Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 108 (2008) 86–96
Food Chemistry
www.elsevier.com/locate/foodchem

Fatty acid composition and oxidative susceptibility of fresh loin and liver from pigs fed conjugated linoleic acid in combination with monounsaturated fatty acids

Diana Martin, Elena Muriel, Teresa Antequera, Trinidad Perez-Palacios, Jorge Ruiz *

Tecnologia de Alimentos, Facultad de Veterinaria, Universidad de Extremadura, Avda. Universidad s/n, 10071 Caceres, Spain

Received 9 July 2007; received in revised form 27 August 2007; accepted 18 October 2007

Abstract

Three levels (0%, 1% and 2%) of conjugated linoleic acid (CLA) were combined with two levels of monounsaturated fatty acids (MUFA) (low: 19% and high: 39%) for pig feeding. The fatty acid profile of neutral lipids (NL) and polar lipids (PL) of loin and liver and their oxidative susceptibility were studied. A dose-dependent enrichment in *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in NL and PL of loin and liver was obtained. This effect was independent of the MUFA supplementation (except for NL of loin in which the CLA accumulation was higher at high MUFA levels). Regardless of the MUFA supplementation, dietary CLA increased the ratio of saturated fatty acids (SFA) to unsaturated fatty acids in both tissues and lipid fractions. The interaction between CLA and MUFA affected the SFA and polyunsaturated fatty acids contents of PL from loin. Regardless of the MUFA level of the diets, CLA supplementation decreased the induced peroxidation values in liver and did not change those of loin.

Keywords: Conjugated linoleic acid; Monounsaturated fatty acids; Oxidation; Loin; Liver; Pig

1. Introduction

Feeding pigs with conjugated linoleic acid (CLA) has been suggested as a potential strategy for obtaining meat and meat products enriched with CLA, since the subsequent accumulation of CLA in pig tissues supplemented with this fatty acid has been reported in most studies (reviewed by Schmid, Collomb, Sieber, & Bee, 2006). The *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 are the main isomers of CLA in commercial CLA-enriched oils used in pig feeding and the predominant isomers found in meat products (Dhiman, Nam, & Ure, 2005). The *cis*-9, *trans*-11 CLA is usually found at higher proportions in animal tissues than the *trans*-10, *cis*-12 CLA, and the biological activity of both isomers has been pointed out to be also different (Dhiman et al., 2005).

CLA feeding has also an effect on the fatty acid composition of animal tissues. Due to an inhibitory effect of CLA isomers on desaturase activity (Lee, Pariza, & Ntambi, 1998), an increase in saturated fatty acids (SFA) and a parallel decrease in monounsaturated fatty acids (MUFA) occur in pig tissues. This leads to less fluid and more consistent lards, which are considered as positive quality characteristics by meat processors (Ruiz & López-Bote, 2002). However, the increase in the ratio of saturated to unsaturated fatty acids could have negative health implications (Department of Health, 1994). Including high levels of MUFA in pig diets when using dietary CLA could be a strategy for counteracting the decrease in MUFA caused by CLA. However, as far as we know, the interaction between CLA and MUFA levels in pig diets on the subsequent CLA isomers accumulation, as well as on the rest of the fatty acid profile of pig tissues, has not been thoroughly considered.

The biological effects attributed to CLA isomers, such as anti-carcinogenic (Pariza & Hargraves, 1985),

* Corresponding author. Tel.: +34 927 257123; fax: +34 927 257110.
E-mail address: jruiz@unex.es (J. Ruiz).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.10.048

anti-atherosclerotic (Lee, Kritchevsky, & Pariza, 1994), immune system enhancing (Haro, Artacho, & Cabrera-Vique, 2006) or body fat reducing (Navarro, Fernández-Quintela, Churruca, & Portillo, 2006) might counteract some of the health risks associated with a higher consumption of SFA. The likely role of CLA isomers as antioxidants has been suggested to explain their beneficial effects in some of these pathologies. CLA was first reported to reduce lipid peroxide formation in a linoleic acid model system (Ha, Storkson, & Pariza, 1990). Later, Ip, Chin, Scimeca, and Pariza (1991) found that dietary CLA decreased the concentrations of thiobarbituric acid reactive substances (TBARS) in rat liver and mammary gland tissues. However, the claimed antioxidant effect of CLA remains still inconclusive, since pro-oxidant effect of CLA isomers or no implication of this fatty acid in oxidative processes have been also reported (Hur, Park, & Joo, 2006). Moreover, due to the polyunsaturated nature of CLA, it could be hypothesised that there should be a higher oxidative susceptibility of tissues containing CLA. Thus, the inclusion of CLA in pig diets enriched in MUFA and the effect on the susceptibility to lipid oxidation of the derived meat and meat products should be more carefully examined.

Therefore, the present work aimed to study the effect of combination of different levels of CLA and MUFA on pig diets on the fatty acid profile and oxidative susceptibility of the fat from the fresh meat and liver of pigs.

2. Materials and methods

2.1. Animals and feeding

Three levels (0%, 1% and 2%) of commercial enriched CLA oil supplement (CLA-60, BASF, Dortmund), 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 feedings (Table 1). All diets were formulated to provide similar protein and energy levels, fulfilling the advised nutritional needs for gilts at considered ages by the National Research Council (NRC, 1998).

The experiment was conducted using 288 finishing gilts (Large white ♂ × Landrace × Large white ♀). Pigs weighing 70 kg and at about 140 days of age were randomly allotted to the 6 different feeding treatments in 4 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) till a final average weight of 107 kg. After fattening (53 days), pigs were slaughtered at a local slaughterhouse by electrical stunning and exsanguination.

2.2. Sampling

Representative samples of mixed diets were taken before the beginning of the trial, in order to determine their chemical and fatty acid composition. The analysis of the composition of the feeds was performed according to the Association of Official Analytical Chemists (AOAC, 2000): nitrogen content (reference 976.05), crude protein (reference 954.01), crude fat (reference 920.39), crude fibre (reference 962.09) and ash (reference 942.05). Feed analysis is shown in Table 1.

Eight animals from each treatment were randomly selected for sampling. Samples from slaughtered animals were taken within 10 min after bleeding. The whole loin and liver from pigs was vacuum packaged and frozen at −80 °C until required.

Table 1
Ingredients and chemical composition of the experimental treatments

	Low MUFA feed			High MUFA feed		
	0% CLA	1% CLA	2% CLA	0% CLA	1% CLA	2% CLA
Ingredient (%)						
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-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 fibre	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
∑SFA	59.7	52.0	43.5	38.8	28.4	20.6
∑MUFA	18.8	18.6	19.2	38.9	38.8	38.7
∑PUFA[a]	21.5	21.9	21.5	22.4	24.4	24.7

[a] Excluding CLA isomers.

2.3. Fatty acid analysis

Total lipids of feeds, loin and liver were extracted with chloroform:methanol (2:1 v/v) according to the method of Folch, Lees, and Stanley (1957). After solvent evaporation under nitrogen, the neutral lipid (NL) and the polar lipid (PL) fractions were isolated according to the method of Ruiz, Antequera, Andres, Petron, and Muriel (2004) using NH_2-aminopropyl cartridges. Fatty acid methyl esters (FAMEs) from lipid fractions were obtained by acidic transesterification, following the method described by Sandler and Karo (1992). FAMEs were analysed by gas chromatography, using a Hewlett-Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionisation detector (FID). Separation was carried out on a polyethylene glycol capillary column (60 m × 0.32 mm i.d. × 0.25 μm film thickness; Supelcowax-10, Supelco, Bellefonte, PA). Oven temperature started at 180 °C. Immediately, it was raised at 5 °C/min till 200 °C; held for 40 min at 200 °C and, increased again at 5 °C/min till 250 °C and held for 21 min at 250 °C. Injector and detector temperatures were 250 °C. Carrier gas was nitrogen at a flow rate of 0.8 ml/min. Individual compounds were identified by comparing their retention times with those of standards (Sigma, St. Louis, MO). Results were expressed as grams of each fatty acid per 100 g of FAMEs detected.

2.4. Measurement of induced lipid oxidation

The susceptibility of muscle and liver tissue homogenates to iron-ascorbate-induced lipid oxidation was determined according to the method of Kombrust and Mavis (1980). Homogenates (1 mg/ml buffer) were incubated at 37 °C in 80 mM tris–malate buffer (pH 7.4) with 5 mM $FeSO_4$ and 2 mM of ascorbic acid in a total volume of 10 ml. At fixed time intervals (0, 50, 100 and 200 min), 1 ml aliquots were removed for measurement of TBARS. TBARS were expressed as nmol malondialdehyde (MDA)/mg protein. Protein was measured by the procedure of Lowry, Rosebrough, Farr, and Randall (1951).

2.5. 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 (Chicago, Il). The effect of CLA and MUFA content of diets and their interaction on the fatty acid profile of NL and PL fractions of loin and liver of pigs was evaluated by a two-way analysis of variance. The effect of the assayed factors on the induced peroxidation values of the samples over reaction time was evaluated by a three-way mixed model repeated-measures (RM) test; CLA, MUFA and CLA × MUFA levels being the between-subject effects and time of induction the within-subject effect. Differences were considered significant at $p \leqslant 0.05$. When the effect of any of the factors was significant, differences between groups were analysed by the Tukey's *post-hoc* test.

3. Results and discussion

3.1. Neutral lipids of loin

The fatty acid profile of the NL fraction of loin from pigs fed different levels of CLA and MUFA is shown in Table 2. A significant dose-dependent enrichment in *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA of NL from loin was obtained ($p = 0.000$ for both isomers). Moreover, the content in both CLA isomers in NL was affected by the dietary MUFA level ($p = 0.002$ for *cis*-9, *trans*-11 CLA and $p = 0.001$ for *trans*-10, *cis*-12 CLA). Thus, regardless of the dietary CLA level, high MUFA supplementation of the pig diets resulted in a higher proportion of both CLA isomers in NL of loin. Furthermore, the content in *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA of NL of intramuscular fat was conditioned also by the interaction CLA × MUFA ($p = 0.000$ for both isomers). Thus, the highest CLA accumulation in NL was obtained in the case of feeding pigs with 2% CLA combined with high MUFA dietary levels. No previous studies about the implication of the dietary MUFA level on the accumulation of CLA isomers in the NL of pork when supplementing CLA to pig diets have been found.

The incorporation of *cis*-9, *trans*-11 CLA on NL of loin was higher than that of the *trans*-10, *cis*-12 CLA isomer. This was observed for all the CLA levels assayed and regardless of the dietary MUFA content. Nevertheless, the ratio *cis*-9, *trans*-11/*trans*-10, *cis*-12 CLA significantly decreased ($p = 0.000$) at increasing doses of CLA in the diets (ratio of 3.1 at 0% CLA, 2.4 at 1% CLA and 2.1 at 2% CLA). The different proportion of CLA isomers has been also reported in other studies in total lipids of pig (Tischendorf, Mockel, Schone, Plonne, & Jahreis, 2002), broiler (Simon, Männer, Schäfe, Sagredos, & Eder, 2002) and mice tissues (Park et al., 1999). In triacylglycerols, DeDeckere, Amelsvoort, McNeill, and Jones (1999), and Watkins et al. (2003) also found a higher incorporation of *cis*-9, *trans*-11 CLA than *trans*-10, *cis*-12 isomer in hamsters tissues and egg yolk, respectively. The less efficient incorporation of *trans*-10, *cis*-12 CLA isomer or its more intensive metabolism have been pointed out as the reason for explaining the lower content of this isomer in animal tissues.

Total contents of SFA and MUFA from NL of loin were significantly modified by the level of CLA used in the pig feeding ($p = 0.000$) (Table 2). Thus, regardless of the MUFA level of the pig diets, SFA proportion of loin was higher in the presence of CLA than in the case of no CLA supplementation. This increase in total SFA content by dietary CLA was mainly due to the significant effect on palmitic acid (C16:0) and stearic acid (C18:0). Moreover, the content in minor SFA such as myristic acid (C14:0), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0), were also affected by dietary CLA. On the contrary, total MUFA proportion of NL

Table 2
Fatty acids of NL from loin of pigs fed different levels of CLA and MUFA (g/100 g FAME)[A]

	Low MUFA			High MUFA			SEM	*p*		
	0% CLA	1% CLA	2% CLA	0% CLA	1% CLA	2% CLA		CLA	MUFA	CLA × MUFA
C14:0	1.08^{b}	1.28^{ab}	1.38^{a}	1.07^{b}	1.30^{ab}	1.33^{ab}	0.03	0.001	0.739	0.891
C14:1 *n*-5	0.17	0.10	0.12	0.15	0.12	0.11	0.01	0.072	0.950	0.598
C15:0	0.05	0.05	0.05	0.04	0.04	0.04	0.00	0.527	0.120	0.852
C16:0	26.10^{ab}	28.12^{a}	27.98^{ab}	25.49^{b}	27.88^{ab}	27.12^{ab}	0.28	0.002	0.258	0.876
C16:1 *n*-7	3.32	3.19	3.40	3.08	2.98	3.48	0.10	0.385	0.574	0.787
C17:0	0.24	0.24	0.22	0.21	0.23	0.23	0.01	0.552	0.406	0.376
C17:1 *n*-7	0.25^{a}	0.18^{b}	0.19^{ab}	0.23^{ab}	0.19^{ab}	0.18^{b}	0.01	0.001	0.653	0.605
C18:0	13.50	14.63	16.65	13.87	15.96	16.29	0.35	0.004	0.492	0.567
C18:1 *n*-9	43.83^{ab}	40.61^{abc}	39.22^{b}	44.55^{a}	40.32^{abc}	39.61^{ab}	0.50	0.000	0.744	0.881
C18:2 *n*-6	8.67	9.46	7.81	8.63	8.80	8.32	0.26	0.255	0.903	0.660
C18:3 *n*-6	0.08	0.05	0.05	0.06	0.06	0.05	0.00	0.052	0.566	0.218
C18:3 *n*-3	0.50	0.59	0.49	0.56	0.56	0.53	0.02	0.304	0.563	0.564
C20:0	0.15^{b}	0.16^{b}	0.21^{a}	0.15^{b}	0.14^{b}	0.17^{ab}	0.01	0.001	0.041	0.282
C20:1 *n*-9	0.64	0.63	0.64	0.65	0.63	0.67	0.01	0.437	0.375	0.872
C20:2 *n*-6	0.30	0.29	0.30	0.31	0.31	0.30	0.01	0.874	0.640	0.836
C20.3 *n*-6	0.18^{a}	0.09^{b}	0.09^{b}	0.16^{ab}	0.12^{ab}	0.16^{ab}	0.01	0.004	0.100	0.070
C20:4 *n*-6	1.12^{a}	1.10^{a}	0.19^{b}	0.68^{ab}	0.62^{ab}	0.84^{ab}	0.08	0.070	0.583	0.003
C21:0	0.07^{b}	0.07^{b}	0.07^{b}	0.07^{ab}	0.09^{a}	0.08^{ab}	0.00	0.086	0.001	0.119
C20:5 *n*-3	0.05^{abc}	0.11^{a}	0.01^{c}	0.03^{bc}	0.09^{abc}	0.10^{ab}	0.01	0.008	0.367	0.006
C22:0	0.01^{ab}	0.01^{ab}	0.01^{b}	0.01^{c}	0.01^{b}	0.02^{a}	0.00	0.002	0.063	0.000
C22:1 *n*-9	0.02^{a}	0.01^{ab}	0.01^{ab}	0.01^{b}	0.01^{ab}	0.02^{a}	0.00	0.087	0.373	0.004
C22:5 *n*-3	0.22^{a}	0.07^{b}	0.09^{b}	0.11^{b}	0.13^{b}	0.14^{ab}	0.01	0.007	0.873	0.000
C24:0	n.d.	n.d.	n.d.	n.d.	0.01	0.01	–	–	–	–
C22:6 *n*-3	0.10^{a}	0.03^{b}	0.04^{b}	0.06^{ab}	0.06^{ab}	0.10^{a}	0.01	0.021	0.184	0.000
C24:1 *n*-9	0.04^{a}	0.02^{b}	0.03^{ab}	0.01^{c}	0.02^{bc}	0.03^{b}	0.00	0.043	0.000	0.000
cis-9, *trans*-11 CLA	0.22^{d}	0.48^{c}	0.70^{b}	0.15^{d}	0.56^{c}	0.93^{a}	0.04	0.000	0.002	0.000
trans-10, *cis*-12 CLA	0.08^{d}	0.20^{c}	0.32^{b}	0.04^{d}	0.24^{c}	0.48^{a}	0.02	0.000	0.001	0.000
∑SFA	41.20^{bc}	44.56^{abc}	46.57^{a}	40.90^{c}	45.65^{a}	45.27^{ab}	0.49	0.000	0.831	0.473
∑MUFA	48.27^{a}	44.73^{ab}	43.61^{b}	48.68^{a}	44.28^{ab}	44.09^{ab}	0.52	0.000	0.871	0.892
∑PUFA[B]	11.23	11.76	9.09	10.61	10.75	10.53	0.34	0.205	0.928	0.296

n.d. (not detected).
[A] Different letters within the same row differed significantly ($p \leqslant 0.05$).
[B] Excluding CLA isomers.

of loin was significantly lower ($p < 0.05$) in the presence of dietary CLA, regardless of the MUFA level of the pig diets. This was mainly due to the significant decrease ($p < 0.05$) in the content of oleic acid (C18:1 *n*-9). The content of minor MUFA, such as heptadecenoic acid (C17:1 *n*-7) or nervonic acid (C24:1 *n*-9), also decreased due to dietary CLA. Total PUFA content of NL from intramuscular fat of loin (excluding CLA isomers) was not affected by dietary CLA, MUFA or the interaction of both factors.

It is interesting to point out that the linear accumulation of CLA isomers in NL with increasing levels of dietary CLA does not seem to be observed in the case of increase/decrease in SFA/MUFA with dietary CLA, since the effect of CLA on the accumulation of these lipid fractions was higher from 0% to 1% than from 1% to 2% CLA levels in the pig diets. This suggests that the effect of dietary CLA on increasing/decreasing SFA/MUFA contents seems to reach saturation at high doses of CLA in the pig diets and higher levels of CLA would not affect proportional higher or lower levels of SFA and MUFA, respectively. This would be of interest, since high levels of CLA could be used in pig feeding without further detriment to SFA/MUFA ratios.

The obtained increase in SFA with a parallel decrease in MUFA contents in NL of loin by dietary CLA agrees with most of the studies found in the scientific literature for total fat of the tissues. The inhibition of Δ9-desaturase by CLA (Smith et al., 2002) has been suggested as the main reason for explaining the modifications in fatty acids. As far as we know, very little has been reported about the effect of feeding CLA on the different fatty acid fractions of intramuscular fat of pork or other experimental animals. Watkins et al. (2003) also found a decrease in MUFA and an increase in SFA contents in NL of egg yolk from CLA-fed hens. The decrease in the C18:1 *n*-9 content of NL related to CLA has been also observed by Livisay, Zhou, Ip, and Decker (2000) in rat muscles fed 2% CLA.

Concerning the effect of dietary MUFA, neither MUFA level nor the combination CLA × MUFA caused significant modifications in total SFA and MUFA contents of NL of loin (Table 2). This result could be surprising, since a higher proportion of total MUFA and lower proportion of SFA might be expected in pork fat from pigs fed a high MUFA diet. The level of MUFA supplementation in the high MUFA treatments was probably not enough for

obtaining a significant enrichment in MUFA of loin. Other authors have reported significant increases in MUFA content of intramuscular fat when supplementing elevated MUFA levels to pig diets. However, MUFA doses were much higher than those used in the present work, such as 80.9% (Miller, Shackelford, Hayden, & Reagan, 1990) or 63% (Myer et al., 1992).

Dietary MUFA level and its interaction CLA × MUFA neither affected the total PUFA content (excluding CLA isomers) of NL of loin, but a significant effect of CLA × MUFA was observed in the accumulation of several long chain fatty acids in NL, especially long chain PUFA. Thus, increasing levels of dietary CLA in low MUFA diets led to a decrease in the content in arachidonic acid (C20:4 *n*-6), eicosapentaenoic acid (C20:5 *n*-3), docosapentaenoic acid (C22:5 *n*-3) and docosahexaenoic acid (C22:6 *n*-3), but this did not happen in high MUFA treatments.

3.2. Polar lipids of loin

The fatty acid profile of the PL fraction of loin from pigs fed different levels of CLA and MUFA is shown in Table 3. Dietary CLA positively affected the *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA contents of PL from intramuscular fat ($p = 0.000$ for both CLA isomers). Contrary to NL of loin, dietary MUFA level and the interaction CLA × MUFA did not affect the deposition of CLA isomers in PL.

As was observed in the case of NL, the content of *cis*-9, *trans*-11 CLA in PL of loin was higher than the proportion of *trans*-10, *cis*-12 CLA. However, while a decrease in the ratio *cis*-9, *trans*-11/*trans*-10, *cis*-12 with increasing levels of dietary CLA was found in NL, that ratio increased at 1% dietary CLA in the case of PL (1.7 at 0% CLA, 2.0 at 1% CLA and 1.8 at 2% CLA). These results might suggest that the different proportion of accumulation of both CLA isomers would depend on the fatty acid fraction, following different behaviour for triacylglycerols and phospholipids, and the dietary CLA and MUFA levels being involved in such differences. DeDeckere et al. (1999) and Watkins et al. (2003) also showed a higher incorporation of *cis*-9, *trans*-11 CLA than *trans*-10, *cis*-12 CLA into triacylglycerols and phospholipids in tissues of hamsters and egg yolk of hens, respectively.

On the other hand, both CLA isomers accumulated at higher proportion in PL than in NL of loin. Thus,

Table 3
Fatty acids of PL from loin of pigs fed different levels of CLA and MUFA (g/100 g FAME)[A]

	Low MUFA			High MUFA			SEM	*p*		
	0% CLA	1% CLA	2% CLA	0% CLA	1% CLA	2% CLA		CLA	MUFA	CLA × MUFA
C14:0	2.23	2.01	1.50	1.73	2.08	1.95	0.12	0.515	0.979	0.269
C14:1 *n*-5	1.61	1.47	1.15	1.05	1.68	1.48	0.09	0.441	0.993	0.121
C15:0	0.06^{b}	0.06^{b}	0.05^{b}	0.11^{a}	0.07^{b}	0.06^{b}	0.00	0.001	0.001	0.046
C16:0	20.10^{a}	17.81^{ab}	16.93^{b}	18.49^{ab}	18.60^{ab}	19.54^{ab}	0.31	0.242	0.296	0.019
C16:1 *n*-7	0.52^{a}	0.42^{abc}	0.30^{c}	0.48^{ab}	0.37^{bc}	0.37^{bc}	0.02	0.000	0.936	0.082
C17:0	0.27	0.25	0.25	0.26	0.27	0.29	0.01	0.953	0.337	0.302
C17:1 *n*-7	1.39	1.31	1.05	0.47	0.79	0.62	0.13	0.788	0.017	0.711
C18:0	9.04	9.40	9.76	9.29	9.61	9.27	0.09	0.226	0.989	0.202
C18:1 *n*-9	12.18^{bc}	12.15^{bc}	10.43^{c}	15.13^{a}	14.16^{ab}	14.26^{ab}	0.35	0.147	0.000	0.394
C18:2 *n*-6	33.80^{bc}	35.68^{ab}	37.56^{a}	32.79^{c}	34.84^{abc}	33.88^{bc}	0.34	0.002	0.002	0.068
C18:3 *n*-6	0.33^{ab}	0.31^{ab}	0.26^{b}	0.36^{a}	0.27^{b}	0.27^{b}	0.01	0.000	0.954	0.281
C18:3 *n*-3	0.68^{b}	0.79^{ab}	0.79^{ab}	0.68^{b}	0.80^{ab}	0.86^{a}	0.02	0.000	0.377	0.606
C20:0	0.04	0.05	0.05	0.04	0.04	0.04	0.02	0.124	0.120	0.325
C20:1 *n*-9	0.16	0.18	0.18	0.21	0.19	0.16	0.01	0.455	0.292	0.076
C20:2 *n*-6	0.47	0.52	0.58	0.48	0.49	0.46	0.01	0.319	0.073	0.106
C20.3 *n*-6	1.37	1.36	1.27	1.41	1.15	1.19	0.03	0.117	0.224	0.290
C20:4 *n*-6	11.56^{ab}	10.90^{ab}	10.50^{ab}	11.79^{a}	9.89^{ab}	9.60^{b}	0.22	0.005	0.170	0.388
C21:0	0.11	0.10	0.12	0.11	0.11	0.09	0.01	0.828	0.335	0.026
C20:5 *n*-3	0.77	0.78	0.84	0.85	0.75	0.80	0.02	0.433	0.898	0.349
C22:0	0.01^{bc}	0.02^{abc}	0.03^{a}	0.01^{c}	0.01^{abc}	0.02^{ab}	0.00	0.000	0.247	0.896
C22:1 *n*-9	0.01	0.01	0.01	0.01	0.02	0.01	0.00	0.082	0.182	0.527
C22:5 *n*-3	1.66	1.57	1.87	1.73	1.59	1.41	0.07	0.808	0.400	0.264
C24:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–
C22:6 *n*-3	0.57	0.80	0.98	0.74	0.82	0.80	0.05	0.141	0.985	0.342
C24:1 *n*-9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–
cis-9, *trans*-11 CLA	0.17^{c}	0.84^{b}	1.46^{a}	0.15^{c}	0.81^{b}	1.36^{a}	0.08	0.000	0.184	0.759
trans-10, *cis*-12 CLA	0.10^{c}	0.42^{b}	0.80^{a}	0.10^{a}	0.41^{b}	0.78^{a}	0.04	0.000	0.701	0.992
∑SFA	31.81	29.69	28.69	30.03	30.80	31.27	0.35	0.494	0.342	0.033
∑MUFA	15.87^{a}	15.53^{ab}	13.11^{b}	17.35^{a}	17.21^{a}	16.91^{a}	0.32	0.033	0.000	0.144
∑PUFA[B]	51.20^{ab}	52.71^{ab}	54.65^{a}	50.81^{ab}	50.61^{b}	49.28^{b}	0.43	0.565	0.001	0.030

n.d. (not detected).
[A] Different letters within the same row differed significantly ($p \leqslant 0.05$).
[B] Excluding CLA isomers.

according to Sugano et al. (1997) and Kramer et al. (1998), it seems that the total incorporation of CLA in lipids differs in the lipid classes. Similar to our findings, both studies reported that CLA isomers were incorporated in a higher proportion in phospholipids.

Dietary CLA significantly affected the total content of MUFA in PL of loin ($p = 0.033$), causing its decrease mainly due to the lower content of C16:1 *n*-7 ($p = 0.000$) (Table 3). Livisay et al. (2000) and Watkins et al. (2003) also found a lower MUFA content in PL from muscle tissue of rats and from egg yolks of CLA-fed hens, respectively. Besides the decrease in C16:1 *n*-7, these authors also detected a significant decrease in C18:1 *n*-9 in PL. In our case, despite the non-significant result ($p = 0.147$), the content in C18:1 *n*-9 of PL also tended to decrease with increasing dietary CLA levels. As it was explained in the case of NL of loin, the decrease in C16:1 *n*-7 and C18:1 *n*-9 in PL of loin could be due to the inhibition of the desaturation of their precursors C16:0 and C18:0 by CLA.

The total contents in SFA and PUFA (excluding CLA isomers) of PL of loin were affected by the interaction CLA × MUFA (Table 3). Thus, when low MUFA diets were supplemented with CLA, total SFA content of PL tended to decrease (the major SFA, C16:0, significantly decreased) and total PUFA content (excluding CLA) tended to increase (the major PUFA, C18:2 *n*-6, significantly increased); whereas when high MUFA diets were supplemented with CLA, total SFA content of PL did not change and some PUFA contents decreased (C18:3 *n*-6, C20:4 *n*-6) or increased (C18:3 *n*-3). Phospholipids composition is less influenced by diet than that of triacylglycerols, as they are constituents of cell membranes. Large changes in the fatty acid profile of the cell membrane would alter membrane properties and other physiological functions. Thus, the saturated to unsaturated ratio has been reported to be highly constant in phospholipids of membranes and changes in the fatty acid composition are mainly limited to an exchange between PUFA and MUFA (Scheeder, Glaser, Eichenberger, & Wenk, 2000). Therefore, it could be thought that the observed modifications in PL of loin from pigs fed the different treatments might mainly be the result of homeostatic mechanisms, which maintain an appropriate level of membrane fluidity (Scheeder et al., 2000).

Table 4
Fatty acids of NL from liver of pigs fed different levels of CLA and MUFA (g/100 g FAME)[A]

	Low MUFA			High MUFA			SEM	*p*		
	0% CLA	1% CLA	2% CLA	0% CLA	1% CLA	2% CLA		CLA	MUFA	CLA × MUFA
C14:0	0.40	0.46	0.49	0.39	0.48	0.38	0.02	0.368	0.501	0.531
C14:1 *n*-5	0.19	0.18	0.19	0.15	0.14	0.16	0.01	0.699	0.002	0.705
C15:0	0.09	0.08	0.08	0.08	0.08	0.08	0.01	0.280	0.354	0.794
C16:0	18.68	17.39	17.41	17.00	17.96	17.22	0.30	0.776	0.484	0.323
C16:1 *n*-7	0.91	0.85	0.86	0.99	1.01	0.75	0.04	0.244	0.578	0.346
C17:0	0.50	0.44	0.46	0.46	0.44	0.50	0.01	0.419	0.974	0.501
C17:1 *n*-7	0.22^{a}	0.15^{ab}	0.16^{ab}	0.20^{ab}	0.16^{ab}	0.14^{b}	0.01	0.001	0.356	0.698
C18:0	23.01	23.00	22.21	22.99	23.37	24.41	0.47	0.967	0.390	0.619
C18:1 *n*-9	18.74	18.99	16.73	21.19	20.25	18.21	0.48	0.061	0.062	0.850
C18:2 *n*-6	18.22^{ab}	19.23^{ab}	19.27^{a}	16.96^{b}	17.75^{ab}	19.05^{ab}	0.24	0.021	0.030	0.466
C18:3 *n*-6	0.36	0.34	0.33	0.36	0.25	0.22	0.02	0.058	0.044	0.323
C18:3 *n*-3	0.93	1.04	0.72	0.76	0.82	0.76	0.05	0.227	0.202	0.454
C20:0	0.06^{c}	0.18^{a}	0.15^{ab}	0.06^{c}	0.09^{bc}	0.06^{c}	0.01	0.000	0.000	0.018
C20:1 *n*-9	0.38^{ab}	0.46^{a}	0.35^{abc}	0.35^{bc}	0.29^{bc}	0.27^{c}	0.01	0.038	0.000	0.065
C20:2 *n*-6	0.55	0.54	0.52	0.56	0.50	0.53	0.01	0.309	0.743	0.519
C20.3 *n*-6	0.51	0.44	0.38	0.49	0.42	0.41	0.02	0.050	0.918	0.818
C20:4 *n*-6	13.00	11.65	11.11	13.19	11.42	10.88	0.27	0.003	0.858	0.923
C21:0	0.17	0.15	0.20	0.13	0.17	0.22	0.01	0.021	0.970	0.261
C20:5 *n*-3	0.37	0.37	0.46	0.46	0.43	0.50	0.02	0.119	0.048	0.811
C22:0	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.723	0.019	0.449
C22:1 *n*-9	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.380	0.204	0.531
C22:2 *n*-6	0.04^{a}	0.03^{b}	0.03^{b}	0.03^{ab}	0.03^{ab}	0.02^{b}	0.01	0.000	0.401	0.584
C23:0	0.71^{a}	0.66^{ab}	0.53^{b}	0.59^{ab}	0.57^{ab}	0.52^{b}	0.02	0.007	0.037	0.320
C22:5 *n*-3	1.14	1.22	1.22	1.19	1.18	1.18	0.03	0.874	0.877	0.831
C22:6 *n*-3	0.73^{b}	0.88^{ab}	0.99^{ab}	0.95^{ab}	0.96^{ab}	1.07^{a}	0.03	0.049	0.046	0.560
C24:1	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.384	0.719	0.464
cis-9, *trans*-11 CLA	0.35^{cd}	0.92^{b}	1.87^{a}	0.29^{d}	0.78^{bc}	1.61^{a}	0.10	0.000	0.099	0.651
trans-10, *cis*-12 CLA	0.07^{cd}	0.37^{b}	0.78^{a}	0.05^{d}	0.31^{bc}	0.72^{a}	0.05	0.000	0.325	0.920
∑SFA	43.63	42.36	41.53	41.71	43.16	43.41	0.54	0.977	0.825	0.368
∑MUFA	20.49	20.67	18.32	22.91	21.88	19.57	0.51	0.055	0.102	0.846
∑PUFA[B]	35.84	35.71	35.02	34.94	33.75	34.62	0.25	0.488	0.030	0.407

n.d. (not detected).
[A] Different letters within the same row differed significantly ($p \leqslant 0.05$).
[B] Excluding CLA isomers.

3.3. Neutral lipids of liver

The fatty acid profile of the NL fraction of liver from pigs fed different levels of CLA and MUFA is shown in Table 4. Enrichment in *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA of NL from liver with increasing levels of supplemented CLA was obtained ($p = 0.000$ for both CLA isomers). The final content in both CLA isomers of NL from liver was approximately twice as high as those of NL from loin. Therefore, it seems that the deposition of CLA isomers is more favoured in NL of liver than loin. This was also observed by Tischendorf et al. (2002) in total fat of liver and muscle from CLA-fed pigs. On the other hand, contrary to NL from loin, the MUFA level of the diet or its interaction with dietary CLA did not affect the content in CLA isomers of the NL of liver.

As it was found in the case of the NL of loin, the incorporation of both CLA isomers in NL of liver followed a dose-dependent behaviour, the accumulation of *cis*-9, *trans*-11 CLA being higher than that of *trans*-10, *cis*-12 CLA. In agreement with NL of loin, the different ratio of CLA isomers in NL of liver significantly decreased ($p = 0.000$) when using CLA in the diets (ratio *cis*-9, *trans*-11/*trans*-10, *cis*-12 of 6.7 at 0% CLA, 2.6 at 1% CLA and 2.4 at 2% CLA), this effect being more marked from 0% to 1% of dietary CLA in the case of NL of liver. The MUFA level did not affect these values.

Contrary to the results found in the case of NL from loin, total SFA, MUFA and PUFA (excluding CLA) contents of NL from liver were unaffected by CLA supplementation or the interaction CLA × MUFA (Table 4). Nevertheless, dietary CLA was involved in the content of the major fatty acids of NL of liver. CLA supplementation increased the C18:2 *n*-6 proportion and decreased that of the C20:4 *n*-6. The same effect on these two fatty acids (increase and decrease, respectively, due to CLA) in total lipids of liver from pigs fed CLA was observed by Tischendorf et al. (2002). Belury and Kempa-Steczko (1997) also found a decrease in C20:4 *n*-6 in NL from mouse liver fed 1.5% CLA. The biosynthetic pathway of C20:4 *n*-6 implies the desaturation of C18:2 *n*-6 to C18:3 *n*-6 by a Δ6-desaturase enzyme and the subsequent elongation of C18:3 *n*-6 to C20:3 *n*-6. After that, a Δ5-desaturase enzyme takes part in the final formation of C20:4 *n*-6. A likely inhibitory effect of dietary CLA on the activity of the implied Δ5- and/or Δ6-desaturases might explain the lower proportion of C20:4 *n*-6 found and the higher proportion of its precursor. Furthermore, the increase in C18:2 *n*-6 in total liver from mice has been found to be related to dietary *cis*-9, *trans*-11 CLA, whereas dietary *trans*-10, *cis*-12 CLA was associated with the decrease in C20:4 *n*-6 in total liver lipids (Kelley, Bartolini, Newman, Vemuri, & Mackey, 2006), suggesting an inhibitory effect on the Δ6-desaturase activity of liver, mainly due to *cis*-9, *trans*-11 CLA, or an inhibitory effect on Δ5-desaturase activity, mainly due to *trans*-10, *cis*-12 CLA. The likely inhibitory effect of Δ6- or Δ5-desaturase activities by dietary CLA has been also hypothesised by other authors (Lo Fiego, Macchioni, Santoro, Patorelli, & Corino, 2005; Simon et al., 2002). Dietary CLA also increased other minor fatty acids contents in NL of liver, such as C21:0 and C22:6 *n*-3, and decreased C17:1 *n*-7, C20:3 *n*-6, C22:2 *n*-6 and C23:0. These opposite effects of CLA on increasing and decreasing several SFA, MUFA and PUFA in liver NL would explain the lack of effect of dietary CLA on the subsequent total contents in the different groups of fatty acids. Therefore, the obtained results suggest that the effect of CLA on fatty acids from NL of liver does not seem to follow a clear pattern. Furthermore, since no effects of dietary CLA on C16:0, C18:0 and their derivates (C16:1 *n*-7 and C18:1 *n*-9) were observed in NL of liver, the likely inhibitory effect of dietary CLA on Δ9-desaturase found in loin seems not to be relevant in the case of liver. On the contrary, a probable inhibition of Δ5- and Δ6-desaturases by CLA could be more important in the subsequent fatty acid profile of NL from liver.

With respect to the effect of MUFA level of the diets, high MUFA supplementation suggested lower accumulation of PUFA in NL from liver. This result was mainly due to the significant decrease ($p < 0.05$) in the contents of C18:2 *n*-6 and C18:3 *n*-6 at high MUFA levels in the diets.

3.4. Polar lipids of liver

The fatty acid profile of the PL fraction of liver from pigs fed different levels of CLA and MUFA is shown in Table 5. In agreement with PL of loin, a dose-dependent enrichment in *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA of PL from liver was obtained, the accumulation of the *trans*-10, *cis*-12 CLA being lower than that of the *cis*-9, *trans*-11 CLA. Similarly to NL of liver and loin, the ratio *cis*-9, *trans*-11/*trans*-10, *cis*-12 CLA of PL of liver decreased when using dietary CLA (4.2 at 0% CLA, 3.4 at 1% CLA and 3.3% at 2% CLA), but the interaction CLA × MUFA also affected these ratios in the PL of liver ($p = 0.010$). Thus, the highest ratio of CLA isomers was found at zero CLA supplementation in high MUFA diets (ratio of 4.9), the ratios for the rest of treatments being lower.

Dietary MUFA level and the interaction CLA × MUFA did not affect the deposition of CLA isomers in the PL of liver, as was found in PL of loin and in NL of liver. The proportion of both CLA isomers in liver was approximately the same in NL and PL. This might suggest that there was not a favoured distribution of CLA isomers through phospholipids in liver, as was previously observed in intramuscular fat. According to Sugano et al. (1997) and Kramer et al. (1998), the incorporation of individual CLA isomers differs not only in the lipid fractions but also in the lipid tissues. In the present work, a higher proportion of *cis*-9, *trans*-11 CLA was found in PL of liver than in PL of pork fat. On the contrary, *trans*-10, *cis*-12 CLA accumulated more efficiently in the PL of intramuscular fat than in the PL of liver. Curiously, these differences resulted in the

Table 5
Fatty acids of PL from liver of pigs fed different levels of CLA and MUFA (g/100 g FAME)[A]

	Low MUFA			High MUFA			SEM	*p*		
	0% CLA	1% CLA	2% CLA	0% CLA	1% CLA	2% CLA		CLA	MUFA	CLA × MUFA
C14:0	0.17	0.15	0.15	0.12	0.15	0.12	0.01	0.601	0.129	0.526
C14:1 *n*-5	0.11^{a}	0.09^{ab}	0.05^{b}	0.07^{ab}	0.06^{b}	0.07^{ab}	0.01	0.048	0.075	0.005
C15:0	0.04	0.04	0.04	0.04	0.05	0.04	0.01	0.617	0.773	0.079
C16:0	13.72	12.73	12.64	11.41	13.19	12.74	0.35	0.898	0.418	0.234
C16:1 *n*-7	0.33	0.31	0.29	0.32	0.33	0.25	0.01	0.117	0.570	0.554
C17:0	0.61	0.49	0.45	0.49	0.45	0.48	0.02	0.150	0.258	0.273
C17:1 *n*-7	0.12^{a}	0.09^{b}	0.08^{b}	0.10^{ab}	0.08^{b}	0.08^{b}	0.01	0.000	0.056	0.373
C18:0	31.27	32.26	32.20	34.44	32.41	32.19	0.34	0.687	0.097	0.092
C18:1 *n*-9	11.38^{bc}	11.11^{bc}	10.46^{c}	13.08^{a}	12.34^{ab}	11.91^{abc}	0.18	0.016	0.000	0.797
C18:2 *n*-6	17.02^{ab}	17.99^{a}	18.67^{a}	15.55^{b}	16.95^{ab}	17.90^{a}	0.25	0.002	0.013	0.797
C18:3 *n*-6	0.23	0.22	0.24	0.26	0.19	0.22	0.01	0.275	0.671	0.411
C18:3 *n*-3	0.50	0.49	0.49	0.49	0.52	0.52	0.01	0.979	0.564	0.822
C20:0	0.04^{c}	0.07^{b}	0.10^{a}	0.04^{c}	0.07^{b}	0.11^{a}	0.01	0.000	0.683	0.454
C20:1 *n*-9	0.12	0.12	0.10	0.12	0.12	0.11	0.01	0.163	0.671	0.684
C20:2 *n*-6	0.40	0.37	0.36	0.39	0.38	0.41	0.01	0.769	0.437	0.371
C20.3 *n*-6	0.56	0.46	0.44	0.45	0.45	0.47	0.02	0.401	0.351	0.227
C20:4 *n*-6	17.07^{ab}	16.18^{abc}	14.99^{bc}	18.07^{a}	15.72^{bc}	14.31^{c}	0.28	0.000	0.921	0.264
C21:0	0.23^{bc}	0.30^{ab}	0.32^{ab}	0.19^{c}	0.28^{b}	0.38^{a}	0.01	0.000	0.972	0.045
C20:5 *n*-3	0.52	0.60	0.73	0.64	0.63	0.69	0.02	0.045	0.402	0.359
C22:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.096	0.718	0.356
C22:1 *n*-9	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.157	0.139	0.245
C22:2 *n*-6	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.004	0.481	0.947
C23:0	0.87^{a}	0.80^{ab}	0.68^{bc}	0.65^{bc}	0.71^{abc}	0.63^{c}	0.02	0.021	0.001	0.114
C22:5 *n*-3	1.81	1.94	2.00	1.77	1.96	1.83	0.04	0.300	0.481	0.662
C22:6 *n*-3	1.33^{b}	1.71^{ab}	2.03^{a}	1.45^{b}	1.79^{ab}	1.64^{ab}	0.06	0.001	0.512	0.065
C24:1	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.772	0.701	0.894
cis-9, *trans*-11 CLA	0.20^{c}	0.98^{b}	1.64^{a}	0.15^{c}	0.81^{b}	1.69^{a}	0.09	0.000	0.313	0.277
trans-10, *cis*-12 CLA	0.06^{c}	0.29^{b}	0.49^{a}	0.03^{c}	0.25^{b}	0.59^{a}	0.03	0.000	0.673	0.092
∑SFA	46.95	46.86	46.59	47.37	47.32	46.70	0.29	0.745	0.580	0.965
∑MUFA	12.07^{bc}	11.73^{bc}	10.99^{c}	13.70^{a}	12.94^{ab}	12.43^{abc}	0.19	0.008	0.000	0.846
∑PUFA[B]	39.45	39.98	39.95	39.09	38.59	38.00	0.29	0.880	0.034	0.510

n.d. (not detected).
[A] Different letters within the same row differed significantly ($p \leqslant 0.05$).
[B] Excluding CLA isomers.

same total percent of CLA in the PL of liver and in the PL of intramuscular fat. A discrimination of *trans*-10, *cis*-12 CLA isomer as a constituent of membranes in liver or a higher rate of oxidation of this isomer in that tissue might explain the lower accumulation of this isomer in phospholipids of liver (Tischendorf et al., 2002).

Similar to the findings in PL from intramuscular fat, dietary CLA was not involved in the content of total SFA and PUFA (excluding CLA isomers) of PL from liver (Table 5), whereas CLA supplementation positively affected the total MUFA content of PL of liver ($p = 0.008$). This result was mainly due to the significant decrease ($p < 0.05$) in the content of C18:1 *n*-9. Furthermore, as was observed in the case of PL from loin and NL from liver, the content in C18:2 *n*-6 in PL of liver increased with a parallel decrease in C20:4 *n*-6 due to dietary CLA. These findings might suggest that the effect of CLA supplementation on the major fatty acids of liver (C18:2 *n*-6 and C20:4 *n*-6) seems to be independent of the lipid fraction, following similar behaviour in phospholipids and triacylglycerols from liver.

Similar to PL of loin, the MUFA level of the diet was involved in the total contents of MUFA and PUFA (excluding CLA isomers) in the PL of liver (Table 5). Thus, high MUFA supplementation implied a higher MUFA content and a lower accumulation of PUFA in PL of liver. This was mainly due to the significantly higher contents of C18:1 *n*-9 and lower contents of C18:2 *n*-6 at high MUFA treatments. The interaction CLA × MUFA did not affect the values of total SFA, MUFA and PUFA (excluding CLA isomers) of PL of liver, contrary to the case of PL in intramuscular fat.

3.5. Measurement of induced lipid oxidation

A significant increase in TBARS values in both type of samples was detected during the assay [p(time) = 0.000 for loin and liver)] (Fig. 1). Lipid oxidation was only influenced by CLA supplementation [p(time × CLA) = 0.003 for loin and p(time × CLA) = 0.011 for liver)], whereas MUFA level and interaction CLA × MUFA did not affect oxidation of the samples over the 200 min of the assay. Therefore, the results shown in Fig. 1 are only those corresponding to the effect of CLA on the induced peroxidation of loin and liver.

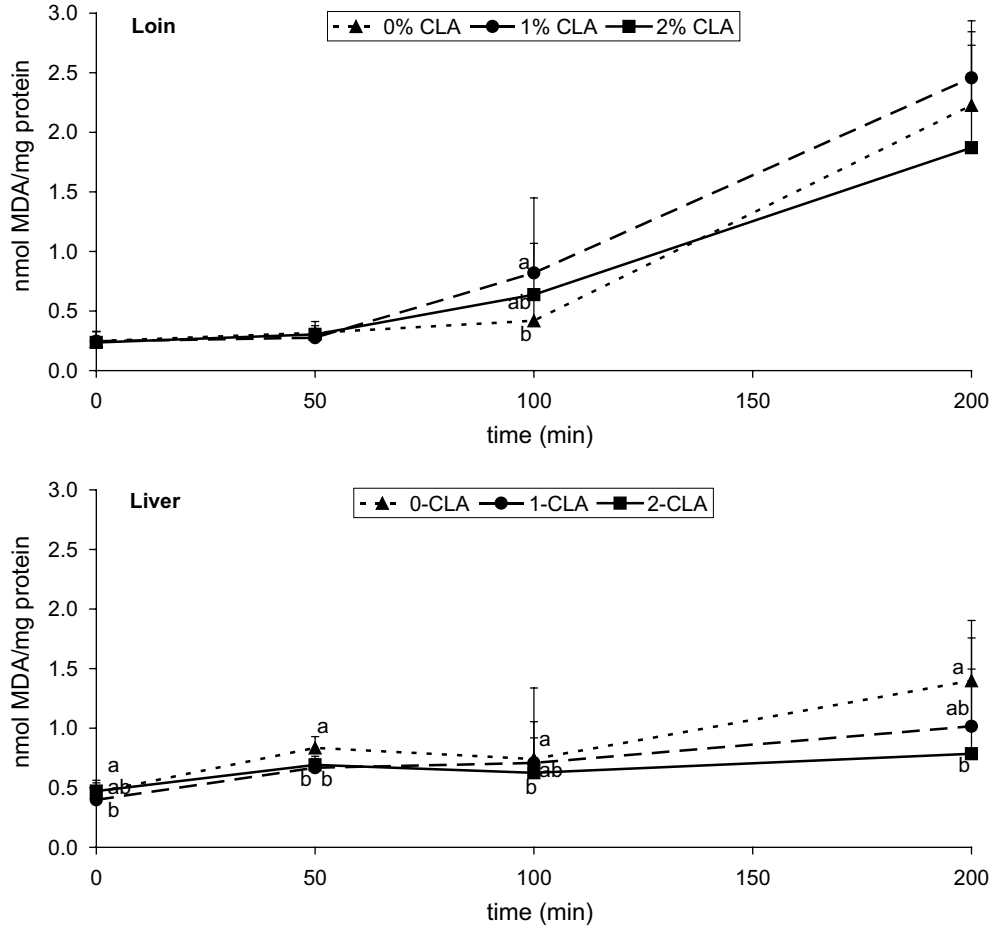

Fig. 1. TBARS values (nmol MDA/mg protein) from induced peroxidation assay of loin and liver from pigs fed different levels of CLA and MUFA.

In the case of loin, a significantly higher susceptibility to oxidation was observed after 100 min of induction when supplementing 1% CLA in pork (0.82 nmol malondialdehyde (MDA)/mg protein), whereas the lowest TBARS values were measured when no CLA was supplemented to the pig diets (0.42 nmol MDA/mg protein), the oxidation value at 2% being in between (0.64 nmol MDA/mg protein). However, no significant differences between the treatments were observed after 200 min of the trial.

In the case of liver, the highest susceptibility to oxidation was obtained when no CLA was supplemented, whereas dietary CLA resulted in a lower susceptibility to oxidation. This was observed from the beginning of the trial up to the end. The samples from pigs fed 2% CLA diets showed the lowest final oxidation values (1.40 nmol MDA/mg protein at 0% CLA and 0.78 nmol MDA/mg protein at 2% CLA), the oxidation at 1% CLA being in between (1.02 nmol MDA/mg protein).

CLA isomers have been suggested both *in vivo* and *in vitro* studies as antioxidants in many pathological situations such as cancer or atherosclerosis. This likely antioxidant role of CLA has been also suggested in foodstuffs (Hur et al., 2006). However, due to the polyunsaturated nature of CLA it could be hypothesised a higher oxidative susceptibility of samples containing CLA. In the present work, dietary CLA did not reduce oxidative susceptibility in the case of loin, but did not increase the overall oxidation process either. In the case of liver, CLA supplementation even caused a lower susceptibility to oxidation for this tissue. Therefore, the obtained results suggest that CLA could have a protective effect on susceptibility to oxidation of liver. These findings agree with those found by Livisay et al. (2000). These authors reported a significant lower MDA formation in liver microsomes from rats fed 1% CLA than in controls, while the MDA formation in muscle homogenates was not different to that of the control.

The antioxidant mechanism of CLA still remains unclear. If CLA acted as an antioxidant by a direct mechanism, the higher total content of CLA found in liver than in loin (NL plus PL) might explain the significant dietary CLA effect found in liver but not in loin. Moreover, it is known that the highest concentration of PUFA in phospholipids and their association in the cell membrane make phospholipids much more susceptible to oxidation (reviewed by Monahan et al., 1990). Since the total content of CLA in PL was the same for loin and liver in the present work, it could be possible that the higher proportion of *cis*-9, *trans*-11 CLA in the case of PL of liver than in PL of loin was related to the lower susceptibility to oxidation of liver than loin. This would imply different antioxidant abilities for both CLA isomers.

On the other hand, CLA could act as antioxidant by an indirect mechanism derived from the ability of dietary CLA in modifying the fatty acid profile of the tissues, through an increase in the ratio of SFA to unsaturated fatty acids. MDA is primarily formed from the oxidation of fatty acids with three or more double bonds, with the amount of MDA formation increasing with the increasing double bond number (Sinnhuber & Yu, 1977). Since dietary CLA decreased the proportion of several PUFA with three or more double bonds in both types of samples (C18:3 *n*-6 and C20:4 *n*-6 in PL of loin; C20:3 *n*-6 in NL of liver and C20:4 *n*-6 in NL and PL of liver), this could be related to the observed decrease in the yield of MDA with the increase in dietary CLA.

4. Conclusions

CLA supplementation of pig diets results in a dose-dependent CLA enrichment of loin and liver, but to a higher extent in liver, both in triacylglycerols and phospholipids. In intramuscular fat, CLA deposition is higher in phospholipids than in triacylglycerols, whereas in liver, there is not a preferential accumulation of CLA isomers in the lipid fractions. In both tissues, the accumulation of *cis*-9, *trans*-11 CLA is higher than that of *trans*-10, *cis*-12 CLA. All these effects seems to be independent of the MUFA level of the pig diets, except in the case of the accumulation of CLA isomers in triacylglycerols of loin.

Regardless of the level of MUFA in the pig diets, dietary CLA modifies the fatty acid profile of both lipid fractions and in both tissues though an increase in the ratio of SFA to unsaturated fatty acids. This might be considered as an indirect antioxidant mechanism that could be related to the protective effect of CLA against lipid oxidation of liver, but not being relevant in the case of loin. A direct antioxidant effect of CLA should not be discarded. The dietary combination of CLA with low or high MUFA levels did not interfere in the protective effect of CLA against oxidation of loin and liver.

Acknowledgments

This research was supported by the Ministerio de Educación y Ciencia, Spain (AGL 2003-03538). CLA was generously provided by BASF. The valuable cooperation of Dr. Clemente López-Bote and the collaboration of I+D Agropecuaria in designing the experimental diets, sampling and pig management are also acknowledged. Diana Martín thanks the Ministerio de Educación y Ciencia for funding her research.

References

Association of Official Analytical Chemist (2000). *Official methods of analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists.

Belury, M. A., & Kempa-Steczko, A. (1997). Conjugated linoleic acid modulates hepatic lipid composition in mice. *Lipids, 32*, 199–204.

DeDeckere, E. A. M., Amelsvoort, J. M. M., McNeill, G. P., & Jones, P. (1999). Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster. *British Journal of Nutrition, 82*, 309–317.

Department of Health (1994). Nutritional Aspects of Cardiovascular Disease. Report on Health and Social Subjects No. 46. London: HMSO.

Dhiman, T., Nam, S., & Ure, A. (2005). Factors affecting conjugated linoleic acid content in milk and meat. *Critical Reviews in Food Science and Nutrition, 45*, 463–482.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–508.

Ha, Y. L., Storkson, J., & Pariza, M. W. (1990). Inhibition of benzo[*a*]pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Research, 50*, 1097–1101.

Haro, A. M., Artacho, R., & Cabrera-Vique, C. (2006). Linoleic conjugated acid: Current interest in human nutrition. *Medicina Clinica, 127*, 508–515.

Hur, S., Park, G., & Joo, S. (2006). Biological activities of conjugated linoleic acid (CLA) and effects of CLA on animal products. *Livestock Science*. doi:10.1016/j.livsci.2006.11.002.

Ip, C., Chin, S. F., Scimeca, J. A., & Pariza, M. W. (1991). Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Research, 51*, 6118–6124.

Kelley, D., Bartolini, G., Newman, J., Vemuri, M., & Mackey, B. (2006). Fatty acid composition of liver, adiposes tissue, spleen, and heart of mice fed diets containing t10, c12-, and c9, t11-conjugated linoleic acid. *Prostaglandines, Leukotrienes and Essential Fatty Acids, 74*, 331–338.

Kombrust, D. J., & Mavis, R. D. (1980). Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: Correlation with vitamin E content. *Lipids, 15*, 315–322.

Kramer, J. K. G., Sehat, N., Dugan, M. E. R., Mossoba, M. M., Yurawecz, M. P., Roach, J. A. G., et al. (1998). Distributions of conjugated linoleic acid (CLA) isomers in tissue lipid classes of pigs fed commercial mixture determined by gas chromatography and silver ion high-performance liquid chromatography. *Lipids, 33*, 549–558.

Lee, K. N., Kritchevsky, D., & Pariza, M. W. (1994). Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis, 108*, 19–25.

Lee, K. N., Pariza, M. W., & Ntambi, J. M. (1998). Conjugated linoleic acid decreases hepatic stearoyl-CoA desaturase mRNA expression. *Biochemical and Biophysical Research Community, 248*, 817–821.

Livisay, S., Zhou, S., Ip, C., & Decker, E. (2000). Impact of dietary conjugated linoleic acid on the oxidative stability of rat liver microsomes and skeletal muscle homogenates. *Journal of Agricultural and Food Chemistry, 48*, 4162–4167.

Lo Fiego, D. P., Macchioni, P., Santoro, P., Patorelli, G., & Corino, C. (2005). Effect of dietary conjugated linoleic acid (CLA) supplementation on CLA isomers content and fatty acid composition of dry-cured Parme ham. *Meat Science, 70*, 285–291.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

Miller, M. F., Shackelford, S. D., Hayden, K. D., & Reagan, J. O. (1990). Determination of the alteration in fatty acid profiles, sensory characteristics and carcass traits of swine fed elevated levels of monounsaturated fats in the diet. *Journal of Animal Science, 68*, 1624–1631.

Monahan, F. J., Buckley, D. J., Gray, J. I., Morrissey, P. A., Asghar, A., Hanrahan, T. J., et al. (1990). Effect of dietary vitamin E on the stability of raw and cooked pork. *Meat Science, 27*, 99–108.

Myer, R. O., Johnson, D. D., Knauff, D. A., Gorbet, D. W., Brendemuhl, J. H., & Walker, W. R. (1992). Effect of feeding high-oleic-acid peanuts to growing–finishing swine on resulting carcass fatty acid profile and on carcass and meat quality characteristics. *Journal of Animal Science, 70*, 3734–3741.

National Research Council (1998). *Nutrient requirements of swine* (10th ed.). Washington, DC: National Academy Press.

Navarro, V., Fernández-Quintela, A., Churruca, I., & Portillo, M. P. (2006). The body fat-lowering effect of conjugated linoleic acid: A comparison between animal and human studies. *Journal of Physiology and Biochemistry, 62*, 137–147.

Pariza, M. W., & Hargraves, W. A. (1985). A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[*a*]anthracene. *Carcinogenesis, 6*, 591–593.

Park, Y., Albright, K. J., Liu, W., Storkson, J. M., Lin, M. E., Cook, M. E., et al. (1999). Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. *Lipids, 34*, 243–248.

Ruiz, J., Antequera, T., Andres, A. I., Petron, M. J., & Muriel, E. (2004). Improvement of a solid phase extraction method for analysis of lipid fractions in muscle foods. *Analytica Chimica Acta, 520*, 201–205.

Ruiz, J., & López-Bote, C. (2002). In Fidel Toldrá (Ed.), *Research advances in the quality of meat and meat products* (pp. 255). Trivandrum, India: Research Signpost.

Sandler, S., & Karo, W. (1992). *Sourcebook of advanced organic laboratory preparations*. San Diego: Academic Press.

Scheeder, M., Glaser, K., Eichenberger, B., & Wenk, C. (2000). Influence of different fats in pig feed on fatty acid composition of phospholipids and physical meat quality characteristics. *European Journal of Lipid Science and Technology, 102*, 391–401.

Schmid, A., Collomb, A., Sieber, R., & Bee, G. (2006). Conjugated linoleic acid in meat and meat products: A review. *Meat Science, 73*, 29–41.

Simon, O., Männer, K., Schäfe, K., Sagredos, A., & Eder, K. (2002). Effects of conjugated linoleic acids on protein to fat proportions, fatty acids, and plasma lipids in broilers. *European Journal of Lipid Science and Technology, 102*, 402–410.

Sinnhuber, R. O., & Yu, T. C. (1977). Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. *Journal of Japanese Society of Fishery Science, 26*, 259–267.

Smith, S. B., Hively, T. S., Cortese, G. M., Han, J. J., Chung, K. Y., Casteñada, P., et al. (2002). Conjugated linoleic acid depresses the Δ9 desaturase index and steroyl coenzyme A desaturase enzyme activity in porcine subcutaneous adipose tissue. *Journal of Animal Science, 80*, 2110–2115.

Sugano, M., Tsujita, A., Yamasaki, M., Yamada, K., Ikeda, I., & Kritchevsky, D. (1997). Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. *Journal of Nutritional Biochemistry, 8*, 38–43.

Tischendorf, F., Mockel, P., Schone, F., Plonne, M., & Jahreis, G. (2002). Effect of dietary conjugated linoleic acids on the distribution of fatty acids in serum lipoprotein fractions and different tissues of growing pigs. *Journal of Animal Physiology and Animal Nutrition, 86*, 313–325.

Watkins, B., Feng, S., Strom, A., De Vitt, A., Yu, L., & Li, Y. (2003). Conjugated linoleic acids alter the fatty acid composition and physical properties of egg yolk and albumen. *Journal of Agricultural and Food Chemistry, 51*, 6870–6876.