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Utilization of diets with different fish oil content in common octopus (*Octopus vulgaris* Cuvier, 1797) and resulting changes in its biochemical composition

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Abstract

The aim of the present work was to obtain the lipid utilization of *Octopus vulgaris* supplying formulated semi-moist diets with different contents in cod oil (reduced from water content): 0 g kg^{-1} (A0, 138 g kg^{-1} lipids DW; $N = 4$), 100 g kg^{-1} (A100, 286 g kg^{-1} lipids DW; $N = 6$) and 200 g kg^{-1} (A200, 388 g kg^{-1} lipids DW; $N = 6$). The rest of the ingredients were constant in the three diets: 200 g kg^{-1} gelatin, 100 g kg^{-1} egg yolk powder, 150 g kg^{-1} freeze-dried *Todarodes sagittatus* and 50 g kg^{-1} freeze-dried *Sardinella aurita*). Survival was 100% with the three diets. The highest absolute feeding (15.8 ± 1.2 g day^{-1}), growth (9.6 ± 1.4 g day^{-1}; 0.91% BW day^{-1}) and feed efficiency rates (60.3%) were obtained with diet A0. This diet also showed greater retention of lipid and protein than A100 and A200. Protein digestibility was above 95% in all of the diets. Only diet A0 led to a high lipid digestibility coefficient (81.25%), which fell drastically to 12.3% in A200. It was notable the high polar lipid digestibility rates (83–89%) respect to neutral lipids (2–87%) in all diets. The best results were obtained with lipid feeding rates of around 1 g day^{-1} and a suitable lipid content on 130–140 g kg^{-1} DW in formulated diets for *O. vulgaris*.

Keywords: *Octopus vulgaris*, formulated diet, lipids, nutrition, digestibility, fish oil

Introduction

The recent type feeds developed for common octopus (*Octopus vulgaris*), which have shown suitable levels of acceptability (Quintana, Domingues & García 2008; Cerezo Valverde, Hernández, Aguado-Giménez & García García 2008; Cerezo Valverde, Hernández, Aguado-Giménez, Morillo-Velarde & García García 2013; García, Domingues, Navarro, Hachero, Garrido & Rosas 2010; Estefanell, Roo, Guirao, Alfonso, Fernández-Palacios, Izquierdo & Socorro 2012; Morillo-Velarde, Cerezo Valverde, Hernández, Aguado-Giménez & García García 2012), allow us to make formulated diets with a known nutritional composition to improve our knowledge of octopus nutritional requirements.

It is known that cephalopods are exclusively carnivorous and, while they commonly use proteins, it was traditionally considered that they rarely used carbohydrates or lipids as energy source (Lee 1994). The body composition of cephalopods is less than 2% lipids on a wet weight basis, but they contain high levels of phospholipids, cholesterol and polyunsaturated fatty acids (PUFA), especially series n-3 HUFA (Sinanoglou & Miniadis-Meimaroglou 1998; Navarro & Villanueva 2000, 2003), suggesting a predominantly structural role for lipids rather than their use for energetic functions (O'Dor, Mangold, Boucher-Rodoni, Wells & Wells 1984; Moltschaniwskyj & Johnston 2006). However, lipase enzymes are found throughout the digestive tract of cephalopods (Boucher-Rodoni 1982; Caruso, Giordano, Mancuso & Genovese 2004; Moltschaniwskyj & Johnston 2006) and there are reports on this group being able to have capability to store the lipid in the form of triglycerides in substantial quantities in the digestive gland (Sieiro, Aubourg & Rocha 2006; Cerezo Valverde, Hernández,

García-Garrido, Rodríguez, Estefanell, Gairín, Rodríguez, Tomás & García García 2012). Recently, Morillo-Velarde, Cerezo Valverde, Serra Llinares & García García (2013) estimated that lipids provided 26% of the daily energy of the animals during a short-term starvation period, the rest being supplied by other sources, e.g. proteins (Lee 1994; García-Garrido, Hachero-Cruzado, Rosas & Domingues 2013) or carbohydrates (Morillo-Velarde, Cerezo Valverde, Serra Llinares & García García 2011). Lipid reserves in *O. vulgaris* during starvation are efficiently mobilized, especially neutral lipids, including steryl esters, triglycerides and free fatty acids (García-Garrido, Hachero-Cruzado, Garrido, Rosas & Domingues 2010; Morillo-Velarde *et al.* 2013). Lipids have also been cited as a potential energy source in other species of cephalopods (Semmens 1998; Moltschaniwskyj & Johnston 2006).

Some research groups have recently obtained good growth results and high feed efficiency rates using diets with high lipid content. For example, Estefanell *et al.* (2012) obtained excellent rearing results using fresh bogue (*Boops boops*) as food and using this species as a raw material source in formulated feeds (187–263 g kg^{-1} lipids dry weight), suggesting that lipids in the diet are efficiently used. The same authors obtained worse results after using bogue with a very high lipid content in tanks (465 g kg^{-1}; Estefanell, Socorro, Tuya, Izquierdo & Roo 2011), which suggests that there is an optimal range for the lipid content of the diet. Cerezo Valverde, Hernández *et al.* (2013) obtained better growth, feed efficiency and protein productive values using formulated diets containing 300–330 g kg^{-1} lipid (dry weight) compared with other diets containing 40–50 g kg^{-1}. García García and Aguado Giménez (2002) had previously confirmed the influence of lipid of different natural diets has on octopus growth, obtaining better growth and feed conversion ratio in octopus fed with *B. boops* (201 g kg^{-1} lipids) than in the same species fed *Sardina pilchardus* (497 g kg^{-1} lipids). In a subsequent article, an optimum lipid content of 70–100 g kg^{-1} dry weight was suggested for natural diets for *O. vulgaris* (García García & Cerezo Valverde 2006).

The aim of this work was to throw further light on the lipid requirements of *O. vulgaris*, by monitoring the changes that take place in growth, feeding efficiency and digestibility of the diet, together with the nutritional composition of the animals after feeding with formulated diets containing different proportions of fish oil in the format diet proposed by Morillo-Velarde, *et al.* (2012).

Material and methods

Experimental animals and maintenance

Common octopus (*O. vulgaris*) were caught in the Mediterranean Sea (Murcia, S.E. Spain) by trawling and kept in 2000-L tanks in the laboratory. The experiment began when the octopus had acclimatized (2 weeks) and fed with round sardinella (*Sardinella aurita*) and crab (*Carcinus mediterranus*) on alternate days, according to García García and Aguado Giménez (2002) and Aguado-Giménez and García García (2002). Subsequently, 16 male animals weighing 714–922 g were placed individually in 262-L circular tanks in which the experiments were carried out. The water temperature was maintained constant (18.4 ± 0.7°C) and rearing system and experimental conditions have been previously described by Morillo-Velarde *et al.* 2012.

Preparation and water stability of the diets

Three different diets were prepared with different proportions of fish oil (Cod liver oil, Acofarma, Terrassa, Barcelona, Spain) representing 0 g kg^{-1} (group A0), 100 g kg^{-1} (group A100) and 200 g kg^{-1} (group A200) in weight of the diet after reducing from the water content (Table 1). The rest of ingredients were maintained constant in the three diets: 200 g kg^{-1} gelatin as binder, 100 g kg^{-1} egg yolk powder, 150 g kg^{-1} freeze-dried European flying squid (*Todarodes sagittatus*) and 50 g kg^{-1} freeze-dried round sardinella (*S. aurita*). Squid and round sardinella were caught by traditional fishing methods from the same zone as the octopus. Both of them were cleaned of fishbones and viscera, triturated and refrigerated at −20°C. After that, all the ingredients were freeze-dried and triturated in a blender to obtain a fine powder (<200 μm), which was vacuum packed and kept refrigerated at 4°C until use. Egg yolk powder, gelatin and fish oil were bought in a commercial form (Table 1). To prepare the feeds all the ingredients were mixed in a domestic food mixer (Mycook® 1.8; Electrodomésticos Taurus, S.L. Lleida, Spain). First the gelatin was dissolved in water and fish oil at 40°C, and subsequently all the ingredients were added until they were

Table 1 Composition in weight (g kg^{-1}) of diets formulated with different fish oil contents

Diets	Water	Gelatin*	Egg yolk†	Round sardinella‡ (*Sardinella aurita*)	European flying squid‡ (*Todarodes sagittatus*)	Cod oil§
A0	500	200	100	50	150	0
A100	400	200	100	50	150	100
A200	300	200	100	50	150	200

*Granulated Gelatin, Bloom 220, supplied by Productos Sur, S.A. (Pol. Ind. Oeste, San Ginés, Murcia, Spain).
†Egg yolk powder, supplied by Avícola San Isidro S.L. (Los Belones, Cartagena, Murcia, Spain).
‡Freeze-dried ingredients.
§Cod liver oil, Acofarma (Terrassa, Barcelona, España).

homogenized. The homogenized mixture was allowed to cool to 4°C in an aluminium mould for 24 h, and then refrigerated at −4°C until use.

The water stability was determined from the loss of dry matter in three samples of each feed after soaking in water for 24 h. With the data obtained, the mean values of the following indices were calculated:

$$\mathrm{VDW}(\%) = (\mathrm{DW_f} - \mathrm{DW_i})/\mathrm{DW_i} \times 100,$$

which expresses the variation in dry weight of the diet after soaking in water, where DW_i and DW_f are the initial and final dry weights respectively.

$$F = \mathrm{DW_i}/\mathrm{DW_f},$$

which represents a correction factor. The dry weight of uneaten diet was multiplied by this correction factor to account for disintegration.

Experimental design

After acclimation, the animals were weighed and sexed before dividing into 4 groups: CI or initial control (three individuals sacrificed at the beginning of the experiment), group A0 (four individuals were fed with diet A0), group A100 (six individuals fed with diet A100) and group A200 (six individuals with diet A200). All of individuals were male in order to avoid any influence of reproductive processes and were individually kept. The diets were supplied for 56 days of the experiment (November 2011–February 2012). Mean initial weights were 803 ± 91 g (719–922 g) for diet A0, 817 ± 79 g (738–956 g) for diet A100 and 802 ± 88 g (714–946 g) for diet A200. At the beginning of the experiment, there were no significant differences between the mean initial weights of the three groups and a survival rate was 100% in all groups. Water temperature varied between 17.0 and 20.8°C during the experimental period (18.4 ± 0.7°C) The feeds were weighed and provided to in the form of one cube shaped piece, corresponding to 5% of the body weight of each individual on the first day and after that readjusted to exceed the demands of each individual. The octopus were fed at 09.00 h 6 days a week (García García & Cerezo Valverde, 2006) and any remaining food was collected after 24 h using a small net. The remaining food was dried at 105 ± 1°C for 48 h until constant weight (AOAC 1997; Method no. 930.15) to calculate the daily intake of each individual. On the last day of the experiment, all the animals were weighed and anaesthetized subsequently by immersion in cold seawater before sacrifice conforming to the ethical principles of reduction, replacement and refinement and avoiding or minimizing any suffering, pain and anxiety in accordance with Directive 2010/63/EU.

Sample collection and preservation

The individuals of each group were dissected to obtain the digestive gland and carcass (animal excluding digestive gland), along with their respective weights. This procedure was necessary to obtain the analytical of the composition of the whole animals, from the results of the digestive gland and carcass. Each of these parts was triturated and mixed to obtain a homogeneous mixture per animal, which was vacuum packed and frozen at −20°C for less than 1 month before carrying out the biochemical analyses. The faeces from each group were collected daily using a small net and frozen at −80°C for lyophilization (Heto, PowerDry LL3000, Allerød, Denmark). All the faeces from each group were collected together to provide sufficient amount for analysis. For the

analysis diets were carried out with a homogeneous pool of fractions of remaining in the course if experiment.

Analytical methods

The analyses were carried out in triplicate of samples of diets and octopus. A 1-g sample was used to determine the ash, moisture and crude protein contents and a 2-g sample for the crude lipid content. Moisture was extracted and determined by drying at 105 ± 1°C for 24 h to reach constant weight (AOAC 1997; Method 930.15) and ash by incineration at 450 ± 1°C for 24 h in a muffle oven (HOBERSAL, HD-230, Caldes de Montbui, Barcelona, Spain). The total lipid content (TL) was obtained using ethyl ether in a SOXTEC AVANTI 2058 (AOAC 1997; Method 920.39). Based on TL, the quantity of sample necessary to extract 10 mg of lipids was calculated according to Folch, Lees and Sloane-Stanley (1957). The lipids were kept dissolved in chloroform:methanol (2:1 v/v) containing butylated hydroxytoluene (0.01%) as antioxidant at −80°C (Christie 1982), adjusting the concentration to 10 μg lipids μL^{-1} before analysis. The lipid classes were separated using high performance thin layer chromatography (HPTLC) according to Olsen and Henderson (1989). Lipids (15 μg) were applied to 20 × 10 cm silica gel plates (Merck) using an automatic injector (Linomat 5; CAMAG, Muttenz, Switzerland) according to Cerezo Valverde, Hernández *et al.* (2013); Cerezo Valverde *et al.* (2012). Crude protein was determined using the Kjeldhal method using a conversion factor of 6.25, and while nitrogen-free extracts (NFE) was determined by difference. Gross energy and the protein energy ratio (P/E in MJ) were estimated using the Miglavs and Jobling (1989) following energy coefficients: protein 23.6 kJ g^{-1}, lipid 38.9 kJ g^{-1} and carbohydrate 16.7 kJ g^{-1}.

Total body proximate composition (TBC) was calculated using the following equation:

$$\mathrm{TBC}(\%) = \left[((\mathrm{DGW} \times \%\mathrm{DGM}) + (\mathrm{CW} \times \%\mathrm{CM})) \times 100\right]/\mathrm{BW},$$

where TBC is the content of macronutrients in the whole animal, DGW is the weight of the digestive gland, CW is the weight of carcass (animal excluding digestive gland), DGM is the content of macronutrients in digestive gland, CM the content of macronutrients in the carcass and BW is the total weight of the whole animal.

Determination of the digestibility

The apparent digestibility coefficients were calculated for the dry matter (ADCDM), protein (ADCPROT) and lipids (ADCL) using the standard equation according to Maynard and Loosli (1969):

$$\mathrm{ADC} = 100 - (100 \times \%\mathrm{Mdiet}\%\mathrm{Mfaeces}) \times (\%\mathrm{Nfaeces}/\%\mathrm{Ndiet}),$$

where M is the inert marker and N the nutrient.

Acid insoluble ash (AIA) was used as inert marker, following the method described by Atkinson, Hilton and Slinger (1984). The final percentage of each lipid class in the sample was obtained as the product of the percentage of total lipids and that representing each class in HPTLC. 5 g of sample were used to obtain the acid insoluble ash (AIA) content, 0.2 g to calculate the lipid content (both faeces and the lyophilized diets) and 0.2 and 0.5 g to calculate proteins for diets and faeces respectively. Each analysis was carried out in triplicate.

Parameters calculated and data analysis

All samples were weighed at the beginning (W_i, initial weight in g), on day 28 from the start of the experiment and end of the experiment (day 56; W_f, final weight in g). The following indices were calculated:

$$\text{Average weight} : W_a\ (\mathrm{g}) = (W_i + W_f/2);$$

$$\text{Weight gain} : W_g\ (\mathrm{g}) = W_f - W_i;$$

$$\text{Absolute feeding rate} : \mathrm{AFR}\ (\mathrm{g/day}) = \mathrm{IF}/t;$$

$$\text{Specific feeding rate} : \mathrm{SFR}\ (\%\mathrm{BW/day}) = (\mathrm{AFR}/W_a) \times 100;$$

$$\text{Absolute growth rate} : \mathrm{AGR}(\mathrm{g/day}) = (W_f - W_i)/t;$$

$$\text{Specific growth rate} : \mathrm{SGR}(\%\mathrm{BW/day}) = (\mathrm{Ln}W_f - \mathrm{Ln}W_i)100/t;$$

$$\text{Feed efficiency} : \mathrm{FE}(\%) = (W_f - W_i)100/\mathrm{IF};$$

$$\text{Feed conversion ratio} : \mathrm{FCR} = \mathrm{IF}/(W_f - W_i);$$

$$\text{Absolute protein feeding rate : APFR(g/day)} = \text{IP}/t;$$

$$\text{Absolute lipid feeding rate : ALFR(g/day)} = \text{IL}/t;$$

$$\text{Protein productive value : PPV(\%)} = 100 \times (\text{Retained protein/IP});$$

$$\text{Lipid productive value : LPV(\%)} = 100 \times (\text{Retained lipid/IL});$$

$$\text{Digestive gland index : DGI(\%)} = (\text{DGW}/W_f) \times 100;$$

where IP is the ingested protein in g and IL the ingested lipid in g; DGW = digestive gland weight and IF is the feed ingested in g, corrected by taking into account the disaggregation rate in water and calculated according to the following formula:

$$\text{IF (wet weight in g)} = (\text{dry feed supplied in g} - \text{uneaten dry feed in g} \times F) + \text{Moisture feed supplied in g},$$

where F values were around 1 (0.99–1.02) for all the formulated diets.

The results obtained were expressed as means ± standard deviation (SD). To analyse the differences, a one way analysis of variance (ANOVA) was carried out. The significant differences obtained between the mean values were analysed using Duncan's test, which permits groups with a different number of samples to be compared, using a level of significance of $P < 0.05$. A Neperian logarithmic transformation of the indices and content was made before the ANOVA.

Table 2 Macronutrient composition (g kg^{-1} diet as dry weight) and the different lipid classes (g kg^{-1} total lipids detected) of diets formulated without fish oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200)

	A0	A100	A200
Moisture	536.6 ± 8.0^a	444.7 ± 3.3^b	348.0 ± 0.9^c
Crude protein	815.7 ± 6.1^a	667.3 ± 4.1^b	568.3 ± 2.3^c
Crude lipid	137.7 ± 6.2^a	286.4 ± 7.6^b	388.3 ± 1.8^c
Polar lipids (total)	138.9 ± 2.7	147.3 ± 24.3	117.0 ± 9.1
LPC	22.5 ± 1.2	17.3 ± 8.7	12.0 ± 2.1
SM	11.5 ± 3.8^a	19.3 ± 4.0^{ab}	5.5 ± 1.1^b
PC	54.9 ± 0.6	68.1 ± 1.1	62.6 ± 9.2
LPE	13.1 ± 2.1	10.0 ± 0.1	8.9 ± 2.9
PS/PI	11.5 ± 1.1	9.3 ± 1.6	6.1 ± 1.7
PE	32.2 ± 4.2^a	22.7 ± 1.8^b	21.5 ± 0.2^b
Neutral lipids (total)	861.2 ± 2.6	852.7 ± 24.5	883.2 ± 9.0
MG	48.8 ± 8.3	40.3 ± 0.7	42.8 ± 1.8
DG	23.7 ± 0.9^a	43.0 ± 4.8^b	50.3 ± 5.1^b
CHO	123.2 ± 1.1^a	95.0 ± 7.2^b	85.4 ± 5.9^b
FFA	248.4 ± 4.4^a	156.3 ± 5.4^b	134.4 ± 6.2^c
TG	374.8 ± 5.2^a	450.7 ± 2.0^b	490.7 ± 9.6^b
SE/WE	47.6 ± 0.2^a	72.3 ± 1.6^b	79.6 ± 0.4^c
Ash	43.8 ± 0.9^a	34.3 ± 0.9^b	29.7 ± 0.3^c
NFE	6.3 ± 4.1	12.1 ± 1.1	13.6 ± 0.4
AIA	1.4 ± 0.1^a	0.7 ± 0.1^b	0.7 ± 0.0^b
Energy (KJ 100 g^{-1})*	2471	2709	2874
P/E (g MJ^{-1})	33.00	24.63	19.77

LPC, Lysophosphatidylcholine; SM, Sphingomyelin; PC, Phosphatidylcholine; LPE, Lysophosphatidylethanolamine; PS/PI, Phosphatidylserine and Phosphatidylinositol; PE, Phosphatidylethanolamine; MG, Monoacylglycerols; DG, Diacylglycerols; CHO, Cholesterol; FFA, Free Fatty Acids; TG, Triacylglycerols; SE/WE, Steryl Esters and Waxes; NFE, Nitrogen-free extract, calculated by difference; AIA, Acid Insoluble Ash; P/E, protein/energy ratio.

Data are expressed as mean ± SD; Values on the same line and different superscripts are significantly different ($P < 0.05$).

*Energy coefficients: protein 23.6 kJ g^{-1}, lipid 38.9 kJ g^{-1} and carbohydrate 16.7 kJ g^{-1} according to Miglavs and Jobling (1989).

Results

The formulated diets had a firm texture before being put into water. Diet A0 lost 1.75%, diet A100 lost 0.0% and diet A200 lost 0.22% of their respective dry weights after 24 h in water, confirming their great stability. There were significant differences in the content of all the macronutrients analysed, diminishing percentage of moisture, protein and ash and increasing percentage of lipids, as the oil content of each diet increased (Table 2). As regards the different lipid classes (% of total lipids), there were no significant differences in the total NLs or total PLs, although the percentage of DG, TG and SE/WE increased and those of SM, PE, CHO and FFA decreased significantly as the content of oil in the diets increased. The energy content was higher in diets A100 and A200 than in diet A0, whereas, the P/E ratio (g MJ^{-1}) was lower in diets A100 and A200 than in diet A0 (Table 2).

All the animals found the diets acceptable, and there was a 100% survival rate in all three groups. During the first month (days 1–28) A0 was the best accepted diet, with an SFR of 1.78 ± 0.15% BW day^{-1} ($P < 0.05$). However, there were no

significant differences in growth (10–11 g day^{-1}) or feed efficiency between groups. Group A0 showed a significantly lower ALFR than A100 and A200 groups (Table 3). During the second month (days 29–56) there was no significant difference in the acceptability of the diets, the SFR being similar. However, animals fed diet A0 showed significantly better growth rates, with AGR of 8.11 ± 1.32 g day^{-1} – double and four times the equivalent rates of the groups fed the A100 and A200 diets (4.38 ± 1.56 g day^{-1} and 2.65 ± 1.88 g day^{-1} respectively; Table 3). Similarly, the indices of feed efficiency (FE, FCR) were significantly better with A0 than in A100. The FE and FCR values for diet A200 were not calculated in the second month because one individual showed a negative value. When the complete period was analysed (days 1–56), animals fed diet A0 showed significantly better growth rates (W_g, AGR, SGR), emphasizing a W_g higher (535 ± 77 g) than those fed with diets A100 and A200 (408 ± 95 g and 361 ± 63 g respectively). ALFR was significantly lower for diet A0, (1.01 g day^{-1}) which doubled in A100 and tripled in A200. The best feed efficiency was detected in A0, although the differences were not significant (for FE, FCR and PPV). Neither were there any differences in DGI. The lipid productive value (LPV) was significantly higher for group A0 (Table 3).

The ADCDM and ADCL values for diet A0 were higher than those obtained for diets A100 and A200. The ADCPROT were similar in the three diets (95–98%; Table 4). According to our results, polar lipids showed higher apparent digestibility coefficients (>83% for the three diets) than neutral lipids (2–87%), whose digestibility decreased with increasing content of lipid in the diet, reaching 0% in MG and FFA in the diets containing fish oil. A similar pattern was observed for all the neutral lipids analysed (Fig. 1). In contrast, no diminution in the digestibility of any of the polar lipids was observed with increasing lipid content in the diet (Fig. 1).

The main differences as regards the nutritional composition of the animals were observed in the digestive gland, in which all of the experimental groups showed higher content of lipids and lower content of protein and ash than initial control animals ($P < 0.05$). The animals fed diets A100 and A200 showed significantly higher content of lipids and a lower content of protein than those fed diet A0 (Table 5). The differences were not significant for the nutritional composition of the carcass. The content of protein in the whole animal was lower in the groups fed diet A100 (730.2 g kg^{-1}) and A200 (740.4 g kg^{-1}) than in those group CI (772.8 g kg^{-1}). The lipid content was greater in the animals fed the experimental diets than in the CI group (Table 5). NL predominated in the digestive gland (877–919 g kg^{-1}) compared with PL (81–133 g kg^{-1}), with TG, FFA and SE/WE being the major lipid classes. In the carcass PL was higher than NL, with PC, PE and CHO being the main lipid classes (Table 6). In both the digestive gland and the carcass, the content of NL increased and the content of PL decreased as the content of oil in the diet rose ($P < 0.05$). These changes can be mainly attributed to a greater content of TG in the digestive gland and CHO, FFA and TG in the carcass ($P < 0.05$).

Discussion

The three formulated diets used were based exclusively on dry and freeze-dried ingredients to feed *O. vulgaris.* The diets used the same base (Morillo-Velarde *et al.* 2012) and only differed in the content of oil, so that any differences seen from using the feeds can be attributed to this last ingredient. Cod liver oil was used as it is rich in Omega 3 essential oils (EPA and DHA), fundamental components of fish and molluscs (Turchini, Torstensen & Ng 2009).

All three formulated diets showed good acceptability, the highest absolute feeding rates and growth corresponding to diet A0. The feeding rates as dry weight obtained with A0 were higher than other experimental diets (Cerezo Valverde *et al.* 2008; Estefanell *et al.* 2012; Morillo-Velarde *et al.* 2012) and better than those estimated in similar experimental conditions for fish-based natural diets (Aguado Giménez & García García 2002; García García & Aguado Giménez 2002). Our results highlight octopus can intake and assimilate the three diets up to 4 weeks, with high and similar SGR among diets. However, of note was the substantial reduction in ingestion and growth recorded in the second month, especially in the two diets with the highest lipid content. These results suggest a poor nutritive balance of the feed or a gradual progressive effect of rejection/aversion to the diet, a phenomenon seen before in studies using artificial diets, in which the animals tended to reduce their ingestion following administration of one format feed for several weeks

Table 3 Mean values ± standard deviation for growth and feed efficiency indices in *Octopus vulgaris* fed formulated diets without fish oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200)

	Days 1–28			Days 29–56			Total period (days 1–56)		
	Diet A0	Diet A100	Diet A200	Diet A0	Diet A100	Diet A200	Diet A0	Diet A100	Diet A200
N	4	6	6	4	6	6	4	6	6
W_i (g)	803 ± 91	817 ± 79	802 ± 88	1111 ± 173	1102 ± 117	1089 ± 152	802 ± 91	817 ± 79	802 ± 88
W_f (g)	1111 ± 173	1102 ± 117	1089 ± 152	1338 ± 140	1225 ± 152	1163 ± 132	1338 ± 140	1225 ± 152	1163 ± 132
W_g (g)	308 ± 105	285 ± 59	287 ± 67	227 ± 37	123 ± 44	74 ± 53	535 ± 77^{a}	408 ± 95^{b}	361 ± 63^{b}
AFR (g day^{-1})	16.97 ± 2.13	13.96 ± 1.52	14.03 ± 3.85	14.60 ± 0.90	12.33 ± 1.16	12.13 ± 3.17	15.79 ± 1.19	13.14 ± 1.32	13.08 ± 3.15
APFR (g day^{-1})	6.42 ± 0.80	5.17 ± 0.56	5.20 ± 1.43	5.52 ± 0.34	4.57 ± 0.43	4.49 ± 1.18	5.97 ± 0.45	4.87 ± 0.49	4.85 ± 1.17
ALFR (g day^{-1})	1.08 ± 0.14^{a}	2.22 ± 0.24^{b}	3.55 ± 0.97^{c}	0.93 ± 0.06^{a}	1.96 ± 0.18^{b}	3.07 ± 0.80^{c}	1.01 ± 0.08^{a}	2.09 ± 0.21^{b}	3.31 ± 0.80^{c}
SFR (%BW day^{-1})	1.78 ± 0.15^{a}	1.46 ± 0.16^{b}	1.47 ± 0.25^{b}	1.20 ± 0.15	1.07 ± 0.10	1.08 ± 0.29	1.48 ± 0.09	1.29 ± 0.13	1.32 ± 0.24
AGR (g day^{-1})	11.01 ± 3.73	10.19 ± 2.10	10.26 ± 2.40	8.11 ± 1.32^{a}	4.38 ± 1.56^{b}	2.65 ± 1.88^{b}	9.56 ± 1.37^{a}	7.29 ± 1.70^{b}	6.45 ± 1.12^{b}
SGR (%BW day^{-1})	1.15 ± 0.29	1.07 ± 0.18	1.08 ± 0.12	0.68 ± 0.18^{a}	0.37 ± 0.10^{b}	0.24 ± 0.19^{b}	0.91 ± 0.11^{a}	0.72 ± 0.12^{b}	0.66 ± 0.09^{b}
FE (%)	63.58 ± 13.81	72.57 ± 9.65	74.24 ± 10.16	55.65 ± 0.80^{a}	35.12 ± 10.10^{b}	n.c.*	60.34 ± 4.68	54.99 ± 8.52	50.29 ± 7.59
FCR	1.63 ± 0.35	1.40 ± 0.21	1.37 ± 0.19	1.84 ± 0.33^{a}	3.05 ± 0.87^{b}	n.c.*	1.67 ± 0.14	1.86 ± 0.31	2.02 ± 0.28
PPV (%)	–	–	–	–	–	–	27.39 ± 3.29	23.39 ± 6.56	21.81 ± 4.16
LPV (%)	–	–	–	–	–	–	14.09 ± 3.62^{a}	9.83 ± 5.06^{ab}	6.48 ± 4.01^{b}
DGI (%)	–	–	–	–	–	–	5.57 ± 0.93	5.11 ± 1.08	5.23 ± 0.68

W_i, initial weight; W_f, final weight; W_g, weight gain; AFR, absolute feeding rate; APFR, absolute protein feeding rate; ALFR, absolute lipid feeding rate; SFR, specific feeding rate; AGR, absolute growth rate; SGR, specific growth rate; FE, feed efficiency; FCR, feed conversion ratio; PPV, protein productive value; LPV, lipid productive value; DGI, digestive gland index.
*N.c.: Not calculated for detecting negative values in 1 individual. Values on the same line and different superscripts are significantly different ($P < 0.05$).

(Domingues, López, Muñoz, Maldonado, Gaxiola & Rosas 2007; Quintana *et al.* 2008), suggesting, in turn, that different diets could be alternated to obtain best results during ongrowing.

As regards the effect of the different lipid contents on growth, the worse results were obtained in individuals fed the diets formulated with 286 and 388 g kg^{-1} lipids DW and ALFR of 2.1 and 3.3 g day^{-1} for diets A100 and A200 respectively (Fig. 2) compared with diet A0. Cerezo Valverde, Hernández *et al.* (2013) obtained better growth using formulated diets containing 300–330 g kg^{-1} lipids DW with similar ALFR (1.2–1.4 g day^{-1}) compared with others containing 40–55 g kg^{-1} lipids DW (ALFR 0.2 g day^{-1}). Estefanell *et al.* (2012) obtained excellent results using 263 g kg^{-1} lipids DW with ALFR 1.5 g day^{-1} (discarded *B. boops* from marine sea-cages agglutinated with alginates) compared with those obtained using a diet with 62 g kg^{-1} lipids DW with ALFR de 0.2 g day^{-1} (*B. boops* caught using traditional methods; Fig. 2) suggesting high efficacy in lipid utilization. These studies coincide in that the best results were obtained with diets formulated with 130–270 g kg^{-1} lipid DW. These results also point to the need to take into account the acceptability of the diet and the use of polar lipid-rich ingredients.

On comparing lipid content and specific growth rate in different natural diets (Fig. 3), the best results were obtained with diets with a low or moderate lipid content (24–187 g kg^{-1} DW; see Fig. 3), but which, nevertheless, showed high ingestion rates and therefore moderate ALFR (0.2–1.8 g day^{-1}). The best growth results were obtained by García García & Cerezo Valverde 2006 with natural diets of crab (24 g kg^{-1} lipids DW; ALFR 0.5 g day^{-1}) and with mixed diets of fish and crab (41–117 g kg^{-1} lipids DW; ALFR 0.7–1.3 g day^{-1}), coinciding with values described for formulated diets. Estefanell *et al.* (2012) also obtained good results with wild bogue (46 g kg^{-1} lipids DW; ALFR 0.2 g day^{-1}) and discarded bogue (187 g kg^{-1} lipids DW; 1.8 g day^{-1}), although the results were worse as the lipid content of the bogue increased (465 g kg^{-1} lipids DW; ALFR 5.4 g day^{-1}; Estefanell *et al.* 2011). These differences could have been due to the different lipid composition and protein content of the natural diets, which would depend on their origin (Estefanell,

Table 4 Apparent digestibility coefficients (%) obtained with formulated diets without fish oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200)

	A0	A100	A200
ADCPROT	95.16	97.48	96.22
ADCL	81.25	26.78	12.27
ADCDM	87.78	70.98	57.44

ADCPROT, apparent digestibility coefficients of the protein; ADCL, apparent digestibility coefficients of the lipids; ADCDM, apparent digestibility coefficients of the dry matter.

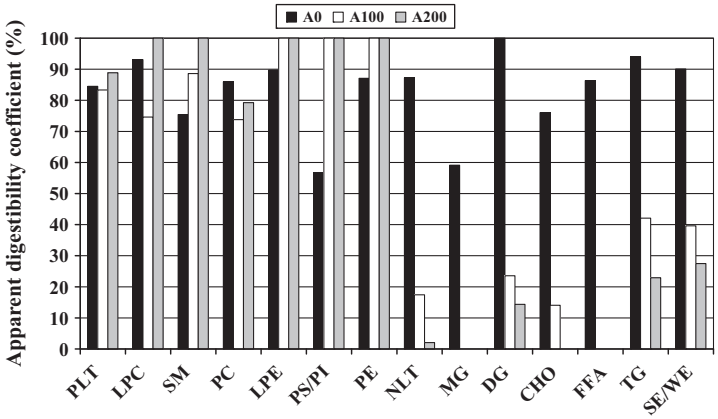

Figure 1 Comparison of apparent digestibility coefficients of the different lipid classes obtained with the diets formulated without fish oil (A0), and with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200). PLT: Polar Lipids Total; LPC: Lysophosphatidylcholine; SM: Sphingomyelin; PC: Phosphatidylcholine; LPE: Lysophosphatidylethanolamine; PS/PI: Phosphatidylserine and Phosphatidylinositol; PE: Phosphatidylethanolamine; NLT: Neutral Lipids Total; MG: Monoacylglycerols; DG: Diacylglycerols; CHO: Cholesterol; FFA: Free Fatty Acids; TG: Triacylglycerols; SE/WE: Steryl Esters and Waxes.

Table 5 Macronutrient composition (g kg^{-1} dry weight) of the different fractions of *Octopus vulgaris* fed with natural diets at the beginning of the experiment (CI) or diets formulated without fish oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200)

	CI ($N = 3$)	A0 ($N = 4$)	A100 ($N = 6$)	A200 ($N = 6$)
Digestive gland				
Moisture	658.2 ± 40.2	622.5 ± 36.7	584.9 ± 47.6	595.4 ± 56.4
Ash	57.5 ± 16.0	35.0 ± 1.7*	33.3 ± 7.3*	29.6 ± 5.7*
Crude protein	582.0 ± 78.6	491.1 ± 52.0[a]	364.0 ± 47.4[b,*]	380.2 ± 79.1[b,*]
Crude lipid	213.5 ± 64.6	366.9 ± 46.1[a,*]	516.5 ± 51.4[b,*]	512.6 ± 75.9[b,*]
NFE	147.0 ± 16.6	107.0 ± 12.4	88.0 ± 33.3	77.9 ± 33.2
Carcass				
Moisture	817.9 ± 12.1	805.9 ± 6.6	803.3 ± 10.9	808.2 ± 6.6
Ash	117.9 ± 15.6	98.5 ± 8.9	107.6 ± 9.9	111.9 ± 7.0
Crude protein	786.1 ± 20.4	797.2 ± 14.7	763.4 ± 18.4	784.0 ± 20.1
Crude lipid	7.0 ± 2.9	3.8 ± 2.7	4.1 ± 1.5	5.5 ± 4.3
NFE	89.0 ± 2.1	100.5 ± 8.9	124.9 ± 18.4	98.6 ± 25.0
Whole animal				
Moisture	811.2 ± 9.4	795.9 ± 6.6	791.9 ± 12.9	796.8 ± 9.6
Ash	112.9 ± 13.3	92.1 ± 8.8	99.9 ± 10.1	103.3 ± 8.6
Crude protein	772.8 ± 18.3	765.9 ± 8.4	730.2 ± 23.6*	740.4 ± 29.2*
Crude lipid	21.1 ± 5.5	40.8 ± 6.9*	57.2 ± 18.3*	60.4 ± 21.5*
NFE	93.1 ± 3.5	101.1 ± 7.5	121.1 ± 19.4	95.9 ± 21.9

NFE, Nitrogen-free extract, calculated by difference.
Data are expressed as mean ± standard deviation; Values on the same line and different superscripts are significantly different ($P < 0.05$).
*$P < 0.05$ respect to initial control (CI).

Socorro, Guirao, Ferna′ndez-Palacios, Izquierdo & Roo 2010).

Of particular note in our results is the high feed efficiency of diet A0 (FE del 60.3%) compared with other natural and experimental diets (<55% in natural diets; e.g. García García & Cerezo Valverde 2006; Biandolino, Portacci & Prato 2010; Prato, Portacci & Biandolino 2010; Estefanell *et al.* 2011; <28% in experimental diets; e.g. Cerezo Valverde *et al.* 2008; Quintana *et al.* 2008; Cerezo Valverde, Hernández *et al.* 2013). The PPV and LPV values for diet A0 were moderate (27.39 and 14.09% respectively), the values for PPV being similar to those obtained with crab (28%) but lower than those obtained for mixed diets of bogue and crab (33%) and bogue alone (36%; García García & Cerezo Valverde 2006). These results may be due to the fact that the main source of protein in our formulated feeds was gelatin, whose protein is deficient in most essential and non-essential amino acids (Karim & Bhat 2009) compared with the amino acid content of *O. vulgaris* (Cerezo Valverde, Martínez-Llorens, Tomás Vidal, Jover, Rodríguez, Estefanell, Gairín, Domingues, Rodríguez & García García 2013). It is therefore necessary to continue research into the formulation of feeds supplemented with pure amino acids or specific raw materials to improve protein performance (Cerezo Valverde, Martínez-Llorens *et al.* 2013).

Protein digestibility was above 95% for all the diets assayed, the value coinciding with those found for natural diets (Hernández & García García 2004; Mazón, Piedecausa, Hernández & García García 2007; Sánchez, Hernández, Cerezo Valverde & García García 2009) and experimental diets (Seiça Neves, Cerezo Valverde & García García 2010; Morillo-Velarde *et al.* 2012), underlining the efficient enzymatic ability of *O. vulgaris* to digest these nutrients (Boucher-Rodoni 1982; Aguila, Cuzon, Pascual, Domíngues, Gaxiola, Sánchez, Maldonado & Rosas 2007; Hamdan, Tomás-Vidal, Martínez, Cerezo Valverde, Moyano & Moyano 2014). Our results suggest that the ADCPROT were not significantly affected by the content of lipids in the diet (Fig. 4) because ADCPROT remains practically constant in all studies regardless of the lipid content of the diet. In contrast, the lipid digestibility coefficients varied with content of lipids in the diet. Only diet A0, which contained 138 g kg^{-1} lipids DW led to a high ADCL (81.25%), a value which fell drastically to 22.3% when the diet contained 388 g kg^{-1} lipids

Table 6 Lipid class content (g kg^{-1} total lipids detected) in carcass and digestive gland in *Octopus vulgaris* fed formulated diets without fish oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200)

	A0 (*N* = 4)	A100 (*N* = 6)	A200 (*N* = 6)
Digestive gland			
Polar lipids (total)	123.0 ± 7.7[a]	133.0 ± 29.3[a]	81.4 ± 23.9[b]
LPC	9.8 ± 1.4	8.7 ± 3.4	n.d.
PC	44.4 ± 2.7	52.0 ± 14.3	42.3 ± 17.4
LPE	8.2 ± 6.8	7.9 ± 4.0	8.1 ± 4.8
PS/PI	33.4 ± 3.3[a]	33.3 ± 9.0[a]	16.2 ± 7.7[b]
PE	35.7 ± 2.9	28.8 ± 9.9	27.3 ± 8.3
Neutral lipids (total)	877.0 ± 7.7[a]	877.9 ± 29.7[a]	918.6 ± 23.9[b]
MG	33.2 ± 4.2	38.1 ± 7.0	42.3 ± 11.6
DG	45.1 ± 3.3	53.7 ± 10.1	44.3 ± 7.0
CHO	52.8 ± 5.5[a]	39.5 ± 11.5[b]	42.6 ± 4.5[ab]
FFA	155.0 ± 11.9	119.9 ± 24.0	151.6 ± 41.6
TG	461.0 ± 30.4[a]	522.3 ± 65.5[ab]	588.7 ± 64.4[b]
SE/WE	124.7 ± 12.4	124.7 ± 13.8	106.7 ± 21.2
Carcass			
Polar lipids (total)	671.0 ± 16.4[a]	635.0 ± 11.6[b]	616.4 ± 22.0[b]
LPC	12.5 ± 4.7[a]	9.4 ± 2.3[ab]	7.5 ± 1.7[b]
SM	21.1 ± 4.5	22.7 ± 2.0	25.2 ± 1.4
PC	244.1 ± 10.3[a]	224.0 ± 19.2[ab]	213.2 ± 13.2[b]
LPE	14.5 ± 5.3	13.9 ± 5.1	14.3 ± 1.6
PS	83.0 ± 2.3	79.8 ± 7.2	84.6 ± 7.6
PI	124.6 ± 7.1	125.0 ± 5.4	122.4 ± 8.1
PE	171.3 ± 8.2	165.8 ± 8.0	162.5 ± 6.6
Neutral lipids (total)	329.0 ± 16.4[a]	365.0 ± 11.6[b]	383.6 ± 21.9[b]
MG	29.9 ± 4.2	33.7 ± 3.7	29.9 ± 2.5
CHO	158.8 ± 4.6[a]	157.9 ± 5.7[a]	173.3 ± 2.2[b]
FFA	52.2 ± 4.9[a]	60.3 ± 8.7[a]	75.0 ± 13.0[b]
TG	11.0 ± 3.1[a]	12.9 ± 4.0[a]	29.2 ± 9.1[b]
SE/WE	62.8 ± 8.7[a]	96.0 ± 16.5[b]	77.9 ± 6.6[ab]

LPC, Lysophosphatidylcholine; SM, Sphingomyelin; PC, Phosphatidylcholine; LPE, Lysophosphatidylethanolamine; PS/PI, Phosphatidylserine and Phosphatidylinositol; PE, Phosphatidylethanolamine; MG, Monoacylglycerols; DG, Diacylglycerols; CHO, Cholesterol; FFA, Free Fatty Acids; TG, Triacylglycerols; SE/WE, Steryl Esters and Waxes.

Values on the same line and different superscripts are significantly different ($P < 0.05$)

DW (Fig. 5), ADCDM falling at the same time (Fig. 6). These results show that octopus has a limited capacity of lipid digestion and catabolization, which is borne out by previous results obtained for cephalopods (Ballantyne, Hochachka & Mommsen 1981; Mommsen & Hochachka 1981; Lee 1994). O'Dor *et al.* (1984) did obtain a high lipid digestibility (95%), although this result was obtained by injecting a pure fatty acid that does not require digestion to be absorbed in the digestive tract and therefore this nutrient use outside the context of a formulated diet and the present study.

It was of note that as the lipid content of the diet rose, so the digestibility of neutral lipids fell, reaching zero digestibility for MG and FFA in diets A100 and A200 (Fig. 1). This zero digestibility factor needs greater consideration and suggests that most of the triacylglycerols in the diets had dedoubled into free fatty acids and monoacylglycerols, which were therefore more abundant in the faeces than in the diet consumed. In contrast, the digestibility coefficients of the polar lipids were high, probably due to the presence of phospholipids in the diet since these improve lipid emulsification and facilitate the transport of dietary fatty acids and lipids from the gut to the rest of the body, possibly through enhanced lipoprotein synthesis, as has been suggested for both *O. vulgaris* and several fish species (Craig & Gatlin 1997; Kasper & Brown 2003; Tocher, Bendiksen, Campbell & Bell 2008). Therefore, the digestive capacity for absorption of these nutrients was not affected in the present study. The predominant role of phospholipids in the tissues of cephalopods (Sinanoglou & Miniadis-Meimaroglou 1998; Navarro & Villanueva 2000, 2003; Cerezo Valverde *et al.* 2012) and their mobilization, especially during growth phases (Morillo-Velarde *et al.* 2013) suggest that future ongrowing studies in *O. vulgaris* could well use formulated diets supplemented with this lipid class.

According to our results and those of other authors (Hernández & García García 2004; Mazón *et al.* 2007; Sánchez *et al.* 2009; Seiça Neves *et al.* 2010), the best ADCL are obtained with diets that contain 20–140 g kg^{-1} of lipid DW, since higher percentage lead to much lower ADCL. This agrees with the growth results of our study which results suggest that diets should contain less than 140 g kg^{-1} lipids DW if ADCDM is not to fall below 90% and if a high quantity of nutrients are not to be lost in the faeces (Figs. 5 and 6).

Unlike the findings of other studies in which the lipid class composition of the carcass and the whole animal was more constant than in the digestive gland (García-Garrido *et al.* 2010; Morillo-Velarde *et al.* 2012), the content of the different lipids was affected by the diet consumed. NL levels in the carcass increased and PL decreased as lipid ingestion rose, which agrees with the findings of Morillo-Velarde *et al.* (2013), who suggested that triglycerides

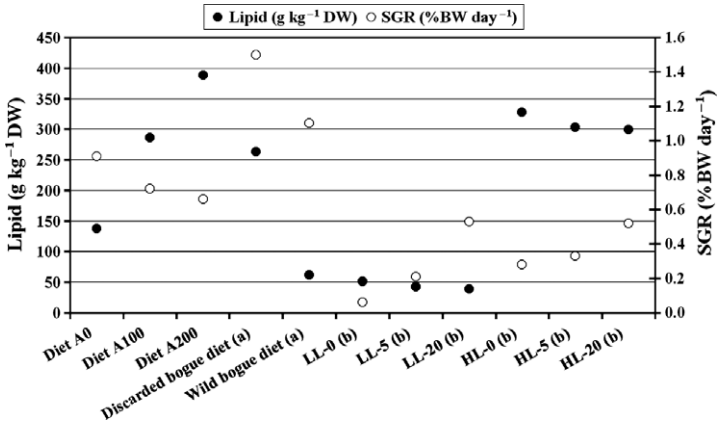

Figure 2 Lipid content (g kg^{-1} dry weight) and specific growth rate (SGR,%BW day^{-1}) in different formulated diets without fish oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200) and other experimental diets: (a) Estefanell *et al.* 2012; (b) Cerezo Valverde, Hernández *et al.* 2013.

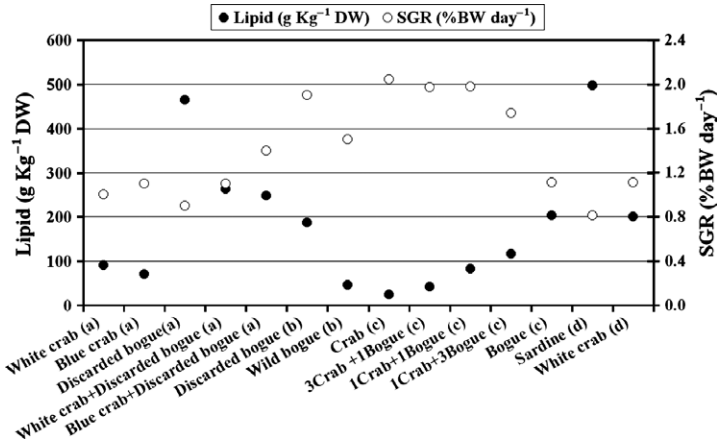

Figure 3 Lipid content (g kg^{-1} dry weight) and specific growth rate (SGR,%BW day^{-1}) in different natural diets: (a) Estefanell *et al.* 2011; (b) Estefanell *et al.* 2012; (c) García García & Cerezo Valverde 2006; (d) García García & Aguado Giménez 2002.

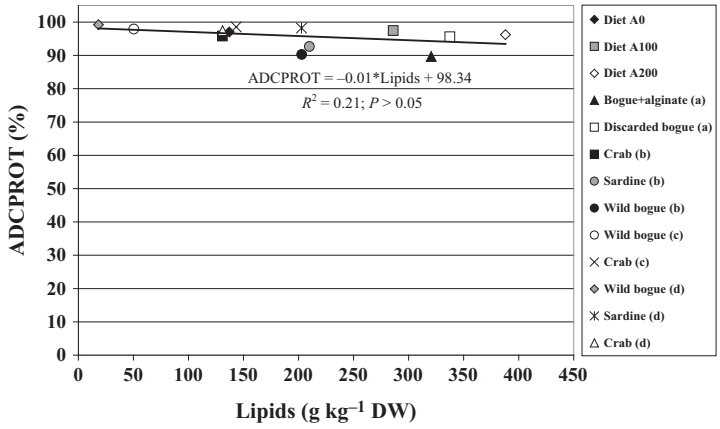

Figure 4 Apparent digestibility coefficients for protein (ADCPROT %) as a function of the lipid in the diet (g kg^{-1} dry weight) for the different diets formulated without fish oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200) and other experimental diets: (a) Seiça Neves *et al.* 2010; (b) Sánchez *et al.* 2009 (c) Mazón *et al.* 2007; (d) Hernández & García García 2004.

and cholesterol are transported simultaneously from the digestive gland to the muscular tissues during starvation. The results also suggest that diets with high lipid content may produce metabolic disorders and provoke changes in the composition of the tissues or an alteration in the form of lipid storage and transport. The higher levels of lipid in diets A100 and A200 were also reflected in the high content of lipid in the octopus digestive gland, which has been seen to serve as lipid and energy store (Sie-

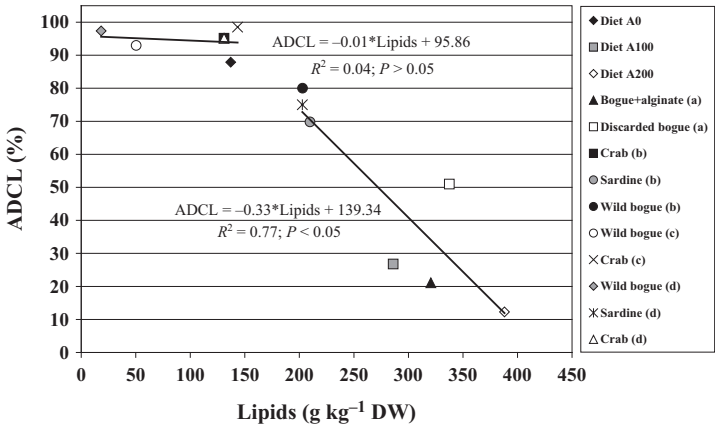

Figure 5 Apparent digestibility coefficients for lipids (ADCL%) as a function of the lipid in the diet (g kg^{-1} dry weight) for the different diets formulated without fish oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200) and other experimental diets: (a) Seiça Neves *et al.* 2010; (b) Sánchez *et al.* 2009 (c) Mazón *et al.* 2007; (d) Hernández & García García 2004.

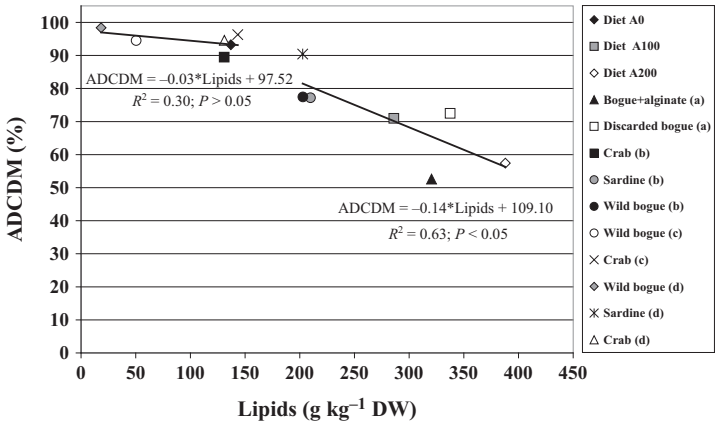

Figure 6 Apparent digestibility coefficients for dry matter (ADCDM%) as a function of the lipid in the diet (g kg^{-1} dry weight) for the different diets formulated without oil (A0), with 100 g kg^{-1} (A100) and 200 g kg^{-1} (A200) and other experimental diets: (a) Seiça Neves *et al.* 2010; (b) Sánchez *et al.* 2009 (c) Mazón *et al.* 2007; (d) Hernández & García García 2004.

iro *et al.* 2006; Cerezo Valverde, Hernández *et al.* 2013; Morillo-Velarde *et al.* 2013).

The results obtained in the present study represent a great stride forward in the search for formulated *O. vulgaris* diets suitable for commercial purposes. First, the format used in the study showed high degree of acceptability, leading to high ingestion and growth rates. Second, the best results were obtained with lipid feeding rates of around 1 g day^{-1} and lipid content of around 130–140 g kg^{-1} DW in the formulated diets. A lower lipid content would diminish the performance of diet and increase protein use, while a higher content would decrease diet digestibility and perhaps lead to metabolic disorders associated with the consumption of excess lipids. The results also point to the interest of carrying out further studies into diets supplemented with phospholipids because of their high digestibility and their major role in the composition of cephalopod tissues. They will also require the study of metabolic pathways and determination of essential and non-essential fatty acids with marking of given molecules. In light of this, the content of lipid in the diet of octopus could vary as a function of the lipid classes present in their composition.

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