

Individual Phospholipid Classes from Iberian Pig Meat As Affected by Diet

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The main objectives of this study were to (1) determine the individual phospholipid (PL) classes content of fresh meat from Iberian pigs and their respective fatty acid and dimethylacetal composition and (2) assess the effect of different diets (acorn and grass vs oleic acid enriched concentrates) on these lipid species. First, it was found that phosphatidylcholine was the major PL, followed by phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol in decreasing order. Each PL class showed a different lipid profile. Second, the feeding regimen influenced the quantity and the fatty composition of the individual PL classes. Meat from pigs fattened with high oleic acid concentrates had higher amounts of most phospholipid classes and polyunsaturated fatty acids, which is an indication of lipid oxidation instability. Lastly, these differences in PL species and fatty acid composition could be used to differentiate meats from Iberian pigs with different feeding regimens.

KEYWORDS: Phospholipid classes; fatty acid composition; dimethylacetals; Iberian pig; muscle; feeding

INTRODUCTION

Phospholipids (PL) are the key components of all biological membranes. Each tissue exhibits its own pattern of PL classes; phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) are the more representative classes in mammal skeletal muscle (1). Each PL class shows a pattern of acyl and alkyl chains in the sn-1 and sn-2 positions (2). Both PC and PE are key components of membrane bilayers, PC making up a very high proportion of the outer leaflet of the cell membrane. PC has a role in signaling via the generation of diacylglycerols, whereas PE has a specific involvement in supporting active transport. PS is located entirely on the inner monolayer surface of the cells, and it is involved in the blood coagulation process. PI is the primary source of the arachidonic acid (C20:4 n-6), which is required for the biosynthesis of eicosanoids (3).

However, the number of studies concerning polar lipids in muscle and muscle foods is much smaller in comparison to those on neutral lipids, and most of these studies have been aimed to study the fatty acid (FA) profile of total polar lipids (4, 5). Nevertheless, over the past decade there has been growing interest in analyzing in more detail this lipid fraction in muscle foods, because it has been suggested by several researchers that lipid oxidation in muscle and muscle foods initiates and mainly takes place in membrane PL (6). The high sensitivity of PL to oxidation

in meat and meat products has primarily two causes: the high proportion of long-chain polyunsaturated FA (PUFA), which are very susceptible to oxidation, and the close contact of PL with catalysts of lipid oxidation located in the aqueous phase of the muscle cell (7). Besides, these compounds are subject to degradation throughout the processing of Iberian hams. In fact, FA released during the ripening process mainly arise from this fraction (4). In addition, the rate and extension of the lipolysis that takes place during the ripening of Iberian ham are related to the features of the raw material and the ripening conditions (4).

It is generally accepted that the FA composition of PL can be altered due to nutritional or environmental factors, such as temperature changes (8). In fact, several works have demonstrated that the FA composition of muscle PL from pig, beef, or chicken is strongly influenced by the FA composition of dietary FA (5–9). Moreover, Sánchez et al. (10) and Pérez-Palacios et al. (11) showed the diet effect on the FA profile of individual PL classes from different rat tissues. Similarly, the proportion of dimethylacetals (DMA) in different PL classes has been shown to be influenced by dietary FA composition (11, 12). DMAs are linked to the sn-1 position of the PL by a vinyl ether linkage and seem to play a certain antioxidant role (13).

Iberian meat products from animals fed outdoors on natural resources reach the highest prices in the market because of their quality characteristics, which are mainly attributed to the outdoor rearing, which implies the consumption of acorns and grass (5, 14). Acorn and grass production is seasonal and restricted, and thus a considerable number of Iberian pigs are fattened with concentrates,

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77 which implies lower quality and market acceptance (14, 15).
78 Currently, monounsaturated FA (MUFA) enriched diets,
79 through the inclusion of high oleic acid sunflower oil, are being
80 used for feeding Iberian pigs to imitate the FA profile of those
81 animals fattened on acorns.

82 Most studies in Iberian ham focused on the effect of the diet on
83 the FA composition have been mainly devoted to the study of the
84 FA profile of neutral lipids, free fatty acids, and PL (4, 5), whereas
85 its influence on the content of muscle individual PL classes or the
86 FA profile of each muscle PL class remains unstudied. Thus, this
87 study was aimed to analyze the amount of individual PL classes in
88 muscle from Iberian pigs as well as their FA composition.
89 Moreover, the effect of feeding Iberian pigs different diets
90 (acorn and grass vs oleic acid enriched concentrates) on both
91 the quantity and lipid composition of the different muscle PL
92 classes was also studied.

93 MATERIALS AND METHODS

94 **Experimental Design.** This study was carried out with 30 pure Iberian
95 pigs, which were divided into two groups according to the feeding regimen
96 during the fattening period prior to slaughter. One group of pigs (AG) ($n =$
97 15) was reared outdoors in a 30 ha extension land with free availability of
98 acorns (*Quercus ilex*, *Quercus rotundifolia*, and *Quercus suber*) and grass.
99 The other group of pigs (HO) ($n = 15$) was also fattened outdoors in a 1 ha
100 extension land, with an oleic acid enriched concentrate (4.120 kg/day) and
101 free availability of grass but mainly fed. The chemical and FA composi-
102 tions of the feeds have been previously published (15). At the beginning
103 of the fattening period weights were 99.2 ± 3.1 and 96.3 ± 1.7 kg for Iberian
104 pigs of the AG and HO groups, respectively. All of the animals were
105 fattened for 110 days and slaughtered the same day at the age of 16 months
106 by electrical stunning and exsanguination at a local slaughterhouse.
107 Slaughter weights were 158.9 ± 3.2 and 162.5 ± 2.9 kg for AG and HO
108 pigs, respectively. One ham of each animal was taken, and their biceps
109 femoris and semimembranosus muscles were dissected and stored at -80°C
110 until analysis. The quantity of each PL class was analyzed in both muscles,
111 whereas the biceps femoris was the only muscle used for studying the FA
112 and DMA compositions of each PL class.

113 **Intramuscular Fat Extraction.** Samples were ground using a com-
114 mercial grinder immediately before fat extraction. Intramuscular total
115 lipids were extracted with chloroform/methanol (2:1, v/v), according to
116 the method described by Folch et al. (16) and modified by Pérez-Palacios
117 et al. (17).

118 **Quantification of Phospholipid Classes.** Quantification of PL
119 classes was carried out by fractionation using HPLC coupled to evapora-
120 tive light scattering detector (ELSD), following the method described by
121 Rombaut et al. (18) with slight modifications. Lipids (24 mg) were
122 dissolved in 1.6 mL of chloroform/methanol solvent (88:12, v/v). Analysis
123 was carried out using an HPLC Shimadzu (LC-20AT prominence liquid
124 chromatography) instrument equipped with a pump (DGU-20A5 promi-
125 nence degasser) and a SIL-20AC autosampler. The analytical column
126 (150 mm \times 30 mm i.d.) was packed with a silica normal-phase Preval Silica
127 3u (GRACE) thermostated in an oven (Shimadzu CTO-20AC prominence
128 column oven) at 40°C . The chromatographic separation was carried out
129 using a linear gradient according to the following scheme: $t = 0$ min, 87.5%
130 A–12% B–0.5% C; $t = 12$ min, 2% A–90% B–8% C for 2 min. The
131 mobile phase was brought back to the initial conditions at $t = 16$ min, and
132 the column was allowed to equilibrate until the next injection at $t = 25$ min.
133 Eluent A consisted of chloroform, eluent B of methanol, and eluent C of
134 triethylamine buffer (pH 3, 1 M formic acid). The flow was maintained at
135 0.7 mL/min. The injection volume was 10 μL . HPLC was coupled with an
136 ELSD (Alltech 3300). The nebulizing gas was N_2 , at a flow rate of 1.6 L/min
137 and a nebulizing temperature of 65°C . The gain was set at 1. Individual PL
138 classes were identified by comparing their retention times with those
139 of external standards (Spectral Services GMBH, Köln, Germany). For
140 quantification purposes, calibration curves of individual PL classes were
141 prepared.

142 **Fatty Acid Methyl Ester (FAME) and Dimethylacetal (DMA)**
143 **Preparation and Analysis.** To analyze the FA composition of each PL
144 class, the PL fractionation was carried out in NH_2 -aminopropyl minicolumns

(500 mg) from Varian (Harbor City, CA). Briefly, minicolumns were
activated with 7.5 mL of *n*-hexane. Twenty milligrams of lipids dissolved in
150 μL of *n*-hexane/chloroform/methanol (95:3:2, v/v/v) was loaded onto
the column. Neutral lipids were eluted with 5 mL of chloroform and free
FA with 5 mL of diethyl ether/acetic acid (98:2, v/v). In this way,
minicolumns retained the PL, being further separated into PL classes in the
same minicolumn in which they had been retained, following the method
used for muscle PL fractionation into PC, PE, PS, and PI described by
Pérez-Palacios et al. (20). PC, PE, PS, and PI were eluted with 30 mL of
acetonitrile/*n*-propanol (2:1, v/v), 10 mL of methanol, 7.5 mL of iso-
propanol/3 N methanolic HCl (4:1, v/v), and 17.5 mL of chloroform/
methanol/37% HCl (200:100:1, v/v/v), respectively. The vacuum was
adjusted to generate a flow of 1 mL/min.

Fatty acid methyl esters (FAME) and DMA from alkenyl chains were
prepared by transesterification in the presence of 0.1 N sodium metal in
methanol and sulfuric acid in methanol at 80°C (21). FAME were
analyzed by gas chromatography, using an Agilent 6890N gas chromato-
graph, equipped with a flame ionization detector (FID). Separation was
carried out on a polyethylene glycol capillary column (60 m long, 0.32 mm
i.d., and 0.25 mm film thickness) (Supelcowax-10, Supelco, Bellefonte,
PA). Oven temperature programming started at 180°C . Immediately, it
was raised at 5°C min^{-1} to 200°C , held for 40 min at 200°C , increased
again at 5°C min^{-1} to 250°C , and held for the last 21 min at 250°C .
Injector and detector temperatures were 250°C . The carrier gas was
helium at a flow rate of 0.8 mL/min. Individual FAME peaks were
identified by comparing their retention times with those of a standard
(Sigma, St. Louis, MO) containing a mix of 37 FAME saturated,
monounsaturated, and polyunsaturated (from C4 to C24). To confirm
identification, selected samples were subjected to gas chromatography
coupled to mass spectrometry (GC-MS) in a HP-5890GC series II gas
chromatograph (Hewlett-Packard) coupled to a mass selective detector
(HP-5971 A, Hewlett-Packard). FA and DMA were separated using the
same column as that used for GC-FID, with helium operating at 41.3 kPa
of column head pressure, resulting in a flow of 1.45 mL min^{-1} at 180°C .
The injector and oven program temperatures were the same as for the GC-
FID analysis. The transfer line to the mass spectrometer was maintained at
 280°C . The mass spectra were obtained by electronic impact at 70 eV, a
multiplier voltage of 1756 V, and collecting data at a rate of 1 scan s^{-1} over
the m/z range of 30–500. Compounds were tentatively identified by
comparing their mass spectra with those contained in the NIST/EPA/
NIH and Wiley libraries.

Statistical Analysis. The effects of pig feeding, muscle, and their
interaction on the content of each individual muscle PL were analyzed
using a two-way analysis of variance with interaction by the General
Linear Model procedure. The effect of pig feeding on the FA and DMA
composition of each individual PL class from the biceps femoris muscle
was analyzed by a one-way analysis of variance (ANOVA) using the General
Linear Model procedure. Mean and standard deviation of the percentages
of DMA, saturated FA (SFA), MUFA, and PUFA were also calculated.
Statistical analyses were performed using the SPSS (v. 15.0) package
software.

RESULTS AND DISCUSSION

Quantification of Individual PL Classes. Iberian pigs fattened
on acorn and grass were considered as the control group because
this group of animals was fattened following the traditional
procedure.

Four different PL classes were determined in the biceps femoris
and semimembranosus muscles of this study (Figure 1). The major
PL was PC, followed by PE and PS, and PI being the minor one
(Table 1), which is basically in agreement with results found by
other authors studying different mammal muscle tissues (2).

The effect of the muscle did not lead to significant difference in
any PL class. On the other hand, there was a higher IMF content
in the biceps femoris (29.07 ± 5.91 g/100 g of muscle dry matter
(DM)) than in the semimembranosus muscle (17.08 ± 4.03 g/
100 g of muscle DM). These results are not in agreement with
those in previous studies in which higher PC and PE contents were
detected in muscles with a higher IMF content (22).

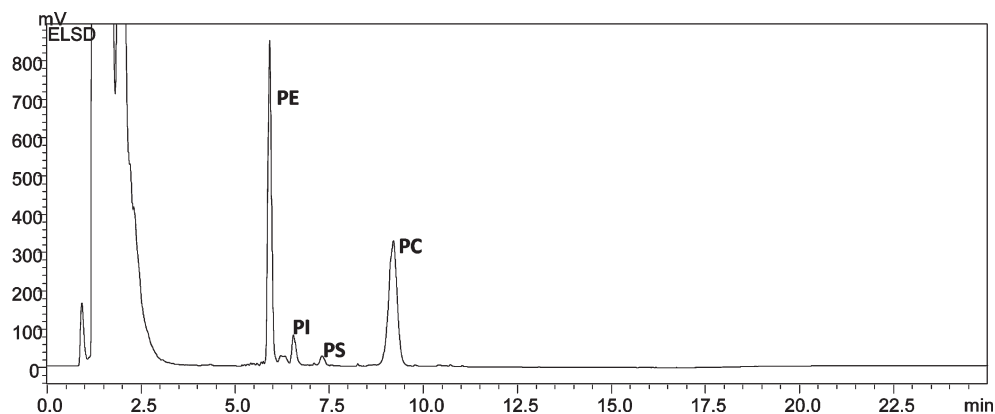


Figure 1. High-performance liquid chromatogram of phospholipid classes in raw thighs of Iberian pigs.

Table 1. Phospholipid Class Content (Expressed as Milligrams of Phospholipid per 100 g of Muscle Dry Matter \pm Standard Deviation) in the Biceps Femoris and Semimembranosus Muscles of Fresh Iberian Hams from Pigs Fattened with Different Diets: Acorn and Grass (AG) and High Oleic Acid Enriched Concentrate (HO)^a

	biceps femoris		semimembranosus		<i>p</i>		
	AG	HO	AG	HO	F	M	F \times M
PC	976.10 \pm 108.05	1444.33 \pm 80.16	1215.57 \pm 70.06	1683.62 \pm 181.65	<0.001	0.143	0.180
PE	352.71 \pm 61.03	499.44 \pm 41.16	524.61 \pm 36.45	608.82 \pm 14.59	0.017	0.146	0.710
PS	76.96 \pm 6.73	81.70 \pm 8.09	79.53 \pm 9.16	75.29 \pm 11.30	0.894	0.697	0.740
PI	58.91 \pm 2.59	86.66 \pm 5.33	63.73 \pm 6.04	80.20 \pm 6.82	<0.001	0.361	0.120
Σ PL	1529.20 \pm 82.65	2061.12 \pm 51.06	1903.96 \pm 21.12	2409.73 \pm 157.05	<0.001	0.114	0.229

^a F, feeding effect; M, muscle effect; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; Σ PL, sum of the content of the different phospholipid classes.

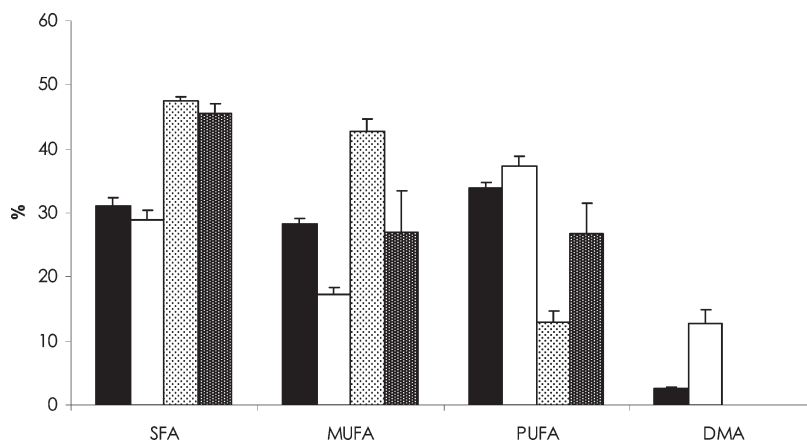


Figure 2. Percentage of dimethylacetals (DMA) and saturated, monounsaturated, and polyunsaturated fatty acids (SFA, MUFA, and PUFA, respectively) in phosphatidylcholine (black bars), phosphatidylethanolamine (white bars), phosphatidylserine (white bar with black dots), and phosphatidylinositol (black bar with white dots) from fresh Iberian hams. Error bars display standard deviations.

213 The effect of diet on the amount of each PL class in biceps
 214 femoris and semimembranosus muscles from Iberian pigs is
 215 shown in **Table 1**. The quantity of PC, PE, and PI was significantly
 216 higher ($p < 0.001$, $p = 0.017$, and $p < 0.001$, respectively) in HO
 217 than in AG muscles, whereas PS was not influenced by pig feeding.
 218 As a consequence, the sum of the content of the four PL classes
 219 was also higher ($p < 0.001$) in HO than in AG biceps femoris and
 220 semimembranosus muscles. The amount of PL has been related to
 221 the type, diameter, and mitochondria content of muscular fibers
 222 (22), which could explain the differences in the content of
 223 individual PL classes between AG and HO Iberian pigs. In fact,
 224 although both groups of Iberian pigs were fattened outdoors, the
 225 area of the land in which AG pigs were reared was larger than
 226 that of HO ones. Thus, AG Iberian pigs should have experienced
 227 more intense physical exercise than HO pigs, which may influence

228 muscle fiber characteristics. Contrarily, Petró et al. (23) and
 229 Tejada et al. (24) found similar total PL contents in muscles from
 230 Iberian pigs fed different diets. Other studies have shown that the
 231 relative percentage of PL classes was not influenced by the diet in
 232 either fish (25) or mammary tissue and erythrocytes from rats (26).

233 **Fatty Acid and Dimethylacetal Compositions of Individual PL**
 234 **Classes.** Total SFA, MUFA, PUFA, and DMA in the four PL
 235 classes from the biceps femoris muscle of raw hams is shown in
 236 **Figure 2**. The highest proportions of SFA were found in PS
 237 (47.55 \pm 0.47%) and PI (41.45 \pm 1.57%), whereas these FA were
 238 lower in PC (31.14 \pm 1.19%) and PE (28.94 \pm 1.53%). Thus, PS
 239 showed a high content of palmitic acid (C16:0) (**Table 4**), whereas
 240 high levels of stearic acid (C18:0) were found in PI (**Table 5**). In rat
 241 muscle, the highest contents of C16:0 and C18:0 were found in PC
 242 and PS, respectively (2).

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Table 2. Fatty Acid and Dimethylacetal Composition (Percent of Total FAME and DMA Detected \pm Standard Deviation) of Phosphatidylcholine of the Biceps Femoris Muscle from Iberian Pigs Fed Different Diets: Acorn and Grass (AG) and High Oleic Acid Enriched Concentrate (HO)^a

	AG	HO	<i>p</i>
C16:0	22.23 \pm 0.76	21.97 \pm 0.73	0.591
C16:1	1.50 \pm 0.14	1.28 \pm 0.19	0.066
C18:0	9.11 \pm 0.66	8.99 \pm 0.53	0.753
C18:1 n-9	23.32 \pm 1.01	17.74 \pm 0.75	<0.001
C18:1 n-7	4.30 \pm 0.22	4.60 \pm 0.33	0.123
C18:2 n-6	28.08 \pm 0.55	27.65 \pm 1.09	0.450
C18:3 n-6	0.20 \pm 0.01	0.20 \pm 0.01	0.328
C18:3 n-3	0.87 \pm 0.14	0.47 \pm 0.03	<0.001
C20:3 n-6	0.92 \pm 0.03	0.93 \pm 0.06	0.653
C20:4 n-6	4.31 \pm 0.81	7.50 \pm 0.49	<0.001
C20:3 n-3	0.48 \pm 0.23	0.62 \pm 0.16	0.300
C20:5 n-3	0.24 \pm 0.04	0.51 \pm 0.14	0.004
C22:1 n-9	0.16 \pm 0.02	0.30 \pm 0.07	0.003
C22:2	0.20 \pm 0.09	0.18 \pm 0.02	0.733
C24:0	0.89 \pm 0.08	0.70 \pm 0.06	0.003
C22:6 n-3	0.48 \pm 0.15	0.61 \pm 0.09	0.136
Σ SFA	32.23 \pm 0.71	31.66 \pm 0.92	0.300
Σ MUFA	29.28 \pm 1.10	23.92 \pm 1.14	<0.001
Σ PUFA	35.03 \pm 0.63	38.67 \pm 1.30	<0.001
C16:0 DMA	1.86 \pm 0.17	3.67 \pm 1.09	0.006
C18:0 DMA	0.31 \pm 0.02	0.73 \pm 0.04	<0.001
C18:1 DMA	0.55 \pm 0.05	1.36 \pm 0.11	<0.001
Σ DMA	2.71 \pm 0.19	5.75 \pm 1.09	<0.001

^a Σ SFA, total amount of saturated fatty acids; Σ MUFA, total amount of monounsaturated fatty acids; Σ PUFA, total amount of polyunsaturated fatty acids; Σ DMA, total amount of dimethylacetals.

Table 3. Fatty Acid and Dimethylacetal Composition (Percent of Total FAME and DMA Detected \pm Standard Deviation) of Phosphatidylethanolamine of the Biceps femoris Muscle from Iberian Pigs Fed Different Diets: Acorn and Grass (AG) and High Oleic Acid Enriched Concentrate (HO)^a

	AG	HO	<i>p</i>
C16:0	11.66 \pm 2.95	5.54 \pm 0.73	0.002
C16:1	0.80 \pm 0.48	1.16 \pm 0.23	0.167
C18:0	15.99 \pm 1.48	18.11 \pm 0.58	0.018
C18:1 n-9	15.04 \pm 2.79	10.87 \pm 0.56	0.011
C18:1 n-7	2.04 \pm 0.47	1.74 \pm 0.12	0.208
C18:2 n-6	15.57 \pm 1.23	15.66 \pm 0.70	0.895
C18:3 n-3	0.56 \pm 0.12	0.29 \pm 0.06	0.002
C20:3 n-6	1.07 \pm 0.09	1.26 \pm 0.12	0.025
C20:4 n-6	19.87 \pm 1.62	34.30 \pm 0.76	<0.001
C20:3 n-3	0.21 \pm 0.02	0.37 \pm 0.11	0.010
C20:5 n-3	0.54 \pm 0.18	0.83 \pm 0.14	0.021
C22:1 n-9	0.26 \pm 0.07	0.42 \pm 0.12	0.035
C22:2	0.57 \pm 0.12	0.91 \pm 0.06	0.001
C24:0	3.36 \pm 0.25	2.84 \pm 0.41	0.045
C22:6	0.77 \pm 0.14	0.66 \pm 0.05	0.113
Σ SFA	31.01 \pm 3.49	26.49 \pm 1.18	0.025
Σ MUFA	18.14 \pm 3.19	14.19 \pm 0.77	0.027
Σ PUFA	39.17 \pm 2.59	54.27 \pm 0.85	<0.001
C16:0 DMA	4.10 \pm 1.18	3.07 \pm 1.31	0.228
C18:0 DMA	4.27 \pm 1.01	1.44 \pm 0.09	<0.001
C18:1 DMA	3.32 \pm 1.36	0.53 \pm 0.12	0.002
Σ DMA	11.68 \pm 3.06	5.05 \pm 1.25	0.002

^a Σ SFA, total amount of saturated fatty acids; Σ MUFA, total amount of monounsaturated fatty acids; Σ PUFA, total amount of polyunsaturated fatty acids; Σ DMA, total amount of dimethylacetals.

Table 4. Fatty Acid and Dimethylacetal Composition (Percent of Total FAME Detected \pm Standard Deviation) of Phosphatidylserine of the Biceps Femoris Muscle from Iberian Pigs Fed Different Diets: Acorn and Grass (AG) and High Oleic Acid Enriched Concentrate (HO)^a

	AG	HO	<i>p</i>
C16:0	29.33 \pm 0.88	17.65 \pm 1.25	<0.001
C16:1	4.32 \pm 0.76	12.84 \pm 1.18	<0.001
C18:0	16.18 \pm 1.27	12.38 \pm 0.36	<0.001
C18:1 n-9	31.64 \pm 2.81	30.12 \pm 0.89	0.280
C18:1 n-7	5.16 \pm 0.44	7.05 \pm 0.46	<0.001
C18:2 n-6	5.12 \pm 0.95	6.83 \pm 0.47	0.007
C18:3 n-3	0.23 \pm 0.01	0.36 \pm 0.00	<0.001
C20:3 n-6	0.25 \pm 0.00	0.29 \pm 0.11	0.402
C20:4 n-6	1.43 \pm 0.30	2.28 \pm 0.59	0.022
C20:3 n-3	1.19 \pm 0.83	3.47 \pm 1.09	0.006
C20:5 n-3	2.61 \pm 0.98	3.70 \pm 0.66	0.071
C22:1 n-9	0.96 \pm 0.22	1.96 \pm 0.53	0.005
C22:2	2.57 \pm 1.46	2.76 \pm 1.22	0.824
Σ SFA	45.51 \pm 1.76	30.03 \pm 1.55	<0.001
Σ MUFA	42.09 \pm 2.38	51.97 \pm 1.50	<0.001
Σ PUFA	12.92 \pm 3.79	19.04 \pm 2.35	0.015

^a Σ SFA, total amount of saturated fatty acids; Σ MUFA, total amount of monounsaturated fatty acids; Σ PUFA, total amount of polyunsaturated fatty acids.

243 The highest content of MUFA was found in PS, followed by
244 PC and PI, whereas PE showed the lowest proportion. This is the
245 consequence of the high levels of C18:1 n-9 observed in PS, which
246 is in agreement with the results found by Pérez-Palacios et al. (2)
247 in rat muscle.

248 PC and PE showed the highest levels of PUFA (33.84 and
249 37.25%, respectively), due to the high proportion of linoleic acid
T2 250 (C18:2 n-6) in PC (Table 2) and the high content of both C18:2 n-6
T3 251 and C20:4 n-6 in PE (Table 3). Similarly, Alasnier et al. (27) and
252 Cambero et al. (28) showed a higher content of C20:4 n-6 in PE
253 than in PC of rabbit muscle. On the other hand, PI showed the
254 highest proportion of C20:4 n-6 in the longissimus dorsi of rat (2).

255 The high oxidation susceptibility of PL compared to neutral
256 lipids (29) is well established, due to their location in membranes
257 close to heme pigments and oxidant systems and due to their high
258 PUFA content (30). In relation the fatty profile of the PL classes,
259 PC and PE would be more susceptible to oxidation than PS and
260 PI because of their higher content of PUFA. On the other hand,
261 the relative oxidation rates of PUFA containing two, three, four,
262 five, or six double bonds are 1, 2, 4, 6, and 8, respectively (31).
263 Thus, taking into account the proportion of individual PUFA of
264 each PL, PE would be the most susceptible to oxidation, followed
265 by PC and PI, with PS being the least prone to oxidation.

266 PE showed the highest proportion of DMA, followed by PC,
267 whereas these compounds were not found in PS and PI. Hexa-
268 decanal dimethylacetal (C16:0 DMA), octadecanal dimethyl-
269 acetal (C18:0 DMA), and octadecanal dimethylacetal (C18:1 n-9)
270 were detected in both PC (Table 2) and PE (Table 3). Thus, the
271 low content of SFA and MUFA in PC and PE could be due, at
272 least in part, to the high levels of DMA found, because one of the
273 pathways for the biosynthesis of plasmalogens involves a desa-
274 turation process of the FA esterified in the analogue PL (32).
275 Accordingly, Pérez-Palacios et al. (2) showed that PE was the PL
276 class containing the highest level of DMA in the longissimus dorsi

of rats. However, these authors also found DMA in PC, PS, and
PI of rat muscle.

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Table 5. Fatty acid and Dimethylacetal Composition (Percent of Total FAME Detected \pm Standard Deviation) of Phosphatidylinositol of the Biceps Femoris Muscle from Iberian Pigs Fed Different Diets: Acorn and Grass (AG) and High Oleic Acid Enriched Concentrate (HO)^a

	AG	HO	<i>p</i>
C16:0	18.76 \pm 0.49	17.15 \pm 0.55	0.006
C16:1	9.02 \pm 0.46	19.02 \pm 0.40	<0.001
C18:0	24.41 \pm 1.70	20.81 \pm 1.73	0.028
C18:1 n-9	11.27 \pm 2.58	12.47 \pm 2.60	0.550
C18:1 n-7	7.35 \pm 0.31	10.57 \pm 1.34	0.007
C18:2 n-6	11.68 \pm 0.02	12.74 \pm 1.57	0.301
C20:4 n-6	7.40 \pm 0.11	7.25 \pm 1.43	0.867
C20:5 n-3	1.51 \pm 0.50		
C22:1 n-9	3.02 \pm 1.23		
C22:2	5.58 \pm 1.12		
Σ SFA	43.17 \pm 1.28	37.95 \pm 1.96	0.007
Σ MUFA	30.67 \pm 2.17	42.06 \pm 2.64	0.001
Σ PUFA	24.66 \pm 1.04	19.99 \pm 1.55	0.004

^a Σ SFA, total amount of saturated fatty acids; Σ MUFA, total amount of monounsaturated fatty acids; Σ PUFA, total amount of polyunsaturated fatty acids.

enriched concentrates showing lower SFA content than acorns and grass (15). Moreover, the incorporation of FA into each PL class is a selective process (13), which can be explained by specific differences in the acylation process for each individual PL (32).

The percentage of MUFA was influenced by the feeding background but showed a variable behavior depending on the PL class. Thus, PC and PE from fresh meat of AG Iberian pigs showed higher proportions of total MUFA and C18:1 n-9 than those from HO pigs, despite the scarce differences in the content of C18:1 n-9 between the feeds consumed by AG (60.44% in acorns) and HO pigs (55.97% in high oleic enriched concentrate) (15). On the other hand, muscle PS and PI from HO Iberian pigs showed higher MUFA proportions than those of AG ones, as a consequence of a higher content of vaccenic (C18:1 n-7) and palmitoleic (C16:1 n-7) acids in PS and PI of HO thighs. The high levels of C16:1 n-7 in muscle PS and PI of HO Iberian pigs could be related to the desaturation of C16:0 to C16:1 n-7 by Δ^9 -desaturase (33). In fact, as described above, PS and PI from HO animals showed a lower content of C16:0 than those of AG ones. The presence of higher substrate (C16:0) content would lead to an increase in desaturase enzyme activity (34) and, consequently, to higher levels of C16:1 n-7.

In PC, PE, and PS, the proportion of total PUFA was significantly higher in HO than in AG fresh meat, as a result of the higher levels of C20:4 n-6 (in PC, PE, and PS) and C18:2 n-6 (only in PS) in HO than in AG animals. These results are in agreement with the FA profile of the feeding, because the high oleic enriched concentrate showed a higher content of C18:2 n-6 than acorns and grass (15). The pathway for biosynthesis of C20:4 n-6 involves the desaturation and elongation of dietary C18:2 n-6 (32). On the other hand, muscle PI showed a statistically higher proportion of total PUFA in AG than in HO pigs, due to the presence of eicosapentaenoic (C20:5 n-3), erucic (C22:1), and docosadienoic (C22:2 n-6) acids, whereas these FA were not found in HO ones. However, no differences in C18:2 n-6 and C20:4 n-6 were found in muscle PI between AG and HO Iberian pigs. In fact, studying the influence of the diet on the FA and DMA composition of PL classes from animal muscles, other authors have shown that PI was the less affected PL (9, 11, 26), which could be related to the role of PI as a second messenger in cell signal transduction mechanism and also to the fact that the maintenance of the FA composition of PI is an important feature of membrane homeostatic mechanisms (26).

The effect of the diet on the content of DMA did not follow the same trend in PC and PE. Higher levels of C16:0 DMA, C18:0 DMA, C18:1 DMA, and, consequently, total DMA were found in muscle PC of HO than in AG pigs. On the other hand, in muscle PE the proportions of C18:0 DMA, C18:1 DMA, and total DMA were higher in AG than in HO. The different influence of the feeding background on the content of DMA in PC and PE is not easily addressed. It could be related to particular PL characteristics, such as the abundance and situation of the PL in membrane, the physiological function of a particular PL, or the role as precursor of several FA for the biosynthesis of biologically active compounds.

Several authors have also shown the effect of the diet on FA composition of PL classes in different mammal muscles (11, 26, 28). As far as Iberian pig is concerned, Muriel et al. (5) also found differences in the FA profile of total PL from the longissimus dorsi muscle between animals fed acorn and grass and those fed high oleic acid enriched concentrates.

Thus, it can be concluded that Iberian pig feeding (acorn and grass vs high oleic acid concentrates) leads to differences both in the quantity of muscle PL classes and in the FA and DMA composition of such PL classes, those from pigs fattened with high oleic acid concentrates showing higher amounts of PL and PUFA. These differences could make the meat from HO animals more prone to lipid oxidation, because PL are very sensitive to oxidation, mainly due to their high PUFA content (7), which in turn could lead to a lower quality in meat products from HO animals. Decomposition of hydroperoxides generated during lipid oxidation creates a wide range of compounds contributing to flavor deterioration (35). Moreover, differences found in the quantity and lipid composition of the different PL classes could be used as tools for differentiating meat from Iberian pigs with different feeding backgrounds.

ABBREVIATIONS USED

PL, phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; AG, acorn and grass; HO, high oleic acid enriched concentrates; FA, fatty acid; FAME, fatty acid methyl esters; DMA, dimethylacetal; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

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