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Changes in the Fatty Acid Profile of the Subcutaneous Fat of Swine throughout Fattening As Affected by Dietary Conjugated Linoleic Acid and Monounsaturated Fatty Acids

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The fatty acid profile of the subcutaneous fat of pigs and its evolution throughout fattening as affected by dietary conjugated linoleic acid (CLA), monounsaturated fatty acids (MUFA), and their interaction (CLA × MUFA) were studied. Three levels (0, 1, and 2%) of an enriched CLA oil (28% *cis*-9,*trans*-11 and 28% *trans*-10,*cis*-12 CLA) were combined with two levels of MUFA (low, 19% average; and high, 39% average) for pig feeding (288 gilts). Subcutaneous shot-biopsies were taken from 48 animals at the beginning of the trial (S1, 70 kg), 14 days later (S2, 80 kg), and at slaughter (S3, 107 kg). Inclusion of CLA in the diet caused an increase during fattening in *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, and saturated fatty acids (SFA) contents of pig backfat and a decrease in MUFA and polyunsaturated fatty acids (PUFA). MUFA supplementation also led to a MUFA enrichment of backfat. The interaction CLA × MUFA affected the SFA content. The rates of accumulation of CLA isomers, SFA, and MUFA throughout the trial did not follow a linear behavior, such rates being higher from S1 to S2 than from S2 to S3. These rates were also influenced by dietary CLA and MUFA levels. The increase in the ratio of saturated to unsaturated fatty acids of backfat caused by dietary CLA might be balanced by supplementation of pig diets with MUFA.

KEYWORDS: Conjugated linoleic acid; monounsaturated fatty acids; pig; fatty acid profile; subcutaneous adipose tissue; rate of accumulation

INTRODUCTION

Fat sources with high proportions of polyunsaturated fatty acids (PUFA) or monounsaturated fatty acids (MUFA) are commonly used or at least have been tested for swine feeding (*1*, *2*). These feeding strategies allow a substantial modification of the fatty acid profile of pork meat and meat products (*3*, *4*), leading to more unsaturated fats, which follows health advice about the decrease in the consumption of saturated fats due to their implication in cardiovascular diseases (*5*). However, these changes involve several negative effects on meat and carcass quality, such as soft adipose tissue, difficult slicing, higher susceptibility to lipid oxidation with the generation of toxic reactive compounds, and low technological quality in the case of cured meat products (*6*).

Conjugated linoleic acid (CLA) is a collective term to describe positional and geometric isomers of linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid) with conjugated double bonds. The *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLAs are the major CLA isomers in nature (*7*). In recent decades, CLA has gained an enormous scientific significance due to its beneficial implication in different pathologies in experimental animals (*8*). In addition, due to its physiological effects, CLA supplementation of pig feeding has been suggested as an interesting approach to improve productive traits, such as reduced feed conversion or decreased backfat thickness (*9*). Feeding pigs with CLA also leads to modifications of the fatty acid profile, increasing the proportion of saturated fatty acids (SFA) in different fat depots while decreasing the proportion of MUFA (*9*). In turn, this leads to less fluid and more consistent lards, which are considered as quality characteristics by meat processors (*6*). Although the increase in SFA could have negative health implications, there is a simultaneous increase in CLA isomers in lipids of pigs fed CLA-enriched diets, which could counteract this negative effect. Moreover, the decrease in MUFA content of swine fat due to dietary CLA

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Table 1. Ingredients and Chemical Composition of the Experimental Treatments Used in Pig Feeding

	low MUFA feed			high MUFA feed		
ingredient (%)	0% CLA	1% CLA	2% CLA	0% CLA	1% CLA	2% CLA
barley	53.3	53.3	53.3	53.3	53.3	53.3
wheat	15.0	15.0	15.0	15.0	15.0	15.0
bran	8.0	8.0	8.0	8.0	8.0	8.0
soybean meal 44%	16.0	16.0	16.0	16.0	16.0	16.0
palm oil	1.6	1.1	0.6	1.0	0.5	0.0
soy olein	0.4	0.4	0.4	0.0	0.0	0.0
olive olein	0.0	0.0	0.0	3.0	3.0	3.0
hydrogenated stearin palm	3.0	2.5	2.0	1.0	0.5	0.0
CLA	0.0	1.0	2.0	0.0	1.0	2.0
carbonate	1.2	1.2	1.2	1.2	1.2	1.2
phosphate	0.4	0.4	0.4	0.4	0.4	0.4
salt	0.4	0.4	0.4	0.4	0.4	0.4
L-lysine 50	0.17	0.17	0.17	0.17	0.17	0.17
L-threonine	0.03	0.03	0.03	0.03	0.03	0.03
choline 75	0.04	0.04	0.04	0.04	0.04	0.04
vitamin and mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
chemical composition (%)						
dry matter	89.2	89.6	89.4	89.3	89.5	89.6
ash	4.9	5.1	5.0	5.1	5.6	5.3
crude fiber	4.2	4.3	4.1	4.7	4.3	4.6
crude fat	7.7	6.9	7.3	7.2	7.1	6.8
crude protein	16.4	16.0	15.8	16.7	16.5	15.8
nitrogen free extractives	62.8	64.1	64.0	62.4	62.7	63.8
fatty acid composition (%)						
C14:0	0.8	0.6	0.5	0.5	0.3	0.3
C16:0	35.3	30.4	25.6	25.4	19.7	15.0
C16:1	0.1	0.1	0.1	0.5	0.4	0.4
C18:0	22.8	20.1	16.6	11.4	7.6	4.6
C18:1 n-9	18.1	18.0	18.7	37.8	37.9	37.8
C18:2 n-6	19.9	20.2	19.8	20.6	22.2	22.5
C18:3 n-3	1.8	1.7	1.6	1.8	2.1	2.1
cis-9,*trans*-11 CLA	0.0	3.9	8.0	0.0	4.3	7.9
trans-10,*cis*-12 CLA	0.0	3.7	7.9	0.0	4.2	8.1
ΣSFA	59.7	52.0	43.5	38.8	28.4	20.6
ΣMUFA	18.8	18.6	19.2	38.9	38.8	38.7
ΣPUFA[a]	21.5	21.9	21.5	22.4	24.4	24.7

[a] Excluding CLA isomers.

supplementation could be partially or totally balanced by including higher levels of MUFA in pig feeding. However, no information about the combined effect of dietary MUFA and CLA on the fatty acid profile of subcutaneous adipose tissue of pigs has been found in the available scientific literature. There is also scarce information about the rate of deposition of CLA isomers and other fatty acids in pig lipids as affected by dietary CLA or by its interaction with dietary MUFA. This information could be of interest because it would allow a prediction of the achieved fatty acid profile of pig lipids at slaughter depending on the CLA inclusion level and the length of the fattening with a particular diet.

Thus, the present work aimed to study the effect of feeding CLA- and MUFA-enriched diets on the fatty acid profile of swine subcutaneous adipose tissue throughout the fattening period.

MATERIALS AND METHODS

Animals and Feeding. Three levels (0, 1, and 2%) of commercial enriched CLA oil supplementation (CLA-60, BASF, Dortmund, The Netherlands), containing approximately 56% of CLA isomers (28% *cis*-9,*trans*-11 and 28% *trans*-10,*cis*-12), and two levels of MUFA (low, 19%; and high, 39%) were combined for pig feeding (**Table 1**). All diets were formulated to provide similar protein and energy levels, fulfilling the advised nutritional needs for female pigs at considered ages by the National Research Council (*10*).

The experiment was conducted using 288 finishing gilts (Large white ♀). Pigs weighing 70 kg and at about 140 days of age were randomly allotted to the six different feeding treatments in four replicates of each treatment (12 pigs per replicate). Pigs were housed in an environmentally controlled experimental grower/finisher shed. Pigs were group-housed (12 pigs per pen) and had ad libitum access to feed (single-space dry feeders) and water (nipple drinkers) to a final average weight of 107 kg. After fattening (53 days), pigs were slaughtered at a local slaughterhouse by electrical stunning and exsanguination.

Sampling Procedure. Two subcutaneous shot-biopsies were taken during the trial for the determination of the fatty acid profile evolution on the subcutaneous fat tissue. Two animals were randomly selected from each box (8 animals for treatment, making a total of 48 sampled pigs) at the initial weight. The first biopsy (S1) was taken at this moment (140 days live time, day 0), and the second one (S2) was taken 14 days later (about 80 kg live weight). In addition, a third backfat sample (S3) was taken following the same procedure immediately after slaughter (53 days from the beginning of the experiment, about 107 kg live weight).

Biopsy samples of subcutaneous fat were taken at 6 cm beside the backbone at the level of the last rib using a Czech gun (PPB-2 Biotech, Nitra, Slovakia) with an adapted cannula (diameter = 0.25 cm) with a sharpened edge. All necessary precautions were taken to prevent animal discomfort during and after the in vivo sampling processes. This included tranquilization with 40 mg of azaperon (Stressnill, Labopica, Madrid, Spain) 1 h before the biopsy was taken and local anesthesia with 2% lidocaine-HCl immediately prior to sample collection. Biopsies always contained the whole backfat thickness, including both the outer and the inner layers. Afterward, animals received a 2 mL penicillin intramuscular injection (300000 IU mL^{-1}, Labopica). All samples were vacuum packaged in plastic bags and frozen at −80 °C until analysis.

Chemical Analyses. Analysis of the feeds was performed according to the Association of Official Analytical Chemists (*11*): crude protein (reference 954.01), crude fat (reference 920.39), crude fiber (reference 962.09), and ash (reference 942.05). The obtained composition of the diets is shown in **Table 1**.

Subcutaneous fat tissue was separated from the skin of the biopsies and washed with distillate water to remove dirt, hair, and blood that could affect results. Total lipids were extracted from subcutaneous fat samples by homogenization with 2 mL of diethyl ether (0.05% butylhydroxytoluene, BHT) in an Omni mixer during 1 min at 3000 rpm and subsequent filtration.

Fat from mixed diets for fatty acid analysis was extracted with a mixture of chloroform/methanol (2:1 v/v) following the procedure described by Folch et al. (*12*).

Fatty acid methyl esters (FAMEs) of subcutaneous fat and of lipids from diets were obtained by acidic transesterification following the method described by Sandler and Karo (*13*). Briefly, 20 mg of extracted lipids placed in a glass vial was thoroughly mixed with 1 mL of 5% sulfuric acid in methanol and kept for 30 min at 80 °C in an oven. Afterward, FAMEs were extracted with 1 mL of hexane. Hexane was evaporated to dryness under a nitrogen stream, and FAMEs were subsequently dissolved in 1 mL of hexane. FAMEs were analyzed by gas chromatography using a Hewlett-Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethyleneglycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA) (60 m × 0.32 mm i.d. × 0.25 μm film thickness). Gas chromatograph oven program temperature was as follows: initial temperature of 180 °C, 5 °C/min to 200 °C; 40 min at this temperature and thereafter 5 °C/min to 250 °C, and then kept for an additional 21 min. Injector and detector temperatures were 250 °C. Carrier gas was nitrogen at a flow rate of 0.8 mL/min. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO). Tridecanoic acid was used as internal standard. Results were expressed as grams per 100 g of detected FAMEs.

Table 2. Fatty Acid Profile (Grams per 100 g of FAMEs) of Subcutaneous Fat from Slaughtered Pigs after Fattening at Different CLA and MUFA Levels ($n = 8$)[a]

	low MUFA			high MUFA				*p*		
fatty acid	0% CLA	1% CLA	2% CLA	0% CLA	1% CLA	2% CLA	SEM	CLA	MUFA	CLA × MUFA
C12:0	0.04ab	0.04a	0.05a	0.03b	0.05a	0.04a	0.00	0.000	0.196	0.021
C14:0	1.01a	1.01a	1.22a	0.71b	1.18a	1.15a	0.03	0.000	0.182	0.002
C14:1n-5	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.543	0.247	0.047
C15:0	0.07	0.06	0.06	0.06	0.06	0.06	0.00	0.973	0.456	0.276
C16:0	23.71a	23.56a	24.34a	20.68b	23.79a	22.81a	0.30	0.002	0.000	0.003
C16:1 n-7	1.83a	1.33b	1.36ab	1.50ab	1.42ab	1.41ab	0.05	0.016	0.394	0.058
C17:0	0.45	0.42	0.43	0.38	0.38	0.41	0.01	0.813	0.151	0.750
C17:1 n-7	0.38a	0.27b	0.27b	0.32ab	0.25b	0.25b	0.01	0.002	0.094	0.500
C18:0	13.70bc	15.82ab	17.02a	13.18c	15.51abc	15.47abc	0.29	0.000	0.106	0.488
C18:1 n-9	37.95b	34.88cd	32.78d	41.68a	35.75bd	35.97bc	0.50	0.000	0.000	0.044
C18:2 n-6	15.97	16.44	15.21	16.21	15.13	15.05	0.19	0.058	0.353	0.129
C18:3 n-6	0.08ab	0.08ab	0.07b	0.09a	0.07b	0.08ab	0.00	0.003	0.465	0.079
C18:3 n-3	1.13ab	1.18ab	1.06b	1.23a	1.11ab	1.15ab	0.02	0.078	0.139	0.040
C20:0	0.16ab	0.18ab	0.18ab	0.18a	0.15b	0.16ab	0.00	0.248	0.412	0.008
C20:1 n-9	0.75b	0.76b	0.65b	1.04a	0.75b	0.73b	0.03	0.000	0.000	0.001
C20:2 n-6	0.51b	0.56b	0.52b	0.66a	0.54b	0.49b	0.01	0.002	0.059	0.000
C20:3 n-6	0.10b	0.10b	0.09b	0.12a	0.09b	0.08b	0.00	0.000	0.584	0.006
C20:4 n-6	0.24a	0.22b	0.19b	0.29a	0.19b	0.18b	0.01	0.000	0.733	0.010
C21:0	0.13	0.15b	0.14b	0.18a	0.15b	0.13b	0.00	0.021	0.003	0.000
cis-9,*trans*-11 CLA	0.25c	1.08b	1.85a	0.19c	1.14b	1.96a	0.11	0.000	0.626	0.561
trans-10,*cis*-12 CLA	0.13c	0.69b	1.23a	0.08c	0.72b	1.38a	0.08	0.000	0.453	0.283
ΣSFA	39.33b	41.30ab	43.48a	35.46c	41.34ab	40.30b	0.47	0.000	0.000	0.010
ΣMUFA	40.92b	37.24cd	35.06d	44.54a	38.19bcd	38.37bc	0.54	0.000	0.000	0.080
ΣPUFA[b]	18.03	18.58	17.14	18.60	17.12	17.02	0.22	0.027	0.554	0.073

[a] Different letters within the same row indicate significant difference ($p \geq 0.05$). [b] Excluding CLA isomers.

Statistical Analysis. An individual pig was the experimental unit for analysis of all data. Statistical analyses were performed by means of the general linear models procedure of the SPSS (v. 15.0) statistical software. The effect of CLA and MUFA contents of diets and their interaction on the proportion of each individual fatty acid from subcutaneous fat was evaluated by a two-way analysis of variance (ANOVA). The effect of time of sampling and its respective interaction with CLA, MUFA, and CLA × MUFA was evaluated by a three-way mixed-model repeated-measures test; CLA, MUFA, and CLA × MUFA levels being the between-subject effects and time of sampling the within-subject effect. Differences were considered to be significant at $p \leq 0.05$. When the effect of any of the factors was significant, differences between groups were analyzed by using Tukey's posthoc test.

RESULTS AND DISCUSSION

Experimental Diets. Ingredients, chemical composition, and fatty acid profile of experimental diets are shown in **Table 1**. All diets showed similar levels of crude fat, crude protein, nitrogen free extractive substances, and dry matter. Fat content was considerably high (around 7%) compared to commercial pig diets, due to the inclusion of 5% of different fat sources in each experimental diet.

As a consequence of including different levels of commercial CLA oil in the feeds, increasing proportions of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA were achieved in concentrates. Thus, the content of each CLA isomer approximately was 0, 4, and 8% at 0% CLA, 1% CLA, and 2% CLA treatments, respectively. Such levels are within commercial recommendations for CLA supplementation of swine diets and are similar to the levels considered by other authors who have previously studied the effect of CLA supplementation on swine productive parameters (*9*).

Major fatty acids in experimental diets in addition to CLA isomers were palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9), and linoleic acid (C18:2 n-6). The proportion of MUFA in diets enriched with olive oleins (high MUFA diets) reached almost 39% of total fatty acids, whereas in low MUFA mixed diets such levels were around 19%. The proportions of PUFA (excluding CLA isomers) were similar in all groups (between 21.5 and 24.7%), whereas SFA content was lower in high-MUFA than in low-MUFA experimental diets. To keep constant the fat content and the levels of MUFA (within each MUFA level) and PUFA of the diets, while increasing their content in CLA, the formulation of the feeds necessarily implied the decrease in the content of the saturated fat sources (palm oil and hydrogenated palm stearin). Thus, the proportion of total SFA in experimental diets decreased with increasing proportions of CLA.

CLA Isomers in Subcutaneous Fat. The fatty acid profile of subcutaneous fat from finished pigs (S3) fed diets containing different levels of CLA and MUFA is shown in **Table 2**. The use of *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA in pig feeding led to increasing levels of both CLA isomers in subcutaneous fat ($p = 0.000$), the extent of accumulation being higher with the level of dietary CLA. Neither MUFA level nor the interaction CLA × MUFA showed any effect on the observed accumulation of any of the two detected CLA isomers in the adipose tissue. Therefore, accumulation of CLA in pig backfat seems to be independent of dietary MUFA supplementation. As far as we know, the effect of the fatty acid profile of the diet on that of pig tissues when CLA is supplemented to pigs has not been thoroughly studied. In a previous work (unpublished data), we also found a lack of effect of MUFA and its interaction with CLA on the accumulation of CLA isomers in the intramuscular fat of pigs fed different levels of CLA and MUFA. Gatlin et al. (*14*) did not find a different CLA accumulation in belly fat from pigs fed CLA-enriched diets when combined with either saturated (tallow) or less saturated (yellow grease) fat sources.

The accumulation of *cis*-9,*trans*-11 CLA was slightly higher than that of *trans*-10,*cis*-12 CLA. This finding might suggest that the *trans*-10,*cis*-12 CLA isomer would either be less efficiently incorporated or have a different metabolism in subcutaneous fat. This different accumulation of CLA isomers has been also reported for other tissues in pigs (*15, 16*), broilers

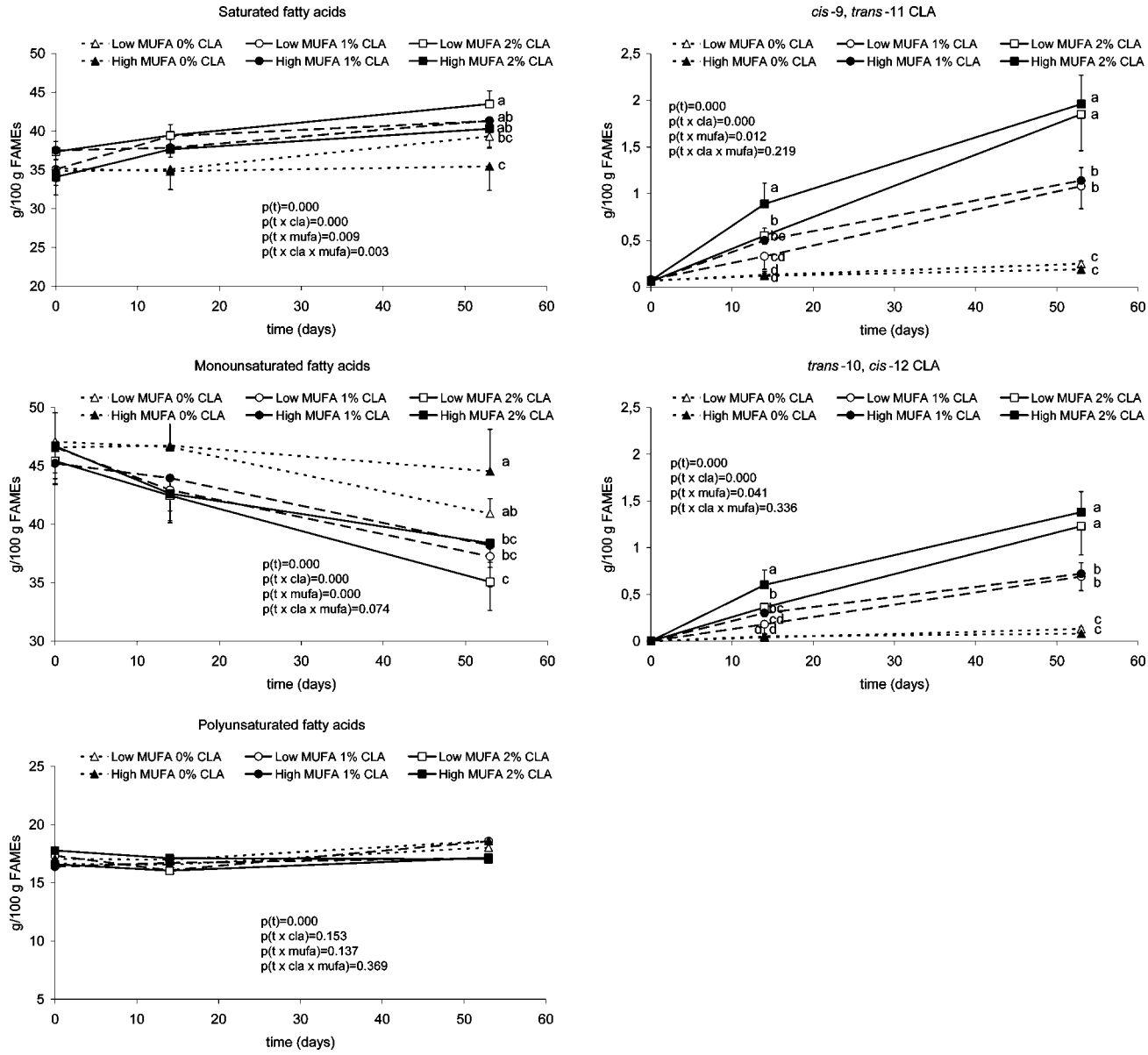

Figure 1. Changes in the main fatty acid groups and CLA isomers of subcutaneous fat of swine throughout fattening.

(*17*), and mice (*18*). Nevertheless, other authors (*19*) have found the opposite trend in adipose tissue of pigs, the *trans*-10,*cis*-12 CLA isomer reaching higher levels than the *cis*-9,*trans*-12 CLA one.

The evolution of the level of the CLA isomers in subcutaneous backfat of pigs throughout the fattening is shown in **Figure 1**. The levels of CLA isomers at the beginning of the trial (S1) were the same for all treatments (0.07% of average for *cis*-9,*trans*-11 CLA and not detected in the case of *trans*-10,*cis*-12 CLA). Subsequently, there was an increasing accumulation of *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA [$p(t) = 0.000$ for both isomers] in subcutaneous adipose tissue. Such deposition throughout the time of feeding was different as a consequence of the level of dietary CLA [$p(t \times \text{CLA}) = 0.000$ for both isomers]. Thus, the higher the dietary CLA level, the greater the accumulation of CLA isomers during the feeding. In most scientific studies, a dose-dependent accumulation of CLA in animal tissues has been demonstrated (*15*, *20*). In addition, the present work reveals that this dose-dependent accumulation of CLA with dietary CLA seems to remain during the length of the feeding. Nevertheless, the linearity of the accumulation of CLA isomers versus the time of feeding was affected by the dietary MUFA level [$p(t \times \text{MUFA}) = 0.012$ for *cis*-9,*trans*-11 CLA and $p(t \times \text{MUFA}) = 0.041$ for *trans*-10,*cis*-12 CLA]. Thus, curiously, when CLA was combined with high MUFA levels in experimental diets, the deposition of both CLA isomers in backfat tissue with the time of feeding did not seem to follow a linear behavior, because the accumulation of CLA isomers was higher in the first period evaluated (from S1 to S2, 70–80 kg) than in the last one (from S2 to S3, 80–107 kg). This was observed for all assayed CLA levels. In fact, the estimated rates of accumulation (average Δ % CLA/days) were 0.031 from S1 to S2 and 0.016 from S2 to S3 for *cis*-9,*trans*-11 CLA and 0.022 from S1 to S2 and 0.011 from S2 to S3 for *trans*-10,*cis*-12 CLA. However, in low-MUFA treatments, the increase in CLA isomers in adipose tissue throughout the fattening seemed to follow a linear behavior. Thus, the estimated rates of accumulation were 0.019 from S1 to S2 and the same value from S2 to S3 for *cis*-9,*trans*-11 CLA, and 0.014 from S1 to S2 and 0.013 from S2 to S3 for *trans*-10,*cis*-12 CLA. Therefore, although MUFA supplementation did not affect the final CLA enrichment (S3, **Table 2**), the MUFA level of the diet seemed to determine the rate of accumulation of CLA isomers in subcutaneous adipose tissue throughout the feeding.

Table 3. Desaturase Indices of Subcutaneous Adipose Tissue from Slaughtered Pigs Fed Different Levels of CLA and MUFA ($n = 8$)[a]

	low MUFA			high MUFA				p		
	0% CLA	1% CLA	2% CLA	0% CLA	1% CLA	2% CLA	SEM	CLA	MUFA	CLA × MUFA
Δ5 (C20:4 n-6/C20:3 n-6)	2.38	2.21	2.24	2.30	2.16	2.20	0.04	0.155	0.614	0.992
Δ6 (C18:3 n-6/C18:2 n-6)	0.005	0.005	0.005	0.006	0.005	0.005	0.00	0.049	0.218	0.560
Δ9 (C14:1 n-5/C14:0)	0.011a	0.007ab	0.007b	0.009ab	0.008ab	0.007b	0.00	0.002	0.520	0.373
Δ9 (C16:1 n-7/C16:0)	0.08a	0.06c	0.06c	0.07ab	0.06bc	0.06abc	0.00	0.000	0.486	0.544
Δ9 (C18:1 n-9/C18:0)	2.81ab	2.27c	1.93c	3.17a	2.32bc	2.35bc	0.08	0.000	0.005	0.221

[a] Different letters within the same row indicate significant difference ($p \leq 0.05$).

There is little information about the rate of deposition of dietary CLA isomers through the fattening of pigs. Similarly to our results, D'Arrigo et al. (*21*) found a linear plus quadratic response in the accumulation of n-3 fatty acids into subcutaneous fat of pigs fed diets with different n-3 fatty acid contents. Thus, there was a linear increase in the amount of these fatty acids, and afterward, this amount plateaued. In our experiment, there was a significantly lower accumulation of CLA isomers in the last period of fattening at high-MUFA diets, which points to a final plateau value that was not finally reached.

SFA, MUFA, and PUFA in Subcutaneous Fat. The total content in SFA of subcutaneous fat of finished pigs (S3) was determined by the level of dietary CLA ($p = 0.000$) (**Table 2**). Thus, the higher the dietary CLA content, the higher the SFA proportion of backfat tissue. This was mainly due to the significant increases in the proportions of palmitic acid (C16:0) (for high-MUFA diets) and stearic acid (C18:0) (for both high- and low-MUFA diets) with the level of CLA fed. Moreover, dietary CLA also caused an increase in the proportions of minor SFA, such as lauric acid (C12:0) or myristic acid (C14:0). On the contrary, the total content of MUFA in backfat samples from finished pigs was significantly lowered by dietary CLA level ($p < 0.05$). This was mainly due to significant decreases in the contents of palmitoleic acid (C16:1 n-7) and oleic acid (C18:1 n-9). Minor MUFA, such as heptadecenoic acid (C17:1 n-7) and eicosenoic acid (C20:1 n-9), were also lowered by CLA supplementation. Dietary CLA also affected the total content of PUFA (excluding CLA isomers) of backfat tissue ($p = 0.027$). Thus, a trend to a lower PUFA proportion with increasing levels of CLA supplementation was observed (no significant differences by Tukey's test). Dietary CLA significantly decreased the contents of linolenic acid (C18:3 n-6), eicosadienoic acid (C20:2 n-6), eicosatrienoic acid (C20:3 n-6), and arachidonic acid (C20:4 n-6).

The increase in SFA and the decrease in MUFA and PUFA contents of subcutaneous adipose tissue of CLA-fed pigs have been also found by other authors (*19*). The inhibition of the Δ9 desaturase by CLA (*22–24*) has been suggested as the main reason explaining the modifications in total SFA and MUFA contents obtained in most of the studies. Thus, the estimation of the ratios C18:1/C18:0 or C16:1/C16:0 has been used by several authors as a tool for predicting the desaturase enzyme activity in response to dietary CLA (*22, 23*). On the other hand, the inhibitory effect of dietary CLA on other desaturase activities could be also the reason explaining the observed decrease in some PUFA contents. Therefore, Δ5, Δ6, and Δ9 desaturase indices were estimated according to their product/precursor ratios, as can be observed in **Table 3**. Dietary CLA significantly depressed all Δ9 indices. Thus, regardless the dietary MUFA level, at 2% CLA, such decreases were 33.7% for Δ9 (C14:1 n-5/C14:0), 22.2% for Δ9 (C16:1 n-7/C16:0), and 28.3% for Δ9 (C18:1 n-9/C18:0) indices with respect to the values at 0% dietary CLA. Dietary CLA also seemed to affect the Δ6 index ($p = 0.049$), but no differences were found by Tukey's test. Dietary CLA, MUFA, and their interaction were not involved in the Δ5 index of subcutaneous adipose tissue. Therefore, the modification of the fatty acid profile of pig adipose tissue due to dietary CLA seems to be related to a likely inhibition of the desaturation of major fatty acids, such as C18:2 n-6, C16:0, or C18:0. Curiously, the highest depression of the desaturase index was observed for C14:0, a minor fatty acid. Furthermore, our findings reveal that this inhibitory effect of CLA seems to be independent of the MUFA level of the diet, as the lack of interaction CLA × MUFA showed.

MUFA supplementation significantly affected the contents of total SFA and MUFA of subcutaneous adipose tissue (**Table 2**). Thus, the backfat from pigs fed the high-MUFA experimental diets reached the highest proportions of MUFA and the lowest of SFA (40.35% of MUFA and 39.04% of SFA, average value regardless of CLA level), whereas those animals fed the low MUFA treatments showed the lowest MUFA and the highest SFA contents (37.74% of MUFA and 41.37% of SFA, average value regardless of CLA level). Furthermore, the combination of CLA and MUFA significantly affected the achieved values of total SFA in backfat tissue ($p = 0.010$). Thus, the highest SFA content (43.48%) was obtained by combining 2% CLA with low-MUFA diets, whereas the lowest content of SFA (35.46%) was observed in the case of high-MUFA diets without CLA supplementation. Total PUFA content of subcutaneous adipose tissue was not affected by the MUFA level of the diets or by the interaction CLA × MUFA.

Therefore, the obtained results show that dietary CLA modifies the fatty acid profile of subcutaneous fat toward an increase in the ratio of saturated to unsaturated fats. This could be desirable from a technological point of view, because less fluid and more consistent lards are considered of higher quality. In addition, pig fats less prone to lipid oxidation could be obtained, which could lead to a lower formation of derived toxic reactive compounds (*6*). On the contrary, it would be less suitable from the human nutritional standpoint, due to the negative implication of a high consumption of saturated fats in cardiovascular diseases (*5*). Nevertheless, our findings show that the decrease in MUFA content in adipose tissue caused by dietary CLA could be counteracted by the increase in the MUFA level of pig diets. Thus, when 2% dietary CLA was combined with high MUFA levels in the swine feeds, the MUFA proportion of adipose tissue was 9.4% higher than in the case of low-MUFA diets with 2% CLA. Furthermore, the achieved MUFA and CLA enrichments of subcutaneous adipose tissue would balance the ratio of saturated to unsaturated fatty acids from a nutritional point of view. Moreover, the final enrichment in CLA of backfat would not be negatively affected by the inclusion of high MUFA levels in the pig diets; on the contrary, MUFA supplementation seems to favor the rate of deposition of CLA isomers throughout the feeding.

The evolution of the accumulation of total SFA, MUFA, and PUFA in subcutaneous adipose tissue of pigs throughout the trial is shown in **Figure 1**. Levels of SFA and MUFA in backfat

were similar at the beginning of the trial (S1) for all treatments (35.7% average for SFA and 46.3% for MUFA). Subsequently, an increase in SFA and a parallel decrease in MUFA contents throughout the fattening were observed [$p(t) = 0.000$ for SFA and MUFA]. This increase/decrease of SFA/MUFA was conditioned by the levels of dietary CLA [$p(t \times \text{CLA}) = 0.000$ for SFA and MUFA]. Thus, the higher the level of CLA supplementation, the higher the increase/decrease of SFA/MUFA throughout the feeding. However, these trends in the contents of SFA and MUFA throughout the trial did not seem to follow a linear behavior, similarly to the observed results for the accumulation of CLA isomers. In the case of SFA and MUFA, dietary CLA was the factor involved in the different rates of deposition of SFA and MUFA throughout the feeding. Thus, the rates of accumulation of SFA and MUFA were proportionately higher for the first 14 days of the trial and, after that, these rates decreased. At 2% CLA, the rates of increase in SFA were 0.202 from S1 to S2 and 0.086 from S2 to S3, and the rates of decrease in MUFA were −0.251 from S1 to S2 and −0.150 from S2 to S3. On the contrary, when no CLA was included in the pig diets, the rates of increase/decrease in SFA/MUFA throughout the trial were higher in the second evaluated period (from S2 to S3) than in the first one (from S1 to S2). The rates of increase in SFA at 0% CLA were −0.004 from S1 to S2 and 0.063 from S2 to S3, and the decreasing rates in the case of MUFA were −0.011 from S1 to S2 and −0.102 from S2 to S3.

Dietary MUFA level was also involved in the evolution of SFA and MUFA accumulation in subcutaneous fat throughout the trial [$p(t \times \text{MUFA}) = 0.009$ for SFA and $p(t \times \text{MUFA}) = 0.000$ for MUFA]. Moreover, the interaction CLA × MUFA affected the SFA deposition throughout the fattening [$p(t \times \text{CLA} \times \text{MUFA}) = 0.003$]. Thus, in agreement with the composition of the diets, the highest increase in SFA during the pig fattening took place in animals fed the low-MUFA diets, whereas high-MUFA diets caused the lowest increase in SFA in subcutaneous adipose tissue throughout the pig feeding. The PUFA content (excluding CLA isomers) of subcutaneous adipose tissue also changed throughout the trial [$p(t) = 0.000$] (**Figure 1**). These fatty acids increased from 16.95% (average value at S1) to 17.75% at the end of the fattening (average value at S3). In this case, dietary CLA, MUFA, and their interactions were not involved in the evolution of the accumulation of PUFA in backfat.

These findings reveal for the first time that CLA and MUFA supplementation of pig diets seems to affect the rate of accumulation of CLA isomers, SFA, and MUFA in subcutaneous adipose tissue during pig feeding. Thus, the rate of deposition of CLA isomers seems to be conditioned by the MUFA level of the diets, whereas the level of CLA supplementation seems to affect the rate of accumulation of SFA and MUFA in backfat. This knowledge is of interest because it would allow a prediction of the fatty acid profile of pig backfat reached at slaughter depending on the CLA inclusion level, the MUFA supplementation, and the length of the fattening period with a specific diet. Therefore, all of these factors should be taken into account to establish the most correct strategies in the CLA supplementation practice for obtaining the desired results with regard to the fatty acid profile.

ABBREVIATIONS USED

CLA, conjugated linoleic acid; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; FAMEs, fatty acid methyl esters; SEM, standard error of the mean; ANOVA, analysis of variance; BHT, butylhydroxytoluene.

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