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Performance of Marine Lecithin Supplemented Feeds for the Common Octopus (*Octopus vulgaris*) Ongrowing: Changes in Proximate Composition and Lipid Classes' Profile

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Abstract: The development of artificial diets is considered vital for feasible cephalopods' culture. *Octopus vulgaris* need a diet with a high protein content but also lipids are important at a lower quantity, as polar lipids and LC-PUFA are essential for development during early stages. In the present study the suitability of marine lecithin as a dietary supplement for *O. vulgaris* juveniles' formulated feeds was tested for 56 days, assessing the performance, changes in proximate composition, and lipid classes' profile in the digestive gland and carcass. Sixteen octopus were fed one of two semi-moist feeds based on dry ingredients: either CALPRO (N = 4) as control or CALPRO-LM (N = 8); which differed from the first, due to the inclusion of 20 g/kg of marine lecithin as a phospholipid dietary supplement. Results showed that marine lecithin did not enhance feed intake, growth, protein or lipid incorporation, nutrients digestibility or feed efficiency. Moreover, at this level of inclusion, the composition of tissues (digestive gland and carcass) regarding macronutrients and lipid classes' profile presented only a small amount of differences. In conclusion, the inclusion of marine lecithin did not promote beneficial effects on performance, making necessary further research related to the nutritional requirements of common octopus.

Keywords: common octopus; *Octopus vulgaris*; diet performance; dietary supplementation; formulated feed; growth; marine lecithin; marine phospholipid

1. Introduction

The development of artificial sustainable diets and the control of reproduction were identified as the main bottlenecks of cephalopods' culture by Villanueva et al. [1]. Efforts have been made to progress in the development of pellets for some important species, such as the common octopus (*Octopus vulgaris*). The first attempt in the development of formulated feeds for the common octopus tested moist diets made by mixing a fish or crustacean paste with binders [2–5]. Then, dry ingredients (freeze-dried or conventional meals) were included in semi-moist [6–13] or pelleted diets [14–16]. *O. vulgaris* preferably chose low-temperature processed feeds [11,12] and either rejected or showed low performance when fed other high-temperature processed ones [14–16]. Recently, Rodríguez–González et al. [17] observed that applying a high drying temperature (100 °C) promoted changes in the polar lipid pool, while applying freeze-drying or a low dehydration temperature (<60 °C) did not, to some extent, when compared with frozen raw materials.

In general terms, *O. vulgaris* requires an artificial diet with a high protein percentage and amino acid content to sustain growth, while lipids are also essential but should be included at a lower quantity [18]. Still, O'Dor et al. [19] suggested that lipids could be a limiting factor even in natural diets.

Despite the multitude of studies regarding ongrowing (supplying natural or formulated diets with different lipid content [3,10,20,21]) and starvation [22,23]; the amount and profile of the lipid fraction considered in the feed formulation for cephalopods still considers the body composition of both wild cephalopods and prey [18].

The low lipid content of cephalopods in general and that of *O. vulgaris* muscle in particular; which is characterized by a high polar lipid fraction compared to the neutral one [24,25], is remarkable. The species hatchlings [26–31] and adults [25,32–34] display similar lipid class profiles, which are characterized by a polar lipid fraction rich in phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylethanolamine (PE), while cholesterol (CHO) is the main class found in neutral lipids. According to Almansa et al. [35] and Navarro and Villanueva [26,36], phospholipids, CHO, and long-chain polyunsaturated fatty acids (LC-PUFA) play a pivotal role in cephalopod development at early stages. In fact, LC-PUFAs, such as docosahexaenoic (DHA), eicosapentaenoic (EPA), and arachidonic (ARA) acids have been considered as essential fatty acids (EFA; [30,31,37]) for *O. vulgaris* hatchlings. The species incorporates these fatty acids (FA) by esterifying them into specific phospholipid substrates [31] which display characteristic FA, or EFA, profiles in major phospholipids [29]. Recent studies reported an enzymatic characterization of the biosynthetic pathways of LC-PUFA and EFA for the species [38,39]. The similarity between the major lipid classes of different cephalopod species and the recent knowledge regarding lipids physiology and requirements points to the possibility that the lipid class's profile of feeds might be a limiting factor for growth performance of *O. vulgaris* when fed artificial diets.

Natural diets, rich in neutral lipids, are commonly associated with restrictions in nutrient absorption, due to a saturation of the digestive system, as a consequence of the absence of emulsifiers or low enzyme activity in the digestive tract of cephalopods [19,20,40]. Moreover, a reduction in lipids' apparent digestibility coefficients related to increasing fish oil in formulated diets composition was observed by Morillo–Velarde et al. [10]. In fact, some authors observed differences between the FA profile of incorporated lipids in *O. vulgaris* hatchlings, namely a preferential esterification of PUFA and EFA into polar lipids while monounsaturated FA were usually esterified into neutral lipids [29,31,41], depending on availability. A higher digestibility of prepared diets, observed when including a higher polar lipid fraction, has been reported [10,42,43]. This sustains the hypothesis that a diet with a lower total lipid but with a higher polar lipid fraction might prevent the lipid digestion issues reported to occur in cephalopods.

There are multiple reports regarding the inclusion of soybean lecithin or marine phospholipids as dietary supplementation in feeds for larvae and juvenile fish to cover the limited capacity for phospholipid synthesis [44], promoting positive effects in growth, survival, digestive functions, and skeletal development [45–49]. In contrast, there is only one manuscript [50] regarding the inclusion of phospholipids in diets for octopus, namely for paralarvae. Guinot et al. [50] used either soybean lecithin or marine phospholipids as enrichment for *Artemia* sp. metanauplii as an attempt to obtain a lipid profile that would cover octopus paralarvae requirements. To the best of our knowledge, until present, there is not a single study on the use of marine phospholipid dietary supplementation of feeds for *O. vulgaris* or for any cephalopod.

Phospholipids used as supplement for artificial diets are commonly extracted from vegetable (soybean), non-marine (egg yolk, milk or brain) or marine animal sources [51,52]. Contrary to other sources, marine phospholipids (usually obtained from the roe of various fish species and krill oil, i.e., 38%–75% and 40%, respectively; [51]) are highly rich in n-3 LC-PUFA, namely EPA and DHA [53]. In both fish and krill oil, PC is the main class found in extractions, followed by PE, PI, and Sphingomyelin (SM) [52]. Since marine lecithin is rich in these EFA and lipid classes, its inclusion as a supplement could be a suitable way to enhance the feed performance of prepared diets given to octopus. In this sense, the present study tested the suitability of marine lecithin (at 20 g/kg) as a dietary supplement for *O. vulgaris* formulated feeds regarding changes in feed intake, growth, nutrients digestibility, and animals' composition (macronutrients and lipid classes' profile).

2. Results

2.1. Formulated Feeds

All tested feeds had a consistent texture before being placed in seawater. The supplemented feed (CALPRO-LM) presented a higher disintegration rate in seawater after 24 h when compared to CALPRO ($p < 0.05$; Table 1).

Table 1. Basal composition (g/kg) of non-supplemented (CALPRO) and marine lecithin supplemented formulated feeds (CALPRO-LM).

Basal Composition (g/kg)	CALPRO	CALPRO-LM
Water	400	400
Gelatin [1]	100	100
Squid (*Todarodes sagittatus*)	150	150
Round sardinella (*Sardinella aurita*)	50	50
Little rockfish (*Boops* sp., *Diplodus* sp.)	50	50
Crab (*Carcinus mediterraneus*)	20	20
Egg yolk [2]	50	50
Starch [3]	100	80
Glucose [4]	20	20
Arginine [4]	5	5
Glutamate [4]	5	5
Marine lecithin [5]	0	20
Gum PRS-T [1]	25	25
Gum PRS-30 [1]	25	25
WSI [6] (g/kg dry matter loss)	42.1 ± 0.5	207.6 ± 5.4 *

[1] Productos Sur, S.A (Pol. Ind. Oeste, San Ginés, Murcia); [2] Avícola San Isidro, S.L. (Los Belones, Cartagena, Murcia); [3] Distriver Hernández S.L. (Alcantarilla, Murcia); [4] Panreac Química S.L.U., (Castellar del Vallés, Barcelona, España); [5] Lecimarine F30, Standard, Innovafood S.L., (Barcelona); [6] WSI—Water stability index. * Statistical difference for $p < 0.05$.

Both feeds presented about 445–466 g/kg of moisture, thus being classified as semi-moist diets (Table 2). The protein, ash, and energy contents were similar between both feeds ($p > 0.05$; Table 2). On the other hand, the inclusion of marine lecithin raised the crude lipid and energy content, and reduced the P/E ratio, in CALPRO-LM ($p < 0.05$). Contrariwise, CHOT was higher in CALPRO due to its high starch content in the basal composition ($p < 0.05$; Table 1). Both feeds presented higher content of absolute TNL than TPL, but no differences were found in either TPL or TNL between the CALPRO and CALPRO-LM feeds regarding g/kg of total lipid ($p > 0.05$, respectively).

Table 2. Macronutrient composition (g/kg dry weight) and lipid class profile (g/kg total lipid) of non-supplemented (CALPRO) and marine lecithin supplemented formulated feeds (CALPRO-LM).

Diet	CALPRO	CALPRO-LM
Moisture	448.2 ± 5.1	466.3 ± 1.8 *
Crude protein	672.5 ± 6.9	674.3 ± 8.8
Crude lipid	71.9 ± 1.7	101.6 ± 3.0 *
CHOT [1]	196.7 ± 5.6	165.8 ± 9.2 *
Ash	58.9 ± 0.7	58.3 ± 1.3
AIA [2]	0.587 ± 0.015	0.476 ± 0.017 *
Energy (MJ/kg)	22.00 ± 0.04	22.67 ± 0.09 *
P/E [3]	30.41 ± 0.09	29.59 ± 0.43 *
LPC [4]	21.0 ± 9.4	20.8 ± 4.3
SM [5]	19.9 ± 1.0	23.2 ± 7.7
PC [6]	58.8 ± 11.4	53.1 ± 9.8
LPE [7]	12.1 ± 1.3	4.8 ± 4.4 *
PS+PI [8]	13.7 ± 6.5	19.8 ± 11.0

Table 2. *Cont.*

Diet	CALPRO	CALPRO-LM
PA [9]	9.8 ± 2.8	4.9 ± 8.5
PE [10]	2.9 ± 5.1	2.1 ± 3.6
UPL [11]	2.9 ± 2.7	n.d.
MG [12]	67.7 ± 12.7	86.6 ± 6.1
DG [13]	10.3 ± 1.0	8.3 ± 3.7
CHO [14]	92.9 ± 5.3	61.6 ± 13.6 *
FFA [15]	248.3 ± 13.7	236.8 ± 20.8
TAG [16]	350.1 ± 13.6	376.4 ± 26.0
SE [17]	89.5 ± 5.9	101.6 ± 7.6
TLP [18]	141.2 ± 21.9	128.7 ± 6.0
TLN [19]	858.8 ± 21.7	871.4 ± 6.1

[1] CHOT—total carbohydrates, calculated by difference; [2] AIA—insoluble acid ash; [3] P/E—protein/energy ratio. [4] LPC: Lysophosphatidylcholine, [5] SM: Sphingomyelin, [6] PC: Phosphatidylcholine, [7] LPE: Lysophosphatidylethanolamine, [8] PS+PI: Phosphatidylserine and Phosphatidylinositol; [9] PA: Phosphatidic acid, [10] PE: Phosphatidylethanolamine, [11] UPL: Unknowkn polar lipids Pigments, [12] MG: Monoacylglycerols, [13] DG: Diacylglycerols, [14] CHO: Cholesterol, [15] FFA: Free fatty acids, [16] TAG: Triacylglycerols, [17] SE: Sterol esters; [18] TPL: Total Polar lipids, [19] TNL: Total Neutral lipids. n.d.—Not detected. * Statistical difference for $p < 0.05$.

2.2. Feed Performance

In the first month of the experiment (days 0–28), all tanks showed 100% survival (Table 3). No differences were observed between the mean individual weights at the beginning of the trial (Table 3; $p > 0.05$). The weight gain (Wg) and final weight (Wf) were higher in octopus fed with CALPRO ($p < 0.05$, respectively). As for the absolute feeding rates, AFR and AFRdw were also higher in the CALPRO treatment compared to CALPRO-LM ($p < 0.05$). In contrast, the specific feeding rate (SFR) was similar between groups ($p > 0.05$). Protein ingestion (APFR) was higher in octopus fed with CALPRO ($p < 0.05$), while both groups displayed similar lipid ingestion (ALFR; $p > 0.05$). The CALPRO diet promoted higher absolute and specific growth (AGR and SGR, respectively; $p < 0.05$) and better use, in terms of feed efficiency (FE; $p < 0.05$) and conversion (FCR; $p < 0.05$), of the prepared formulation by the individuals.

Table 3. Mean values (± standard deviation) for growth, ingestion, and feed efficiency indexes in *O. vulgaris* fed with non-supplemented (CALPRO) or the marine lecithin supplemented feed (CALPRO-LM) during the first month (days 0–28), the second month (days 29–56), and total experimental period.

Period	1st Month (Days 0–28)		2nd Month (Days 29–56)		Total (Days 0–56)	
Diet	**CALPRO**	**CALPRO-LM**	**CALPRO**	**CALPRO-LM**	**CALPRO**	**CALPRO-LM**
Survival (%)	100.0	100.0	100.0	87.5	100.0	87.5
Wi [1] (g)	707 ± 31	704 ± 80	1152 ± 64	910 ± 88 *	707 ± 31	704 ± 80
Wf [2] (g)	1152 ± 64	914 ± 82 *	1474 ± 46	1143 ± 142 *	1474 ± 46	1143 ± 142 *
Wg [3] (g)	445 ± 63	210 ± 71 *	322 ± 53	233 ± 84	767 ± 33	441 ± 123 *
AGR [4] (g/day)	15.89 ± 2.24	7.50 ± 2.55 *	11.48 ± 1.89	8.32 ± 3.00	13.69 ± 0.58	7.88 ± 2.19 *
SGR [5] (%BW/day)	1.74 ± 0.22	0.94 ± 0.34 *	0.88 ± 0.16	0.81 ± 0.25	1.31 ± 0.06	0.87 ± 0.22 *
AFR [6] (g/day)	25.63 ± 4.02	18.56 ± 3.04 *	26.61 ± 3.87	23.51 ± 6.67	26.12 ± 3.82	20.91 ± 4.39
AFRdw [7] (g/day)	14.14 ± 2.22	9.91 ± 1.62 *	14.69 ± 2.14	12.55 ± 3.56	14.41 ± 2.11	11.16 ± 2.34
APFR [8] (g/day)	9.44 ± 1.48	6.65 ± 1.09 *	9.80 ± 1.43	7.36 ± 3.71	9.62 ± 1.41	7.48 ± 1.57
ALFR [9] (g/day)	1.04 ± 0.16	1.03 ± 0.17	1.08 ± 0.16	1.14 ± 0.58	1.06 ± 0.15	1.16 ± 0.24
SFR [10] (%BW/day)	2.76 ± 0.48	2.30 ± 0.35	2.03 ± 0.33	2.30 ± 0.68	1.32 ± 0.19	2.27 ± 0.45
FE [11] (%)	63.22 ± 13.87	39.93 ± 10.47 *	43.12 ± 3.58	37.36 ± 14.08	53.07 ± 6.44	38.69 ± 10.78
FCR [12]	1.64 ± 0.36	2.68 ± 0.80 *	2.33 ± 0.19	3.10 ± 1.35	1.91 ± 0.24	2.82 ± 1.01
PPV [13] (%)	-	-	-	-	25.35 ± 3.08	14.82 ± 4.47 *
LPV [14] (%)	-	-	-	-	13.86 ± 1.72	9.22 ± 1.91 *
DGI [15] (%)	-	-	-	-	5.87 ± 0.57	6.47 ± 1.07

[1] Wi—initial weight, [2] Wf—final weight, [3] Wg—weight gain, [4] AGR—absolute growth rate, [5] SGR—specific growth rate, [6] AFR—absolute feeding rate, [7] AFRdw—absolute feeding rate in dry weight, [8] APFR—absolute protein feeding rate, [9] ALFR—absolute lipid feeding rate, [10] SFR—specific feeding rate, [11] FE—feed efficiency, [12] FCR—food conversion ratio, [13] PPV—protein productive value, [14] LPV—lipid productive value, [15] DGI—digestive gland index. * Statistical difference for $p < 0.05$.

During the second month, the group fed with the lecithin-supplemented diet registered a 12.5% mortality. This was due to an overnight individual escape from the experimental tank at day 46. The CALPRO group showed a significant higher final weight ($p < 0.05$) but there were no differences in feeding rates ($p > 0.05$), growth rates ($p > 0.05$), and feed efficiency ($p > 0.05$; Table 3).

During the whole experimental period (day 0–56), food ingestion (AFR, SFR), feed efficiency and conversion (FE, FCR), as well as animal condition (i.e. DGI) were similar between treatments ($p > 0.05$; Table 3). The CALPRO diet promoted higher growth rates (AGR and SGR; $p < 0.05$) and better nutrient use (PPV and LPV; $p < 0.05$).

The feces of octopus fed with CALPRO-LM presented a higher lipid content and lower acid insoluble ashes (AIA) than those collected from animals fed with CALPRO ($p < 0.05$; Table 4). All the apparent digestibility coefficients (ADC diets, proteins, and lipids) were similar between both treatments ($p > 0.05$). Both polar (TPL) and neutral (TNL) lipid fractions were similar and efficiently digested ($p > 0.05$). However, there were differences regarding lipid classes' digestibility. Octopus fed with CALPRO presented higher ADCs for LPE, PA, PE, CHO, and TAG while LPC, PC, and PS + PI were better digested by those fed CALPRO-LM ($p < 0.05$).

Table 4. Macronutrient composition of feces (g/kg dry weight) and Apparent Digestibility Coefficients (ADC) of non-supplemented (CALPRO) or marine lecithin supplemented feeds (CALPRO-LM).

	CALPRO	CALPRO-LM
Feces		
Moisture (g/kg)	74.4 ± 0.2	78.2 ± 14.1
Crude protein (g/kg)	163.0 ± 0.8	164.5 ± 38.8
Crude lipid (g/kg)	33.5 ± 0.9	38.5 ± 0.5 *
Ash (g/kg)	141.9 ± 1.5	167.9 ± 20.7
CHOT [1] (g/kg)	661.7 ± 2.0	629.2 ± 59.9
AIA [2] (g/kg)	2.837 ± 0.383	1.910 ± 0.153 *
Nutrients Digestibility		
ADC [3] diet	0.79 ± 0.03	0.75 ± 0.02
ADC [3] protein	0.95 ± 0.01	0.94 ± 0.01
ADC [3] lipid	0.90 ± 0.02	0.91 ± 0.01
Lipid Classes Digestibility		
ADC [3] LPC [4]	0.93 ± 0.01	0.96 ± 0.01 *
ADC [3] SM [5]	0.88 ± 0.02	0.89 ± 0.01
ADC [3] PC [6]	0.90 ± 0.01	0.93 ± 0.01 *
ADC [3] LPE [7]	0.98 ± 0.01	0.83 ± 0.01 *
ADC [3] PS+PI [8]	0.76 ± 0.03	0.83 ± 0.01 *
ADC [3] PA [9]	0.97 ± 0.01	0.93 ± 0.01 *
ADC [3] PE [10]	1.00 ± 0.00	0.97 ± 0.01 *
ADC [3] MG [11]	0.71 ± 0.04	0.74 ± 0.02
ADC [3] DG [12]	1.00 ± 0.00	1.00 ± 0.00
ADC [3] CHO [13]	0.88 ± 0.02	0.83 ± 0.01 *
ADC [3] FFA [14]	0.85 ± 0.02	0.87 ± 0.01
ADC [3] TAG [15]	0.99 ± 0.01	0.99 ± 0.00 *
ADC [3] SE [16]	0.87 ± 0.02	0.88 ± 0.01
ADC [3] TPL [17]	0.90 ± 0.01	0.91 ± 0.01
ADC [3] TNL [18]	0.91 ± 0.01	0.91 ± 0.01

[1] CHOT—total carbohydrates, calculated by difference; [2] AIA—insoluble acid ash; [3] ADC—apparent digestibility coefficient. [4] LPC: Lysophosphatidylcholine, [5] SM: Sphingomyelin, [6] PC: Phosphatidylcholine, [7] LPE: Lysophosphatidylethanolamine, [8] PS+PI: Phosphatidylserine and Phosphatidylinositol; [9] PA: Phosphatidic acid, [10] PE: Phosphatidylethanolamine, [11] MG: Monoacylglycerols, [12] DG: Diacylglycerols, [13] CHO: Cholesterol, [14] FFA: Free fatty acids, [15] TAG: Triacylglycerols, [16] SE: Sterol esters; [17] TPL: Total Polar lipids, [18] TNL: Total Neutral lipids. * Statistical difference for $p < 0.05$.

The proximate composition of the digestive gland at the end of the experiment was similar between both treatments ($p > 0.05$; Table 5). The use of 20 g/kg marine lecithin supplementation did

not produce differences in the crude lipid content in the carcass ($p > 0.05$). In the latter tissue, octopus fed with CALPRO-LM presented higher moisture and ash but lower protein content than those fed CALPRO ($p < 0.05$).

Table 5. Macronutrient composition (g/kg dry weight) of tissues (digestive gland, carcass, and whole animal) of wild *Octopus vulgaris* (Initial) and fed non-supplemented (CALPRO) or marine lecithin supplemented feeds (CALPRO-LM).

	Initial	CALPRO	CALPRO-LM
N	4	4	4
Digestive gland			
Moisture (g/kg)		631.3 ± 21.5	646.9 ± 47.3
Crude protein (g/kg)		528.9 ± 26.8	571.9 ± 58.4
Crude lipid (g/kg)		364.4 ± 37.9	321.3 ± 62.0
Ash (g/kg)		32.9 ± 4.4	27.4 ± 4.3
CHOT [1] (g/kg)		73.8 ± 15.2	79.4 ± 12.4
Carcass			
Moisture (g/kg)		802.8 ± 6.5	817.6 ± 4.6 *
Crude protein (g/kg)		828.7 ± 11.7	793.8 ± 16.3 *
Crude lipid (g/kg)		15.1 ± 4.9	17.9 ± 1.7
Ash (g/kg)		104.3 ± 3.6	117.3 ± 8.5 *
CHOT [1] (g/kg)		51.9 ± 15.1	71.2 ± 10.6
Whole animal			
Moisture (g/kg)	808.9 ± 4.5 ^{a}	792.7 ± 5.9 ^{b}	806.0 ± 4.7 ^{a}
Crude protein (g/kg)	835.8 ± 10.2 ^{a}	811.1 ± 11.5 ^{b}	778.6 ± 14.1 ^{c}
Crude lipid (g/kg)	20.2 ± 2.3 ^{b}	35.6 ± 6.9 ^{a}	38.5 ± 2.8 ^{a}
Ash (g/kg)	112.5 ± 5.4 ^{a}	100.1 ± 3.4 ^{b}	111.1 ± 8.5 ^{ab}
CHOT [1] (g/kg)	31.5 ± 9.0 ^{b}	53.2 ± 14.3 ^{ab}	71.8 ± 9.2 ^{b}

[1] CHOT—total carbohydrates, calculated by difference. * Statistical difference for $p < 0.05$. Different alphabetical superscripts across the rows indicate significant difference between treatments, $p < 0.05$.

Regarding the final macronutrient composition of whole octopus, it is remarkable that there was a higher protein content in those fed with CALPRO ($p < 0.05$; Table 5), while the lipid, ash, and total carbohydrates (CHOT) contents were similar between experimental treatments ($p > 0.05$). Both octopus fed with either CALPRO or CALPRO-LM showed some differences in proximate composition when compared with wild octopus. At the end of the trial, both groups showed a decrease in protein and an increase in the lipid content ($p < 0.05$; Table 5).

The lipid class profile in tissues was practically not modified with the dietary marine lecithin supplementation ($p > 0.05$; Table 6). Minor differences were found regarding FFA and SE in the digestive gland between both treatments, where those fed with CALPRO showed a higher FFA and lower SE content than the ones fed with CALPRO-LM ($p < 0.05$; Table 6). Both the digestive gland and carcass presented similar total polar (TPL; $p > 0.05$) and total neutral lipids (TNL; $p > 0.05$) regardless of the ingested diet.

Table 6. Lipid class composition (% total lipids) of octopus carcass and digestive gland.

	Carcass		Digestive Gland	
	CALPRO	CALPRO-LM	CALPRO	CALPRO-LM
LPC [1]	1.06 ± 0.33	1.30 ± 0.15	0.18 ± 0.25	0.04 ± 0.06
SM [2]	2.27 ± 0.18	2.15 ± 0.27	0.54 ± 0.19	0.36 ± 0.14
PC [3]	25.03 ± 0.22	25.40 ± 2.11	7.30 ± 0.40	6.60 ± 0.83
LPE [4]	0.55 ± 0.36	0.43 ± 0.24	0.30 ± 0.08	0.24 ± 0.34
PS+PI [5]	13.92 ± 0.99	13.55 ± 1.49	3.06 ± 0.61	2.34 ± 0.77
PA [6]	1.40 ± 0.07	1.14 ± 0.43	0.30 ± 0.14	0.41 ± 0.14
PE [7]	17.62 ± 0.81	16.55 ± 1.15	3.98 ± 0.48	3.12 ± 0.74

Table 6. *Cont.*

	Carcass		Digestive Gland	
	CALPRO	**CALPRO-LM**	**CALPRO**	**CALPRO-LM**
MG [8]	4.59 ± 0.34	4.53 ± 0.18	4.44 ± 0.17	5.90 ± 1.25
DG [9]	-	-	2.41 ± 0.22	2.30 ± 0.07
CHO [10]	22.54 ± 0.49	23.05 ± 0.94	6.50 ± 1.03	5.84 ± 0.66
FFA [11]	6.17 ± 0.49	7.19 ± 0.94	11.13 ± 0.75	8.68 ± 1.12 *
TAG [12]	2.38 ± 0.36	2.38 ± 1.12	56.72 ± 1.99	57.35 ± 4.34
SE [13]	2.50 ± 0.71	2.32 ± 0.94	3.15 ± 0.60	6.82 ± 2.15 *
TPL [14]	61.84 ± 0.57	60.53 ± 1.62	15.65 ± 0.52	13.10 ± 1.75
TNL [15]	38.17 ± 0.57	39.47 ± 1.62	84.35 ± 0.52	86.90 ± 1.74

[1] LPC: Lysophosphatidylcholine, [2] SM: Sphingomyelin, [3] PC: Phosphatidylcholine, [4] LPE: Lysophosphatidylethanolamine, [5] PS+PI: Phosphatidylserine and Phosphatidylinositol; [6] PA: Phosphatidic acid, [7] PE: Phosphatidylethanolamine, [8] MG: Monoacylglycerols, [9] DG: Diacylglycerols, [10] CHO: Cholesterol, [11] FFA: Free fatty acids, [12] TAG: Triacylglycerols, [13] SE: Sterol esters; [14] TPL: Total Polar lipids, [15] TNL: Total Neutral lipids. * Statistical difference for $p < 0.05$.

3. Discussion

In the present study, the inclusion of 20 g/kg of marine lecithin in the formulation (diet CALPRO-LM) increased the content of total lipids while maintaining the lipid classes' profile of the control diet CALPRO (non-supplemented diet), translating in a higher available amount of polar lipids in the supplemented feed (CALPRO-LM; data not shown). Both diets showed a high content of FFA in their lipid class profiles (Table 2). We believe that this excess of oxidation was related to the state of raw materials used in the preparation of feeds, as all raw materials were purchased from the suppliers up to one month before being used. All raw materials were frozen at −80 °C, freeze-dried, ground to powder, stored in vacuum-pack, and kept at 4 °C to be used in the different formulations. The cold chain was never broken from the time these entered the facilities and until the formulations were prepared. Another remarkable issue was the higher disintegration rates in water (WSI) observed in the CALPRO-LM feed, pointing to a negative effect of lecithin supplementation on the stability of CALPRO formulation. A decrease in stability after being submerged for 24 h in seawater was also observed when soybean lecithin was included in a semi-moist feed tested previously in common octopus [54]. In contrast, the inclusion of fish oil in the same type of feed provided a higher stability [10].

Survival rates were high in both treatments. Higher survival rates (98–100%) were achieved in some marine fish species when fed marine phospholipid supplemented diets [47–49]. In fact, the CALPRO diet did not generate any mortality in the present or in a previous study in which a formulated feed, with a similar basal composition to CALPRO, was supplied to octopus [6]. The only registered mortality was observed in a CALPRO-LM experimental tank at day 46 and had no relation whatsoever to the feeding conditions. A previous experiment performed with fish oil-supplemented diets also showed 100% survival [10], while the inclusion of soybean lecithin generated a 12.5% mortality rate [54].

A similar food intake (SFR of 2.03–2.76%BW/day) was observed between treatments during both the first and second months of the experiment (Table 3). The CALPRO diet promoted higher growth rates than CALPRO-LM during the first month (SGR of 1.74 ± 0.22 and 0.94 ± 0.34%BW/day, respectively). In contrast, during the second month, SGR was similar between both diets (SGR of 0.88 ± 0.16 and 0.81 ± 0.25%BW/day for CALPRO and CALPRO-LM, respectively; $p > 0.05$) but values were lower than in the first month. This decrease in SGR after a given time was previously reported by other authors who were testing prepared diets in the species [6,7,13,54,55]. If this issue is exclusively related to the formulation itself or if it has some relation to the species behavior remains to be determined, as the feeding rates were maintained. A good formulation should promote a progressive increase in food-intake to sustain, or even increase, growth rates in growing octopus.

Considering the whole experimental period (days 0–56), the best growth and feed efficiency were obtained in animals fed the diet without marine phospholipids supplementation (CALPRO). Bearing

in mind the present and the similar results reported by Rodríguez–González et al. [54], it seems that the inclusion of 20g/kg of phospholipids in formulated feeds, from animal marine (marine lecithin) or vegetable (soybean lecithin) sources, do not have a beneficial effect on octopus growth (AGR and SGR), ingestion (AFR and SFR), feed efficiency (FE) or food conversion (FCR). Other studies also showed a negative effect or no benefit related to the inclusion of lipids in formulated feeds [7,10,54] or when octopus were fed natural diets with a high fat content [20,56,57].

In the present study, octopus showed absolute growth rates between 7.88 g/day and 13.69 g/day being fed diets with 71.9–101.6 g/kg crude lipid. The highest growth rates in this species were reported when individuals were fed crustaceans, such as the green crab (*Carcinus mediterraneus*; 16.23–18.8 g/day, 23.9–24.9 g/kg lipids [2,57] or blue and white crabs (*Portunus pelagicus* and *Plagusia depressa*; 14.1–17.1 g/day; 70–91 g/kg lipids; [56]. In general, crustaceans are rich in polar lipids [17,25]. In this sense, a low dietary content of this type of lipid in a prepared diet might have some constraining effect on octopus growth performance. Despite crude lipid and total polar lipids increased in absolute quantity with the addition of marine lecithin (approaching the CALPRO-LM composition to that of crustaceans), the rearing performance was not enhanced. The differences in growth observed between crustaceans and formulated feeds with similar dietary lipid content and lipid classes profile demonstrate that the amount of lipids, and specifically polar lipids, cannot be considered as the sole factor affecting octopus growth. In this study, the low-lipid diet CALPRO promoted AGR results of ≈14 g/day, similar to those obtained with the use of a crustacean-based diet [56].

Diets tested in the present study had a lower efficiency (FE between 39% and 53%) when compared to others reported previously (61–73%; [6,7]). The present results show a lower FE in the supplemented treatment during the first month. In contrast, Morillo–Velarde et al. [10] did not report any effect on FE (in individual reared octopus during the same experimental period) when supplying lipid supplemented feeds with fish oil. In the present study, a decrease of FE was only observed during the second ongrowing month, i.e., in the long term, similar to what was reported when feeding a supplemented soybean lecithin diet [54]. However, in both cases (the present and the latter), this FE reduction was not so acute when compared to those reported to occur when octopus were fed with fish oil supplemented feeds [10]. Altogether, these results might suggest that *O. vulgaris* has a limited tolerance to high-lipid feeds during prolonged periods, as the two referred studies (Morillo–Velarde et al. [10] and Rodríguez–González et al. [54]) and the present showed this FE reduction and share the commonality of feeding octopus on high-lipid diets. The higher metabolic effectiveness of smaller animals, compared with bigger ones, should also explain the decrease in FE during the second month [6,58]. Although the individuals used in the experiment were males (known to be less affected by reproduction processes), maturation could also have affected FE. For this reason the maturation stage of individuals should have been evaluated before and after the trial.

Marine lecithin supplementation did not improve protein or lipid retention. Instead, these were reduced when compared with individuals fed the CALPRO diet (Table 3). The protein retention (PPV) of animals fed the supplemented diet was low (≈15%), while octopus fed CALPRO presented similar PPV to those reported to be fed on crab (25%; [57]) or other formulated feeds containing squid (26–27%; [6]). Moreover, PPV was reported to be not significantly affected with other lipid supplements included in common octopus feeds [10,54]. LPV was also lower in octopus fed the CALPRO-LM diet (≈9%) when compared with CALPRO, other formulated feeds [6,7,12] or natural diets [2]. In this respect, fish oil [10] and soybean lecithin [54] supplemented diets also promoted a significant reduction in LPV when increasing crude lipid content in diets.

Feeds digestibility was lower (0.75–0.79) than previously reported in diets prepared with freeze-dried ingredients and higher dietary crude lipid contents [8,12]. This difference could be mostly related to the null digestibility of complex polysaccharides by *O. vulgaris* [9]. Higher feed digestibility was reported to occur when starch was not included as raw material (0.93; [8]) compared with feeds which included 30 g/kg of starch (0.86; [12]). Undoubtedly, the inclusion of starch in the prepared diets (80–100 g/kg) penalized the total digestibility in the present study.

Protein ADC was not affected by the inclusion of marine lecithin suggesting that 20 g/kg of this supplementation did not interfere in protein digestibility. This observation is in accordance with previous studies, in which the lipid content of diets did not interfere with protein digestibility when either natural [20,21] or fish oil supplemented feeds were supplied to octopus [10]. In contrast, protein ADC diminished significantly when soybean lecithin was added, at a same level of inclusion, in feeds for octopus [54]. In addition, the high protein ADCs obtained in the present study (above 0.94) agree with preceding coefficients (over 0.9) already reported in *O. vulgaris* [9,10,40], verifying a high protein digestion effectiveness in this species [59–62].

A high lipid ADC (above 0.90) was also observed in both polar (0.76–0.98) and neutral (0.71–1.00) lipid classes, as reported in previous studies [10,12,40,54]. Marine lecithin supplementation did not interfere with lipid ADC, while in a previous study [54], the inclusion of soybean lecithin promoted a slight decrease in lipid digestibility. These results contrast with those reporting a limited capacity of cephalopods to metabolize lipids [26,63,64]. High lipase activity has been observed mainly at the digestive gland of cephalopods and, to a lesser degree, in salivary glands [65,66]. This is characterized as a continuous secretion in the digestive gland of opportunistic cephalopod species, such as *Euprymna tasmanica*, while secretion is stimulated by feeding in the remaining [67]. The lipases, produced and accumulated at the digestive gland, are used at once in opportunistic species. When the accrued volume of lipases is finished, its production is slowed down, generating a decrease in lipid digestion efficiency. Although lipases activity was not determined, considering the high lipid ADC, it can be speculated that the low-lipid diets (71.9–101.6 g/kg crude lipid) tested in this study promoted a continuous and effective lipid digestion.

Other marine organisms, i.e., fish species, experienced an enhancement in lipid digestion and nutrient assimilation, due to the emulsifying effect of dietary phospholipids [44]. Contrariwise, the present results (of including 20g/kg of marine lecithin) or previous results of including soybean lecithin [54] in an *O. vulgaris* feed have shown no benefits. In the present study, apart from the high lipid digestibility, no other influence was exerted by the inclusion of marine lecithin. A previous study, in which fish oil [10] was included as the lipid source apart from the naturally prepared ingredients, reported an acute decrease of lipid ADC related with the content of total lipids (from 81.25% to 12.27% in non-supplemented and 200 g/kg supplemented diets, respectively). Both tested diets in the present study presented a lower total lipid content (71.9–101.6 g/kg dry weight) when compared with the non-supplemented fish oil diet (137.7 g/kg dry weight) tested by Morillo–Velarde et al. [54]. In all these diets, lipids proceeded from natural sources (freeze-dried ingredients), egg yolk (half in tested diets of the present study), and lipid supplements (either marine lecithin or fish oil). The CALPRO and CALPRO-LM feeds presented a higher lipid ADC than the non-supplemented diet tested previously [10], suggesting the relevance of a low amount of total lipids in diets formulated for *O. vulgaris*. Still, the polar and neutral lipid fractions digestibility (TPL and TNL ADCs, respectively) displayed high percentual values (above 0.90) and were not influenced by lecithin. Contrariwise, Morillo–Velarde et al. [10] observed a decrease in TNL ADC of high-lipid diets supplemented with fish oil. Altogether, it suggests a better digestibility towards polar lipids when compared to neutral. On the other hand, Rodríguez–González et al. [54] observed a significant enhancement in TPL and TNL ADCs when including 20 g/kg of soybean lecithin as supplement, which further suggest the relevance of the lipid class and/or fatty acids profiles on lipids digestibility. In general, good condition (DGI) values were determined for both groups, similar to those reported to occur when feeding octopus with crab and around 4.97% [2] or formulated feeds (6%; [6,7]). In addition, the present results were similar to those reported by Morillo–Velarde et al. [10] and Rodríguez–González et al. [54], who did not observe any effect on animals' condition related to the inclusion of lipid supplements.

The digestive gland is considered a lipid storage organ [22–24]. Accordingly, we verified a lipid content ranging between 321.3 g/kg and 364.4 g/kg in this organ, while a much lower quantity of 15.1–17.9 g/kg was found in the carcass. Contrary to previous studies [3,10] but similar to the results reported by Rodríguez–González et al. [54], the group fed with the higher lipid diet (CALPRO-LM;

Table 5) did not show a higher accumulation of lipids in the digestive gland. Moreover, the inclusion of marine lecithin did not generate variations in the overall proximate composition of the digestive gland but only in the carcass (Table 5). A higher lipid content was verified in the whole animal composition of experimental octopus compared to those sampled at the beginning of the experiment. Accordingly, Morillo–Velarde et al. [10] and Rodríguez–González et al. [54] also reported differences in the lipid content between experimental and wild animals. Therefore, a diet's effect on animals' proximate composition was verified, which was probably caused by the high-lipid content.

Regarding the lipid profile of tissues, the LC profile of tissues in the present study (PC, PS+PI, PE, FFA, and TAG) was similar to that previously reported in this species [3,22,24,25]. This profile was mostly conserved in tissues between treatments: It was similar in the carcass, but FFA were reduced and SE increased in the digestive gland of octopus fed the CALPRO-LM diet (Table 6). This suggests that the inclusion of dietary marine lecithin (at 20 g/kg) did not disturb either the metabolism or the lipid storage and transport as reported by Morillo–Velarde et al. [10] to occur when octopus were fed with a high-lipid diet. Morillo–Velarde et al. [22] and García–Garrido et al. [23] reported a preferential use of TAG during fasting while the polar lipids content was not modified in the digestive gland. The higher FFA percentage observed in the digestive gland of octopus fed CALPRO compared with those fed CALPRO-LM could suggest a higher TAG catabolism or FFA storage in this organ with a poorer crude lipid content feeds. In any case, the present results indicate that marine lecithin supplementation at 20 g/kg does not promote an increased rearing performance.

4. Materials and Methods

4.1. Ethical Statement

The present study was performed according to Spanish regulations, Law 6/2013 and European Directive 2010/63/EU, for the protection of animals used for experimentation and other scientific purposes. Experiments and procedures with animals were accepted and supervised by the Ethical Committee of the University of Alicante.

4.2. Experimental Conditions

Wild *O. vulgaris* sub-adults were caught by trawling in the Mediterranean Sea (37°49′0″ N: 0°45′0″ W; Murcia, Spain) and transported inside 250 L bins in seawater to the Murcia Institute of Agri-Food Research and Development (IMIDA) facilities. Individuals were acclimatised to captivity for two weeks in 1970L circular tanks (176 cm of diameter and 81 cm of water column) and fed daily to satiety on thawed green crab *Carcinus mediterraneus* [57]. Seven days before the start of the experiment, octopuses were selected and placed into tanks, which were subject to low light intensity (opaque textile covering tanks), had one PVC pipe as a den, and external surrounding nets to prevent the octopus from escaping. The rearing tanks were connected to a recirculated seawater system (5940 L; with mechanical, biological, and UV filtration systems) with a controlled temperature (Air Energy, Heat Pump Inc., Model 400Ti. Fort Lauderdale, Florida, USA). The seawater temperature was 18.7 ± 0.4 °C, within the optimal range according to Aguado Giménez and García García [68]; dissolved oxygen saturation was above 80% [69]; salinity was 37‰; pH was 8.2, and total ammonia nitrogen (TAN) was kept below 0.2 mg L^{-1}. The photoperiod was established at 12L: 12D and controlled by using fluorescent daylight lamps (Wedeco-REX 37W) connected to a digital timer (Orbis Data-Log).

4.3. Preparation, Storage, and Water Stability of Diets

Two diets were prepared using a mixture of freeze-dried ingredients, binders, and pure substances (Table 1). Formulated feeds varied in the dietary marine lecithin supplementation (either 0 g/kg or 20 g/kg): CALPRO (150 g/kg of freeze-dried squid, 50 g/kg of freeze-dried little rockfish, 100 g/kg of starch and 0 g/kg of marine lecithin) as control and CALPRO-LM (obtained by substituting 20 g/kg of

starch by marine lecithin in the CALPRO formula). The basal composition of the control diet (CALPRO) was similar to a previously tested diet by Cerezo Valverde and García García [6].

The commercial dietary supplement used (LECIMARINE F30 Standard, Innovafood S.L, Barcelona) was extracted from wild herring roe (Table 7). The raw ingredients were cleaned and processed, the bones and viscera in bigger fish (*S. aurita*, *Boops* sp., *Diplodus* sp.) and squid (*T. sagittatus*) were removed, and the whole crabs were ground (*C. mediterraneus*). The raw materials were deep-frozen and maintained at −80 °C, in a Thermo Scientific REVCO VALUE PLUS (Thermo Electron Corporation, Asheville, NC, USA) before being freeze-dried. Freeze-drying was performed using a HETO Power Dry LL3000 (Jouan Nordic, Allerød, Denmark). Afterwards, ingredients were ground in a Retsch Grindomix GM200 blender (Retsch GmbH, Haan, Germany) to obtain a <200 µm fine powder, which was vacuum-packed (LeaderVac V 500, TecSelor S.L., Lorca, Spain) and maintained at 4 °C until use.

Table 7. Lipid composition of LECIMARINE F30 Standard (manufacturer data).

Total phospholipids	Minimum 28%
PC	Minimum 24%
Total fatty acids	Minimum 80%
Fatty acids (% of total fatty acids)	
Total omega-3	Minimum 30%
EPA	Minimum 10%
DHA	Minimum 14%

PC: Phosphatidylcholine, EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

Feeds were prepared every 4 days by manually kneading the mixture of dried ingredients with water until a homogeneous mixture was obtained. In the CALPRO-LM formulation, 20 g/kg of paste of marine lecithin was previously dissolved in distilled water using a hand blender. The raw mixture was packed manually into plastic bags before being vacuum-sealed and introduced in a hot-bath (without exceeding 55 °C) until the gelatin was melted (≈1 h). Finally, feeds were stored until use at 4 °C. Diets were used up to 4 days after being prepared.

Feed stability was expressed as the disintegration percentage of dry weight after soaking in seawater for 24 h. The Water Stability Index (WSI) was calculated using the mean values from three replicates according to the following equation: WSI (%) = ((DWi − DWf)/DWi)) × 100, where DWi and DWf are the initial and final dry weights, respectively.

The diets' energy was calculated applying the following energy coefficients: Protein 23.6 kj/g, lipid 38.9 kg/g and carbohydrate 16.7 kJ/g [70].

4.4. Experimental Design

A total of 12 male octopuses were kept individually in 12 plain bottom circular tanks (216 L; 83 cm of diameter and 40 cm of water column) for 56 days (November and December of 2016). Two experimental groups were established according to different formulated feeds: CALPRO (N = 4) and CALPRO-LM (N = 8). The mean initial weights of the octopus were similar ($p < 0.05$) and of 707 ± 31 g and 704 ± 80 g for CALPRO and CALPRO-LM, respectively. Individuals were fed to satiety following a feeding protocol with three non-consecutive days of fasting (Mondays, Wednesdays, and Saturdays) per week [13]. Feeds were supplied as cube-shaped (53.3 ± 13.0 g) portions, at 12 h, surpassing animals' demand. Initial rations corresponded to 5% of the initial body weight and were readjusted along the trial. The collection of leftovers (food remains and feces) and maintenance (cleaning) were always performed before feed supply (between 9 and 11 h, with a duration of about 5 min per tank) to avoid interference with food acceptance. Firstly, feces were aspirated (using a syphon), collected (using a net to remove water excess), and frozen before being freeze-dried. Secondly, the previous day feed wastes were collected and dried for 48 h until a constant weight was achieved (Method n°. 930.15; [71]).

Octopuses were individually weighed at the onset (day 0), at the middle (day 28), and at the end (day 56) of the experiment, to minimize an effect of handling stress in food intake and, consequently, in

growth. Variations in diets' performance was assessed regarding three experimental periods: Days 0 to 28, days 29 to 50, and days 0 to 56. At day 56, octopuses were euthanized by immersion in iced seawater followed by the destruction of the cephalic ganglia.

4.5. Sample Collection and Analytical Method

Samples were collected and determinations performed according to Rodríguez–González et al. [12]. At day 56, after 24 starving hours, all octopus were euthanized to obtain the biometric data (total weight, digestive gland index weight, and carcass weight, i.e., the animal, excluding the digestive gland). Tissues samples (digestive gland and carcass) were obtained from four individuals of each experimental group. Biochemical analyses of moisture (Method 930.15), crude protein (Method 830.15), crude lipid (Method 920.39), and ash (Method 942.05) were performed in tissues, feeds, and feces according to AOAC [71] methodologies. Moisture was determined by drying samples in an oven (Heraeus Typ. UT12) until a constant weight was obtained. Ash was quantified by incineration in a muffle oven (Heraeus Typ. M110). Crude protein was determined by the Kjeldhal method (commercial catalyst) applying a conversion factor of 6.25 for nitrogen transformation. Total lipid was determined using ethyl ether extraction in a SOXTEC AVANTI 2058. Total carbohydrates were determined by subtracting the sum of total protein, lipid, moisture, and ash from the total weights [12]. Digestive gland and carcass samples were homogenized by trituration (Foss Tecator AB Homogenizer 2094, Höganäs, Sweden) and frozen (at −20 °C) until analysis in triplicate in the next days. The composition of whole animals was calculated using the percentage with respect to the total weight represented by each tissue. The collection of feces began one week after the start of the experiment, to avoid interferences from the diet previously supplied during acclimation. Feces were collected from each tank, freeze-dried, and conserved through being vacuum-packed until analysis.

The specific composition of lipid classes was analyzed in formulated feeds, tissue samples, and feces in triplicate. After quantifying the total lipid contents in samples (Method n° 920.39; [71]), the needed quantity of each sample (feeds, digestive gland or carcass) to extract 10 mg of lipids was calculated according to Folch et al. [72]. Lipids were kept dissolved in hexane at −80 °C, adjusting the concentration to 10 μg μL^{-1} with chloroform to methanol (2:1) before analysis. The method described by Olsen and Henderson [73] was followed for HPTLC lipid classes' separation. An automatic injector (Linomat 5, CAMAG, Muttenz, Switzerland) was used to apply lipids (15 μg) to 20 × 10 cm silica gel plates (Merck). The plates were developed using methyl acetate, isopropanol, chloroform, methanol, and 0.25% (*w*/*v*) KCl (10:10:10:4:3.6 by vol.) as the polar solvent system, and hexane, diethyl ether, and glacial acetic acid (32:8:0.8 by vol.) as the neutral solvent system. Lipid classes' visualization was achieved after charring at 160 °C for 15 min the sprayed plate with 30 g L^{-1} (*w*/*v*) cupric acetate in 8% phosphoric acid. The final quantification was made by densitometry in a TLC 3 scanner (CAMAG, Muttenz, Switzerland) at a wavelength of 254 nm. A 10 μg μL^{-1} solution made up with pure standards (SM, PC, PS, PE as polar lipids and MG, CHO, FFA, and TAG as neutral lipids; Larodan Fine Chemicals, Malmo, Sweden) was applied to identify lipid classes, regarding the order of appearance and reference position (lysophosphatidylcholine, LPC; sphingomyelin, SM; phosphatidylcholine, PC; lysophosphatidylethanolamine, LPE; phosphatidylserine, PS; phosphatidylinositol, PI; phosphatidic acid, PA; phosphatidylethanolamine, PE; monoacylglycerols, MG; diacylglycerols, DG; cholesterol, CHO; free fatty acids, FFA; triacylglycerols, TAG; steryl esters, SE).

4.6. Data Analysis and Statistics

Growth, ingestion, and feed efficiency indices were calculated as follows: (1) Average weight (Wa; g) = (Wi + Wf)/2, where Wi = initial weight (g); Wf = final weight (g); (2) weight gain (Wg; g) = Wf − Wi; (3) Absolute growth rate (AGR; g day^{-1}) = (Wf − Wi)/t, where t = time in days; (4) Specific growth rate (SGR; %BW.day^{-1}) = (LnWf − LnWi) × 100/t, where BW refers to body weight; (5) digestive gland index (DGI; %) = DGW × 100/Wf, where DGW refers to digestive gland weight (g); (6) correction factor for ingestion (F) = DWi/DWf, considers disaggregation rate in water, where DWi = initial

dry weight (g) and DWf = final dry weight (g); (7) corrected ingestion (IF; wet weight in g) = (dry feed supplied in g—uneaten dry feed in g × F) + moisture feed supplied in g); (8) absolute feeding rate (AFR; g day^{-1}) = IF/t; (9) absolute protein or lipid feeding rate (AyFR; g day^{-1}) = Iy/t, where y could be either protein or lipid, Iy = ingested protein or lipid (g); (10) specific feeding rate (SFR; %BW day^{-1}) = (AFR × 100)/Wa; (11) feed efficiency (FE; %) = (Wf − Wi) × 100/IF; (12) feed conversion ratio (FCR) = IF/(Wf − Wi); (13) productive value for lipids or proteins (LPV and PPV, respectively; %) = 100 × retained (Lipids or Proteins)/Intake (Lipids or Proteins); (14) apparent digestibility coefficients (Maynard and Loosli, 1962) (ADCN) = 100 − (100 × % Mdiet/% Mfaeces) × (100- % Nfaeces/% Ndiet), where N = nutrient (either dry matter—DM, protein—PROT or lipid—L), M = inert marker, acid insoluble ash (AIA) applying the method described by Atkinson et al. [74]; (15) total polar lipids (TPL, %) were obtained as the sum of all the polar lipid classes percentages; (16) total neutral lipids (TNL, %) were obtained as the sum of all the neutral lipid percentages.

Results are expressed as mean ± standard deviation (S.D.). All data were tested for both normal distribution and homogeneity of variances using the Shapiro–Wilk test and the Levene's test [75,76], respectively. An arcsine square root transformation was applied to data reported as percentage and to that not achieving normality or homogeneity [77].

A *t*-test [75] was used to compare feeds (by their stability in water, macronutrient composition, and lipid class profile), feeding and growth rates, feed efficiency, proximate composition of tissues, apparent digestibility coefficients (of nutrients and lipid classes) and to assess the differences between the lipid class profiles (in digestive gland, carcass, whole animals, and feces samples). One-way ANOVA [75] was used to compare the macronutrient composition between wild and experimental complete animals. The Tukey HSD post-hoc test was used to establish homogeneous subsets among treatments, and the Games–Howell test was used when variances were not homogenous among groups. A significance level of $p < 0.05$ was established for all statistical analyses.

5. Conclusions

In conclusion, the inclusion of marine lecithin at 20 g/kg in formulated diets did not promote beneficial effects on survival, feed intake, growth, efficiency or nutrients digestibility but increased the lipid content in tissues preserving the lipid classes' profile. In spite of the ineffectiveness of the tested dietary supplement, and other lipid sources tested in previous studies, further research is needed to achieve a better knowledge of nutritional requirements in cephalopods.

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