doi: 10.17221/18/2016-CJAS

Effect of Sex and Dietary Treatment on the Composition and Rheological Properties of Dry-cured Ham Subcutaneous Fat

José Francisco Segura Plaza[1], Rosa Escudero[2], María Dolores Romero de Ávila[2], lvaro Olivares[1], María Isabel Cambero[2], Clemente José López-Bote[1]*

[1]Department of Animal Production, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain

[2]Department of Nutrition, Bromatology and Food Technology, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain

**Corresponding author: josesegu@ucm.es*

ABSTRACT

Segura Plaza J.F., Escudero R., Romero de Ávila M.D., Olivares Á., Cambero M.I., López-Bote C.J. (2017): **Effect of sex and dietary treatment on the composition and rheological properties of dry-cured ham subcutaneous fat**. Czech J. Anim. Sci., 62, 110–120.

The effect of sex, dietary fat source (lard vs palm oil), and glycerol inclusion in fattening diet on the composition, fatty acid distribution within the triglyceride (TAG) and slip point and textural parameters was studied on dry-cured hams subcutaneous fat. A marked effect of sex on saturated fatty acids (SFA) percentage was found with barrows showing higher values than gilts. No effect of dietary fat source on subcutaneous SFA or polyunsaturated fatty acids (PUFA) was observed. Dietary glycerol increased monounsaturated fatty acids and decreased total PUFA in subcutaneous fat. Besides, the possibility of altering fatty acid composition at the 2-position of the TAG by dietary intervention during the fattening phase is very limited. Partial restructuration was observed in external positions of the TAG. All these changes affected slip point and textural parameters. An increase of hardness when palm oil was used as dietary fat and a decrease in all textural parameters values when glycerol was included were observed.

Keywords: meat product; fatty acids; positional distribution; textural parameters

Principal efforts of pork industry have been focused on decreasing the total amount of deposited fat in pigs and the modification of its fatty acid (FA) composition to better match the nutritional value for human diet and technological requirements. Quality of dry-cured hams and other meat products depends on lipid composition of the raw material for both their flavour development (Soto et al. 2010) and subsequent industrial processing (Hugo and Roodt 2007).

Fatty acid composition is completely related to meat quality since it influences tissue firmness (Lopez-Bote et al. 2002; Hallenstvedt et al. 2012) and stickiness (Nishioka and Irie 2005), shelf life, eating quality, and flavour (Lopez-Bote et al. 2002). Generally, a high ratio of saturated (SFA) to

Supported by the Spanish Ministry of Economy and Competitiveness (Projects No. AGL2010-19158 and No. AGL2013-48121) and by the Autonomous Community of Madrid and Moncloa Campus of International Excellence (MEDGAN and UCM-UPM S2013/ABI-2913).

doi: 10.17221/18/2016-CJAS

polyunsaturated fatty acids (PUFA), fundamentally linoleic acid (C18:2n-6), improves most aspects of meat quality but may negatively influence the nutritional value. On the contrary, high C18:2n-6 and low SFA levels have been associated with low consistency, oiliness, and soft texture compromising technological and sensory quality of pork meat and meat products. Therefore, a number of feeding practices are implemented in different consortium rules aimed at the production of quality meat products, which emphasizes the importance of C18:2n-6 and SFA maintenance within certain limits in pig tissues (Candek-Potokar and Skrlep 2012).

The subcutaneous fat (SF) is from ca. 95% composed of adipocytes (lipids storage) separated by a network of connective tissue fibres, predominantly collagen and small quantities of elastic and reticular fibres (Sumena et al. 2010), which contribute to the texture features, mainly cohesiveness and toughness. It is fully accepted that FA composition of pig adipose tissue is dependent upon the FA profile of the diet (Mitchaothai et al. 2007). The C18:2n-6 concentration is currently controlled through the incorporation of saturated fats or ingredients that enhance endogenous fat synthesis in fattening diets (Farnworth and Kramer 1987). Among saturated fats, lard and palm oil are frequently used because of their low C18:2n-6 concentration together with a high concentration of SFA and monounsaturated fatty acids (MUFA) (De Blas et al. 2013). Glycerol is a co-product of the bio-fuel industry, which is now fully available for swine nutrition. It has been proven to enhance FA synthesis, thus reducing the concentration of C18:2n-6 in pig tissues (Mourot et al. 1994), and increasing meat firmness (Schieck et al. 2010).

Lard and palm oil are considered to be of similar nutritional value, but it is noticeable that location of FA within the triglyceride (TAG) is markedly different. While lard concentrates most C16:0 in the internal (sn-2) position, palm oil locates C16:0 in the external (sn-1 and sn-3) positions of the TAG (Small 1991; Innis and Nelson 2013). This may be an aspect of interest, since the distribution of FA within the TAG may affect physical properties of adipose tissue (Smith et al. 1998; Segura et al. 2015b) and recently implications with illnesses as obesity, diabetes or hypertension have been detected (Gouk et al. 2013).

This research was undertaken to study the effect of diet on profile and positional distribution of FA within the TAG molecule of SF in dry-cured hams. Diets containing glycerol (which enhances *de novo* FA synthesis) and two fat sources (lard vs palm oil) differing in the FA distribution within the TAG were used in fattening gilts and barrows.

MATERIAL AND METHODS

Experimental design. The experiment was conducted using randomly selected 20 barrows and 20 gilts at 80 kg body weight. The female line (Syra, Gene+, Erin, France) used included blood from Large White, Landrace, and Duroc and the sire line was PIC L65 (PIC, Barcelona, Spain). Four finishing diets differing in the fat source (palm oil or lard) and the concentration of glycerol (0 vs 50 g/kg) were provided for 32 days until slaughter. All diets contained low concentration of C18:2n-6 (10 g/kg). Diets were provided for *ad libitum* consumption and were formulated according to the Spanish Foundation for the Development of Animal Nutrition (FEDNA) (De Blas et al. 2013). The ingredient composition and the calculated (De Blas et al. 2013) nutrient content of the diets are shown in Table 1. Dietary fatty acid distribution within the TAG of fat sources used in this experiment is shown in Table 2.

The pigs were slaughtered at 110 (± 2.98) kg of body weight. The right thighs from each pig were obtained at cutting (24 h after slaughter) and processed in a traditional manner for approximately 12 months to produce a dry-cured ham, and were subsequently deboned. The SF at the level of *biceps femoris* muscle was carefully removed from each deboned dry-cured ham and samples were stored at 4°C until use.

Triglyceride purification. The total lipids of SF were extracted (Segura and Lopez-Bote 2014) and the TAG were purified by thin-layer chromatography (TLC) on silica gel plates (0.25 mm thickness) that were developed with hexane/ethyl ether/acetic acid (75 : 25 : 1 by volume). To detect the position of the TAG, the TLC plates were sprayed with primuline acetone/water (80 : 20 by volume) 0.05% solution. TAG fractions were scraped off the plates and eluted from silica with hexane/ethyl ether (95 : 5 by volume). In each case, the samples of purified TAG were analyzed by both gas chromatography and lipase hydrolysis (Perona and Ruiz-Gutierrez 2004).

Lipase hydrolysis. For the positional analysis of TAG sn-2 fatty acids, 10 mg of purified TAG

doi: 10.17221/18/2016-CJAS

were hydrolyzed with 2 mg of pancreatic lipase in 1 ml of 1M Tris–HCl buffer (pH 8), 0.1 ml $CaCl_2$ (22%), and 0.25 ml deoxycholate (0.1%). The reaction was stopped when approximately 60% of the TAGs were hydrolyzed (1–2 min) by adding 0.5 ml of 6 N HCl. The lipids were extracted three times with 1.5 ml aliquots of ethyl ether, and the reaction products were separated by TLC (see above). Free fatty acids (FFA) and sn-2-monoacylglycerol bands representing the positions sn-1,3 and sn-2 of TAG were scraped off the plate and transmethylated (see below). The validity of the procedure was confirmed by comparing the fatty acid composition of the original TAG and those remaining after the partial hydrolysis.

Lipid analysis. Fatty acid methyl esters (FAME) were obtained from isolated lipids by heating the samples at 80°C for 1 h in 3 ml of methanol/toluene/H_2SO_4 (88 : 10 : 2 by volume). After cooling, 1 ml of hexane was added and the samples were mixed. The FAME were recovered from the upper phase, separated, and quantified using a gas chromatograph (HP 6890 Series GC System; Hewlett Parkard, USA) equipped with flame ionization

Table 1. Ingredients and calculated analysis[1] of diets (g/kg diet as fed basis)

	Pre-experimental diet	Finishing diet			
		palm oil		lard	
		0% Gly	5% Gly	0% Gly	5% Gly
Ingredients					
Barley	300	300	342	300	342
Wheat bran	120	303	200	303	200
Rye	320	150	150	150	150
Glycerol-85	–	–	50	–	50
Rapeseed meal (34%)	60	80	80	80	80
Soyabean meal (47%)	134	107	122	107	122
Soyabean oil	32	–	–	–	–
Lard	–	–	–	32	32
Palm oil	–	32	32	–	–
Lysine(50%)	6	54	48	54	48
DL-Methionine	0.7	0.6	0.6	0.6	0.6
L-Threonine (99%)	1.1	1	0.9	1	0.9
Choline (60%)	0.2	0.2	0.2	0.2	0.2
Calcium carbonate	11.4	11.6	11.6	11.6	11.6
Monocalcium phosphate	1	–	–	–	–
Sodium chloride	4	4	1.2	4	1.2
Phytases	0.2	0.2	0.2	0.2	0.2
Formicacid	2	–	–	–	–
Premix	5	5	5	5	5
Calculated composition					
Net energy (kcal/kg)	2.41	2.41	2.43	2.41	2.43
Crude protein (%)	16.3	16.2	16.2	16.2	16.2
Crude ashes (%)	3.80	3.7	4	3.7	4
C16:0	1.24	1.59	1.58	0.97	0.96
C18:0	0.54	0.17	0.17	0.43	0.43
C18:1	2.26	1.51	1.5	1.64	1.63
C18:2n-6	2.61	0.95	0.92	0.95	0.92

Gly = glycerol

[1]according to the Spanish Foundation for the Development of Animal Nutrition (FEDNA) (De Blas et al. 2013) (supplied per kg of diet)

doi: 10.17221/18/2016-CJAS

detector. Separation was performed with a J&W GC Column, HP-INNOwax Polyethylene Glycol (30 m × 0.316 mm × 0.25 µm). Nitrogen was used as a carrier gas. After injection at 170°C, the oven temperature was raised to 210°C at a rate 3.5°C/min, then to 250°C at a rate of 7°C/min, and held constant for 1 min. The flame ionization was held at 250°C. The split ratio was 1 : 40. FAME peaks were identified by comparing their retention times with those of authentic standards (Sigma-Aldrich, USA).

Slip point determination. Triplicate SF samples were independently collected from each dry-cured ham. Samples were melted at 80°C and drawn 1 cm into capillary tubes while still warm. Capillary tubes containing the samples were stored at 4°C overnight and then placed vertically in a chilled water bath. The temperature was increased gradually in the water bath (2°C/min). The temperature when the lipid began to move up the capillary tube was recorded (ISO 6321-2002).

Moisture and water activity **(a_w)**. Moisture was determined by drying the sample at 110°C to a constant weight and the results were expressed as percentage (AOAC 2006; method 950.46). Water activity (a_w) was measured using a Decagon CX1 hygrometer (Decagon Devices Inc., USA) at 25°C.

Table 2. Fatty acid composition of the whole triglyceride (TAG), position 2 (Sn-2), and position 1,3 (Sn-1,3) of fat sources used in the experiment

	Palm oil	Lard
TAG[1]		
C16:0	45.6	24.5
C18:0	4.7	13.2
C18:1 n-9	39.9	49.8
C18:2 n-6	9.8	12.5
Sn-2[1]		
C16:0	12.5	70.8
C18:0	2.9	9.3
C18:1 n-9	65.6	16.5
C18:2 n-6	19.0	3.4
Sn-1,3[2]		
C16:0	62.2	1.6
C18:0	5.7	15.2
C18:1 n-9	27.0	65.5
C18:2 n-6	5.1	17.7

[1]g/100 g of total present fatty acids

[2]g/100 g of total present fatty acids calculated as Sn-1,3 = (3 × TAG – Sn-2)/2

Texture Profile Analysis **(*TPA*)**. TPA was performed using a TA.XT2i SMS Texture Analyser (Stable Microsystems Ltd., UK) with the Texture Exponent programs. Textural tests were carried out at about 22°C. Briefly, three cylinders of 1 cm height and 1.5 cm diameter were prepared from each sample. A double compression cycle test was performed up to 50% compression of the original portion height with an aluminum cylinder probe of a 2-cm diameter. A time of 5 s was allowed to elapse between the two compression cycles. Force-time deformation curves were obtained with a 30 kg load cell applied at a crosshead speed of 2 mm/s. The following parameters were quantified (Bourne 1978): hardness (N), maximum force required to compress the sample; springiness (m), ability of the sample to recover its original form after deforming force was removed; adhesiveness (N × s), area under the abscissa after the first compression; cohesiveness, extent to which the sample could be deformed prior to rupture; gumminess (N), force to disintegrate a semisolid meat sample for swallowing (hardness × cohesiveness); and chewiness (J), work required to masticate the sample before swallowing (hardness × cohesiveness × springiness).

Statistical analysis. Chemical and TPA analyses were carried out by triplicate. Response data were analyzed as a completely randomized design with dietary fat source, glycerol level and gender as main effects and their interactions, by using the GLM procedure of the SAS software (Statistical Analysis System, Version 9.2, 2009). A factorial arrangement was used for meat quality data (2 fat sources × 2 glycerol levels × 2 sexes). The experimental unit was the individual pig for meat quality traits (five pigs of each sex chosen at random). Duncan's test was used to separate treatment means.

RESULTS AND DISCUSSION

Moisture, a_w, pH, and fatty acid profile. Expectedly, moisture values of the samples were around 4% and a_w and pH around 0.8 and 5.8, respectively (Table 3) and were similar to values obtained by other authors (Romero de Avila et al. 2014). As shown also in Table 3, the three variables remained unaffected by sex (Sex) or dietary fat (Fat) although an interaction Sex × Fat was observed in moisture and a_w. In gilts, an increase of moisture

doi: 10.17221/18/2016-CJAS

Table 3. Effect of sex (Sex), dietary fat source (Fat), and glycerol inclusion (Gly) on moisture, water activity (a_w), pH, and slip point (°C) of subcutaneous fat of dry-cured ham

	Palm oil				Lard				SEM (n = 5)	P-value[1]				
	0% Gly		5% Gly		0% Gly		5% Gly							
	gilt	barrow	gilt	barrow	gilt	barrow	gilt	barrow		Sex	Fat	Gly	Sex × Fat	Sex × Gly	Fat × Gly
Moisture	5.52	4.22	5.24	4.02	3.99	4.26	3.98	5.00	0.569	0.4040	0.2372	0.8612	0.0137	0.5735	0.4172
a_w	0.81^{a-c}	0.81^{a-c}	0.79^{bc}	0.84^{a}	0.80^{bc}	0.78^{c}	0.82^{ab}	0.81^{ab}	0.012	0.5297	0.2350	0.0338	0.0136	0.0356	0.2384
pH	5.97	6.01	5.79	5.81	5.80	5.94	5.88	5.77	0.092	0.7343	0.4155	0.0620	0.8790	0.2702	0.2593
Slip point	29.3^{ab}	28.7^{ab}	29.6^{ab}	29.5^{ab}	29.2^{ab}	29.7^{a}	28.4^{b}	29.5^{ab}	0.406	0.3687	0.8546	0.8745	0.0361	0.3062	0.0479

[1]no significant triple interactions for main effects were detected ($P > 0.05$)

$^{a-c}$different letters within the same row indicate difference between groups ($P < 0.05$)

and a decrease of a_w were observed when animals were lard-fed in comparison to palm oil diet, in barrows the effect was opposite. These results are in agreement with Tabilo et al. (1999) who found higher moisture in hams of female pigs, although Mitchaothai et al. (2007) did not find differences in pH, moisture or drip loss in pigs fed either beef tallow or sunflower oil. Glycerol inclusion in the diet led to a significant increase of a_w and a decreasing tendency of pH of SF of dry-cured hams. Mourot et al. (1994) found a reduction in water and cooking losses in Large White pigs fed 5% glycerol from 30 to 100 kg of live weight. It was attributed to glycerol action on cell osmotic pressure, what could increase the water content. Thus, muscle fibres would be hyperhydrated reducing protein denaturation during heat treatment, which provides a benefit to meat processor. Kosmider et al. (2011), who found a reduction in this parameter in meat from pigs fed glycerol, described similar results. An interaction sex vs glycerol inclusion (Gly) was observed in a_w. Thus, barrows fed with glycerol showed higher values of a_w than gilts.

Differently from fresh fat, the SF composition of dry-cured ham is mainly based on TAG, phosphoglycerides, and a high concentration of monoglycerides and FFA due to lipolytic processes occurring during processing (Narvaez-Rivas et al. 2007). Effects of dietary treatment and sex on FA composition of total SF are shown in Table 4. A marked effect of sex on total SFA was found, barrows showing higher values than gilts. The most marked effect induced by sex on SFA was observed for C14:0, C15:0, C16:0, C17:0, and C20:0 but no differences were observed for C18:0. Total and individual PUFA as C18:2n-6, C18:3n-3, C20:3n-6, and C20:4n-6 concentrations were higher in gilts when compared to barrows. No effect of sex was observed for MUFA and C18:1 isomers. Piedrafita et al. (2001) found that gender affected the proportion of FAs, as gilts showed a higher proportion of C18:2n-6 than barrows and barrows had lower proportion of unsaturated FA than gilts. Latorre et al. (2009) observed that in SF, C16:0, C18:0, and total SFA were higher and C18:2n-6 and total PUFA proportions were lower in barrows than in gilts. Nuernberg et al. (2005) also observed that SF from gilts had lower SFA and higher PUFA proportions than from barrows.

No effect of dietary fat source was observed on majoritarian FAs concentration. Besides, attending

doi: 10.17221/18/2016-CJAS

Table 4. Effect of sex (Sex), dietary fat source (Fat), and glycerol inclusion (Gly) on fatty acid profile of the subcutaneous fat of dry-cured ham

	Palm oil				Lard				SEM (n = 5)	P-value[1]					
	0% Gly		5% Gly		0% Gly		5% Gly								
	gilt	barrow	gilt	barrow	gilt	barrow	gilt	barrow		Sex	Fat	Gly	Sex × Fat	Sex × Gly	Fat × Gly
C14:0	1.12^{cd}	1.29^{ab}	1.07^{d}	1.35^{a}	1.23$^{a–c}$	1.29^{ab}	1.18$^{a–c}$	1.30^{ab}	0.113	0.0001	0.1726	0.8364	0.0543	0.1927	0.6643
C15:0	0.07^{a}	0.04^{d}	0.06$^{a–c}$	0.05^{cd}	0.06$^{a–d}$	0.05$^{b–d}$	0.06^{ab}	0.05$^{a–d}$	0.011	0.0001	0.3976	0.8610	0.0761	0.6130	0.2383
C16:0	22.3^{cd}	23.0$^{a–c}$	22.1^{cd}	23.8^{a}	22.8$^{b–d}$	23.3^{ab}	21.9^{d}	22.6$^{b–d}$	0.717	0.0001	0.4404	0.2597	0.2017	0.1548	0.0110
C16:1n-9	0.28^{ab}	0.17^{d}	0.29$^{a–c}$	0.18^{d}	0.24^{bc}	0.21^{cd}	0.31^{a}	0.28^{ab}	0.040	0.0001	0.0085	0.0016	0.0011	0.8950	0.0092
C16:1n-7	2.01^{b}	2.38^{ab}	2.10^{b}	2.70^{a}	2.16	2.30^{b}	2.08^{b}	2.36^{ab}	0.302	0.0003	0.4055	0.2559	0.1316	0.2985	0.2211
C17:0	0.46^{a}	0.29^{d}	0.39$^{a–c}$	0.28^{d}	0.37$^{b–d}$	0.33^{cd}	0.43^{ab}	0.34$^{b–d}$	0.073	0.0001	0.6035	0.8224	0.0767	0.9156	0.0724
C17:1	0.42^{a}	0.33^{c}	0.40^{ab}	0.33^{c}	0.40^{ab}	0.35^{bc}	0.43^{a}	0.37$^{a–c}$	0.051	0.0001	0.2355	0.6099	0.3102	0.9594	0.2813
C18:0	12.2	11.4	11.5	11.4	11.6	11.9	11.6	11.7	1.102	0.6671	0.8036	0.4386	0.3424	0.7187	0.6852
C18:1n-9	43.0^{b}	45.3^{ab}	46.5^{a}	46.1^{a}	45.4^{ab}	44.7^{ab}	45.2^{ab}	45.0^{ab}	2.016	0.6940	0.7897	0.0610	0.2460	0.3620	0.0710
C18:1n-7	2.25	2.53	2.26	2.59	2.40	2.24	2.31	2.53	0.351	0.1059	0.7198	0.4941	0.1906	0.2969	0.7307
C18:2n-6	13.1^{a}	10.5^{b}	10.8^{b}	8.79^{c}	10.5^{b}	10.5^{b}	11.7^{ab}	10.8^{b}	1.369	0.0014	0.8255	0.1056	0.0250	0.8506	0.0016
C18:3n-3	1.04^{a}	0.85^{bc}	0.84^{bc}	0.70^{c}	0.83^{bc}	0.86^{b}	0.93^{ab}	0.90^{b}	0.114	0.0174	0.5066	0.1182	0.0153	0.9742	0.0007
C18:4n-3	0.13^{ab}	0.11^{c}	0.12^{bc}	0.13^{ab}	0.13^{ab}	0.13$^{a–c}$	0.14^{a}	0.14^{a}	0.012	0.2358	0.0008	0.0332	0.9190	0.0253	0.4371
C20:0	0.20^{c}	0.25^{a}	0.21^{bc}	0.24^{ab}	0.23$^{a–c}$	0.25^{a}	0.2^{bc}	0.23$^{a–c}$	0.025	0.0001	0.4565	0.2018	0.2016	0.4757	0.2444
C20:1n-9	0.96^{d}	1.22^{a}	1.01^{cd}	1.14$^{a–c}$	1.20^{a}	1.17^{ab}	1.10$^{a–d}$	1.05$^{b–d}$	0.107	0.0156	0.1351	0.0446	0.0007	0.2363	0.1235
C20:3n-6	0.21^{a}	0.18^{cd}	0.21^{a}	0.16^{c}	0.20^{ab}	0.18^{cd}	0.21^{a}	0.22^{a}	0.019	0.0001	0.0400	0.0762	0.0022	0.4683	0.0006
C20:4n-6	0.17^{a}	0.17^{a}	0.14^{bc}	0.13^{c}	0.17^{a}	0.15^{ab}	0.17^{a}	0.15$^{a–c}$	0.019	0.0166	0.0399	0.0019	0.2497	0.5094	0.0027
SFA	36.4	36.3	35.3	37.1	36.3	37.2	35.4	36.2	1.452	0.0458	0.9776	0.2394	0.9800	0.2777	0.3436
MUFA	48.9^{b}	51.9^{ab}	52.6^{a}	53.0^{a}	51.8^{ab}	51.0^{ab}	51.5^{ab}	51.6^{ab}	2.358	0.3240	0.8048	0.0684	0.1450	0.5872	0.0980
PUFA	14.7^{a}	11.8^{b}	12.1^{b}	9.91^{c}	11.9^{b}	11.9^{b}	13.1^{a}	12.2^{a}	1.536	0.0012	0.6849	0.0885	0.0295	0.8425	0.0011
UI	0.80^{a}	0.77^{b}	0.78^{b}	0.74^{c}	0.77^{b}	0.76^{b}	0.80^{a}	0.78^{a}	1.956	0.0001	0.6288	0.5553	0.0633	0.3467	0.0005

SFA = total saturated fatty acids, MUFA = total monounsaturated fatty acids, PUFA = total polyunsaturated fatty acids, UI = unsaturation index

[1]no significant triple interactions for main effects were detected ($P > 0.05$)

$^{a–d}$different letters within the same row indicate difference between groups ($P < 0.05$)

doi: 10.17221/18/2016-CJAS

to glycerol dietary inclusion, a numerical increase of total MUFA and C18:1n-9 values and a decrease in total PUFA and C18:2n-6 concentrations were observed. Corresponding statistical tendencies ($0.06 < P < 0.10$) are shown in Table 4. In agreement with this study, Mourot et al. (1994) observed that dietary glycerol increased C18:1n-9 and reduced C18:2n-6 in backfat and *semimembranosus* muscle tissue of pigs. Kijora et al. (1997) did not observe any significant change in the SFA profile of the backfat, but reported a moderate increase in C18:1n-9, accompanied by a decrease in C18:2n-6 and C18:3n-3 concentrations, thus producing a decline in the PUFA/MUFA ratio in backfat. That is to say that interactions Sex × Fat and Fat × Gly in individual and total PUFA concentrations were detected (Table 4). The lower concentration in barrows was more noticeable in animals fed palm oil and the glycerol inclusion in palm oil diet produced a higher decrease of PUFA concentration than the addition to lard diet. These results seemed expectable considering that the SFA/PUFA ratio in palm oil diet was higher than in the lard one.

Positional distribution analysis of fatty acid within triacylglyceride. It is known that FA are not esterified at random by the glycerol hydroxyl groups in animals. In contrast to other species, the TAG's sn-2 position of adipose tissue of pigs and also of human and pig milk is mainly occupied by C16:0 (Innis and Nelson 2013). Table 5 shows the concentration of main FA, total SFA, MUFA, and PUFA and unsaturation index (UI) in the whole TAG, in sn-2 and the average of 1- and 3-positions (sn-1,3). It can be observed that C16:0 is mainly located in the sn-2 position, while C18:0 esterifies the external positions of the TAG. Monounsaturated FAs (mainly C18:1) were preferentially located at the sn-1,3 position and PUFA (C18:2n-6) are almost at random located with slight preference for sn-1,3 position. Similar results were reported in different pig tissues and human milk and substitutes by Innis and Nelson (2013).

It is noticeable that the significant differences in FA profile between sexes showed in Table 4, which are in agreement with the literature, were not reflected in the whole TAG analysis (Table 5). Lipolytic and oxidation reactions occurring during dry-curing process together with an increase of variability produced by the method of analysis could make the observed changes not significant. Nevertheless, the same behaviour was numerically observed when the position, where each FA was more abundant, was analyzed on its own. Interestingly, the increase of C16:0 and total SFA and the decrease of C18:2n-6 and total PUFA (all of them in sn-2) became again significant. On the other hand, no significant effect was observed in SF when dietary fat was considered (Table 4). Nevertheless, as it can be seen in Table 5, C16:0, C18:2n-6, total PUFA, and UI were affected in the whole TAG. It can be assumed that changes in dietary fat source should affect TAG composition (Mitchaothai et al. 2007). After all biochemical reactions happening during dry-curing process, changes in dietary fat must be conserved in residual TAG and, therefore, detectable when TAG were previously isolated. Higher C18:2n-6 and total PUFA concentrations were found in animals fed a lard diet and such increase was fundamentally reflected in sn-1,3. C16:0 was higher in animals fed palm oil diet without any effect on the positions and also remarkable was the increase of C18:0 only in sn-1,3 position.

Although, as shown in Table 2, a marked difference in FA composition in sn-2 exists between dietary lard and palm oil, FA profile of sn-2 position in stored lipids seems to be highly metabolically regulated and positional distribution proportions are constant despite dietary fat differences. Little information exists on this topic. Innis et al. (1997) provided diets differing in total FA composition and distribution within the TAG, they studied the liver lipids of piglets and reported a limited effect of dietary treatment, and therefore suggested metabolic regulation of FA composition in sn-2 position. Segura et al. (2015a) also found a limited effect of either sex or dietary treatment on the FAs located in the internal sn-2 position of pig SF. Gastric and pancreatic lipases hydrolyze FA from the external positions of the TAG. Lard contains about 25% C16:0 mainly located in the internal sn-2 position (approximately 70% of sn-2 FAs). In contrast, the C16:0 in palm oil is predominantly esterified at the 1,3 position, while C18:1 and C18:2n-6 FAs are located preferentially in the internal sn-2 position (Table 2). As a result, formation of FFA and 2-monoglycerides during the digestion of fat is markedly different depending on the dietary fat source (Innis and Nelson 2013) so that the rebuild of the TAG could not be convergent. In bovine tissue, dietary intervention has also been proven to alter FA in the external sn-1,3 position of the TAG rather than in the

doi: 10.17221/18/2016-CJAS

sn-2 location. Smith et al. (1998) observed that a depression in desaturase enzyme activity led to an increase of C18:0 concentration located in the external sn-1,3 position in bovine adipose tissue, but limited response was observed on sn-2.

Inclusion of glycerol in the diet did not produce any effect on positional distribution of FA within TAG (Table 5). This finding supports the theory that there is a limited possibility of altering FA composition at the sn-2 location of the TAG by dietary intervention during the fattening phase (Segura et al. 2015a). This is a matter of interest, because it is believed that SFA in the sn-2 position have a more detrimental health effect than those located in the external sn-1,3 location (Berry 2009), thus negatively affecting pork consumer acceptability. Nevertheless, further studies are needed.

Table 5. Effect of gender (Sex), dietary fat source (Fat), and glycerol (Gly) inclusion on the fatty acid composition of dry-cured ham subcutaneous fat in the whole triglyceride (TAG), position 2 (Sn-2), and position 1,3 (Sn-1,3)

	Sex		Fat		Gly		SEM (n = 5)	P-value[1]		
	gilt	barrow	palm oil	lard	0%	5%		Sex	Fat	Gly
TAG										
C16:0	25.7	25.4	25.9	24.6	25.5	25.2	0.876	0.6534	0.0454	0.5910
C16:1	2.46	2.52	2.35	2.55	2.61	2.45	0.127	0.4798	0.1335	0.0632
C18:0	13.2	13.4	14.0	13.1	13.7	13.6	0.785	0.5434	0.1728	0.7884
C18:1	47.4	47.5	47.5	48.6	48.6	48.0	1.358	0.6548	0.2647	0.4956
C18:2n-6	5.97	5.83	5.76	6.86	5.58	6.31	0.665	0.8924	0.0340	0.0958
SFA	41.7	41.7	42.0	39.8	41.3	40.9	1.535	0.9185	0.0645	0.6927
MUFA	51.5	51.7	52.0	53.0	52.9	52.5	1.377	0.8422	0.3173	0.6304
PUFA	6.83	6.62	6.17	7.33	5.93	6.75	0.710	0.8534	0.0377	0.1796
UI	0.68	0.67	0.65	0.68	0.65	0.67	0.020	0.9870	0.0279	0.2935
Sn-2										
C16:0	43.2	45.6	43.7	42.4	43.0	43.1	0.402	0.0395	0.0439	0.7754
C16:1	3.29	3.28	3.37	3.12	3.32	3.24	0.152	0.9642	0.9846	0.4068
C18:0	7.85	7.54	8.14	7.61	7.52	7.88	0.557	0.3919	0.1802	0.3275
C18:1	32.3	29.7	29.9	31.4	31.4	30.6	1.225	0.0913	0.3227	0.4809
C18:2n-6	4.49	4.21	4.54	4.52	4.16	4.53	0.132	0.0842	0.0537	0.1087
SFA	56.5	59.3	58.4	57.1	58.1	57.8	1.499	0.0396	0.7466	0.6442
MUFA	36.5	34.2	34.7	35.6	35.6	35.1	1.780	0.1129	0.9468	0.7662
PUFA	7.34	6.53	6.92	7.32	6.75	7.12	0.413	0.0301	0.0391	0.0925
UI	0.58	0.52	0.53	0.56	0.55	0.55	0.027	0.2027	0.0984	0.8645
Sn-1,3										
C16:0	17.0	15.3	16.5	15.3	16.4	15.9	1.634	0.1035	0.3499	0.6299
C16:1	2.05	2.14	1.80	2.21	2.19	2.00	0.192	0.4305	0.1550	0.1390
C18:0	15.8	16.3	16.5	15.5	16.2	16.0	1.129	0.4739	0.0396	0.8382
C18:1	55.0	56.4	55.0	55.7	56.0	55.4	2.240	0.3424	0.2982	0.6722
C18:2n-6	6.71	6.64	6.26	7.84	6.30	7.05	0.358	0.1970	0.0293	0.2080
SFA	34.3	32.9	34.4	32.0	33.9	33.2	1.695	0.4115	0.1211	0.6737
MUFA	59.0	60.5	59.3	60.1	59.8	59.7	1.443	0.3308	0.3745	0.9670
PUFA	6.98	6.57	6.43	7.99	6.44	7.21	0.313	0.0499	0.0318	0.0927
UI	0.73	0.74	0.72	0.76	0.73	0.74	0.031	0.5411	0.056	0.4545

SFA = total saturated fatty acids, MUFA = total monounsaturated fatty acids, PUFA = total polyunsaturated fatty acids, UI = unsaturation index

[1]no significant interactions for main effects were detected ($P > 0.05$)

doi: 10.17221/18/2016-CJAS

Slip point and textural parameters. Slip point was not directly affected by dietary fat, sex or glycerol inclusion (Table 3). Daza et al. (2009) found no effect on slip point of outer, inner, and sub-inner subcutaneous backfat in Iberian pigs fed acorns or a formulated diet rich in C18:1n-9. Nevertheless, an interaction Sex × Fat in slip point was found (Table 3). Thus, gilts fed palm oil diet showed higher values of this variable than barrows. The opposite change occurred in lard-fed animals. It has to be noted that the same interaction occurs for C18:2n-6 and total PUFA (Table 4). Opposite to feeding lard, there is a marked decrease of PUFA concentration in barrows fed palm oil. In general, higher proportions of C18:0 and lower of C18:2n-6 led to a harder fat and higher melting point (Lea et al. 1970). Lopez-Bote et al. (2002) also found an increase on slip point when PUFA were substituted by MUFA. In slip point values, also an interaction Fat × Glycerol was detected. In animals fed diets containing palm oil, an increase in slip point value was found when glycerol was included (29.0 to 29.5°C) but the opposite occurred when lard was used. It is remarkable that, again, the same interaction was detected for C16:0, C18:2n-6, total PUFA, and UI (Table 4). In the case of palm oil diet, an increase of C16:0 and decrease of C18:2n-6 and total PUFA concentrations were found in SF when glycerol was present in the diet and in lard, total PUFA concentration remained almost unaltered while C16:0 concentration decreased; such changes could justify the observed slip point values.

Although Hallenstvedt et al. (2012) found that female pigs showed significantly higher firmness scores than male pigs, no differences due to sex in considered TPA properties were found in this study. Attending to dietary fat, hardness values were higher in fat from pigs fed palm oil diet than from those fed lard diet. Despite no effect was found in FA of SF due to different fat source, Segura et al. (2015b) confirmed that hardness and adhesiveness behaviour was influenced by FA positional distribution within the TAG molecule. In Table 5, a plausible redistribution of FA depending on dietary fat was shown. In fact, animals fed diets containing palm oil showed higher concentration of C18:0 and lower of C18:2n-6 in sn-1,3 position, which could cause an increase of hardness value (Smith et al. 1998; Segura et al. 2015b).

Glycerol inclusion in the diet caused a significant decrease in all considered values of rheological

Table 6. Effect of gender (Sex), dietary fat source (Fat), and glycerol (Gly) inclusion on the texture parameters of dry-cured ham subcutaneous fat.

	Palm oil				Lard				SEM (n = 5)	P-value[1]					
	0% Gly		5% Gly		0% Gly		5% Gly								
	gilt	barrow	gilt	barrow	gilt	barrow	gilt	barrow		Sex	Fat	Gly	Sex × Fat	Sex × Gly	Fat × Gly
Hardness (N)	24.9^{a}	26.4^{a}	11.9^{b}	7.93^{b}	22.1^{a}	21.5^{a}	5.74^{b}	6.03^{b}	2.265	0.6476	0.0107	0.0001	0.7058	0.4386	0.9520
Adhesiveness (N × s)	–0.10^{cd}	–0.14^{cd}	–0.04^{a}	–0.03^{a}	–0.15^{d}	–0.15^{d}	–0.04^{a}	–0.05^{ab}	0.018	0.2696	0.0853	0.0001	0.7158	0.5499	0.3081
Springiness (10^{-3}) (m)	3.07^{ab}	2.63^{bc}	1.46^{e}	1.39^{e}	2.43^{c}	3.24^{a}	1.9^{d}	1.57^{ed}	0.173	0.8493	0.1517	0.0001	0.0457	0.0619	0.1200
Cohesiveness	0.24^{bc}	0.31^{a}	0.25^{bc}	0.25^{bc}	0.28^{ab}	0.25^{bc}	0.22^{c}	0.25^{bc}	0.016	0.0615	0.2741	0.0091	0.1488	0.9387	0.7681
Gumminess (N)	4.36^{b}	9.06^{a}	0.24^{c}	0.27^{c}	6.32^{b}	5.54^{b}	1.26^{c}	1.62^{c}	0.863	0.0604	0.7182	0.0001	0.0262	0.1203	0.0847
Chewiness (10^{-2}) (J)	1.63^{a}	1.96^{a}	0.44^{b}	0.29^{b}	1.54^{a}	1.77^{a}	0.24^{b}	0.21^{b}	0.212	0.5054	0.3134	0.0001	0.9742	0.1882	0.9976

[1]no significant triple interactions for main effects were detected ($P > 0.05$)

$^{a–d}$different letters within the same row indicate difference between groups ($P < 0.05$)

doi: 10.17221/18/2016-CJAS

properties (Table 6). Possible factors that could help explain such behaviour could be, in first place, the increase of a_w detected when glycerol was included in the diet. Serra et al. (2005) in *biceps femoris* muscle and Segura et al. (2015b) in SF of dry-cured ham found a negative correlation of hardness and chewiness with a_w. Besides, the observed tendency to a lower final pH could be an indicative of a higher proteolysis that happened during processing when glycerol was added (Candek-Potokar and Skrlep 2012). Moreover, a higher a_w implies less inhibition of enzymatic activity (Morales et al. 2007). In any case, collagen fibres and other protein matrixes would suffer longer proteolysis, which would affect the rheological properties. In springiness, Sex × Fat and Sex × Gly interactions were detected and a Sex × Fat interaction was observed in gumminess. Although there is no available information, such changes could be possibly related to the different behaviour of a_w and total PUFA (the same interactions found) observed for gilts and barrows. Gilts fed palm oil showed higher values of a_w and lower values of total PUFA than barrows; in lard, such differences were not detected. Springiness and gumminess were higher in palm-fed gilts than in barrows. To our knowledge, no direct dependence of springiness or cohesiveness on FAs has been previously described. In fact, Sumena et al. (2010) concluded that the contribution to such texture features was the network of connective tissue fibres, predominantly the collagen fibres and small quantities of elastic and reticular fibres, and not the adipocyte composition itself. Gumminess was also higher in gilts fed diets containing glycerol than in barrows while there were no such differences when glycerol was not included in the diet. More studies should be carried out to clarify such differences.

CONCLUSION

The combination of glycerol with different dietary fat sources could be a regulatory system of rheological properties of subcutaneous fat of dry cured ham without a remarkable effect on positional distribution of the fatty acids of the triglyceride molecule. The use of two fat sources with different fatty acid positional distribution but similar nutritional value (lard vs palm oil) caused a restructuration of fatty acids within the triglyceride molecule and, therefore, a change in rheological properties of subcutaneous fat. Dietary interventions could be another way to modify fat properties of dry-cured hams in order to develop different flavour, odour, and taste attributes. However, more research should be carried out.

Acknowledgement. The authors acknowledge Isabel Martín for her technical assistance in laboratory analysis.

REFERENCES

AOAC (2006): Official Methods of Analysis of AOAC International. 17th Ed. Association of Official Analytical Chemists, Gaithersburg, USA.

Berry S.E. (2009): Triacylglycerol structure and interesterification of palmitic and stearic acid-rich fat: an overview and implications for cardiovascular disease. Nutrition Research Reviews, 22, 3–17.

Bourne M.C. (1978): Texture profile analysis. Food Technology, 32, 62–66.

Candek-Potokar M., Skrlep M. (2012): Factors in pig production that impact the quality of dry-cured ham: a review. Animal, 6, 327–338.

Daza A., Menoyo D., Lopez-Bote C.J. (2009): Carcass traits and fatty acid composition of subcutaneous, intramuscular and liver fat from Iberian pigs fed in confinement only with acorns or a formulated diet. Food Science and Technology International, 15, 563–569.

De Blas C., Gasa J., Mateos G.G. (2013): Nutrient Requirements of Swine. Spanish Foundation for the Development of Animal Nutrition (FEDNA), Madrid, Spain. (in Spanish)

Farnworth E.R., Kramer J.K.G. (1987): Fat metabolism in growing swine: a review. Canadian Journal of Animal Science, 67, 301–318.

Gouk S.W., Cheng S.F., Mok J.S.L., Ong A.S.H., Chuah C.H. (2013): Long-chain SFA at the Sn-1,3 positions of TAG reduce body fat deposition in C57BL/6 mice. British Journal of Nutrition, 110, 1987–1995.

Hallenstvedt E., Kjos N.P., Overland M., Thomassen M. (2012): Changes in texture, colour and fatty acid composition of male and female pig shoulder fat due to different dietary fat sources. Meat Science, 90, 519–527.

Hugo A., Roodt E. (2007): Significance of porcine fat quality in meat technology: a review. Food Reviews International, 23, 175–198.

Innis S.M., Nelson C.M. (2013): Dietary triacyglycerols rich in sn-2 palmitate alter post-prandial lipoprotein and unesterified fatty acids in term infants. Prostaglandins, Leukotrienes and Essential Fatty Acids, 89, 145–151.

Innis S.M., Dyer R.A., Lien E.L. (1997): Formula containing randomized fats with palmitic acid (C16:0) in the 2-position increases 16:0 in the 2-position of plasmas and chylomicron triglycerides in formula-fed piglets to

doi: 10.17221/18/2016-CJAS

levels approaching those of piglets fed sow's milk. Journal of Nutrition, 127, 1362–1370.

Kijora C., Kupsch R.D., Bergner H., Wenk C., Prabucki A.L. (1997): Comparative investigation on the utilization of glycerol, free fatty acids, free fatty acids in combination with glycerol and vegetable oil in fattening of pigs. Journal of Animal Physiology and Animal Nutrition, 77, 127–138.

Kosmider A., Leja K., Czaczyk K. (2011): Improved utilization of crude glycerol by-product from biodiesel production. In: Montero G. (ed.): Biodiesel - Quality, Emissions and By-products. InTech, Rijeka, Croatia, 341–364.

Latorre M.A., Ripoll G., Garcia-Belenguer E., Arino L. (2009): The increase of slaughter weight in gilts as strategy to optimize the production of Spanish high quality dry-cured ham. Journal of Animal Science, 87, 1464–1471.

Lea C.H., Swoboda P.A.T., Gatherum D.P. (1970): A chemical study of soft fat in cross-breed pigs. Journal of Agricultural Science, 74, 279–284.

Lopez-Bote C.J., Isabel B., Daza A. (2002): Partial replacement of poly- with monounsaturated fatty acids and vitamin E supplementation in pig diets: effect on fatty acid composition of subcutaneous and intramuscular fat and on fat and lean firmness. Animal Science, 75, 349–358.

Mitchaothai J., Yuangklang C., Wittayakun S., Vsupen K., Wongsutthavas S., Srenanul P., Hovenier R., Everts H., Everts H., Beynen A.C. (2007): Effect of dietary fat type on meat quality and fatty acid composition of various tissues in growing-finishing swine. Meat Science, 76, 95–101.

Morales R., Serra X., Guerrero L., Gou P. (2007): Softness in dry-cured porcine biceps femoris muscles in relation to meat quality characteristics and processing conditions. Meat Science, 77, 662–669.

Mourot J., Aumaitre A., Mounier A., Peiniau P., Francois A.C. (1994): Nutritional and physiological effects of dietary glycerol in the growing pig. Consequences on fatty tissues and post mortem muscular parameters. Livestock Production Science, 38, 237–244.

Narvaez-Rivas M., Vicario I.M., Constante E.G., Leon-Camacho M. (2007): Changes in the concentrations of free fatty acid, monoacylglycerol, and diacylglycerol in the subcutaneous fat of Iberian ham during the dry-curing process. Journal of Agricultural and Food Chemistry, 55, 10953–10961.

Nishioka T., Irie M. (2005): Evaluation method for firmness and stickiness of porcine perirenal fat. Meat Science, 70, 399–404.

Nuernberg K., Dannenberger D., Nuernberg G., Ender K., Voigt J., Scollan N.D., Wood J.D., Nute G.R., Richardson R. (2005): Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. Livestock Production Science, 94, 137–147.

Perona J.S., Ruiz-Gutierrez V. (2004): Analysis of neutral lipids: triacylglycerols. In: Nollet L.M.L. (ed.): Handbook of Food Analysis. Marcel Decker, New York, USA, 275–312.

Piedrafita J., Christian L.L., Lonergan S.M. (2001): Fatty acid profiles in three stress genotypes of swine and relationships with performance, carcass and meat quality traits. Meat Science, 57, 71–77.

Romero de Avila M.D., Escudero R., Ordonez J.A., Cambero M.I. (2014): Wiebull analysis characterizes the breaking properties of dry-cured ham slices. Meat Science, 97, 451–458.

Schieck S.J., Shurson G.C., Kerr B.J., Johnston L.J. (2010): Evaluation of glycerol, a biodiesel coproduct, in grow-finish pig diets to support growth and pork quality. Journal of Animal Science, 88, 3927–3935.

Segura J., Lopez-Bote C.J. (2014): A laboratory efficient method for intramuscular fat analysis. Food Chemistry, 145, 821–825.

Segura J., Cambero M.I., Camara L., Loriente C., Mateos G.G., Lopez-Bote C.J. (2015a): Effect of sex, dietary glycerol or dietary fat during late fattening on fatty acid composition and positional distribution of fatty acids within the triglyceride in pigs. Animal, 14, 1–8.

Segura J., Escudero R., Romero de Avila M.D., Cambero M.I., Lopez-Bote C.J. (2015b): Effect of fatty acid composition and positional distribution within the triglyceride on selected physical properties of dry-cured ham subcutaneous fat. Meat Science, 103, 90–95.

Serra X., Ruiz-Ramirez J., Arnau J., Gou P. (2005): Texture parameters of dry-cured ham m. biceps femoris samples dried at different levels as a function of water activity and water content. Meat Science, 69, 249–254.

Small D.M. (1991): The effects of glyceride structure on absorption and metabolism. Annual Reviews of Nutrition, 11, 412–434.

Smith S.B., Yang A., Larsen T.W., Tume R.K. (1998): Positional analysis of triacylglycerol from bovine adipose tissue lipids varying in degree of saturation. Lipids, 33, 197–207.

Soto E., de la Hoz L., Ordonez J.A., Hierro E., Herranz B., Lopez-Bote C.J., Cambero M.I. (2010): The lipid composition and texture profile of dry-cured loin as affected by feeding level in the period prior to the late fattening phase and by rearing system of Iberian pigs. Italian Journal of Food Science, 22, 377–385.

Sumena K.B., Lucy K.M., Chungath J.J., Ashok N., Harshan K.R. (2010): Regional histology of the subcutaneous tissue and the sweat glands of Large White Yorkshire pigs. Tamil Nadu Journal of Veterinary and Animal Sciences, 6, 128–135.

Tabilo G., Flores M., Fiszman S.M., Toldra F. (1999): Postmortem meat quality and sex affect textural properties and protein breakdown of dry-cured ham. Meat Science, 51, 255–260.

Received: 2016–02–08
Accepted after corrections: 2016–10–06