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High performance liquid chromatography-tandem mass spectrometry of phospholipid molecular species in eggs from hens fed diets enriched in seal blubber oil

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Abstract

The total lipid fraction of eggs from hens fed diets enriched in seal blubber oil (1.25–5.0% SBO) was directly analysed with normal-phase high performance liquid chromatography coupled on-line with electrospray ionization ion-trap tandem mass spectrometry (HPLC-ESI-MS–MS) for the identification of the molecular species of phospholipids (PLs). The species of phosphatidylethanolamine (PE) and phosphatidylinositol (PI) were all detected as the $[M-H]^-$ ions. The phosphatidylcholine (PC), sphingomyelin (Sph) and lysophosphatidylcholine (LPC) classes, were detected as formate adducts $[M+HCOO]^-$. Tandem MS of PE and PI showed the loss of the carboxylate anions, and, for PI, also the loss of water and inositol. Product ion spectrum of PC, LPC and Sph contained only the $[M-CH_3]^-$ ion fragment. Feeding different levels of SBO for 5 weeks resulted in a significant increase of PE, PC and PI molecular species carrying eicosapentaenoic acid ($C_{20:5\,\omega3}$, EPA), docosapentaenoic acid ($C_{22:5\,\omega3}$, DPA) and docosahexaenoic acid ($C_{22:6\,\omega3}$, DHA), but not Sph nor LPC. The highest increase of the ω3/ω6 ratio occurred for PE and PC. On the contrary, PI was less affected by the increase of SBO in the diet.

Keywords: Egg phospholipids; Tandem mass spectrometry; High performance liquid chromatography; Seal blubber oil; ω3 Fatty acids

1. Introduction

The design of new functional foods [1–3] is the current trend to ensure optimal nutrition, at least for particular groups of subjects with nutritional deficiencies or requirements [4,5]. The impact of functional foods on current regulation was abundantly reviewed [6,7]. In the US, the National Academy of Sciences defines functional food as the one that encompasses potentially healthy products providing health benefit beyond that of traditional nutrients it contains [8]. In the EU law, specific provisions should still be introduced to govern 'functional claims' and 'nutritional claims' of food [9].

Some functional foods are enriched with long-chain polyunsatured fatty acids ω3 (LC-PUFAs ω3), such as eicosapentaenoic acid ($C_{20:5\,\omega3}$, EPA), docosapentaenoic acid ($C_{22:5\,\omega3}$, DPA) and docosahexaenoic acid ($C_{22:6\,\omega3}$, DHA). Epidemiological investigations indicate that LC-PUFAs ω3 have a beneficial effect with respect to coronary heart disease, a major cause of death in most developed countries [10,11] and can be used as cancer chemopreventive agents [12] and against inflammatory lesions [13,14]. Already in 1992 the Scientific Committee for Food of the EU recommended a daily intake of 1 g LC-PUFAs ω3 respectively 0.5% of the total nutritional energy consumption [15,16] for preventing clinical signs of deficiencies in healthy adults. More recently, the European Commission has authorised the placing on the market of DHA-rich microalgal oil as a novel food [17].

Recent studies have proposed seal blubber oil (SBO) as an alternative source of LC-PUFAs ω3 in the formulation of functional food [18], since it is a primary source of DPA,

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which has shown an anticarcinogenic effect on mice [19]. In SBO, LC-PUFAs ω3 are mainly located in the sn-1,3 positions of triacylglycerols (TAGs), unlike fish oils [20]. This could affect the distribution of LC-PUFAs ω3 in the molecular species of egg PLs from hens fed SBO enriched diets, as it has been already reported for TGs of biological samples [21–27].

The characterization of phospholipid classes rich in PUFA ω3 is also interesting because they are used as a source of essential fatty acids in infant formulas, food additives and ingredients of therapeutic and cosmetic products [28]. Moreover, phospholipids from egg yolk have been recently introduced as a novel food or novel food ingredient in the EU [29].

In a previous work [30], the effects of feeding laying hens with refined SBO on the regiospecific distribution of fatty acids in egg TGs, phosphatidylcholine and phosphatidylethanolamine were studied by using a clean-up procedure involving thin layer chromatography, lipase hydrolysis, transmethylation and gas chromatography of fatty acids methyl esters (FAMEs). The hens were fed four diets containing 0% (control), 1.25%, 2.5%, or 5% of SBO for 5 weeks, respectively.

In the present work, the total lipid fraction from the same eggs was directly analysed by means of normal-phase high performance liquid chromatography coupled on-line with electrospray ionization ion-trap tandem mass spectrometry (HPLC-ESI-MS–MS) for the identification of each molecular species of the PLs, including phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), sphingomyelin (Sph) and lysophosphatidylcholine (LPC).

This procedure allowed the separation of each phospholipid molecular species and their unambiguous identification.

2. Experimental

2.1. Materials

HPLC-grade methanol, chloroform and water were purchased from Lab-Scan Analytical Sciences (Dublin, Ireland). All other reagents were of analytical grade. PLs standards (purity greater than 99%), including phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, *N*-palmitoyl-sphingomyelin, 1-oleoyl-glycero-3-phosphocholine (lysophosphatidylcholine), phosphatidylcholine β-docosa-hexaenoyl-γ-stearoyl and 2-linoleoyl-1-palmitoyl-sn-glycero-3-phosphoethanolamine were purchased from Sigma (St. Louis, MO, USA).

SBO was prepared by rendering the thick subcutaneous fat layer of only adult harp seals (*Phoca groenlandica*). The subcutaneous fat was free of all blood vessels, connective tissue and protein and was essentially 100% pure fat.

2.2. Diets

A total of 72, 26-week-old Single Comb White Leghorn (SCWL) laying hens were randomly assigned to 36 cages. Four different diets (A–D) were formulated from a basal mixture consisting of corn, wheat, soybean, a vitamin premix and pure wood cellulose to absorb the fat. Fat was added to the diets as follows: diet A (control), 5% tallow (T); diet B 1.25% SBO + 3.75% T; diet C, 2.5% SBO + 2.5% T; diet D, 5% SBO. Eggs collected after 5 weeks of treatment were analysed.

2.3. Lipid extraction

Three eggs from each cage were pooled and the lipid content was extracted according to Bligh and Dyer [31] on 1 g aliquot of the edible part of the eggs (albumin and yolk combined). Prior to analysis, 1 mg of the lipid extract was redissolved in 5 ml of chloroform/methanol/water/formic acid (5/5/1/0.1, v/v/v).

2.4. HPLC system

A ternary gradient module (Jasco LG-980-02, Tokyo, J) and a pump module (Jasco PU-980) were used. Solvents were degassed using a GT-103 module (Gastorr, Tokyo, J). The column was a Polaris Si-A 3 μ 100 mm × 4.6 mm (Varian, Middelburg, NL) protected with a Silica 4 mm × 3.0 mm I.D. precolumn from Phenomenex (Torrance, USA). Phospholipid classes were separated according to Malavolta et al. [32]. Briefly, the mobile phase was a gradient of solvent A [$CHCl_3$/MeOH/NH_4OH (30%) 80:19.5:0.5, v/v], and solvent B [$CHCl_3$/MeOH/H_2O/NH_4OH (30%) 60:34:5.5:0.5, v/v]. The gradient started at 100% of A, decreased to 0% in 10 min, then was held (100%B) for 15 min; and then reached back 100% A in 5 min. The flow rate was 0.8 ml/min; the analyses were performed with the column kept at room temperature. The injection loop was 5 μl.

2.5. Tandem mass spectrometry

The HPLC system described above was coupled on-line to an LCQ ion-trap mass spectrometer (Finnigan, San José, CA, USA) equipped with an electrospray ionization source (ESI). The HPLC effluent was splitted and 0.3 ml/min entered the MS through a steel ionization needle set at 5.0 kV and a heated capillary set to 200 °C. The sheath gas flow was approx. 90 arbitrary units. The ion source and the ion optic parameters were optimised with respect to the negative molecular related ions of phospholipids standards. The molecular mass peaks from the HPLC effluent were detected using negative ion full-scan ESI-MS analysis. Mass resolution was 0.1 Da. Tandem mass (MS^2) experiments were carried out with relative collision energy of 45%. Integration was performed with the Interactive Chemical Information System (ICIS) peak detection algorithm software provided by Finnigan, after correction for the contribution from the ^{13}C isotope effect.

2.6. Data handling

Results are expressed as mean ± standard deviation (SD). The differences in the PL species composition among dietary

groups were analysed for statistical significance using the Tukey's test. The statistical software was GraphPad InStat version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). Significance was defined as $P < 0.05$.

3. Results and discussion

3.1. Separation of phospholipid classes

The total lipid fraction was injected in HPLC-MS–MS without prior clean-up. This procedure reduced analysis time, manipulations, solvent amounts and consumable parts (e.g. TLC plates and derivatization reagents) to a minimum. Triacylglycerols were eluted from the column within the first 2 min of the HPLC run (data not shown). Thus, the HPLC flow was directed to the waste during the first 4 min through the divert valve of the mass detector. At the fourth minute, the valve automatically shifted to the injection position and the HPLC flow entered the mass detector.

The separation of five main classes from the egg lipid extracts was obtained as shown in Fig. 1. The retention time for the different classes increased in the order PE, PI, PC, Sph and LPC. All classes eluted within 18 min. The chromatographic system mainly separated the different phospholipid classes and also allowed some minor separation among different species within a given class. Thus, Fig. 1 shows the traces obtained when using the MS in negative ionization mode to select the signals from two species of PE ($C_{16:0/C22:6}$ and $C_{16:0/C18:1}$), two species of PI ($C_{18:0}/C_{20:4}$ and $C_{18:0}/C_{22:6}$) and two species of PC ($C_{16:0}/C_{22:6}$ and $C_{16:0}/C_{18:1}$), respectively.

3.2. Characterization of the phospholipid molecolar species within a class

The molecular mass peaks from the different phospholipid classes were detected using negative ion full-scan ESI-MS analysis (Fig. 2). The mass spectra were acquired under peaks obtained from reconstructed negative ion chromatogram in the time expected for the elution of each molecular species. The species of PE and PI were all detected as the $[M-H]^-$ ions. The PC, Sph, LPC classes, having a choline group at the polar head, were detected as formate adducts $[M+HCOO]^-$. It should be noted that only minor peaks of these classes were obtained at $[M+35]^-$ due to the formation of a chlorine adduct. Moreover, the mass spectrum of PC and LPC showed the molecular ions ($[M-15]^-$) resulting from the loss of the methyl group of choline, at m/z 744.5 and 480.2, for PC and LPC, respectively.

In order to achieve the characterization of each egg phospholipid class, negative ion fragments (MS^2) formed after collision activated dissociation (CAD) were investigated. The fragmentations were compared with those obtained from known standards and previous literature data [33,34].

Figs. 3 and 4 show MS^2 spectra of the molecular species of selected PLs detected in the egg samples. The product ion spectrum from MS^2 of PE and PI (Fig. 3) showed the loss of the carboxylate anions. The fragmentation of molec-

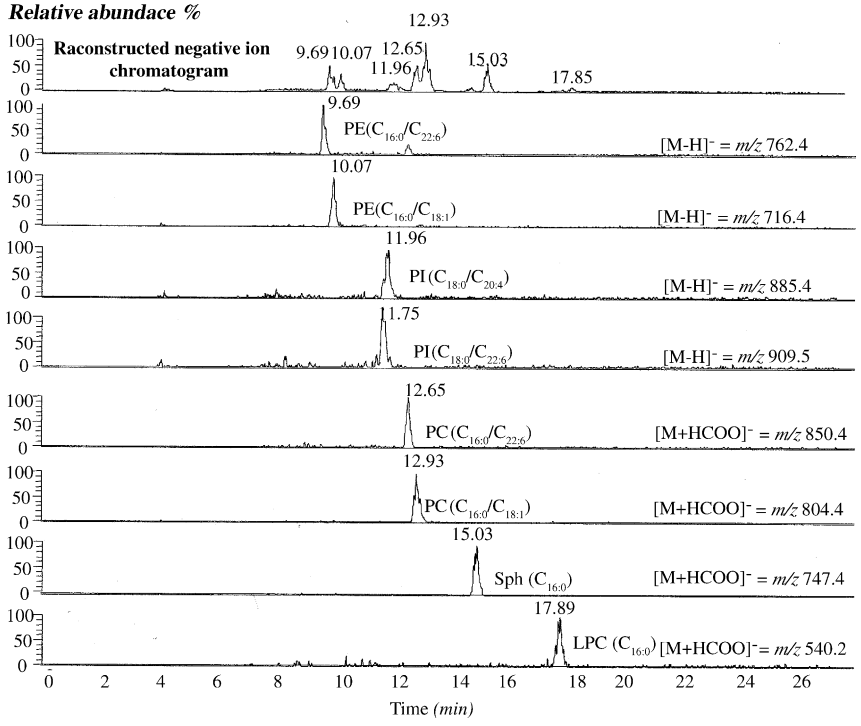

Fig. 1. Negative ion HPLC-ESI-MS analysis of egg phospholipid (samples obtained from hens fed diet D) with the MS operating in scan mode. PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine; Sph, sphingomyelin; LPC, lysophosphatidylcholine.

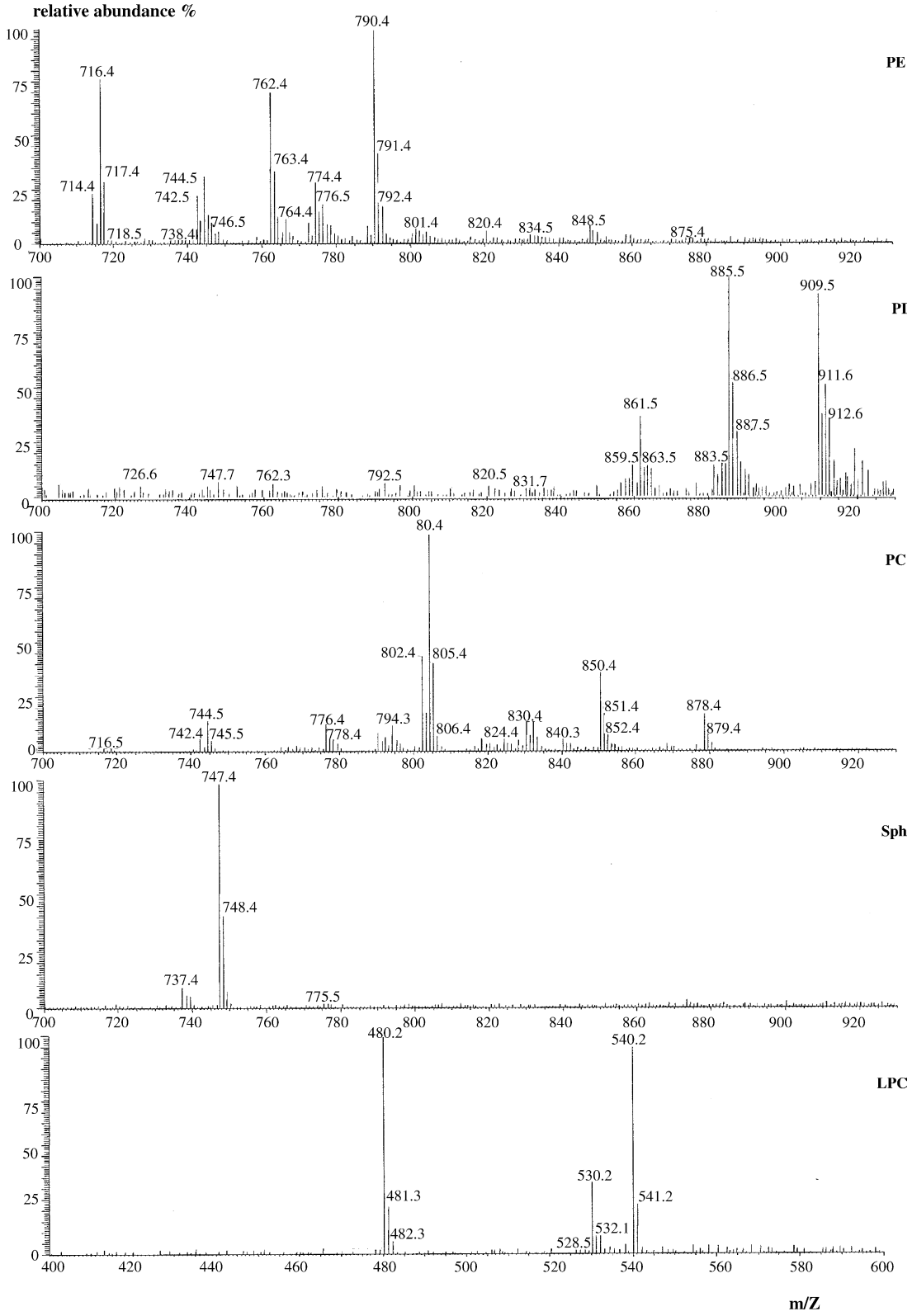

Fig. 2. Negative-ion HPLC-ESI-MS mass spectra obtained from the reconstructed negative ion chromatogram in Fig. 1. The molecular ions of egg phospholipid classes are shown. PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine; Sph, sphingomyelin; LPC, lysophosphatidylcholine.

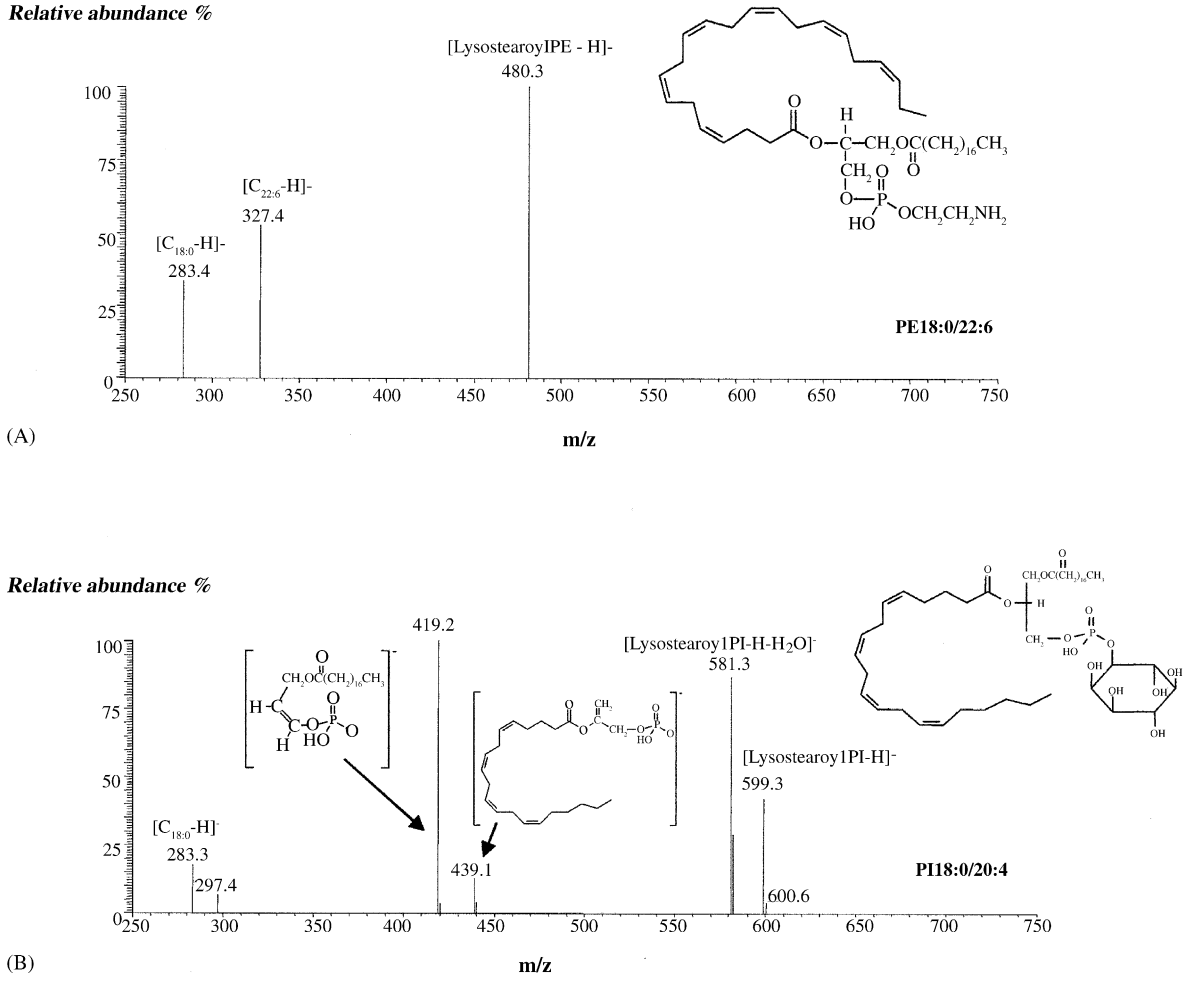

Fig. 3. Negative HPLC-ESI-MS–MS product ion spectra (with tentative identification of fragments) of selected PE and PI molecular species detected in egg samples: (A) CAD of *m/z* 790.4, i.e. PE 18:0/22:6 and (B) CAD of *m/z* 885.5, i.e. PI 18:0/20:4.

ular ion *m/z* 790.4, i.e. PE ($C_{18:0}/C_{22:6}$), displayed as major fragments (Fig. 3A), one ion resulting from the fragmentation at the ester bond (*m/z* 480.3, [LysostearoylPE–H]$^-$) and two carboxylate anions at *m/z* 327.4 ([$C_{22:6}$−H]$^-$) and at *m/z* 283.4 (stearate). Fig. 3B shows the tandem mass spectrum of *m/z* 885.5, i.e. PI ($C_{18:0}/C_{20:4}$). CAD of PI molecular species produced abundant ions resulting from the fragmentation at the ester bond ([lysoPI–H]$^-$) followed by neutral loss of H_2O ([LPI–H–H_2O]$^-$) and inositol ([LPI–H–H_2O–$C_6H_{10}O_5$]$^-$); the latest fragment was found for both fatty acids ([LysostearoylPI–H_2O–$C_6H_{10}O_5$]$^-$ and [LysoarachidoylPI–H_2O–$C_6H_{10}O_5$]$^-$). Only one fatty acid carboxylate anion was detected ([$C_{18:0}$–H]$^-$). These patterns were used for species confirmation of PE and PI.

Product ion spectrum of PC, LPC and Sph formiate adducts ions were shown to contain only the [M − CH_3]$^-$ ion fragment, due to the loss of the methyl group of choline. In Fig. 4, the fragmentation of molecular ion *m/z* 804.4, i.e. PC ($C_{16:0}/C_{18:1}$), *m/z* 747.4, i.e. Sph ($C_{16:0}$) and *m/z* 540.2, i.e. LPC ($C_{16:0}$) are reported.

3.3. Molecular species composition of egg phospholipid classes

The 5-week feeding time was chosen in order to ensure the highest incorporation of ω3 PUFAs in egg PLs. The molecular species composition of each phospholipid class from eggs by hens fed at different levels of SBO are shown in Table 1.

3.3.1. PE molecular species

The feeding at different levels of SBO affected the molecular species composition of PE, markedly. The most abundant molecular species for PE was 18:0/20:4 for samples obtained by feeding diet A (control diet), whereas the major species were 16:0/22:6 and 18:0/22:6 for samples obtained by feeding diets B–D. There was a significant higher amount of the ions *m/z* 762.4 (i.e. PE 16:0/22:6), *m/z* 790.4 (i.e. PE 18:0/22:6) and *m/z* 792.4 (i.e. PE 18:0/22:5) with respect to the control diet. Moreover, a significant lower amount of PE molecular species carrying ω6 PUFA, such as 16:0/20:4 (*m/z* 738.4), 18:0/18:2 (*m/z* 742.5) and 18:0/20:4 (*m/z* 766.5) was

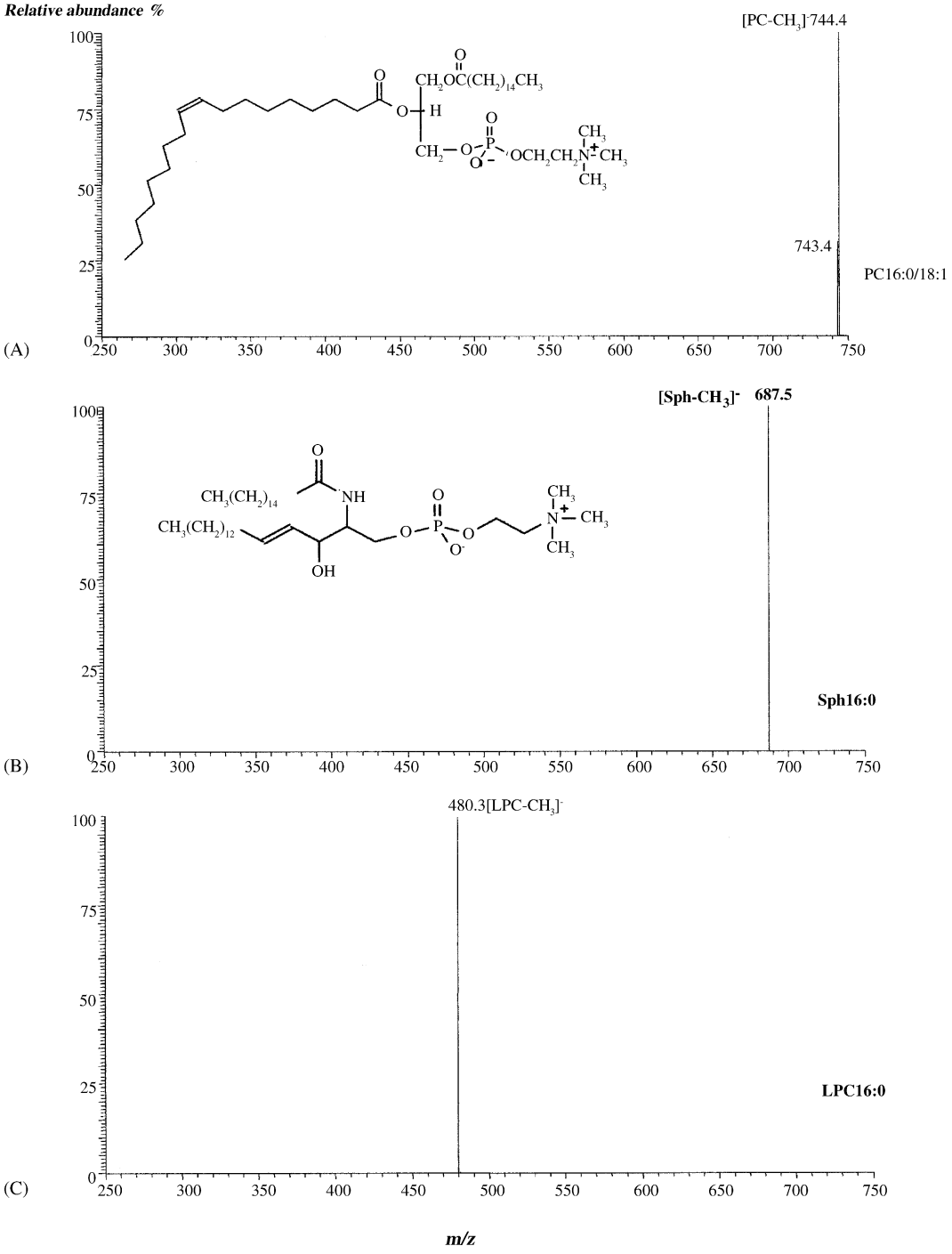

Fig. 4. Negative HPLC-ESI-MS–MS product ion spectra of selected PC, Sph and LPC molecular species detected in egg samples: (A) CAD of *m/z* 804.4, i.e. PC 16:0/18:1; (B) CAD of *m/z* 747.4, i.e. Sph 16:0 and (C) CAD of *m/z* 540.2, i.e. LPC 16:0.

found in the samples obtained with SBO-enriched diets (diets B–D) with respect to the samples obtained with the control diet.

3.3.2. PI molecular species

In contrast with PE, the major molecular species for PI was 18:0/20:4 (*m/z* 885.5) for all samples. Anyway, the SBO supplementation of hens lead to a statistically significant increase ($P < 0.05$, $n = 3$) of PI molecular species carrying DPA and DHA, such as 16:0/22:6 and 18:0/22:5, in the egg samples obtained by feeding hens with diet D, with respect to the remaining samples. For PI 18:0/22:6 there was not a statistically significant increase when feeding hens diet D with respect to diet C. At the same time, a statistically significant decrease ($P < 0.05$, $n = 3$) of PI 16:0/20:4, PI 18:0/18:2 and PI 18:0/20:4 was observed.

Table 1
Molecular species of phospholipids in eggs from hens fed at different levels of SBO during five weeks

PL molecular species		Relative abundance in percentage (mean ± SD)			
Ion (*m*/*z*)	Fatty acids	Diet (% SBO)			
		A (0%)	B (1.25%)	C (2.5%)	D (5%)
PE ($[M-H]^-$)					
714.4	16:0/18:2	4.5 ± 1.1	3.6 ± 0.3	5.0 ± 1.0	2.5 ± 0.9
716.4	16:0/18:1	11.9 ± 1.3 a	12.5 ± 0.1	16.3 ± 1.4 b	14.6 ± 1.3
738.4	16:0/20:4–18:2/18:2	5.0 ± 0.3 a	1.7 ± 0.9 b	1,0 ± 0.2 b	0.6 ± 0.3 b
742.5	18:0/18:2–18:1/18:1	13.8 ± 0.9 a	8.9 ± 1.2 b	8.4 ± 0.2 b	7.1 ± 0.6 b
744.5	18:0/18:1	14.8 ± 1.6	12.0 ± 1.7	11.8 ± 1.0	11.6 ± 0.8
762.4	16:0/22:6	8.0 ± 0.9 a	22.2 ± 1.3 b	22.1 ± 1.8 b	25.4 ± 2.6 b
764.4	18:1/20:4–18:0/20:5-16:0/22:5	5.0 ± 0.5	4.9 ± 1.1	4.2 ± 0.7	4.3 ± 1.0
766.5	18:0/20:4	27.0 ± 0.8 a	10.8 ± 1.0 b	9,9 ± 0.5 b	4.4 ± 1.6 c
790.4	18:0/22:6	7.5 ± 0.1 a	19.9 ± 0.6 b	19.7 ± 1.9 b	25.0 ± 2.2 b
792.4	18:0/22:5	2.4 ± 0.8 a,c	3.6 ± 0.5 a,b	1.8 ± 0.2 c	5.0 ± 0.4 b
PI ($[M-H]^-$)					
833.5	16:0/18:2	8.0 ± 1.7	5.6 ± 0.6	6.4 ± 1.2	5.7 ± 0.2
835.5	16:0/18:1	5.8 ± 0.8	6.9 ± 0.6	6.2 ± 0.9	7.1 ± 1.7
855.5	16:0/20:5	0.2 ± 0.1	0.8 ± 0.2	0.7 ± 0.4	0.7 ± 0.1
857.5	16:0/20:4–18:2/18:2	5.2 ± 0.7 a,b	5.7 ± 0.7 a	3.9 ± 0.7 b	0.1 ± 0.1 c
861.5	18:0/18:2–18:1/18:1	21.0 ± 2.2 a	19.3 ± 1.3 a	17.6 ± 0.6 a,b	14.0 ± 0.4 b
863.5	18:0/18:1	9.3 ± 1.4	9.5 ± 0.7	9.3 ± 0.5	8.4 ± 1.6
881.5	16:0/22:6	0.4 ± 0.1 a	0.7 ± 0.2 a	0.6 ± 0.2 a	2.0 ± 0.7 b
883.5	18:0/20:5–16:0/22:5	0.8 ± 1.5	3.5 ± 0.4	2.6 ± 2.2	4.1 ± 0.8
885.5	18:0/20:4	48.9 ± 3.7 a	26.8 ± 0.8 b	26.3 ± 2.7 b	22.9 ± 1.1 b
909.5	18:0/22:6	0.3 ± 0.2 a	13.9 ± 1.8 b	17.8 ± 1.5 b,c	20.3 ± 3.0 c
911.6	18:0/22:5	0.1 ± 0.1 a	7.4 ± 1.3 b	8.6 ± 1.4 b	14.8 ± 2.4 c
PC ($[M+HCOO]^-$)[a]					
802.4	16:0/18:2	11.5 ± 1.1	10.5 ± 1.2	13.2 ± 1.4	11.2 ± 1.7
804.4	16:0/18:1	32.9 ± 2.6	30.6 ± 2.2	35.0 ± 0.3	34.4 ± 1.1
806.4	16:0/18:0	4.6 ± 1.4	3.1 ± 0.5	4.2 ± 0.3	3.0 ± 0.3
824.4	16:0/20:5–16:1/20:4	0.3 ± 0.3 a	1.3 ± 0.4 b	1.2 ± 0.2 a,b	2.1 ± 0.5 b
826.4	16:0/20:4	3.1 ± 1.2 a	2.2 ± 0.4	1.4 ± 0.3	1.1 ± 0.4 b
830.4	18:0/18:2–18:1/18:1	12.0 ± 0.2 a	8.1 ± 0.5 b	7.6 ± 0.1 b	6.4 ± 0.5 b
832.4	18:0/18:1	10.6 ± 1.3 a	7.2 ± 0.5 b	7.5 ± 0.4 b	6.0 ± 0.9 b
850.4	16:0/22:6–18:1/20:5–18:2/20:4	6.5 ± 0.8 a	16.9 ± 0.8 b	13.6 ± 0.1 c	17.4 ± 0.4 b
852.4	18:1/20:4–18:0/20:5–16:0/22:5	2.2 ± 1.5	3.0 ± 0.9	2.7 ± 0.3	3.7 ± 0.4
854.4	18:0/20:4	11.1 ± 0.9 a	4.3 ± 0.6 b	2.5 ± 0.6 c	1.7 ± 0.6 c
876.4	18:1/22:6	0.6 ± 0.1 a	1.2 ± 0.3 b	1.2 ± 0.1 b	1.2 ± 0.1 b
878.4	18:0/22:6–18:1/22:5	3.1 ± 0.4 a	9.3 ± 0.9 b,c	8.1 ± 0.3 b	9.7 ± 0.4 c
880.4	18:0/22:5	1.3 ± 0.4	1.9 ± 0.2	1.2 ± 0.1	2.0 ± 0.5
Sph ($[M+HCOO]^-$)[a]					
747.4	16:0	95.6 ± 0.6	93.8 ± 1.0	95.6 ± 0.7	94.1 ± 0.8
775.4	18:0	4.4 ± 0.6	6.2 ± 1.0	4.4 ± 0.7	5.9 ± 0.8
LPC($[M+HCOO]^-$)[a]					
540.2	16:0	45.9 ± 2.0	49.6 ± 1.6	41.9 ± 0.8	51.9 ± 4.0
564.2	18:2	n.d.	4.0 ± 1.2	3.8 ± 1.0	n.d.
566.2	18:1	20.5 ± 3.1	20.6 ± 0.2	25.6 ± 3.1	21.2 ± 1.6
568.2	18:0	33.5 ± 2.2	26.0 ± 1.9	28.8 ± 5.0	26.9 ± 2.9

n.d., not detected, %<0.1; values in the same row with different letters are significantly different ($P < 0.05$). Legend for *fatty acids*, total fatty acid carbon number:number of double bonds.

[a] For quantitative analysis of PC, LPC and Sph, also the ions $[M+35]^-$ and $[M-15]^-$ were considered.

3.3.3. PC molecular species

The major molecular species for PC was PC 16:0/18:1 for all samples. It accounted for 32.9%, 30.6%, 35.0% and 34.4% of total PC molecular species in samples from diets A–D, respectively. Feeding hens with SBO-enriched diets resulted in a significant higher amount of PC molecular species carrying EPA, DPA and DHA, and in a significant decrease of PC molecular species carrying ω6 PUFAs, in samples from diets B–D, with respect to the samples from diet A.

3.3.4. Sph molecular species

The different levels of SBO in the four diets had no effect on the molecular species profile of Sph. There were no statistically significant differences among the Sph molecular

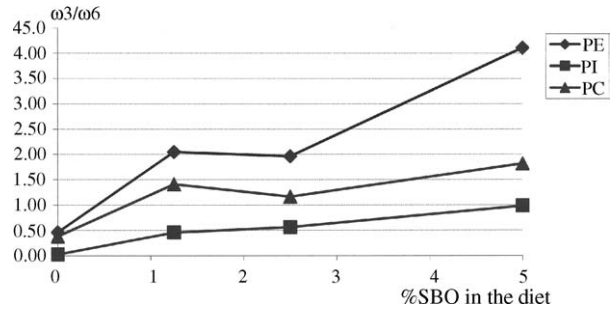

Fig. 5. Effect of the SBO content in the hens diet on the ω3/ω6 ratio of phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylcholine (PC) in hen eggs.

species composition of samples obtained with different feedings. The most abundant ions was *m*/*z* 747.3, corresponding to Sph 16:0, which accounted for approximately 95% of total molecular species; the second most important species was Sph containing stearic acid (about 5% of total molecular species).

3.3.5. LPC molecular species

As it was seen for Sph, the different levels of SBO had no effect on the molecular species profile of LPC and no statistically significant differences were registered among the samples. The most abundant ion was *m*/*z* 540.2, corresponding to LPC 16:0, which accounted for approximately 40%–50% of total LPC molecular species. Minor molecular species were those containing stearic, oleic and linoleic acid. It was also observed that LPC can arise from the hydrolysis of PC when the lipid fraction is not analysed immediately after its extraction.

4. Conclusions

The results showed that feeding different levels of SBO for 5 weeks resulted in a significant increase of PE, PC and PI molecular species carrying EPA, DPA and DHA, but not Sph nor LPC. The increase of the ω3/ω6 ratio versus the SBO concentration of the feeding is plotted in Fig. 5. The ratio was obtained by dividing the sum of PL molecular species containing ω3 fatty acids (16:0/22:6, 16:0/22:5–18:0/20:5, 16:0/20:5, 18:0/22:6, 18:0/22:5 and 18:0/20:5) by the sum of the species containing ω6 (16:0/18:2, 16:0/20:4, 18:0/18:2 and 18:0/20:4). The trend was different according to the different PLs. The highest increase of the ω3/ω6 ratio occurred for PE and PC. On the contrary, PI was less affected by the increase of SBO in the diet.

The HPLC-ESI-MS–MS method was a powerful tool in order to shorten the sample preparation, since the purification of PLs from the total lipid fraction was not necessary; this reduced the formation of artifacts. In addition, a definitive identification of the PL molecular species could be achieved.

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