

Dietary CLA supplementation and gender modify fatty acid composition of subcutaneous and intramuscular fat in Iberian × Duroc finishing heavy pigs

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Abstract

The objective of this research was to evaluate the effect of dietary conjugated linoleic acid (CLA) enrichment for finishing heavy fatty pigs on performance, carcass traits and fatty acid composition in subcutaneous and intramuscular fat (IMF), including CLA main isomers concentration. Forty castrated Iberian × Duroc pigs, half males and half females, with 120 (± 2.83) kg live weight were used. Pigs were fed experimental diets containing two levels (0 and 1%) of CLA. No effect of CLA was observed on backfat thickness or IMF concentration. In subcutaneous backfat, dietary CLA increased the C16:0, C18:0, *c9,t11*-CLA, *t10,c12*-CLA and saturated fatty acids (SFA) proportion and decreased those C18:1 n-9, C18:2 n-6, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) proportions and MUFA/SFA and C18:1 n-9/C18:0 and C16:1 n-7/C16:0 ratios. In the IMF, dietary CLA increased C16:0, SFA, *c9,t11* and *t10,c12* proportions and reduced C18:1 n-9 and MUFA, MUFA/SFA and C18:1 n-9/C18:0 ratios, but showed no effect on C18:2 n-6 and PUFA proportion. Subcutaneous backfat fatty acid profile was affected by gender, but no gender effect on intramuscular fatty acid profile was observed. CLA isomer accumulation was lower in heavy pigs compared to the lean genotype probably as a consequence of the higher carcass fat concentration in the former.

Additional key words: carcass traits; conjugated linoleic acid; fatty acid profile; heavy pigs.

Resumen

La suplementación de CLA en la dieta y el sexo modifican la composición de ácidos grasos de la grasa subcutánea e intramuscular en cerdos Ibéricos × Duroc durante la fase de acabado

El objetivo de esta investigación fue valorar el efecto del enriquecimiento con ácido linoleico conjugado (CLA) en la dieta de cerdos grasos durante la fase de acabado sobre el crecimiento, el rendimiento de la canal y la composición de ácidos grasos en la grasa subcutánea e intramuscular (IMF), incluyendo la concentración de los principales isómeros del CLA. Se usaron cuarenta cerdos castrados Ibérico × Duroc, mitad machos y mitad hembras, con un peso vivo de 120 ($\pm 2,83$) kg. Los cerdos se alimentaron con dietas experimentales que contenían dos niveles (0 y 1%) de CLA. No se observó ningún efecto del CLA sobre el espesor de grasa subcutánea o en la concentración de IMF. En la grasa subcutánea el CLA incrementó la concentración de C16:0, C18:0, *c9,t11*-CLA, *t10,c12*-CLA y la proporción de ácidos grasos saturados (SFA) y redujo el C18:1 n-9, C18:2 n-6, las proporciones de ácidos grasos monoinsaturados (MUFA) y poliinsaturados (PUFA) y las proporciones de MUFA/SFA y C18:1 n-9/C18:0 y C16:1 n-7/C16:0. En la IMF el CLA incrementó el C16:0, y la proporción de SFA, *c9,t11* y *t10,c12* y redujo el C18:1 n-9 y las proporciones de MUFA, MUFA/SFA y C18:1 n-9/C18:0, pero no se mostró ningún efecto sobre la proporción de C18:2 n-6 y los PUFA. El sexo tuvo influencia sobre la concentración de ácidos grasos de la grasa subcutánea, pero no se observó ningún efecto sexo sobre los ácidos grasos intramusculares. Un efecto de dilución de la gran acumulación de grasa en la canal de estos animales grasos, con marcado carácter adipogénico, comparados con los genotipos magros podría ser la explicación de la menor concentración de los isómeros del CLA en animales Ibérico × Duroc.

Palabras clave adicionales: ácido linoleico conjugado; cerdos grasos; perfil de ácidos grasos; rendimiento de la canal.

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Introduction

There is a growing interest in the production of high quality dry-cured meat products for niche market, sometimes associated to the production of autochthonous pig breeds. This requires the production of fat pigs slaughtered at heavy weight (140-160 kg), where fat content and composition play a determinant role in the nutritive, sensory and technological quality of the meat (López Bote, 1998).

Fat pigs accumulate large amount of subcutaneous lipids and are extremely inefficient (Serrano *et al.*, 2008). However, any strategy aimed to increase feed efficiency (reducing slaughter weight, crossing with lean pigs, etc.) has proven to reduce also intramuscular fat (IMF), and therefore have been discarded because of its negative effect on dry cured meat products (Carrapiso *et al.*, 2003; Ventanas *et al.*, 2007).

Several studies have reported that dietary CLA administration to growing-finishing pigs reduces subcutaneous fat and improves feed efficiency, without any effect on IMF concentration (Dugan *et al.*, 1997; Joo *et al.*, 2002). This has raised an interest in feeding CLA to pigs (Ostrowska *et al.*, 1999; Thiel-Cooper *et al.*, 2001).

Moreover, CLA has also beneficial effects such as anti-carcinogenic (Pariza and Hargraves, 1985), anti-atherosclerotic (Lee *et al.*, 1994) and enhance of immune system (Haro *et al.*, 2006), and therefore dietary CLA administration to pigs has proven to be an effective way to concentrate active isomer in pig tissues, thus producing additional benefits to consumers (Pariza *et al.*, 2001; Belury, 2002).

At the moment, most studies with CLA feeding to pigs have been performed on lean (Ostrowska *et al.*, 1999; Thiel-Cooper *et al.*, 2001; Martín *et al.*, 2007; Larsen *et al.*, 2009) or moderately fat pigs (Eggert *et al.*, 2001; Fernández-Figares *et al.*, 2008; Cordero *et al.*, 2010) but there is lack of information of the possible effect of CLA on heavy fat pigs.

The objective of this research was to evaluate the effect of dietary CLA enrichment for finishing heavy fat pigs (castrated males and castrated females) on performance, carcass traits, backfat thickness, IMF content, and fatty acid composition including the concentration of CLA isomers in subcutaneous and IMF.

Material and methods

Animals and diets

The routine of animal care and experiment procedures used in this experiment was approved by the University Complutense of Madrid. Forty Iberian × Duroc pigs, half castrated males (CM) and half castrated females (CF), with 120.0 (\pm 2.83) kg live weight were used. The pigs were randomly distributed and located in eight pens of five animals (CM or CF). Pigs were fed with their respective experimental diets which were formulated to contain either no CLA (0%) or 1% CLA supplementation. The CLA oil supplementation (CLA-60, Loderstar™, Belgium), contained approximately 60% of CLA isomers (30% *c9,t11* and 30% *t10,c12*) (Table 1).

All the diets were formulated to provide the same protein and energy levels. Diets were provided *ad libitum* for 42 days. Before the beginning of the experiment all pigs were subjected to the same feeding and management. Pigs were slaughtered at a local slaughterhouse at 153.2 (\pm 3.0) kg live weight.

Ingredients, chemical composition and main fatty acids of experimental diets are shown in Table 1. Determination of the compositional analysis of feeds (in triplicate) was carried out according to AOAC (2005). Fatty acids of diets were extracted and quantified by the one-step procedure of Sukhija and Palmquist (1988) from lyophilised samples. Pentadecanoic acid (C15:0) (Sigma-Aldrich, Madrid, Spain) was used as internal standard. Fatty acid methyl esters were analysed by gas chromatography using a Hewlett Packard HP-6890 (Avondale, PA, USA) gas chromatograph equipped with a flame ionization detector and a capillary column (HP-Innowax, 100 m × 0.25 mm *i.d.* and 0.2 μ m polyethylene glycol-film thickness) (Kramer *et al.*, 1998).

Sample collection and chemical analysis

At slaughter, carcass weight, carcass yield, hams, forelegs, loins, chops and bacon weight were recorded. Samples of subcutaneous fat and *Longissimus dorsi* at the level of the last rib were taken, weighed, vacuum-packed in low-oxygen permeable film, and kept frozen at -20°C for fatty acid analysis. Liver samples (appro-

Abbreviations used: ADFI (average daily feed intake), ADG (average daily gain), CF (castrated females); CLA (conjugated linoleic acid), CM (castrated males), FCE (feed conversion efficiency), IMF (intramuscular fat), ME (malic enzyme), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acid), SFA (saturated fatty acid).

Table 1. Ingredients, chemical composition and analyzed fatty acid composition of experimental diets

Ingredients (%)	0% CLA	1% CLA
Barley	50	50
Wheat	22	22
Corn	13.6	13.6
Soybean meal (44%)	11	11
Soybean oil	1	0
CLA oil ¹	0	1
Calcium carbonate	1.3	1.3
Calcium phosphate	0.5	0.5
Marine salt	0.4	0.4
Vitamin and mineral premix	0.3	0.3
Calculated composition		
Net energy (kcal kg ⁻¹)	2,222	2,222
<i>Chemical composition (%)</i>		
Ash	4.90	4.90
Crude fiber	4.74	4.74
Crude protein	14.42	14.42
Crude fat	3.80	4.30
<i>Fatty acid composition (%)</i>		
C12:0	0.05	0.07
C14:0	0.29	0.25
C16:0	15.89	15.74
C16:1 n-9	0.06	0.05
C16:1 n-7	0.16	0.15
C18:0	3.28	3.21
C18:1 n-9	21.11	21.77
C18:1 n-7	1.07	0.96
C18:2 n-6	52.02	44.01
C18:3 n-3	5.37	4.26
c9,t11-CLA	0.00	4.45
t10,c12-CLA	0.00	4.38
Σ SFA ²	19.51	17.87
Σ MUFA ²	22.40	22.93
Σ PUFA ²	57.40	57.10

¹ CLA (60% CLA isomers, half cis-9,trans-11 and half trans-10, cis-12) was provided by Loderstar™ (Belgium). ² Σ SFA, Σ MUFA, Σ PUFA: sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

ximately 30 g) were collected at slaughter, placed in liquid nitrogen and stored at -80°C for enzyme activity analyses.

Lipids from subcutaneous backfat and *Longissimus dorsi* muscle were extracted with a mixture of chloroform/methanol (2:1 v/v) following the procedure described by Folch *et al.* (1957). Lipid extracts were methylated in the presence of sodium methoxide (Christie, 1982) and analyzed as described for dietary fatty acids.

Enzyme assays

Malic enzyme (ME) and glucose 6-phosphate dehydrogenase (G6PD) activities were determined in liver extracts as described by Álvarez *et al.* (2000). Weighed quantities of liver tissue sample were homogenised with an Ultra turrax T18basic (IKAw; Labortechnik, Staufen, Germany) in 3 vol. ice-cold buffer [20 mM Tris-HCl, 0.25 M sucrose, 2 mM ethylenediamine-tetraacetate EDTA], pH 7.4 and spun at 20,000 × g for 40 min. G6PD and ME activities were determined using continuous spectrophotometric assays following the NADPH formation at 340 nm. The soluble liver protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as standard. All enzyme assays were conducted at 37°C. Care was taken to ensure that initial rates were being measured in all assays. Control experiments established that the enzyme was stable in the buffer used during the time period of the assay and at the temperature required (Álvarez *et al.*, 1998). All enzyme assays were carried out in triplicate. The enzymatic activity units (IU) defined as μmol substrate converted to product per min at the assay temperature, were expressed per mg liver soluble protein (specific activity). All chemicals were supplied by Amersham Biosciences (Uppsala, Sweden) and by Sigma-Aldrich (Madrid, Spain).

Statistical analysis

Data were analyzed using the GLM procedure of SAS (SAS, 2002). The effects of dietary CLA level, gender and the interaction CLA × Gender were studied. The experimental unit was the pen all the analyses done. The data are presented as the means and the standard errors of means. The initial weight was used as covariate for productive variables and the carcass weight (CW) for carcass characteristics. When the covariates were not significant ($p > 0.05$) they were removed from the model. All differences were considered significant at $p < 0.05$ and p values between 0.05 and 0.10 were considered a trend.

Results

Dietary CLA and gender had no influence ($p > 0.05$) on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion efficiency (FCE). The

ADG, ADFI and FCE values were 785.7 g, 3.59 kg and 4.57 kg kg⁻¹ and 795.2 g, 3.42 kg and 4.31 kg kg⁻¹ for treatment 0% CLA and 1% CLA respectively. The ADG, ADFI and FCE values were, 809.5 g, 3.61 kg and 4.46 kg kg⁻¹ for CM and 771.4 g, 3.40 kg and 4.41 kg kg⁻¹ for CF.

Dietary CLA had no effect on carcass characteristics (Table 2). Dietary CLA increased the sum of hams, forelegs and loins weights (H + F + L) ($p = 0.10$). The forelegs weight (FW) were higher ($p = 0.03$) in CM than in CF, while the bacon weight (BW) tended ($p = 0.05$) to be higher in CF than in CM. The interaction dietary CLA × gender showed no effect ($p > 0.05$) for all carcass traits studied.

The influence of dietary CLA and gender on fatty acid profile of subcutaneous backfat is presented in Table 3. Dietary CLA increased the C12:0, C14:0, C16:0, C18:0 fatty acid proportion. SFA increased from 40.02% to 44.48% in the pigs fed the diet containing 1% CLA when compared to those receiving the control diet. Concentration of C18:1 n-9, MUFA, C18:2 n-6 and PUFA fatty acids decreased in pigs fed the CLA diet. The CLA enhanced the proportion of the *c9,t11*-CLA and *t10,c12*-CLA isomers in subcutaneous backfat and reduced the MUFA/SFA, C18:1 n-9/C18:0 and C16:1 n-7/C16:0 ratios.

The subcutaneous backfat from CM had higher C18:0, SFA ($p < 0.05$) and lower, MUFA and MUFA/SFA and C18:1 n-9/C18:0 ratios than those from the CF, whereas C16:0 ($p = 0.07$) and C18:1 n-9 ($p = 0.06$) proportions

tended to be higher and lower respectively in CM than in CF.

According to results showed in Table 4, in the IMF from *Longissimus dorsi*, dietary CLA inclusion increased C12:0, C14:0, C16:0, C16:1 n-7, SFA, *c9,t11*-CLA and *t10,c12*-CLA proportions, also reduced C18:1 n-9 and MUFA proportions and MUFA/SFA and C18:1 n-9/C18:0 ratios, and produced no effect on C18:2 n-6 and PUFA concentration.

The gender had no effect ($p > 0.05$) on fatty acid profile of IMF (Table 4). However, CM tended ($p < 0.10$) to have higher C18:0, SFA and *c9,t11*-CLA proportions and lower MUFA proportion and MUFA/SFA and C18:1 n-9/C18:0 ratios than CF. The interaction dietary CLA level × gender showed no effect for all fatty acid proportion determined.

Dietary CLA, gender and interaction CLA × gender showed no effect on G6PD and ME activity in the liver (Table 5).

Discussion

The productive results observed in the present experiment are in agreement with data from Serrano *et al.* (2008) obtained in Duroc × Iberian pigs. No effect of dietary CLA administration was observed on ADG, ADFI or FCE, according to Ramsay *et al.* (2001) and Tischendorf *et al.* (2002). However, some other experiments reported a beneficial effect of CLA on ADG

Table 2. Effect of dietary CLA and gender on carcass traits¹

Items ¹	% dietary CLA		Gender		SEM ²	p-value			
	0	1	CM ¹	CF ¹		Diet (D)	Gender (G)	D × G	CW ¹
Initial weight (kg)	121.6	118.4	123.5	116.5	2.83	0.53	0.18	0.56	—
Slaughter weight (kg)	154.6	151.8	157.5	148.9	3.00	0.50	0.05	0.54	—
Carcass weight (kg)	123.1	119.8	124.7	118.3	2.56	0.37	0.09	0.61	—
Carcass yield (%)	79.6	78.9	79.1	79.4	0.36	0.17	0.64	0.85	—
Hams weight (kg)	28.9	29.5	29.1	29.2	0.24	0.12	0.76	0.91	<0.01
Forelegs weight (kg)	22.3	22.4	22.7 ^a	22.0 ^b	0.20	0.57	0.03	0.96	<0.01
Right loin weight (kg)	2.0	2.0	2.9	2.1	0.06	0.97	0.20	0.73	<0.01
Hams + foreleg + loin (kg)	53.2	53.9	53.8	53.3	0.29	0.10	0.32	0.84	<0.01
Chops weight (kg)	5.3	5.3	5.3	5.3	0.10	0.89	0.89	0.75	<0.01
Bacon weight (kg)	12.8	12.7	12.6	12.9	0.13	0.49	0.05	0.17	<0.01
Backfat thickness (cm)	7.5	7.3	7.3	7.5	0.21	0.65	0.41	0.10	<0.01
Ham subcutaneous fat thickness (cm)	7.4	7.3	7.2	7.5	0.20	0.78	0.23	0.96	<0.01
Intramuscular fat in <i>Longissimus dorsi</i> (%)	9.4	10.6	10.2	9.8	0.63	0.21	0.67	0.96	0.97

¹ CM: castrated males; CF: castrated females; CW: carcass weight. ² SEM: error standard of mean. ^{a,b} Means within a row without a common superscript are significantly different ($p < 0.05$).

Table 3. Fatty acids profile (%) of subcutaneous backfat according to CLA levels in the diet and gender

Fatty acid	% dietary CLA		Gender		SEM ²	p-value		
	0	1	CM ¹	CF ¹		Diet (D)	Gender (G)	D × G
C12:0	0.10 ^b	0.13 ^a	0.11	0.12	0.002	<0.01	0.40	0.14
C14:0	1.59 ^b	2.06 ^a	1.83	1.82	0.032	<0.01	0.77	0.93
C16:0	25.52 ^b	27.20 ^a	26.60	26.12	0.19	<0.01	0.07	0.77
C16:1 n-7	2.04	1.99	2.00	2.04	0.044	0.43	0.52	0.69
C18:0	12.82 ^b	15.08 ^a	14.27 ^a	13.63 ^b	0.221	<0.01	0.05	0.88
C18:1 n-9	43.25 ^a	38.94 ^b	40.69	41.49	0.271	<0.01	0.06	0.52
C18:1 n-7	2.45	2.46	2.42	2.51	0.062	0.99	0.42	0.81
C18:2 n-6	10.89 ^a	10.38 ^b	10.52	10.75	0.16	0.03	0.35	0.51
C18:3 n-3	1.34	1.23	1.30	1.27	0.042	0.05	0.60	0.55
c9,t11-CLA	0.00 ^b	0.33 ^a	0.17	0.16	0.009	<0.01	0.87	0.87
t10,c12-CLA	0.00 ^b	0.18 ^a	0.09	0.09	0.005	<0.01	0.89	0.89
Σ SFA ³	40.02 ^b	44.48 ^a	42.81 ^a	41.69 ^b	0.342	<0.01	0.02	0.80
Σ MUFA ³	47.74 ^a	43.39 ^b	45.11 ^b	46.04 ^a	0.293	<0.01	0.03	0.64
Σ PUFA ³	12.23 ^a	11.61 ^b	11.82	12.02	0.162	0.01	0.33	0.75
Σ MUFA/Σ SFA	1.19 ^a	0.98 ^b	1.05 ^b	1.10 ^a	0.014	<0.01	0.02	0.96
C18:1 n-9/C18:0	3.37 ^a	2.58 ^b	2.85 ^b	3.04 ^a	0.05	<0.01	0.04	0.99
C16:1 n-7/C16:0	0.08 ^a	0.07 ^b	0.08	0.08	0.002	<0.01	0.15	0.64

¹ CM: castrated males; CF: castrated females. ² SEM: error standard of mean. ³ Σ SFA, Σ MUFA, Σ PUFA: sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. ^{a,b} Means within a row without a common superscript are significantly different ($p < 0.05$).

Table 4. Fatty acids profile (%) of intramuscular fat from *Longissimus dorsi* according to CLA levels in the diet and gender

Fatty acid	% dietary CLA		Gender		SEM ²	p-value		
	0	1	CM ¹	CF ¹		Diet (D)	Gender (G)	D × G
C12:0	0.05 ^b	0.06 ^a	0.06	0.05	0.001	0.01	0.32	0.16
C14:0	1.09 ^b	1.26 ^a	1.19	1.15	0.033	<0.01	0.25	0.27
C16:0	30.95 ^b	32.76 ^a	32.00	31.70	0.360	<0.01	0.41	0.57
C16:1 n-7	2.53 ^b	2.98 ^a	2.69	2.82	0.110	<0.01	0.26	0.46
C18:0	12.36	12.85	12.96	12.25	0.360	0.19	0.05	0.73
C18:1 n-9	46.62 ^a	43.65 ^b	44.68	45.59	0.592	<0.01	0.12	0.12
C18:1 n-7	2.99	2.97	2.94	3.02	0.110	0.85	0.44	0.91
C18:2 n-6	3.03	3.04	3.05	3.02	0.282	0.96	0.91	0.15
C18:3 n-3	0.37	0.35	0.38	0.34	0.031	0.60	0.26	0.15
c9,t11-CLA	0.00 ^b	0.07 ^a	0.04	0.03	0.004	<0.01	0.07	0.07
t10,c12-CLA	0.00 ^b	0.01 ^a	0.01	0.00	0.002	<0.01	0.18	0.18
Σ SFA ³	44.45 ^b	46.92 ^a	46.21	45.16	0.611	<0.01	0.10	0.54
Σ MUFA ³	52.15 ^a	49.61 ^b	50.32	51.44	0.670	<0.01	0.10	0.23
Σ PUFA ³	3.40	3.47	3.29	3.39	0.182	0.77	0.29	0.15
Σ MUFA/Σ SFA	1.18 ^a	1.05 ^b	1.09	1.14	0.030	<0.01	0.09	0.43
C18:1 n-9/C18:0	3.70 ^a	3.45 ^b	3.47	3.68	0.081	0.01	0.06	0.54
C16:1 n-7/C16:0	0.08 ^b	0.09 ^a	0.082	0.087	0.002	0.03	0.17	0.56

¹ CM: castrated males; CF: castrated females. ² SEM: error standard of mean. ³ Σ SFA, Σ MUFA, Σ PUFA: sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. ^{a,b} Means within a row without a common superscript are significantly different ($p < 0.05$).

Table 5. Influence of dietary CLA and gender on G6PD and ME activity (IU mg⁻¹ soluble protein) in the liver

Enzyme	% dietary CLA		Gender		SEM ²	<i>p</i> -value		
	0	1	CM ¹	CF ¹		Diet (D)	Gender (G)	D × G
G6PD ^a	0.052	0.049	0.055	0.045	0.005	0.65	0.19	0.18
ME ^b	0.013	0.015	0.014	0.014	0.002	0.67	0.87	0.51

¹ CM: castrated males; CF: castrated females. ² SEM: error standard of mean. ^a G6PD: glucose-6-phosphate dehydrogenase. ^b ME: malic enzyme.

(Thiel-Cooper *et al.*, 2001), ADFI (Cook *et al.*, 1998) and FCE in finishing pigs (Ostrowska *et al.*, 1999). According to Dugan *et al.* (2001) heterogeneous response of pig to dietary CLA administration could be explained by the different dietary concentration of CLA isomers, the content of other dietary constituents, duration of the feeding trial and pig genotype.

No effect of dietary CLA administration was observed on carcass characteristics (Table 2), which is in disagreement with most previous research in which CLA was included at similar dietary concentration. Thiel-Cooper *et al.* (1998) in lean pigs fed a diet with 1% of CLA reported a 10% reduction in subcutaneous backfat. Ostrowska *et al.* (2003) reported a 23% reduction in subcutaneous backfat and an increase 10% of firmness (with 1% of CLA). Wiegand *et al.* (2001) with a dose of approximately 1% reported a reduction in subcutaneous backfat and an increase in marbling and firmness. Dunshea *et al.* (1998) and Ostrowska *et al.* (1999) reported up to a 25 to 30% reduction in subcutaneous backfat thickness at higher dietary CLA concentration. However, in other experiments no effect of 1% dietary CLA administration was observed on fat accumulation (Eggert *et al.*, 2001). In this experiment, pigs were slaughtered at higher weights (> 120 kg), thus suggesting that the effectiveness of 1% dietary CLA is lower in fatty pigs than in lean genotypes. According to Ramsay *et al.* (2001), the influence of CLA on carcass fat and lean deposition seems to be affected by pig phase of growth and genotype, being different in lean than obese or fat pigs. In our experiment the CLA level in the diet (1%) did not affect subcutaneous fat deposition although tended to increase the combined weight of H + F + L.

In the present experiment a 1% dietary CLA inclusion did not affect IMF concentration (Table 2). This is contradictory to some previous research in which IMF increased due to dietary CLA administration with a slaughter weight around 105 kg (Joo *et al.*, 2002; Wiegand

et al., 2001) and 113 kg (Averette *et al.*, 2002). However, no effect of 1% dietary CLA on IMF concentration have been found in pigs slaughtered at heavy weights (> 120 kg) (Eggert *et al.*, 2001).

The effects of dietary CLA administration on subcutaneous and intramuscular fatty acids are shown in Table 3 and 4 respectively. Dietary CLA increased *c9,t11*-CLA and *t10,c12*-CLA, which agrees with the findings of Kramer *et al.* (1998), Eggert *et al.* (2001) and Martín *et al.* (2007). On the other hand, the accumulation of *c9,t11*-CLA was higher than *t10,c12*-CLA in subcutaneous and intramuscular fat. This result is also in agreement with data from Eggert *et al.* (2001), Smith *et al.* (2002) and Martín *et al.* (2007) and might suggest that the *t10,c12* isomer would be either less efficiently incorporated or have a different metabolism. On the other hand, Larsen *et al.* (2009) found in lean pigs 1.19 and 0.93% of *c9,t11*-CLA and *t10,c12*-CLA respectively in bacon from pigs fed 1% CLA enriched diets for 56 days. However, it is noticeable that despite similar experimental conditions in our study the concentration of both isomers were considerably lower (0.33 and 0.18% for *c9,t11*-CLA and *t10,c12*-CLA respectively), than in the study of Larsen *et al.* (2009). The main distinctive characteristic between these two experiments is the marked adipogenicity of Iberian pigs (López-Bote, 1998).

A possible explanation of the effect of dietary CLA on CLA isomers accumulation observed in our experiment could be the higher carcass fat concentration in Iberian × Duroc heavy pigs compared to lean genotypes. It has been recently reported lack of fat mobilization induced by CLA in obese humans (Desroches *et al.*, 2005), while a similar dose was shown to reduce body fatness either in healthy exercising subjects (Thom *et al.*, 2001) or in postmenopausal healthy women (Raff *et al.*, 2009).

Previous experiments have shown an increase in C14:0, C16:0, C18:0, SFA proportion and a reduction

of C18:1 n-9 proportion and MUFA fatty acids in subcutaneous backfat due to dietary CLA inclusion (Bee, 2001; Eggert *et al.*, 2001; Ramsay *et al.*, 2001; Averette *et al.*, 2002; Smith *et al.*, 2002; Martín *et al.*, 2007). In the present study subcutaneous backfat SFA increased from 40.0% to 44.5% during 42 days of experiment, but Larsen *et al.* (2009) found that SFA increased from 38.5% to 46% during 56 days, thus suggesting that in finishing heavy pigs 42 days supplementation with CLA is enough to modify backfat SFA. The lowering PUFA concentration of CLA in subcutaneous fat is also a subject of potential interest, since there is a negative relationship between PUFA content and fat consistency. An excessive accumulation of PUFA in pig fat leads to an extension of the processing time of carcass joints due to a low fat consistency and therefore, impaired water migration (López-Bote, 1998), which is of importance in production of quality meat products.

The Δ -9 desaturase index, assessed by the MUFA/SFA, C18:1 n-9/C18:0 and C16:1 n-7/C16:0, decreased with the CLA administration, which is in agreement with previous research in which stearoyl coenzyme A desaturase activity in porcine subcutaneous adipose tissue was either measured (Smith *et al.*, 2002) or estimated (Eggert *et al.*, 2001; Martín *et al.*, 2007). According to Park *et al.* (2000), the *t10,c12*-CLA isomer of CLA has an inhibitory effect on Δ -9 desaturase activity compared to others CLA isomers, whereas *c9,t11*-CLA isomer seems to have not effect on fatty acid desaturation.

Regarding the influence of gender on fatty acid composition of subcutaneous backfat, Pieszka *et al.* (2006) observed that in gilts fed with CLA diets, the SFA content in fat was lower and the content of PUFA, MUFA and PUFA/SFA ratio were higher than in barrows. In agreement with our results, some other experiments also reported that subcutaneous backfat from males is more saturated than that from females (Courboulay and Massabie, 1996; Smithard *et al.*, 1980), although, Serrano *et al.* (2008) did not observe influence of gender on fatty acid composition of subcutaneous backfat from Duroc \times Iberian heavy pigs.

In IMF from *Longissimus dorsi*, Bee (2001), in pigs slaughtered at 105.2 kg live weight, and Cordero *et al.* (2010), in heavy pigs, observed an increase of C16:0 and SFA and a reduction of C18:1 n-9 and MUFA with dietary CLA, whereas on C18:2 n-6, no effect was observed. Eggert *et al.* (2001) also observed that dietary CLA increased C16:0 and SFA concentration, but did not find effect on C18:2 n-6 and PUFA. It is interesting to note that increase in the C16:0 proportion in IMF is

similar or a little bit lower than in subcutaneous backfat. The decreasing effect of CLA on C18:1 n-9, MUFA proportions and Δ -9 desaturase index was of lower magnitude in IMF than in subcutaneous backfat, which suggests lower regulatory effect of Δ -9 desaturase in muscle tissue. In the current experiment this result was likely due to the lower *t10,c12*-CLA isomer concentration in IMF than in subcutaneous fat.

In this study the relationships found between IMF percentage and C14:0, C16:0 and C18:2 n-6 proportions in IMF is of interest. Previous works have also shown a relationship between SFA and intramuscular fat content (Bee, 2001; Eggert *et al.*, 2001; Ramsay *et al.*, 2001), thus suggesting an indirect mechanism of dietary CLA on IMF content. Future research is needed in this topic.

G6PD and ME are the main enzymes involved in supplying NADPH for the reductive biosynthesis of fatty acids (Mourot *et al.*, 1995), but they also contribute to metabolic pathways other than lipogenesis. Both enzyme activities were unaltered by dietary CLA supplementation. The G6PD activity was higher than ME and seemed to be the main producer of NADPH. This observation agrees with comparative studies in newborns (Le Dividich *et al.*, 1994) and growing pigs (Mourot *et al.*, 1995). Bee (2001) did not observe effect of dietary CLA on malic and fatty acid synthesis enzymes activity in subcutaneous backfat and omental fat in finishing pigs, but the same author (Bee, 2000) found a positive effect of CLA on G6PD and ME activity in piglet adipose tissues. According to Daza *et al.* (2007), the effect of these enzymes is more marked in young animals or when animals receive different levels of energy in the diet. We did not find any effect of CLA on lipogenic enzymes and this confirm that the increase of SFA and the decrease of MUFA concentrations in both tissues studied is not due to an increased synthesis, but more likely to a down-regulation of Δ -9 desaturase activity. This is in agreement with Bretillon *et al.* (1999) who reported that both the activity and gene expression of hepatic Δ 9-desaturase were inhibited by dietary CLA.

It is concluded that a 1% dietary CLA inclusion produced no effect on carcass traits and IMF content, but affects fatty acids profile of subcutaneous backfat and IMF. Levels of CLA isomers in subcutaneous and IMF were lower than in lean pigs, which suggest that a higher dietary CLA supplementation is needed to achieve the same levels in tissues for obese heavy pig genotypes, thus indicating that a correct combination of CLA concentration and administration time should

be defined in each productive circumstance according to pig fatness.

The accumulation of the CLA isomers was lower in heavy pigs compared to the lean genotype probably as a consequence of the higher carcass fat concentration in the former. More studies are needed to understand the underlying mechanism of CLA level in the diet on carcass and fat quality in obese pigs.

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