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Individual ~~phospholipid classes~~Phospholipid Classes from Iberian ~~pig meat as affected~~Pig Meat As Affected by ~~diet~~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*~~ 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. ~~Firstly,~~First, it was found that phosphatidylcholine was the major PL, followed by phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol in ~~a~~decreasing order. Each PL class showed a different lipid profile. ~~Secondly,~~Second, the feeding ~~regime~~regimen influenced the quantity and the fatty composition of the individual PL classes. Meat from pigs fattened with high oleic acid concentrates had higher ~~amount~~amounts of most phospholipid classes and ~~of~~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 ~~regimes.~~regimens.

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

~~INTRODUCTION~~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)~~(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 ~~signalling~~signaling via the generation of diacylglycerols, ~~while~~whereas PE has a specific involvement in supporting active transport. PS is located entirely on the inner monolayer surface of the ~~cells~~cells, and it is involved in the blood coagulation process. PI is the primary source of the arachidonic acid (C20:4 ~~n-6)~~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, ~~last~~over the past decade there has been ~~a~~growing interest in analyzing ~~more~~in more detail this lipid fraction in muscle foods, ~~since~~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~~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 ~~is~~are related to the features of the raw material and ~~to~~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, Sanchez et al. ~~(10)~~and Perez-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 ~~its~~their quality characteristics, which are mainly attributed to the outdoor rearing, which implies the consumption of acorns and grass ~~(5, 14). Acorns~~. Acorn and grass production ~~are~~is seasonal and restricted, and thus a considerable number of Iberian pigs are fattened with concentrates, which implies lower quality and market acceptance ~~(14, 15).~~. Currently, monounsaturated FA (MUFA) enriched diets, through the inclusion of high oleic acid sunflower oil, are being used for feeding Iberian pigs ~~in order~~to imitate the FA profile of those animals fattened on acorns.

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

~~MATERIAL AND METHODS~~Materials and Methods

Experimental ~~design~~Design. This study was carried out with 30 pure Iberian pigs, which were divided into two groups according to the feeding ~~regime~~regimen during the fattening period prior to slaughter. One group of pigs (AG) ~~(n=15) was reared outdoors in a 30 Ha extension land with free availability of acorns~~(*Querqus ilex*, *Q. rotundifolian* = 15) was reared outdoors in a 30 ha extension land with free availability of acorns (*Querqus ilex*, *Querqus rotundifolia*, and *Querqus suber*) and grass. The other group of pigs (HO) (*Q. subern* ~~) and grass. The other group of pigs (HO) (n=15)~~= 15) was also fattened outdoors in a 1 ~~Ha~~ha extension land, with an oleic acid enriched concentrate (4.120 ~~Kg/day)~~kg/day) and free availability of grass but mainly fed. The chemical and FA ~~composition~~compositions of the feeds ~~has~~have been previously published ~~(15).~~. At the beginning of the fattening period weights were 99.2 ± 3.1 and 96.3 ± 1.7 kg for Iberian pigs of the AG and HO groups, respectively. All of the animals were fattened for 110 days and slaughtered the same day at the age of 16 months by electrical stunning and exsanguination at a local slaughterhouse. Slaughter weights were ~~158.9±3.2~~158.9 ± 3.2 and ~~162.5±2.9~~162.5 ± 2.9 kg for AG and HO ~~pigs~~pigs, respectively. One ham of each animal was ~~taken~~taken, and their biceps femoris and semimembranosus muscles were dissected and stored at −80 °C until analysis. The quantity of each PL class was analyzed in both muscles, whereas the biceps femoris was the only muscle used for studying the FA and DMA compositions of each PL class.~~*Biceps femoris* and *Semimembranosus* muscles were dissected and stored at −80° C until analysis. The quantity of each PL class was analyzed in both muscles whereas the *Biceps femoris* was the only muscle used for studying the FA and DMA composition of each PL class.~~

Intramuscular ~~fat extraction~~Fat Extraction. Samples were ground using a commercial grinder immediately before fat extraction. Intramuscular total lipids were extracted with chloroform/methanol (2:1, ~~vol/vol),~~v/v), according to the method described by Folch et al. ~~(16)~~and modified by Perez-Palacios et al. ~~(17).~~.

Quantification of ~~phospholipid classes~~Phospholipid Classes. Quantification of PL classes was carried out by fractionation using HPLC coupled to evaporative light scattering detector (ELSD), following the method described by ~~Rombaut,~~Rombaut et al. ~~(18)~~with slight modifications. Lipids (24 mg) were dissolved in 1.6 ~~ml~~mL of ~~chloroform:methanol~~chloroform/methanol solvent (88:12, ~~vol/vol).~~v/v). Analysis was carried out using an HPLC Shimadzu (LC-20AT prominence liquid chromatography) instrument equipped with a pump (DGU-20A5 prominence degasser) and a SIL-20AC autosampler. The analytical column (150 mm × 30 mm ~~I.D.)~~i.d.) was packed with a silica normal-phase Prevail Silica 3u (GRACE) thermostated in an oven (Shimadzu CTO-20AC prominence column ~~over)~~oven) at 40 °C. The chromatographic separation was carried out using a linear gradient according to the following scheme: ~~t = 0 min, 87.5%A 12%B 0.5%C; t = 12 min, 2%A 90%B 8%C for 2 min. The mobile phase was brought back to the initial conditions at t = 16 min and the column was allowed to equilibrate until the next injection at t =~~

25 min. Eluent A consisted of chloroform, eluent B of methanol and eluent C of triethylamine buffer (pH 3, 1 M formic acid). The flow was maintained at 0.7 ml/min. The injection volume was 10 µL. HPLC was coupled with an ELSD (Alltech 3300). The nebulising gas was N*t* = 0 min, 87.5% A−12% B−0.5% C; *t* = 12 min, 2% A−90% B−8% C for 2 min. The mobile phase was brought back to the initial conditions at *t* = 16 min, and the column was allowed to equilibrate until the next injection at *t* = 25 min. Eluent A consisted of chloroform, eluent B of methanol, and eluent C of triethylamine buffer (pH 3, 1 M formic acid). The flow was maintained at 0.7 mL/min. The injection volume was 10 µL. HPLC was coupled with an ELSD (Alltech 3300). The nebulizing gas was N_2, at a flow rate of 1.6 L/min,L/min and a nebulisingnebulizing temperature of 65 °C. The gain was set at 1. Individual PL classes were identified by comparing their retention times with those of external standards (Spectral Services GMBH, Koln, Germany). For quantification purposes, calibration curves of individual PL classes were prepared.

Fatty acid methyl estersAcid Methyl Ester (FAME) and dimethyl acetalsDimethylacetal (DMA) preparationPreparation and analysisAnalysis. For analysingTo analyze the FA composition of each PL 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*-nhexane.-hexane. Twenty milligrams of lipids dissolved in 150 µL of *n*-nhexane:chloroform:methanol-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:aceticether/acetic acid (98:2, v/v) ([19]). 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 Perez-Palacios et al. ([20]). PC, PE, PS, and PI were eluted with 30 mL of acetonitrile:acetonitrile/*n*-propanol (2:1, v/v), 10 mL of methanol, 7.5 mL of isopropanol:3isopropanol/3 N methanolic HCl (4:1, v/v), and 17.5 mL of chloroform:methanol:37%chloroform/methanol/37% HCl (200:100:1, v/v/v), respectively. The vaccumvacuum 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 (80 °C [21]). FAME were analyzed by gas chromatography, using an Agilent 6890N gas chromatograph, equipped with a flame ionization detector (FID). Separation was carried out on a polyethyleneglycolpolyethylene glycol capillary column (60 m long, 0.32 mm id,i.d., and 0.25 mm film thickness) (Supelcowax-10, Supelco, Bellafonte,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.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, monoun-saturatedmonounsaturated, and polyunsaturated (from C4 to C24). To confirm identification, selected samples were subjected to gas chromatography coupled to mass spectrometry (GC-MS) in a HP5890GCHP-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 KPakPa of column head pressure, resulting in a flow of 1.45 mlmL 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* over the m/zrange of 30-500.30−500. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley libraries.

Statistical analysisAnalysis. The effecteffects of pig feeding, musclemuscle, and their interaction on the content of each individual muscle PL waswere 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.*Biceps femoris* muscle was analysed 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. Statistic analyses were performed using the SPSS (v.15.0) package software.

RESULTS AND DISCUSSIONResults and Discussion

Quantification of individualIndividual PL classesClasses. 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 ~~*Biceps femoris* and *Semimembranosus* muscles of this study (Figure~~ 1). The major PL was PC, followed by ~~PE, PS~~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).~~.

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

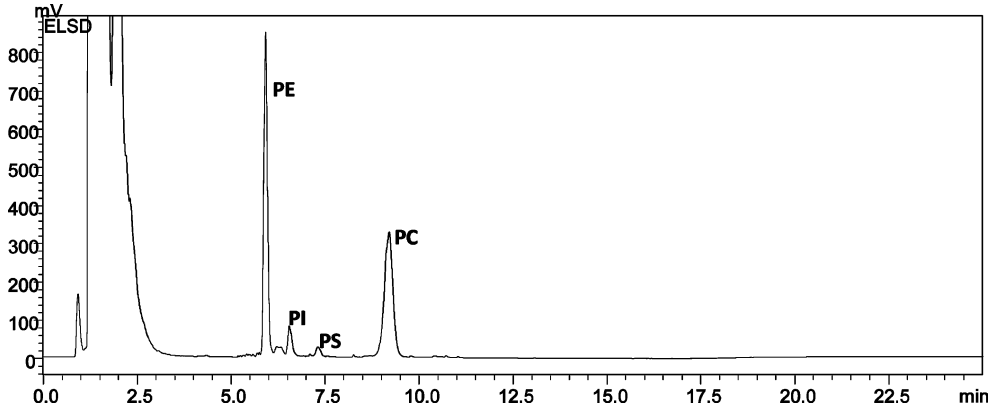

Table 1. Phospholipid Class Contentd (Expressed as Milligrams of Phospholipid per 100 g of Muscle Dry Matter ± Standard Deviation) in the Biceps Femoris and Semimembranosus Muscles of Fresh Iberian Hams from Pigs Fattened with Different Diets: Acorn and Grass (AG) and High Oleic Acid Enriched Concentrate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

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

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

~~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 muscle dry matter (DM)) than in the *Semimembranosus*~~ 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).~~.

The effect of diet on the amount of each PL class in biceps femoris and semimembranosus muscles from Iberian pigs is shown in Table ~~*Biceps femoris* and *Semimembranosus* muscles from Iberian pigs is shown in Table~~ 1. The quantity of PC, ~~PE~~PE, and PI was significantly higher (p ~~<0.001,~~ < 0.001, p ~~=0.017~~= 0.017, and p ~~<0.001,~~< 0.001, respectively) in HO than in AG muscles, whereas PS was not influenced by pig feeding. As a consequence, the sum of

the content of the four PL classes was also higher (p ~~<0.001) in HO than in AG *Biceps femoris* and *Semimembranosus*~~ < 0.001) in HO than in AG biceps femoris and semimembranosus muscles. The amount of PL has been related to the type, ~~the diameter~~diameter, and ~~the~~mitochondria content of muscular ~~fibres (~~fibers [22]~~),~~, which could explain the differences in the content of individual PL classes between AG and HO Iberian pigs. In fact, although both groups of Iberian pigs were fattened outdoors, the area of the land in which AG pigs were reared was larger than that of HO ones. Thus, AG Iberian pigs should have ~~done a~~experienced more intense physical exercise than HO pigs, which may influence muscle ~~fibre~~fiber characteristics. Contrarily, Petron et al. ~~(~~[23]~~)~~and Tejeda et al. ~~(~~[24]~~)~~found similar total PL contents in muscles from Iberian pigs fed ~~on~~different diets. Other studies have shown that the relative percentage of PL classes was not ~~influence~~influenced by the diet in either fish ~~(~~[25]~~)~~or mammary tissue and erythrocytes from rats ~~(~~[26]~~).~~. Fatty ~~acid~~Acid and ~~dimethylacetal composition~~Dimethylacetal Compositions of ~~individual~~Individual PL ~~classes~~Classes. Total SFA, MUFA, ~~PUFA~~PUFA, and DMA in the four PL classes from the biceps femoris muscle of raw hams is shown in Figure ~~*Biceps femoris* muscle of raw hams is shown in Figure~~ 2. The highest proportions of SFA were found in PS (47.55 ± ~~0.47 %)~~0.47%) and PI (41.45 ± ~~1.57 %), while~~1.57%), whereas these FA were lower in PC (31.14 ± ~~1.19 %)~~1.19%) and PE (28.94 ± ~~1.53 %).~~1.53%). Thus, PS showed a high content of palmitic acid (C16:0) (Table 4~~)~~), whereas high levels of stearic acid (C18:0) were found in PI (Table 5). In rat muscle, the highest ~~content~~contents of C16:0 and C18:0 were found in PC and PS, respectively ~~(~~[2]~~).~~.

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.

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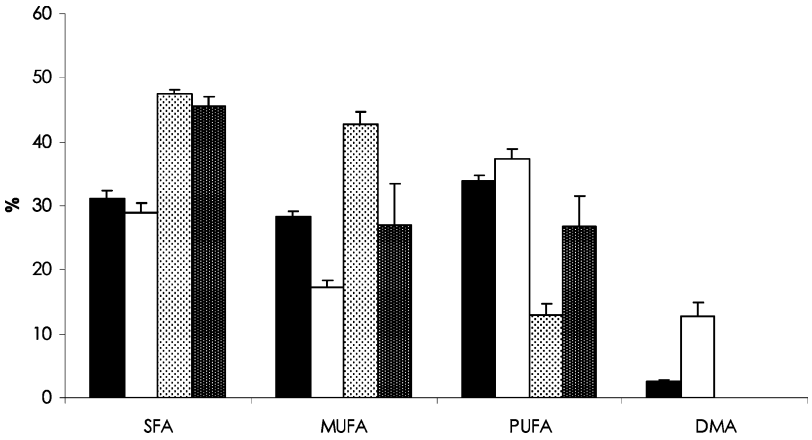

The highest content of MUFA was found in PS, followed by PC and PI, ~~while~~whereas PE showed the lowest proportion. This is the consequence of the high levels of C18:1 n-9 observed in PS, which is in agreement with the results found by Perez-Palacios et al. ~~(~~[2]~~)~~in rat muscle.

PC and PE showed the highest levels of PUFA ~~(33.84%~~(33.84 and 37.25%, respectively), due to the high proportion of linoleic acid (C18:2 n-6) in PC (Table 2~~),~~) and the high content of both C18:2 n-6 and C20:4 n-6 in PE (Table 3). Similarly, Alasnier et al. ~~(~~[27]~~)~~and Cambero et al. ~~(~~[28]~~)~~showed a higher content of C20:4 n-6 in PE than in PC of rabbit muscle. On the other hand, PI showed the highest proportion of C20:4 n-6 in the longissimus dorsi of rat ~~*Longissimus dorsi* of rat (~~[2]~~).~~.

Table 2. Fatty Acid and Dimethylacetal Composition (Percent of Total FAME and DMA Detected ± 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	*p*
C16:0	22.23 ± 0.76	21.97 ± 0.73	0.591
C16:1	1.50 ± 0.14	1.28 ± 0.19	0.066
C18:0	9.11 ± 0.66	8.99 ± 0.53	0.753
C18:1 n-9	23.32 ± 1.01	17.74 ± 0.75	<0.001
C18:1 n-7	4.30 ± 0.22	4.60 ± 0.33	0.123
C18:2 n-6	28.08 ± 0.55	27.65 ± 1.09	0.450
C18:3 n-6	0.20 ± 0.01	0.20 ± 0.01	0.328
C18:3 n-3	0.87 ± 0.14	0.47 ± 0.03	<0.001
C20:3 n-6	0.92 ± 0.03	0.93 ± 0.06	0.653
C20:4 n-6	4.31 ± 0.81	7.50 ± 0.49	<0.001
C20:3 n-3	0.48 ± 0.23	0.62 ± 0.16	0.300
C20:5 n-3	0.24 ± 0.04	0.51 ± 0.14	0.004
C22:1 n-9	0.16 ± 0.02	0.30 ± 0.07	0.003
C22:2	0.20 ± 0.09	0.18 ± 0.02	0.733
C24:0	0.89 ± 0.08	0.70 ± 0.06	0.003
C22:6 n-3	0.48 ± 0.15	0.61 ± 0.09	0.136
Σ SFA	32.23 ± 0.71	31.66 ± 0.92	0.300
Σ MUFA	29.28 ± 1.10	23.92 ± 1.14	<0.001
Σ PUFA	35.03 ± 0.63	38.67 ± 1.30	<0.001
C16:0 DMA	1.86 ± 0.17	3.67 ± 1.09	0.006
C18:0 DMA	0.31 ± 0.02	0.73 ± 0.04	<0.001
C18:1 DMA	0.55 ± 0.05	1.36 ± 0.11	<0.001
Σ DMA	2.71 ± 0.19	5.75 ± 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 ± 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	*p*
C16:0	11.66 ± 2.95	5.54 ± 0.73	0.002
C16:1	0.80 ± 0.48	1.16 ± 0.23	0.167
C18:0	15.99 ± 1.48	18.11 ± 0.58	0.018
C18:1 n-9	15.04 ± 2.79	10.87 ± 0.56	0.011
C18:1 n-7	2.04 ± 0.47	1.74 ± 0.12	0.208

	AG	HO	*p*
C18:2 n-6	15.57 ± 1.23	15.66 ± 0.70	0.895
C18:3 n-3	0.56 ± 0.12	0.29 ± 0.06	0.002
C20:3 n-6	1.07 ± 0.09	1.26 ± 0.12	0.025
C20:4 n-6	19.87 ± 1.62	34.30 ± 0.76	<0.001
C20:3 n-3	0.21 ± 0.02	0.37 ± 0.11	0.010
C20:5 n-3	0.54 ± 0.18	0.83 ± 0.14	0.021
C22:1 n-9	0.26 ± 0.07	0.42 ± 0.12	0.035
C22:2	0.57 ± 0.12	0.91 ± 0.06	0.001
C24:0	3.36 ± 0.25	2.84 ± 0.41	0.045
C22:6	0.77 ± 0.14	0.66 ± 0.05	0.113
Σ SFA	31.01 ± 3.49	26.49 ± 1.18	0.025
Σ MUFA	18.14 ± 3.19	14.19 ± 0.77	0.027
Σ PUFA	39.17 ± 2.59	54.27 ± 0.85	<0.001
C16:0 DMA	4.10 ± 1.18	3.07 ± 1.31	0.228
C18:0 DMA	4.27 ± 1.01	1.44 ± 0.09	<0.001
C18:1 DMA	3.32 ± 1.36	0.53 ± 0.12	0.002
Σ DMA	11.68 ± 3.06	5.05 ± 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.

The high oxidation susceptibility of PL compared to neutral lipids ~~(~~[29]~~)~~is well established, due to their location in membranes close to heme pigments and oxidant systems and due to their high PUFA content ~~(~~[30]~~)~~. In relation the fatty profile of the PL classes, PC and PE would be more susceptible to oxidation than PS and PI because of their higher content of PUFA. On the other hand, the relative oxidation rates of PUFA containing ~~2, 3, 4, 5~~two, three, four, five, or ~~6~~six double bonds are 1, 2, 4, ~~6~~6, and 8, respectively ~~(~~[31]~~)~~. Thus, taking into account the proportion of individual PUFA of each PL, PE would be the most susceptible to oxidation, followed by PC and PI, ~~and~~with PS being the least prone to oxidation.

PE showed the highest proportion of DMA, followed by PC, ~~while~~whereas these compounds were not found in PS and PI. ~~Hexadecanaldimethylacetal~~Hexadecanal dimethylacetal (C16:0 DMA), ~~octadecanaldimethylacetal~~octadecanal dimethylacetal (C18:0 ~~DMA)~~DMA), and ~~octadecenaldimethylacetal~~octadecenal dimethylacetal (C18:1 n-9) were detected in both PC (Table 2) and PE (Table 3). Thus, the low content of SFA and MUFA in PC and PE could be due, at least in part, to the high levels of DMA found, ~~since~~because one of the pathways for the biosynthesis of plasmalogens involves a desaturation process of the FA esterified in the analogue PL ~~(~~[32]~~)~~. Accordingly, Perez-Palacios et al. ~~(~~[2]~~)~~showed that PE was the PL 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. ~~*Longissimus dorsi* of rats. However, these authors also found DMA in PC, PS and PI of rat muscle.~~

Tables 2~~–~~5 ~~show the FA and DMA composition of PC, PE, PS and PI, respectively, of~~ *Biceps femoris* show the FA and DMA composition of PC, PE, PS, and PI, respectively, of biceps femoris muscle from AG and HO Iberian pigs. The effect of feeding Iberian pigs with different diets did not lead to changes in SFA of PC. On the other hand, PE, ~~PS~~PS, and PI showed statistically higher total SFA percentage in AG than in HO thighs, as a consequence of the ~~significantly~~significant differences in the proportions of C16:0 and C18:0 between AG in HO pigs in PE, ~~PS~~PS, and PI. These differences were a consequence of the FA composition of feeding, oleic acid 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).

Table 4. Fatty Acid and Dimethylacetal Composition (Percent of Total FAME Detected ± 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	*p*
C16:0	29.33 ± 0.88	17.65 ± 1.25	<0.001
C16:1	4.32 ± 0.76	12.84 ± 1.18	<0.001
C18:0	16.18 ± 1.27	12.38 ± 0.36	<0.001
C18:1 n-9	31.64 ± 2.81	30.12 ± 0.89	0.280
C18:1 n-7	5.16 ± 0.44	7.05 ± 0.46	<0.001
C18:2 n-6	5.12 ± 0.95	6.83 ± 0.47	0.007
C18:3 n-3	0.23 ± 0.01	0.36 ± 0.00	<0.001
C20:3 n-6	0.25 ± 0.00	0.29 ± 0.11	0.402
C20:4 n-6	1.43 ± 0.30	2.28 ± 0.59	0.022
C20:3 n-3	1.19 ± 0.83	3.47 ± 1.09	0.006
C20:5 n-3	2.61 ± 0.98	3.70 ± 0.66	0.071
C22:1 n-9	0.96 ± 0.22	1.96 ± 0.53	0.005
C22:2	2.57 ± 1.46	2.76 ± 1.22	0.824
Σ SFA	45.51 ± 1.76	30.03 ± 1.55	<0.001
Σ MUFA	42.09 ± 2.38	51.97 ± 1.50	<0.001
Σ PUFA	12.92 ± 3.79	19.04 ± 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.

Table 5. Fatty acid and Dimethylacetal Composition (Percent of Total FAME Detected ± 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	*p*
C16:0	18.76 ± 0.49	17.15 ± 0.55	0.006
C16:1	9.02 ± 0.46	19.02 ± 0.40	<0.001
C18:0	24.41 ± 1.70	20.81 ± 1.73	0.028
C18:1 n-9	11.27 ± 2.58	12.47 ± 2.60	0.550
C18:1 n-7	7.35 ± 0.31	10.57 ± 1.34	0.007
C18:2 n-6	11.68 ± 0.02	12.74 ± 1.57	0.301
C20:4 n-6	7.40 ± 0.11	7.25 ± 1.43	0.867
C20:5 n-3	1.51 ± 0.50		

	AG	HO	*p*
C22:1 n-9	3.02 ± 1.23		
C22:2	5.58 ± 1.12		
Σ SFA	43.17 ± 1.28	37.95 ± 1.96	0.007
Σ MUFA	30.67 ± 2.17	42.06 ± 2.64	0.001
Σ PUFA	24.66 ± 1.04	19.99 ± 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.

The percentage of MUFA was influenced by the feeding background but ~~showing~~showed a variable ~~behaviour~~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 ~~of~~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 ~~the~~Δ^9~~desaturase (~~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~~and, consequently, to higher levels of C16:1 n-7.

In PC, ~~PE~~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~~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, ~~since~~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)~~(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 ~~showed~~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 of~~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~~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 ~~easy to be~~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*~~ also found differences in the FA profile of total PL from the longissimus dorsi muscle between animals fed ~~on~~acorn and grass and those fed ~~with~~high oleic acid enriched concentrates.

Thus, it can be concluded that Iberian pig feeding (acorn and grass ~~vs.~~vs high oleic acid concentrates) leads to differences ~~in~~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 ~~of~~PUFA. These differences could make the meat from HO animals more prone to lipid oxidation, ~~since~~because PL are very sensitive to ~~oxidation~~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 ~~hidroperoxides~~hydroperoxides generated during lipid oxidation ~~create~~creates a wide range of compounds contributing to ~~flavour~~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 USEDAbbreviations Used

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

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

~~**Figure 2.** Percentage of dimethyl acetals (DMA) and saturated, monounsaturated and polyunsaturated fatty acids (SFA, MUFA and PUFA, respectively) in phosphatidylcholine (), phosphatidylethanolamine (□), phosphatidylserine () and phosphatidylinositol () from fresh Iberian hams. Error bars display standard deviations.~~

~~**Table 1.** Phospholipid classes content (expresses as mg phospholipid/100 g muscle dry matter ± 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).~~

	Biceps femoris		*Semimembranosus*		*p*		
	AG	HO	AG	HO	F[z]	M[y]	F * M
PC[t]	976.10 ± 108.05	1444.33 ± 80.16	1215.57 ± 70.06	1683.62 ± 181.65	<0.001	0.143	0.180
PE[u]	352.71 ± 61.03	499.44 ± 41.16	524.61 ± 36.45	608.82 ± 14.59	0.017	0.146	0.710
PS[v]	76.96 ± 6.73	81.70 ± 8.09	79.53 ± 9.16	75.29 ± 11.30	0.894	0.697	0.740
PI[w]	58.91 ± 2.59	86.66 ± 5.33	63.73 ± 6.04	80.20 ± 6.82	<0.001	0.361	0.120
Σ PL[x]	1529.20 ± 82.65	2061.12 ± 51.06	1903.96 ± 21.12	2409.73 ± 157.05	<0.001	0.114	0.229

[z]F: feeding effect[y]M: mucle effect[t]PC: phosphatidylcholine[u]PE: phosphatidylethanolamine[v]PS: phosphatidylserine[w]PI: phosphatidylinositol[x] Σ PL: sum of the content of the different phospholipid classes

Table 2. Fatty acid and dimethyl acetal composition (% of total FAME and DMA detected ± standard deviation) of phosphatidylcholine of the *Biceps femoris* muscle from Iberian pigs fed with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

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

	AG	HO	*p*
C18:1 DMA	0.55 ± 0.05	1.36 ± 0.11	< 0.001
ΣDMA[z]	2.71 ± 0.19	5.75 ± 1.09	< 0.001

v: total amount of saturated fatty acidsx: total amount of monounsaturated fatty acidsy: total amount of polyunsaturated fatty acidsz: total amount of dimethyl acetals

Table 3. Fatty acid and dimethyl acetal composition (% of total FAME and DMA detected ± standard deviation) of phosphatidylethanolamine of the *Biceps femoris* muscle from Iberian pigs fed with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

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

v: total amount of saturated fatty acidsx: total amount of monounsaturated fatty acidsy: total amount of polyunsaturated fatty acidsz: total amount of dimethyl acetals

Table 4. Fatty acid and dimethyl acetal composition (% of total FAME detected ± standard deviation) of phosphatidylserine of the *Biceps femoris* muscle from Iberian pigs fed with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

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

v: total amount of saturated fatty acidsx: total amount of monounsaturated fatty acidsy: total amount of polyunsaturated fatty acidsz: total amount of dimethyl acetals

Table 5. Fatty acid and dimethyl acetal composition (% of total FAME detected ± standard deviation) of phosphatidylinositol of the *Biceps femoris* muscle from Iberian pigs fed with different diets: acorn and grass (AG) and high oleic acid enriched concentrate (HO).

	AG	HO	*p*
C16:0	18.76 ± 0.49	17.15 ± 0.55	0.006
C16:1	9.02 ± 0.46	19.02 ± 0.40	< 0.001
C18:0	24.41 ± 1.70	20.81 ± 1.73	0.028
C18:1 n-9	11.27 ± 2.58	12.47 ± 2.60	0.550
C18:1 n-7	7.35 ± 0.31	10.57 ± 1.34	0.007
C18:2 n-6	11.68 ± 0.02	12.74 ± 1.57	0.301
C20:4 n-6	7.40 ± 0.11	7.25 ± 1.43	0.867
C20:5 n-3	1.51 ± 0.50	–	
C22:1 n-9	3.02 ± 1.23	–	
C22:2	5.58 ± 1.12	–	
ΣSFA[v]	43.17 ± 1.28	37.95 ± 1.96	0.007
ΣMUFA[x]	30.67 ± 2.17	42.06 ± 2.64	0.001
ΣPUFA[y]	24.66 ± 1.04	19.99 ± 1.55	0.004

~~v: total amount of saturated fatty acidsx: total amount of monounsaturated fatty acidsy: total amount of polyunsaturated fatty acidsz: total amount of dimethyl acetals~~