies, energetics helps to understand how nutrients modulate the physiological mechanisms related with the transformation of food energy into biomass. The basic equation is $I=H+U+R+P$ where I is the ingested energy, H the energy lost in faeces, U the energy lost in nitrogen metabolism, R the energy invested in respiratory metabolism and P is the energy invested in production of biomass or gametes. In a practical sense, this equation can provide data regarding how much energy in feeds is necessary for maximum growth rate and how the different proportions of nutrients are used as a source of metabolic energy for growth, or lost as waste products. For several cephalopods, including *Pareledone charcoti* (Daly & Peck, 2000), *Octopus vulgaris* (Petza *et al.*, 2006), *Enteroctopus megalocyathus* (Pérez *et al.*, 2006; Farias *et al.*, 2009), and *Octopus maya* (Rosas *et al.*, 2007; Farias *et al.*, 2009) from total ingested energy (100%), U ranged from 2 to 14% and R between 23 and 68%. Bioenergetic models are commonly used to estimate growth or consumption in aquatic animals and are very useful to study how types of food modulate the destination of ingested energy. In fact, energetic models allow us to estimate food digestibility, important data for balanced food designs (Lucas, 1993). In aquatic animals, the use of energy from food changes with body weight, mainly because nutritional requirements change during different phases of the life cycle and are also modulated by temperature (Katsanevakis *et al.*, 2005; André *et al.*, 2009; Farias *et al.*, 2009). From an aquaculture point of view, energetic models can be used to calculate the required supply of energy (E) as a function of body mass (B). To make an adequate calculation of that value in cephalopods it is necessary to take into account that part of the ingested energy is lost through feces, just before food enters into the hepatopancreas to be digested (Boucher-Rodoni *et al.*, 1987; Lucas, 1993). The differences between energy intake rate (I) and energy lost in faeces (H) is defined as energy absorbed and include respiratory metabolism (R) and biomass production (P). In that sense, E(B) can be calculated as $Ab(B) - R(B) - P(B)$, where E(B) must remain positive in order for an individual to survive. The use of this model to test diets for cephalopods could

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be very useful because through E(B) it is possible to integrate the relationship between the digestive and energetic properties of the food and the energetic demands for metabolism and biomass production (André *et al.*, 2009).

The digestive gland (DG) lipid content is considerably higher when compared to the muscle or flesh (Semmens, 1998). This organ plays an important role in lipid digestion and metabolism in cephalopods (Semmens *et al.*, 1995), and there is evidence that it is not used for long term lipid storage (Semmens, 1998; Fluckiger *et al.*, 2008). This organ is also considered to be a good indicator of nutritional status, and is a good candidate to study the effects of different diets in lipid composition of cephalopods, while the mantle is not affected in such magnitude when animals are fed diets that do not fulfill their nutritional requirements. Furthermore, recent studies indicate that fatty acids (FA) play a critical role on nutrition of *Octopus vulgaris* both during paralarval stages and during growth of juveniles with emphasis in DHA/EPA proportion (docosahexanoic acid (22:6 n-3); EPA, eicosapentanoic acid (20:5 n-3)) as a key factor to explain differences in survival and growth (Navarro & Villanueva, 2003; Miliou *et al.*, 2005; Okumura *et al.*, 2005) .

The objective of this research was to determine the effects of two binders (gelatine and alginate) on growth, partial energy balance and lipid composition of the mantle and the digestive gland of *O. vulgaris* fed each diet. Frozen squid were used as a control diet, since it has delivered good growth for this species, from previous research (Quintana *et al.*, 2008).

Material and methods

Artisan bottom trawl nets were used to capture juvenile octopuses (<1 kg) in coastal waters of Huelva (South Spain). These were immediately brought to the research facility (Centro IFAPA Agua del pino, Cartaya, Spain). Thirty animals were randomly distributed in 30 cylindrical tanks of 80 L each. Octopuses were fed with squid at 10% (wet weight of food/wet weight of the animal) (% bw d⁻¹) once a day at 09:00 h. The three diets tested were: 1) CON, squid (*Loligo gahi*) as a control diet, 2) GEL, composed of squid paste (*Loligo gahi*) (30%), fish hydrolyse CPSP® (10%), and high quality fish flour

(50%), agglutinated with 10% of gelatine and 3) ALG, composed of squid paste (*L. gahi*) (30%), fish hydrolyse CPSP® (10%), and high quality fish flour (50%), all agglutinated with 10% of alginate.

The high quality fish flower was purchased from PIENSOS MAQUEDA S.L. (Seville, Spain), fish hydrolise (CPSP®) was supplied by Sopropeche, (France), while gelatine (Aditio 80-100 Blooms) was purchased from Panreac® (Spain), and alginate (Pokel Merl® and Pokel Cal®) was purchased from Prosur (Murcia, Spain). There were no differences ($p>0.05$) in average weights of octopuses fed CON, and the GEL and ALG diets (713.0 ± 92.9 g, 708.3 ± 152.3 g and 717.8 ± 144.3 g, respectively) at the start of the experiment. The uneaten remains and faeces from each tank were removed, separated and dried every morning, before feeding. Octopuses were weighted individually every ten days; for every weighing interval, data was used to calculate: 1) Instantaneous growth rate (IGR) ($\%bw\ d^{-1}$) = $((\ln W_2 - \ln W_1)/t) \times 100$, where W_2 and W_1 are the final and initial weight of the octopuses, respectively, $\ln$ the natural logarithm, t the number of days of the experimental period and $\%bw\ d^{-1}$ percentage of the body weigh per day, 2) Feeding rate (FR) ($\%bw\ d^{-1}$) = $((FI/Average\ W(t)) \times 100$, where FI is the food ingested and $Average\ W(t)$ is the average weight of the octopus during that period, and 3) Food conversion (FC) = $(W_2 - W_1)/FI$, where $W_2 - W_1$ is the weight gained by the octopus during that period of time.

Water temperature was 15 ± 1 °C, with the natural photoperiod between February and March of 2008. Water flow, of $25\ L\ h^{-1}$, was adjusted in all tanks to maintain oxygen concentration close to saturation levels. The experiment lasted for 30 days. Afterwards, three octopuses fed each diet were placed in respiratory chambers, in order to determine oxygen consumption and nitrogen excretion. At the end of these trials, samples of mantle and DG of 3 octopuses fed each diet were collected in order to calculate lipid composition.

Partial energy balance

The energy balance equation that represents the energy flow through *O. vulgaris* is $I = P + R_{tot} + U_{tot} + F$ where I is the total ingested energy, P is the energy equivalents of somatic and gonadal growth respectively, R_{tot} is the energy invested in respiration, U_{tot} is the energy lost as nitrogenous and other

waste components excreted and F is the energy unabsorbed voided with the faeces (Lucas, 1993). Total ingested energy was calculated as $I = IR \times EFC$, where IR is the ingestion rate $\text{g day}^{-1} \text{Kg}^{-1}$ of animal and EFC is the energy food content (Kjoules g^{-1}). The absorbed energy (Ab) was obtained by calculation of the absorbed efficiency (Ab , %) defined as: $AE = [(I' - F') / (1 - F')I'] \times 100$ (Condrey *et al.*, 1972), where I' is the ratio of the ash free dry weight (afdwt) to the dry weight (dw) of the food and F' is the ratio of the afdwt to dw of the faeces. The dw and afdwt of food and faeces were obtained by placing food and faeces samples at 60°C until constant weight and by placing samples in a muffle furnace at 500°C for 4h, respectively. The energy absorbed was calculated as: $Ab = I \times AE$.

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Energy produced (P) was calculated using the actual growth rate of the octopus obtained during experimental time. The value of $18.8 \pm 0.72 \text{ Kj g}^{-1} \text{ dw}$ was used to transform the growth data into production units (P ; $\text{Kj g}^{-1} \text{ dw d}^{-1}$). This value was obtained from analyzing energy content applied to 10 whole animals by means of a calorimeter (Parr®), previously calibrated with benzoic acid.

The oxygen consumption rate and ammonia excretion (mg h^{-1}) were measured in 9 fasting octopuses, at the end of the experiment. Oxygen consumption and ammonia excretion were determined individually by a continuous flow metabolic chamber connected to a flow through system. Measurements were done during 9 hours between 8:30 and 17:30 h. A mean oxygen consumption value was calculated as $VO_2 = O_{2e} - O_{2ex} \times Fr$, where VO_2 is oxygen consumption ($\text{mg O}_2 \text{ h}^{-1} \text{ animal}^{-1}$), O_{2e} indicates oxygen concentration at entrance to the chamber (mg l^{-1}), O_{2ex} is oxygen concentration at exit (mg l^{-1}), and Fr is the flow rate (L hr^{-1}). Oxygen concentration was measured using a digital oxymeter (YSI 50B digital, USA) with a polarographic sensor ($\pm 0.01 \text{ mg l}^{-1}$), previously calibrated with oxygen-saturated seawater at 15°C . Ammonia excretion was calculated as $N\text{-NH}_3 = NH_{3ex} - NH_{3e} \times Fr$, where $N\text{-NH}_3$ indicates ammonia excretion ($\text{mg N-NH}_3 \text{ h}^{-1} \text{ animal}^{-1}$), NH_{3ex} concentration at the exit of the chamber (mg l^{-1}), NH_{3e} concentration at the entrance of the chamber (mg l^{-1}) Fr is the flow rate (L hr^{-1}). A 14.3 and 20.5 Kj mg^{-1} conversion factors of oxygen consumption and

ammonia excretion were used to transform unfed and fed VO_2 and $N-NH_3$ values into energetic units ($Kj\ h^{-1}\ animal^{-1}$) R and U, respectively (Lucas 1993). Taking into consideration the octopus weight R) and U were expressed as $Kj\ Kg^{-1}\ d^{-1}$ wet weight (ww) Recorded values of oxygen consumption and ammonia excretion were transformed to $\mu g-At\ Kg^{-1}\ h^{-1}$ and the atomic O:N ratio was estimated.

Assimilated, respiratory and production gross efficiencies were calculated as $R/I \times 100$, $U/I \times 100$ and $P/I \times 100$, respectively. Respiratory (R) and production net efficiencies (PE) were calculated as $R/Ab \times 100$ and $P/Ab \times 100$, respectively. Required supply of energy (E) as a function of body mass ($B = 1\ kg$) was calculated as: $E(B) = Ab - R - P$ were all values were expressed as $Kj\ kg^{-1}\ day^{-1}$.

Lipid composition

Moisture from diets and octopus mantle and DG, as well as total lipids (TL) and fatty acids of total lipid from mantle and digestive gland of octopus were analysed. Moisture content was determined using the method of Horwitz (1980). 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. Total lipid (TL) extracts were subjected to acid-catalyzed transmethylolation for 16 h at $50\ ^\circ C$, using 1 ml of toluene and 2 ml of 1% sulphuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) were purified by thin layer chromatography (TLC), and visualized by spraying with 1% (w/v) iodine in $CHCl_3$ (Christie, 1982). FAME were separated and quantified by using a Shimadzu GC-2010 gas chromatograph equipped with a flame ionization detector ($250\ ^\circ C$) and a fused silica capillary column Tecnokroma Superwax-280TM (10 m x 0.10 mm I.D.). Hydrogen was used as carrier gas and samples were applied by on-column injection at an initial temperature of $50\ ^\circ C$. During each analysis, the oven was programmed to rise from 60 to $150\ ^\circ C$ at a rate of $39\ ^\circ C\ min^{-1}$, and then to a final temperature of $230\ ^\circ C$ at $2.5\ ^\circ C\ min^{-1}$. Individual FAMEs were identified by reference to authentic standards and to a well-characterized fish oil (PUFA-3,

Biosigma, Spain). BHT, potassium chloride, potassium bicarbonate, iodine were supplied by Sigma Chemical Co (St. Louis, MO). TLC (20x20 cm x 0.25 mm) and HPTLC (10x10 cm x 0.15 mm) plates, pre-coated with silica gel (without fluorescent indicator) were purchased from Macherey-Nagel (Düren, Germany). All organic solvents for GC used were of reagent grade and were purchased from Panreac (Barcelona, Spain).

Statistics

Differences in growth and lipid composition of the mantle and digestive gland among the three diets were analyzed with one-way ANOVA. When differences were found, a Tukey multiple comparison test was performed. Homogeneity of variances was verified with the Cochran test. Means obtained during the treatment were compared by using Duncan's multiple range test.

Since in the case of fatty acid composition, it is frequently difficult to interpret the results from the tables, an additional analysis of principal components was performed to determine differences between diets, mantle and DG composition of octopuses fed each diet. Principal components analysis is a statistical method that is normally used to simplify or reduce the number of variables losing the minimum amount of information possible, without the need to confirm normality and distribution. This makes it easier to understand and explain the results obtained. The new factors or principal components are a lineal combination of the original variables, and are independent between them (Carrasco and Hernán, 1993). A key factor from this type of analysis is to determine the variables that explain the highest variance percentage, and can explain an acceptable proportion of total variance. Therefore, fatty acid data were further chemometrically analysed with Principal Component Analysis (PCA). The score plots obtained after the generation of the two first principal components were used to ascertain groups based on anatomical (mantle, DG) and dietary (ALG, GEL, CON) adscription. The most representative fatty acids and groups, i.e. 14:0, 16:0, 16:4, 18:1n-9, 20:1n-9, 20:2n-6, 20:4n-3, 20:4n-6, 20:5n-3, 22:1n-11, 22:5n-3, 22:6n-3, Saturates, monoenes, PUFA, total n-3 and total n-6, were selected and introduced in the PCA model. PCA was run iteratively selecting variables

based on the analysis of the anti image matrix and higher (>0.4) communalities.

All statistical essays were done with the program Spss 17, with a 95% confidence interval (Zar, 1999).

Results

Animals fed CON grew significantly higher ($p<0.05$) compared to the two artificial diets, which were not different between them (Fig. 1; $p>0.05$). Final weight of octopuses fed CON, GEL and CAL was 1075.8 ± 127.8 g, 712.3 ± 113.4 g, and 667.8 ± 134.7 g, respectively. CON also promoted higher ($p<0.05$) growth rates, while no differences ($p>0.05$) were found between octopus fed the two artificial diets. Overall growth rates for the experiment were of 1.4 ± 0.2 %bw d^{-1} , 0.2 ± 0.2 %bw d^{-1} , and -0.2 ± 0.2 %bw d^{-1} , for octopuses fed the CON, GEL and ALG diets, respectively. Growth rates were different between all diets ($p<0.05$), being higher ($p=0.000$) for the control, while octopuses fed the GEL diet had higher ($p=0.002$) growth rates than the ones fed the ALG diet. Average feeding rates for octopuses fed CON, GEL and CAL were of 6.9 ± 0.3 %bw d^{-1} , 7.1 ± 0.3 %bw d^{-1} , and 7.3 ± 0.3 %bw d^{-1} , respectively, and were not significantly different ($p>0.05$). Food conversions for animals fed the ALG diet were negative, while values of 21.5 ± 1.5 % and 3.2 ± 1.1 % were found for octopuses fed CON and the GEL diet, respectively.

Mortality was 10% for octopuses fed the GEL diet, while no casualties occurred for animals fed CON or the ALG diet.

Partial energy balance

The type of diet did not affect the ingestion rate; values between 83 to 89.6 g kg^{-1} day^{-1} were recorded in octopuses fed experimental diets (Table 1; $P > 0.05$). Calorimetric data showed that experimental diets offered different amount of energy; elaborated diets had higher energy content than CON (Table 1; $P < 0.05$). As a consequence, the type of diet affected the ingested energy with higher values on animals fed ALG bound diet than GEL and control diet, respectively (Table 1; $P < 0.05$). Absorption efficiency (%) was also affected by type of diet, with higher values in animals fed diet GEL and

control diet (79.5% and 82.3%, respectively) than octopuses fed ALG (41%) (Table 1; $P < 0.05$).

Fasting and feeding oxygen consumption and ammonia excretion measurements were obtained from animals fed three experimental diets (Fig. 2). The oxygen consumption of fasting octopuses fed ALG were lower than registered of animals fed GEL and control diets (Table 2; $P < 0.05$). After feeding, oxygen consumption of octopuses fed the three diets reached the peak 3 h after; newly, a higher post prandial oxygen consumption was observed in animals fed GEL and control diets than in animals fed ALG (Fig. 2; $P < 0.05$).

There were no differences between ammonia excretion values registered in animals fed the three experimental diets. A mean value of $307.8 \text{ mg N-NH}_3 \text{ Kg}^{-1} \text{ h}^{-1}$ was calculated (Table 2; $P > 0.05$). After feeding, an increment on ammonia excretion was observed (Fig. 2). Animals fed ALG reached the ammonia excretion peak 2 h after feeding. In contrast, octopuses fed GEL and CON showed a peak 4 and 6 h after feeding, respectively (Fig. 2). Post prandial ammonia excretion registered in animals fed the control diet resulted 46% lower than in animals fed elaborated diets (Table 2; $P < 0.05$). Fasting and feeding O:N ratio values were between 4 to 15 indicating protein metabolism in animals fed experimental diets (Mayzaud, P. & Conover, R.J., 1988). Higher values ($p < 0.05$) were recorded in animals fed CON as compared to those fed the elaborated diets (Table 2).

Animals fed the ALG diet were energetically affected by the diet. A negative value of P was recorded in those animals ($P = -59.8 \text{ Kj Kg}^{-1} \text{ day}^{-1}$). In contrast, octopuses fed GEL and CON showed positive P values. However animals fed GEL showed a P value 18 times lower than when fed CON (Table 3; $P < 0.05$). Once the energetic balance was integrated, it was noticeable that octopuses fed ALG channelled 58% of ingested energy to R, 11% to U and lost 4% as P. Animals fed GEL channelled 82% to R, 11% to U and 0.9% to P and octopuses fed CON channelled 77% to R, 17% to U and 24% to P (Fig 3A). The required supply of energy as a function of body mass $E(B)$ was -32, 84.5 and $154 \text{ Kj Kg}^{-1} \text{ day}^{-1}$ for octopuses fed ALG, GEL and CON, respectively (Fig. 3B).

Lipid composition

Moisture of the three diets (Table 4) and of octopus DG fed these diets (Table 6) was significantly different ($p<0.05$). Moisture of CON was higher ($p<0.05$) compared to the artificial diets, while moisture of the GEL diet was also higher ($p<0.05$) than that of the ALG diet (Table 4). Animals fed the two artificial diets had similar DG moisture ($p=0.773$), while this was lower in the DG of animals fed CON ($p<0.05$) (Table 6). Moisture of octopus mantle fed the three diets was similar ($p>0.05$) (Table 4).

Total lipid content in % of dry weight ($\%dw^{-1}$) for the mantle of octopuses fed the three diets was similar ($p>0.05$) at the end of the experiment. Results are presented both in percentage of total fatty acids (Table 4) and content ($\mu g/mg$ DW) (Table 5).

Saturates and monoenes in the mantle of animals fed CON and the GEL diet were similar ($p>0.05$), and were higher ($p<0.05$) compared to the ALG diet, in content (Table 5), although in % of total FA there were no differences in saturates between all diets. For monoenes, only for the ones fed ALG its importance was lower (Table 4). The highest saturated and monoenes were 16:0 and 20:1 n-9, respectively (Tables 4 and 5), and they were lower for animals fed the ALG diet.

The most important n-3 HUFA's were DHA and EPA, and they were similar ($p>0.05$) for octopuses fed the three diets (Tables 4 and 5). The n-3 series represent more than 1/3 in percentage of total FA (Table 4).

The most abundant n-6 was arachidonic acid (20:4 n-6, ARA) for all diets, being higher ($p<0.05$) in those fed the ALG diet, in % of total FA (Table 4), while there were no differences in content (Table 5). The ratio between DHA/EPA was higher ($p<0.05$) for octopuses fed the ALG diet, while the EPA/ARA and DHA/ARA ratios were higher ($p<0.05$) for octopuses fed CON (Tables 4).

As for the mantle fatty acid composition, results for DG are presented both in percentage of total fatty acids (Table 6) and in content ($\mu g/mg$ DW) (Table 7). Total lipid content in % of dry weight ($\%dw^{-1}$) for the DG of

octopuses fed CON was higher ($p<0.05$) than for GEL and ALG, while for the ones fed these two artificial it was similar ($p>0.05$) (Table 6).

Concentrations of almost all FA in content were higher in the DG of animals fed CON, compared to the two artificial diets (Table 7). Octopuses fed CON had higher concentration ($p<0.05$) of monoenes and saturates (Table 7). Contrary, in % of total fatty acids, monoenes were higher in those fed GEL, while saturates were higher in animals fed ALG (Table 6). The highest saturate was 16:0 and monoenes were 18:1 n-9 (most important in the artificial diets) and 20:1 n-9 (most important in CON) (Tables 6 and 7). The concentration of 16:0 was higher ($p<0.05$) in animals fed CON (Table 7). Contrary, they were similar ($p>0.05$) in % of total FA, for the ones fed CON and ALG, both higher than for GEL (Table 6).

Higher concentrations ($p<0.05$) of n-3 HUFA were found for the octopuses fed CON, (Tables 6 and 7). This can be explained by the differences in DHA and EPA content. The most important n-3 HUFA were DHA and EPA (Tables 6 and 7). The n-3 series represent almost half of total FA in percentage for animals fed CON, while they account for about 1/3 in DG of animals fed the artificial diets (Table 6).

The n-6 series was more relatively abundant for animals fed the artificial diets ($p<0.05$) (Table 6). The most important n-6 was ARA for all diets, being higher in animals fed CON (Table 7). Contrary, in relative abundance, it was higher for the ALG diet (Table 6).

The DHA/EPA ratio was similar for octopuses fed the three diets. Contrary, the EPA/ARA and DHA/ARA ratios were higher ($p<0.05$) for octopuses fed CON, and also higher ($p<0.05$) for octopuses fed GEL, compared to those fed ALG (Tables 6).

Principal components analysis

Data selected were well suited for PCA since a value of 0.6 was obtained for the Kaiser- Meyer-Olkin test (KMO) and Bartlett's test for sphericity was highly significant ($P<0.000$). The first two principal components generated explained 80% of total variance (44.5% the first component or factor score 1, and 35.5% the second or factor score 2) (Figure 4). The analysis of the component matrix after Varimax rotation allowed to associate

first component to 14:0, monoenes and n-9 fatty acids on the positive side, and to n-6 fatty acids on the negative side. PUFA, especially n-3 were strongly associated to the positive side of the second component.

The score plot revealed two distinct groups on the first component mainly identifiable with the DG and Mantle composition, although the DG fatty acid pattern of the ALG group was classified with the mantle results of all the dietary treatments. The second component separated clearly digestive gland fatty acids pattern of CON (associated to higher PUFA3) from that of the GEL group (Figures 4 and 5).

Discussion

Cephalopods have been fed with artificial diets, with negative or low growth. Domingues *et al.* (2005, 2008) and Ferreira *et al.* (2009) have fed semi-humid artificial diets to cuttlefish (*Sepia officinalis*). These diets promoted negative growth. Contrary, moderate growth was obtained using artificial diets for the same species by Domingues (1999), Castro *et al.* (1993), Castro & Lee (1994) and Domingues *et al.* (2005). Also, recently two species of octopuses (*O. vulgaris* in Spain and *O. maya* in México) have been fed artificial diets. *O. vulgaris* were fed prepared diets based on shrimp or squid paste agglutinated with gelatine (Quintana *et al.*, 2008), with the diet based on squid paste promoting acceptable growth ($1.5\% \text{bw d}^{-1}$). This artificial diet was a non-profitable one, from a practical point of view, since it was exclusively based on squid paste, but it demonstrated that gelatine is a good binder for octopus diets.

Recently, Rosas *et al.* (2008) fed *O. maya* with an artificial diet based on crab paste agglutinated with either gelatine or alginate. High mortality was observed for octopuses fed the alginate diet, with acceptable growth rates obtained for the diet agglutinated with gelatine ($1.91 \pm 0.14\% \text{bw d}^{-1}$), which were higher than the control used (crab meat, $1.72 \pm 0.18\% \text{bw d}^{-1}$). In the present experiment, higher growth rates (although low, compared to the control) were obtained with the artificial diet agglutinated with gelatine, while animals fed the diet agglutinated with alginate lost weight.

In the present study, genuine prepared ingredients (fish powder, CPSP), were used to feed octopuses, the squid paste (30%) being the only

natural component. As in other studies, experimental diets produced lower growth rates in comparison to control groups. Domingues *et al.* (2007) fed *O. maya* with an artificial diet based on squid paste, CPSP and a pellet used to feed prawns. Octopuses accepted well the artificial diets, but did not grow ($0.0\pm0.3\%bw\ d^{-1}$). Similarly, Rosas *et al.*, (2007) fed the same species with an artificial diet also based in squid paste, CPSP and a pellet used to feed prawns, with good acceptance, but negative growth ($-0.08\%bw\ d^{-1}$).

Aguila *et al.* (2007), also working with *O. maya*, used different percentages of CPSP in diets agglutinated with alginate, obtaining growth rates below $1\%bw\ d^{-1}$; this diet delivered better results to the ones obtained here, also agglutinated with alginate and similar concentrations of CPSP. We have several hypotheses to possibly explain the marginal growth or no-growth rates obtained when feeding octopuses with prepared diets. One could be due to changes in protein structure during elaboration, due to denaturation by heating (Domingues *et al.*, 2009), avoiding the enzyme attack during the chymo formation, which could affect the digestibility. There are evidences that demonstrate that meat cooking affected myofibrillar protein susceptibility to proteases, reducing, the meat digestibility (Santé-Lhoutellier *et al.*, 2008; Gatellier & Santé-Lhoutellier, 2009).

Also another hypothesis could be related with the during food elaboration. During protein processes, amino acids and some lipids could also be lost, reducing the nutritional quality of the diet and affecting the growth rate, not via the digestibility, but via the changes of nutritional characteristics. In fact, results from energetic balance demonstrate that physiologically useful energy for animal maintenance $E(B)$ was affected by type of diet. A negative value of $E(B)$ was observed in animals fed ALG, while positive (85 and 154 $Kj\ Kg^{-1}\ day^{-1}$) were observed in octopuses fed GEL and CON, respectively. It is evident that the ALG diet did not cope the physiological requirements for octopus growth. In contrast positive values were observed for animals fed GEL and CON. Value of $E(B)$ of animals fed the GEL diet was 44% lower than the $E(B)$ value observed in animals fed CON, suggesting that nutritional characteristics of the diet affect in different forms the physiologically useful energy of experimental animals. In the case of the GEL diet, it is evident that although that diet was well accepted by animals, they invested a lot of energy

into the respiratory metabolism (82%) reducing the energy available to biomass production. This is typical of animals fed with diets that do not cover the nutritional requirements, as demonstrated by Pérez *et al.* (2006) for *Enteroctopus megalocyathus*.

Although lipids usually account for less than 2% in cephalopods (Almansa *et al.*, 2006), their importance is much higher in the DG. This organ contains 4 to 5 times more lipids and lipase activity compared to the mantle (Moltschanlowskyj & Johnston, 2006). The DG plays an important role in lipid digestion and metabolism, with several functions as enzyme secretion, digestion and absorption (Semmens *et al.*, 1995). Contrary, there is evidence that the DG is not used to store lipids (Moltschanlowskyj & Johnston, 2006; Fluckiger *et al.*, 2008), which makes it a good candidate to study the effects of different diets in lipid composition of this organ for cephalopods.

Absolute concentrations of almost all FA in content obtained in the present study were higher ($p < 0.05$) in the DG of animals fed CON, compared to the two artificial diets (Table 7). This can be explained by the much larger amounts of lipids, almost 4 times higher, compared to the ones of octopuses fed the prepared diets. For the mantle, there are a few differences when comparing some FA in qualitative (Table 4) and quantitative data (Table 5), mainly in saturates, ARA and n-6.

The most abundant fatty acids (both in content and % of total FA) in the mantle of octopuses fed either the natural diet (CON) or the artificial diets in this study (Tables 4 and 5) were palmitic acid (16:0), EPA (20:5 n-3) and DHA (22:6 n-3). This has also been reported for cuttlefish (Sinanoglou and Miniadis-Meimaroglou, 1998; Zlatanov *et al.*, 2006; Ferreira *et al.*, 2009) *Eledone moschata* (Sinanoglou and Meimaroglou, 1998), *O. vulgaris* (Miliou *et al.*, 2006; Zlatanov *et al.*, 2006), *Todarodes sagittatus* (Sinanoglou & Miniadis-Meimaroglou, 1998), and *Loligo vulgaris* (Zlatanov *et al.*, 2006). Similarly, and in agreement with Ferreira *et al.* (2009), these three FA were the most abundant in the DG.

The mantle of cuttlefish fed artificial diets did not show differences (in absolute concentration) between monoenes, saturates and PUFA, compared to natural diets (Ferreira *et al.*, 2009). This pattern was different in the present experiments for monoenes and saturates using *O. vulgaris*, since animals fed

the ALG diet (which promoted negative growth) had lower concentrations of these fatty acid groups (Table 5). The DG of cuttlefish fed artificial diets showed lower contents of saturates, monoenes and PUFA, compared to natural diets (Ferreira *et al.*, 2009), and this pattern was confirmed in the present experiments (Table 7). Marine species use monoenes as energy substrates (Sargent *et al.*, 1995), contrary to cephalopods that present an amino acid based metabolism (Domingues, 1999). This could explain the lower concentration of these fatty acids in the composition of the mantle (<12%) and DG of cuttlefish (22 to 33% of total FA) (Ferreira *et al.*, 2009) and in the present experiment the mantle (10 to 13%) (Table 4) and DG of octopuses fed CON (less than 25%, Table 6).

The DHA/EPA ratio varied between 1.4 and 1.6 in the mantle, being higher for animals fed the ALG diet which promoted negative growth, and could indicate a most extensive use of EPA by these animals, that were in worse condition due to their feeding on a deficient diet. This ratio is similar to those reported for the mantle of *O. vulgaris* by Zlatanov *et al.*, (2006) (1.5), Miliou *et al.*, (2006), (between 1.5 and 1.9) and by Sinanoglou & Miniadis-Meimaroglou (1998) (1.5).

The DHA/EPA ratio varied between 1.8 and 2.1 in the DG of *O. vulgaris* in this study (Table 6), being similar between the natural and artificial diets, which can be explained by the simultaneous decrease of both FA in DG of animals fed the two deficient diets. We have not found information in the bibliography consulted about dietary effects on the DHA/EPA ratios for *O. vulgaris*. Nevertheless, lower ratios in the DG of *S. officinalis* when feeding crustaceans (between 0.3 and 0.6), or when feeding on fish (between 1.5 and 1.8) were reported by Fluckiger *et al.*, (2008). Ferreira *et al.* (2009) also indicates lower ratios for DG of *S. officinalis*, (<0.5), with significant differences between those fed crustaceans (between 0.2 and 0.5) and artificial diets (between 0.9 and 1.0).

Increase in ARA is related to growth in *O. vulgaris* mantle (Miliou *et al.*, 2006). Results obtained here showed no differences in ARA absolute concentrations between diets (Table 5), and an opposite pattern in % of total FA, since octopuses with higher growth rates had less ARA than those with lower growth rates (Table 4).

In conclusion, none of the two artificial diets promoted satisfactory growth, but gelatine proved to be a more adequate binder. Acceptance was good, and growth was comparable to the one obtained by other authors using higher percentages of natural feeds such as fish, squid or prawn, in the elaboration of the artificial diets.

The lower growth obtained with the artificial diets was greatly reflected in total lipid composition of the DG, and consequently the fatty acid composition of this organ. It is interesting to verify that the FA composition of the mantle of octopuses fed all diets was similar, according to the principal components analysis (Figure 4). This can be explained by the fact that the mantle is a much more metabolically stable organ, and even in periods of underfeeding or feeding on nutritional deficient diets, FA are not removed rapidly from this tissue. This fact was also observed for cuttlefish by Ferreira *et al.*, (2009), that reported no differences in FA mantle composition when fed different diets, even some that promoted negative growth. Also, octopuses that were fed the ALG diet and lost weight had DG composition that was similar to their mantle and mantle of octopuses fed the other diets (Figure 4). This also confirms that those animals fed the ALG diet were indeed in worse condition. Further confirmations are obtained when analyzing the principal components for octopuses fed the three diets (Figure 5). In this case, diet did not affect mantle composition in FA, but it greatly affected DG composition, and in a greater extent for animals fed the ALG diet. The majority of the most important fatty acid groups, such as n-3 PUFA, as well as important fatty acids, such as EPA and DHA decreased in the DG of animals fed the artificial diets, which indicates that these animals were in worse condition, compared to the ones fed CON. However differences in energetic balance were observed between diets, indicating that GEL was adequately digested but probably failed in nutritional characteristics (as fatty acids proportion; amino acids, etc.) that affect the biomass production.

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Table 1 - Ingestion rate ($\text{g kg}^{-1} \text{ day}^{-1}$), absorbed energy and ingested energy of *O. vulgaris* fed squid bound alginate (ALG) and gelatin (GEL). Fresh squid used as control diet (CON). Values as Mean $\pm$ S.E. Different letters indicate statistical differences at $P < 0.05$ level.

	Ingestion Rate $\text{g kg}^{-1} \text{ day}^{-1}$	Energy of Food kJ g^{-1}	Ingested energy $\text{KJ kg}^{-1} \text{ day}^{-1}$	Absorption Efficiency %
ALG	89.56 $\pm$ 0.92 ^a	17.35 $\pm$ 0.26 ^b	1553.82 $\pm$ 16.04 ^c	40.80 $\pm$ 3.71 ^a
GEL	83.26 $\pm$ 1.34 ^a	17.00 $\pm$ 0.31 ^b	1415.42 $\pm$ 22.81 ^b	79.50 $\pm$ 6.36 ^b
CON	87.38 $\pm$ 0.51 ^a	14.48 $\pm$ 0.21 ^a	1265.30 $\pm$ 6.96 ^a	82.30 $\pm$ 6.50 ^b

Table 2 - Oxygen consumption and ammonia excretion of *O. vulgaris* fed squid bound alginate (ALG) and gelatin (GEL). Fresh squid used as control diet (CON). Values are Mean $\pm$ S.E. Different letters indicate statistical differences at $P < 0.05$ level.

Oxygen consumption	Fasting mgO ₂ h ⁻¹ kg ⁻¹	Feeding mgO ₂ h ⁻¹ kg ⁻¹	TRP h	RRut Kj kg ⁻¹ day ⁻¹	RAHI Kj kg ⁻¹ day ⁻¹	RTot Kj kg ⁻¹ day ⁻¹
ALG	2821.38 $\pm$ 83.80 ^a	4983.37 $\pm$ 125.03 ^a	3	793.94	86.91	880.85
GEL	3145.20 $\pm$ 107.10 ^b	5235.82 $\pm$ 224.29 ^b	3	885.06	84.04	969.10
CON	2385.28 $\pm$ 132.11 ^b	6519.23 $\pm$ 572.63 ^b	3	671.22	166.18	837.40
Ammonia excretion	mgNH ₃ h ⁻¹ kg ⁻¹	mgNH ₃ h ⁻¹ kg ⁻¹	TRP h	U _{rut} Kj kg ⁻¹ day ⁻¹	U _{pp} Kj kg ⁻¹ day ⁻¹	U _{tot} Kj kg ⁻¹ day ⁻¹
ALG	271.158 $\pm$ 41.75 ^a	1089.65 $\pm$ 237.64 ^b	2	122.89	44.89	167.78
GEL	388.209 $\pm$ 56.70 ^a	1285.29 $\pm$ 305.07 ^b	6	143.95	158.86	302.81
CON	264.218 $\pm$ 112.56 ^a	540.998 $\pm$ 43.565 ^a	4	108.86	44.58	153.44
O:N						
ALG	11.04	4.85				
GEL	8.60	4.32				
CON	9.58	12.79				

TRP = Time to reach the peak. RRut = Routine Respiratory metabolism, RAHI = Apparent heat increment Respiratory metabolism. RTot = Total respiratory metabolism (RRut + RAHI), U_{rut} = Routine Nitrogen metabolism. U_{pp} = Post prandial nitrogen metabolism, U_{tot} = Total Nitrogen metabolism (U_{rut} + U_{pp}).

Table 3 - Energy production (P, Kj Kg⁻¹ day⁻¹) of *O. vulgaris* juveniles fed experimental diets: Alginate (ALG), Gelatine (GEL) y Squid (CON). Values as mean $\pm$ S.E. Different letters indicate statistical differences at P < 0.05 level.

	ALG	GEL	CON
Initial weight, g	717.77 $\pm$ 45.63 ^a	692.34 $\pm$ 42.9 ^a	713.0 $\pm$ 42.9 ^a
Final weight, g	618.16 $\pm$ 65.88 ^a	712.32 $\pm$ 35.9 ^b	1075.8 $\pm$ 35.9 ^c
Experimental Days	30	30	30
Production Kj Kg ⁻¹ day ⁻¹	-59.76 $\pm$ 8.51 ^a	12.0 $\pm$ 5.62 ^b	217.6 $\pm$ 4.68 ^c

Table 4 – Diet moisture (%ww), Mantle moisture (%ww), Total Lipids (% DW) of octopus mantle and Fatty acids of total lipids (% weight) of octopuses mantle fed squid (CON) and two artificial diets (GEL, ALG).

Diet composition	CON	GEL	ALG
Diet moisture	80,5 $\pm$ 1,9 ^a	58,4 $\pm$ 2,1 ^b	49,9 $\pm$ 1,6 ^c
Mantle moisture	78,6 $\pm$ 2,5	80,2 $\pm$ 2,0	81,6 $\pm$ 2,5
Mantle Total Lipids	5,2 $\pm$ 0,6	4,6 $\pm$ 0,7	4,1 $\pm$ 0,6
14:0	1,87 $\pm$ 0,38 ^a	1,45 $\pm$ 0,43 ^{ab}	1,06 $\pm$ 0,06 ^b
15:0	0,43 $\pm$ 0,01	0,37 $\pm$ 0,03	0,39 $\pm$ 0,10
UK 1	1,64 $\pm$ 0,11 ^a	1,45 $\pm$ 0,16 ^{ab}	1,36 $\pm$ 0,12 ^b
16:0	23,88 $\pm$ 3,31 ^a	21,84 $\pm$ 0,55 ^{ab}	19,58 $\pm$ 1,91 ^b
16:1 n-7	1,18 $\pm$ 0,11	1,39 $\pm$ 0,34	0,94 $\pm$ 0,21
16:1 n-5	0,32 $\pm$ 0,02	0,28 $\pm$ 0,03	0,31 $\pm$ 0,05
17:0	1,12 $\pm$ 0,08 ^a	1,41 $\pm$ 0,14 ^{ab}	1,46 $\pm$ 0,17 ^b
16:4	3,40 $\pm$ 0,42 ^a	5,02 $\pm$ 0,64 ^b	5,43 $\pm$ 0,61 ^b
18:0	7,19 $\pm$ 0,88	8,98 $\pm$ 1,57	9,07 $\pm$ 0,58
18:1 n-9	2,77 $\pm$ 0,36 ^{ab}	3,07 $\pm$ 0,59 ^a	2,16 $\pm$ 0,30 ^b
18:1 n-7	1,74 $\pm$ 0,16	1,77 $\pm$ 0,22	1,42 $\pm$ 0,18
18:2 n-6	0,46 $\pm$ 0,01	0,47 $\pm$ 0,08	0,49 $\pm$ 0,18
20:1 n-9	3,55 $\pm$ 0,31 ^a	3,42 $\pm$ 0,27 ^a	2,82 $\pm$ 0,16 ^b
20:2 n-6	0,41 $\pm$ 0,03	0,36 $\pm$ 0,04	0,37 $\pm$ 0,07
20:4 n-6	2,97 $\pm$ 0,90 ^a	4,47 $\pm$ 1,31 ^{ab}	6,17 $\pm$ 1,06 ^b
20:4 n-3	0,19 $\pm$ 0,03 ^a	0,13 $\pm$ 0,04 ^{ab}	0,11 $\pm$ 0,03 ^b
20:5 n-3	15,65 $\pm$ 1,25	13,32 $\pm$ 0,90	13,95 $\pm$ 2,08
22:1 n-11	0,12 $\pm$ 0,01 ^{ab}	0,35 $\pm$ 0,16 ^a	0,06 $\pm$ 0,05 ^b
22:1 n-9	1,45 $\pm$ 0,26	1,81 $\pm$ 0,23	1,57 $\pm$ 0,23
22:5 n-3	0,86 $\pm$ 0,08 ^a	0,89 $\pm$ 0,07 ^a	1,08 $\pm$ 0,12 ^b

22:6 n-3	21,77 ± 2,38	18,43 ± 1,55	21,65 ± 2,15
UK	3,66 ± 0,54	5,01 ± 0,94	4,83 ± 1,18
Totals			
Saturates	34,60 ± 3,96	34,21 ± 1,27	31,74 ± 2,09
Monoenes	12,13 ± 1,03 ab	13,14 ± 1,63 a	10,28 ± 0,93 b
PUFA	44,57 ± 4,42	41,17 ± 2,51	46,36 ± 3,80
n-3	39,54 ± 3,51	33,85 ± 2,31	37,75 ± 4,24
n-6	4,40 ± 1,06 a	6,37 ± 1,16 ab	7,98 ± 1,12 b
n-9	7,77 ± 0,79 a	8,30 ± 0,78 a	6,54 ± 0,30 b
n-3 HUFA	38,68 ± 3,56	33,08 ± 2,36	37,10 ± 4,24
n-3/n-6	9,25 ± 1,68 a	5,43 ± 0,94 b	4,84 ± 1,20 b
DHA/EPA	1,39 ± 0,10 ab	1,38 ± 0,07 a	1,54 ± 0,09 b
EPA/ARA	5,56 ± 1,40 a	3,15 ± 0,80 b	2,34 ± 0,68 b
DHA/ARA	7,73 ± 1,95 a	4,34 ± 1,11 b	3,62 ± 0,89 b
Mon/n-3 H	0,32 ± 0,05 ab	0,40 ± 0,07 a	0,28 ± 0,05 b
Mon/PUFA	0,28 ± 0,05 ab	0,32 ± 0,06 a	0,22 ± 0,04 b
Mon/Sat	0,35 ± 0,03	0,38 ± 0,05	0,32 ± 0,02
18:1n-9/n3 HUFA	0,07 ± 0,01 ab	0,09 ± 0,02 a	0,06 ± 0,01 b

UK, unknown fatty acids; 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. Letters show significant differences (p<0.05). Values as mean ± S.D.

Table 5 – Fatty acids of total lipids (µg/mg DW) of the mantle of octopuses fed the squid (CON) and two artificial diets (GEL, ALG).

Diet composition	CON	GEL	ALG
14:0	0,39 ± 0,13 a	0,26 ± 0,07 ab	0,15 ± 0,03 b
15:0	0,09 ± 0,01 a	0,07 ± 0,01 b	0,05 ± 0,01 b
UK 1	0,33 ± 0,03 a	0,26 ± 0,02 ab	0,20 ± 0,06 b
16:0	4,87 ± 0,70 a	3,96 ± 0,53 a	2,77 ± 0,38 b
16:1 n-7	0,24 ± 0,06 a	0,25 ± 0,05 a	0,13 ± 0,01 b
16:1 n-5	0,07 ± 0,01 a	0,05 ± 0,00 b	0,04 ± 0,01 b
17:0	0,23 ± 0,02	0,26 ± 0,04	0,21 ± 0,02
16:4	0,69 ± 0,04	0,91 ± 0,16	0,78 ± 0,22
18:0	1,46 ± 0,04	1,63 ± 0,36	1,31 ± 0,33
18:1 n-9	0,57 ± 0,14 a	0,55 ± 0,07 a	0,30 ± 0,04 b
18:1 n-7	0,36 ± 0,07 a	0,32 ± 0,04 a	0,20 ± 0,03 b
18:2 n-6	0,10 ± 0,02 a	0,08 ± 0,00 ab	0,07 ± 0,02 b
20:1 n-9	0,73 ± 0,16 a	0,62 ± 0,07 a	0,40 ± 0,07 b
20:2 n-6	0,08 ± 0,02 a	0,07 ± 0,01 ab	0,05 ± 0,00 b
20:4 n-6	0,61 ± 0,21	0,82 ± 0,30	0,89 ± 0,30
20:4 n-3	0,04 ± 0,01 a	0,02 ± 0,01 ab	0,02 ± 0,01 b
20:5 n-3	3,23 ± 0,64	2,43 ± 0,47	2,04 ± 0,67

22:1 n-11	0,02 ± 0,01 a	0,06 ± 0,02 b	0,00 ± 0,01 a
22:1 n-9	0,29 ± 0,04	0,33 ± 0,05	0,23 ± 0,08
22:5 n-3	0,18 ± 0,04	0,16 ± 0,04	0,16 ± 0,05
22:6 n-3	4,52 ± 1,20	3,38 ± 0,75	3,15 ± 0,92
% UK	0,76 ± 0,14	0,91 ± 0,20	0,67 ± 0,12
Totals			
Saturates	7,06 ± 0,88 a	6,20 ± 0,78 a	4,52 ± 0,75 b
Monoenes	2,50 ± 0,51 a	2,36 ± 0,21 a	1,45 ± 0,18 b
PUFA	9,22 ± 2,20	7,53 ± 1,50	6,74 ± 1,92
n-3	8,18 ± 1,94	6,19 ± 1,27	5,50 ± 1,66
n-6	0,91 ± 0,26	1,16 ± 0,31	1,15 ± 0,34
n-9	1,60 ± 0,32 a	1,49 ± 0,11 a	0,93 ± 0,16 b
n-3 HUFA	8,01 ± 1,90	6,07 ± 1,26	5,40 ± 1,64

UK, unknown fatty acids; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid. Letters show significant differences ($p < 0.05$). Values as mean $\pm$ S.D.

Table 6 – Digestive gland moisture (%ww), Total Lipids (%dw) of octopus digestive gland and Fatty acid of total lipids (% dw) of octopuses digestive gland fed squid (CON) and two artificial diets (GEL, ALG).

Diet composition	CON	GEL	ALG
DG moisture	66,7 ± 1,2 a	74,6 ± 2,3 b	75,7 ± 2,1 b
DG Total Lipids	36,5 ± 6,7 a	12,9 ± 2,1 b	9,7 ± 2,2 b
14:0	3,87 ± 0,11 a	3,77 ± 0,74 a	1,64 ± 0,34 b
15:0	0,58 ± 0,03 a	0,73 ± 0,10 b	0,35 ± 0,05 c
UK 1	0,73 ± 0,12 a	1,00 ± 0,34 ab	1,27 ± 0,13 b
16:0	20,71 ± 0,56 a	18,83 ± 0,84 b	19,72 ± 0,61 ab
16:1 n-7	2,06 ± 0,15 a	4,75 ± 0,53 b	1,92 ± 0,36 a
16:1 n-5	0,32 ± 0,02 a	0,38 ± 0,04 a	0,24 ± 0,03 b
17:0	0,58 ± 0,04 a	0,89 ± 0,11 b	1,02 ± 0,07 b
16:4	0,59 ± 0,05 a	1,55 ± 0,34 b	3,49 ± 0,79 c
18:0	3,89 ± 0,26 a	6,43 ± 0,62 b	10,77 ± 1,24 c
18:1 n-9	3,99 ± 0,24 a	6,89 ± 0,79 b	3,95 ± 0,89 a
18:1 n-7	2,36 ± 0,13 a	2,61 ± 0,10 a	1,92 ± 0,23 b
18:2 n-6	0,46 ± 0,02 a	0,92 ± 0,09 b	0,54 ± 0,01 a
20:1 n-9	4,94 ± 0,39	5,18 ± 2,54	2,70 ± 0,26
20:2 n-6	0,56 ± 0,02 a	0,42 ± 0,10 b	0,33 ± 0,02 b
20:4 n-6	1,49 ± 0,07 a	2,43 ± 0,30 b	5,12 ± 0,52 c
20:4 n-3	0,31 ± 0,02 ab	0,43 ± 0,10 a	0,17 ± 0,07 b
20:5 n-3	14,64 ± 0,25 a	9,79 ± 1,74 b	11,15 ± 1,41 b
22:1 n-11	0,31 ± 0,08	2,61 ± 2,57	0,64 ± 0,07

22:1 n-9	0,94 ± 0,07		1,13 ± 0,36		1,10 ± 0,11
22:5 n-3	0,62 ± 0,02	a	1,03 ± 0,19	b	1,00 ± 0,11 b
22:6 n-3	29,10 ± 1,25	a	18,09 ± 4,50	b	23,18 ± 1,76 ab
% UK	2,69 ± 0,28	a	5,47 ± 1,75	b	3,81 ± 1,63 ab
Totals					
Saturates	29,71 ± 0,90	a	30,76 ± 0,79	a	33,70 ± 0,41 b
Monoenes	15,93 ± 0,67	a	24,79 ± 6,00	b	13,29 ± 0,33 a
PUFA	50,34 ± 1,69	a	36,42 ± 7,43	b	44,46 ± 2,60 ab
n-3	46,42 ± 1,53	a	30,56 ± 6,71	b	36,45 ± 3,42 b
n-6	2,84 ± 0,15	a	4,28 ± 0,56	b	6,49 ± 0,46 c
n-9	9,88 ± 0,63	ab	13,20 ± 3,61	a	7,75 ± 0,53 b
n-3 HUFA	44,84 ± 1,41	a	29,46 ± 6,53	b	35,62 ± 3,40 ab
n-3/n-6	16,35 ± 0,40	a	7,08 ± 0,91	b	5,66 ± 0,90 b
DHA/EPA	1,99 ± 0,06		1,83 ± 0,16		2,09 ± 0,12
EPA/ARA	9,85 ± 0,32	a	4,02 ± 0,45	b	2,21 ± 0,47 c
DHA/ARA	19,58 ± 0,09	a	7,38 ± 1,37	b	4,58 ± 0,77 c
Mon/n-3 H	0,36 ± 0,03		0,92 ± 0,51		0,37 ± 0,03
Mon/PUFA	0,32 ± 0,02		0,74 ± 0,39		0,30 ± 0,01
Mon/Sat	0,54 ± 0,01	a	0,81 ± 0,21	b	0,39 ± 0,01 a
18:1n-9/n3 HUFA	0,09 ± 0,01	a	0,25 ± 0,10	b	0,11 ± 0,02 a

DG, digestive gland; UK, unknown fatty acids; 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. Letters show significant differences (p<0.05). Values as mean ± S.D.

Table 7 – Fatty acid of total lipids (µg/mg DW) of octopuses digestive gland fed squid (CON) and two artificial diets (GEL, ALG).

Diet composition	CON		GEL		ALG
14:0	9,55 ± 1,89	a	2,57 ± 0,97	b	0,53 ± 0,22 b
15:0	1,42 ± 0,25	a	0,47 ± 0,12	b	0,11 ± 0,02 b
UK 1	1,76 ± 0,09	a	0,62 ± 0,15	b	0,39 ± 0,08 b
16:0	50,93 ± 8,86	a	12,50 ± 3,51	b	6,24 ± 1,75 b
16:1 n-7	5,14 ± 1,39	a	3,18 ± 1,02	a	0,62 ± 0,25 b
16:1 n-5	0,79 ± 0,16	a	0,25 ± 0,07	b	0,07 ± 0,01 b
17:0	1,42 ± 0,18	a	0,58 ± 0,13	b	0,32 ± 0,09 b
16:4	1,43 ± 0,17	a	0,98 ± 0,11	b	1,06 ± 0,07 b
18:0	9,52 ± 1,44	a	4,18 ± 0,88	b	3,32 ± 0,53 b
18:1 n-9	9,82 ± 1,77	a	4,45 ± 0,83	b	1,29 ± 0,55 c
18:1 n-7	5,80 ± 1,01	a	1,72 ± 0,42	b	0,59 ± 0,09 b
18:2 n-6	1,16 ± 0,24	a	0,62 ± 0,19	b	0,17 ± 0,05 c
20:1 n-9	12,08 ± 1,67	a	3,10 ± 0,43	b	0,84 ± 0,15 b
20:2 n-6	1,39 ± 0,33	a	0,29 ± 0,12	b	0,10 ± 0,02 b

20:4 n-6	3,69 ± 0,88 a	1,64 ± 0,58 b	1,59 ± 0,27 b
20:4 n-3	0,76 ± 0,12 a	0,30 ± 0,12 b	0,06 ± 0,03 c
20:5 n-3	36,22 ± 7,81 a	6,69 ± 2,56 b	3,58 ± 1,27 b
22:1 n-11	0,74 ± 0,17 ab	1,40 ± 0,83 a	0,20 ± 0,07 b
22:1 n-9	2,31 ± 0,42 a	0,70 ± 0,06 b	0,34 ± 0,06 b
22:5 n-3	1,54 ± 0,27 a	0,70 ± 0,27 b	0,32 ± 0,10 b
22:6 n-3	72,24 ± 17,10 a	12,50 ± 5,43 b	7,39 ± 2,32 b
% UK	6,76 ± 1,26 a	3,52 ± 1,07 b	1,12 ± 0,14 c
Totals			
Saturates	73,07 ± 12,63 a	20,38 ± 5,51 b	10,59 ± 2,61 b
Monoenes	39,15 ± 6,69 a	15,60 ± 1,29 b	4,20 ± 1,15 c
PUFA	124,87 ± 28,68 a	25,00 ± 10,03 b	14,13 ± 4,26 b
n-3	115,13 ± 26,39 a	21,02 ± 8,66 b	11,65 ± 3,83 b
n-6	7,07 ± 1,71 a	2,91 ± 1,03 b	2,02 ± 0,40 b
n-9	24,21 ± 3,83 a	8,26 ± 0,53 b	2,47 ± 0,76 c
n-3 HUFA	111,16 ± 25,29 a	20,27 ± 8,40 b	11,39 ± 3,75 b

UK, unknown fatty acids; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid. Letters show significant differences ($p < 0.05$). Values as mean $\pm$ S.D.

Figure 1. Weights (g) of common octopus fed with squid (CON) and two artificial diets (GEL and ALG) for the different weighing periods. Bars indicate standard deviations.

Figure 2. Oxygen consumption ($\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) and ammonia excretion ($\text{mg N-NH}_3 \text{ kg}^{-1} \text{ h}^{-1}$) of *O. vulgaris* juveniles fed experimental diets. Data as mean $\pm$ S.E. Arrow indicates time when octopuses were fed.

Figure 3A. Energetic balance of *O. vulgaris* juveniles fed three experimental diets (ALG, GEL, CON). Values into the columns are as $\text{Kj Kg}^{-1} \text{ h}^{-1}$, while percentage indicate proportion of each value as Ingestion rate ($I = 100\%$).

Figure 3B. Effect of type of diet on dietary supply of energy $E(B)$ as a function of body mass $E(B)$ for octopuses fed CON, GEL and ALG.

Figure 4. Results for the principal components analysis for FA composition of the DG and mantle of octopuses used in the experiment.

Figure 5. Results for the principal components analysis for FA composition of the octopuses fed the tree diets tested.