CONJUGATED OCTADECADIENOIC ACID ISOMERS (CLA) IN PORTUGUESE VEAL FROM AROUQUESA AND BARROSÃ AUTOCHTHONOUS BREED CALVES SLAUGHTERED IN EARLY AUTUMN

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Background

The conjugated linoleic acids (CLA), a group of positional and geometric octadecadienoic isomers with conjugated double bonds, are produced either in the rumen by the activity of microbial enzymes on polyunsaturated fatty acids (linoleic acid, 18:2 and linolenic acid, 18:3) or by endogenous desaturation (via delta-9 desaturase) of rumen derived octadecenoate isomers. Recent research has focused on the nutritional role of the CLA isomers because of their health benefits. These effects include potential anticarcinogenic, antiatherosclerotic and immune-modulating properties (Prates and Mateus, 2002; Demirel *et al*., 2004). Rumenic acid (*cis*-9,*trans*-11 CLA) has been associated with anticarcinogenic effects, while *trans*-10,*cis*-12 CLA isomer possesses an important role in the lipid metabolism (Fritsche and Fritsche, 1998). Meats from national demarcated regions with Protected Designation of Origin (PDO) are traditional meats (extensive production systems) produced in a delimited region whose quality is essentially due to the geographic environment. Furthermore, these PDO meats have supposedly unique characteristics, mainly in lipid fraction, linked to the local production systems and animal breeds.

Objectives

The aim of this study was to characterise the total CLA contents and CLA isomeric profiles of Portuguese traditional Arouquesa-PDO and Barrosã-PDO veal, both obtained from autochthonous calves fed extensively during summer (with the least abundant green pastures) and slaughtered in early autumn (October).

Materials and methods

Arouquesa-PDO and Barrosã-PDO veal were obtained from Arouquesa (7-8 months of age, 230 ± 28 kg live weight) and Barrosã (8-9 months of age, 192 ± 35 kg live weight) breed calves, produced in the north of Portugal (Fig. 1). The calves were reared on a traditional production pasture-based system according to the rules established in the product specifications. Muscles samples were collected from the ribeye (T1-T3 *longissimus thoracis* muscle, LT) and loin (L4-L6 *longissimus lumborum* muscle, LL) portions of *longissimus dorsi* muscle and from the distal region of *semitendinosus* muscle (ST) 2-3 days after slaughter (+1 °C). All muscle samples were ground using a food processor (3 × 5 s), vacuum packed and stored at -80 °C until analysed.

Total lipids were extracted from meat (dry matter) by ultrasonication, using methylene-chloride (4:1 v/v) (3×) and *n*-hexane (1×), as was previously described in Fritsche *et al.* (2000). Lipid contents of the test samples were calculated, in duplicate, by weighing the residues obtained after solvent evaporation under a stream of nitrogen. Methyl ester solutions of fatty acids were obtained by alkaline transesterification with sodium methoxide.

The methyl esters of CLA isomers were individually separated and quantified by triple column silver-ion (ChromSpher 5 Lipids, 4.6 mm ID × 250 mm, 5 µm particle size, Chrompack, Bridgewater, NJ, USA), using an HPLC system (HP 1100 Series, Hewlett-Packard, Palo Alto, CA, USA) equipped with autosampler and diode array detector adjusted at 233 nm, with a solvent (0.1 % acetonitrile in hexane) flow rate of 1 ml/min and injection volumes of 20-30 µl. The CLA isomers identification and quantification (external standard technique) were performed as described by Alfaia *et al.* (2003).

The data were analysed using the GLM procedure of SAS (1989). Total lipids and total CLA contents and the proportion of each CLA isomer were studied by analysis of variance, including the effects of breed and muscle type and their interaction. When the *F*-test was significant, the least-squares means were compared at a significance level of 5% ($P<0.05$).

Results and discussion

The intramuscular fat contents of the ribeye (T1-T3, LT) and loin (L4-L6, LL) portions of *longissimus dorsi* muscle and the distal region of *semitendinosus* (ST) muscle of Arouquesa-PDO and Barrosã-PDO veal are presented in Table 1. These data do not allow us to identify breed differences "*per se*" but the overall effect of breed and local production system (traditional production system). The content of total lipids (mg/g muscle) was similar ($P>0.05$) in the three muscles of both breeds. Our values were lower or similar to those reported by Roseiro *et al.* (2002) for Barrosã veal (2.97%, in *longissimus dorsi* muscle) but much lower than those reviewed by Chizzolini *et al*. (1999) for beef (6.3%, in *longissimus dorsi*, and 3.9%, in *semitendinosus* muscle). According to the criteria set by Food Advisory Committee (1990) (less than 5% of fat) these meats-PDO may be considered lean.

The isomeric distribution of individual CLA isomers (Fig. 2), the total content of CLA (mg/g muscle), as well as the content of some specific CLA (mg/g fat), are depicted on Table 1.

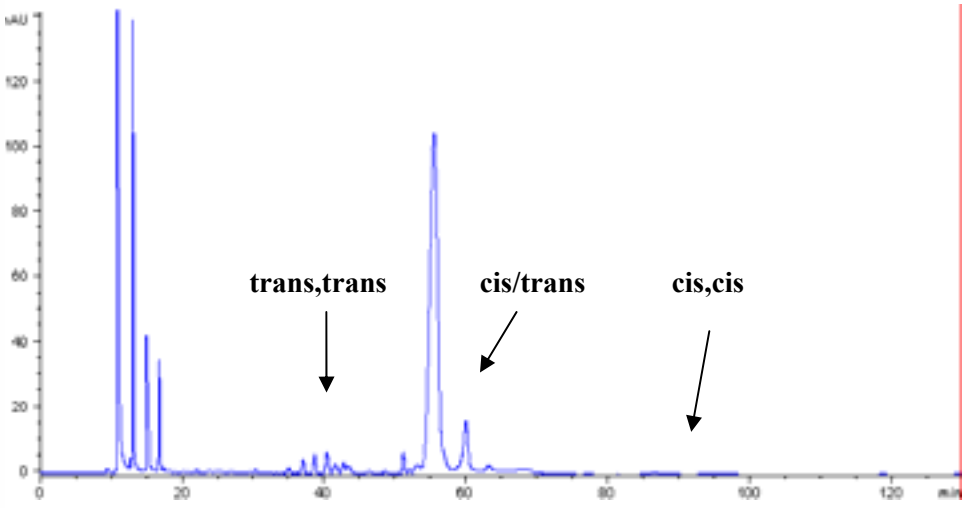

Figure 2. Typical Ag^{+}-HPLC chromatogram of CLA isomeric profile in veal from Arouquesa and Barrosã breed calves slaughtered in early autumn.

Figure 1. Geographical distribution of Arouquesa (A) and Barrosã (B) breed calves. Adapted from Direcção-Geral de Veterinária website.

The total CLA content was higher ($P<0.05$) in Arouquesa-PDO veal than in Barrosã-PDO veal, yet no significant differences were observed among the muscles within a breed. Furthermore, no significant differences were observed on specific CLA content in meat from LT, LL and ST muscles compared traditional veal obtained from calves of a different breed. The sum of *cis,trans* CLA isomers contributed around 93% (Barrosã breed) and 90.5% (Arouquesa breed) to total CLA. Total *trans,trans* CLA isomers contributed only with 9.0% and 6.4% in Arouquesa-PDO and Barrosã-PDO veal, respectively. The percentage of total *cis,trans* isomers is mainly due to the amounts of *cis*-9,*trans*-11 CLA isomer (84.7% to 85.5% for Barrosã-PDO and 79.3% to 80.6% for Arouquesa-PDO veal). The predominant CLA isomer, *cis*-

9,*trans*-11, showed significant differences between Arouquesa-PDO and Barrosã-PDO veal, and the variations in the amount of *cis*-9,*trans*-11 probably reflects either differences in the pasture composition or breed differences in delta-9 desaturase expression (Taniguchi *et al*., 2004). The interaction between breed and muscle type was significant for the proportions of *cis/trans*-12,14 and *trans*-11,*cis*-13 isomers. Finally, no significant differences in total *cis,cis* CLA isomers within each muscle and between the breeds were observed, and the mean amounts detected, mainly composed of the *cis*-9,*cis*-11 isomer, were lower than 0.6%.

Table 1. Analysis of variance and least-square means of total lipids and total CLA contents (mg/g muscle), specific CLA contents (mg/g muscle) and its individual isomers (% CLA) of intramuscular fat of the ribeye (T1-T3, LT) and loin (L4-L6, LL) portions of *longissimus dorsi* and the distal region of *semitendinosus* (ST) muscles of two traditional Portuguese breed calves slaughtered in early autumn.

	Arouquesa-PDO veal (n=15)			**Barrosã-PDO veal (n=12)**			Level of significance		
	LT (T1-T3)	LL (L4-L6)	ST (distal region)	LT (T1-T3)	LL (L4-L6)	ST (distal region)	B	M	B × M
Total lipids (mg/g muscle)	**23.6 ± 6.58**	**23.9 ± 10.00**	**24.6 ± 10.16**	**25.0 ± 6.29**	**20.0 ± 4.71**	**20.4 ± 6.42**	**ns**	**ns**	**ns**
Total CLA contents (mg/g muscle)	**0.206± 0.072**	**0.205± 0.102**	**0.221 ± 0.131**	**0.194 ± 0.120**	**0.164 ± 0.062**	**0.145 ± 0.046**	*	**ns**	**ns**
Specific CLA contents (mg/g fat)	**8.73 ± 1.77**	**8.66 ± 2.23**	**8.84 ± 2.57**	**7.56 ± 3.19**	**8.29 ± 2.49**	**7.28 ± 1.63**	**ns**	**ns**	**ns**
CLA isomers (% CLA)									
t12,t14	1.49 ± 0.88	1.69 ± 1.04	1.52 ± 0.47	0.97 ± 0.58	1.61 ± 0.68	1.21 ± 0.46	ns	ns	ns
t11,t13	2.24 ± 1.39	2.04 ± 1.24	1.52 ± 0.47	1.81 ± 0.64	1.09 ± 0.75	1.21 ± 0.60	**	*[#]	ns
t10,t12	0.49 ± 0.40	0.73 ± 0.72	0.44 ± 0.37	0.40 ± 0.36	0.52 ± 0.52	0.49 ± 0.49	ns	ns	ns
t9,t11	3.29 ± 2.05	3.58 ± 2.40	3.15 ± 1.88	1.88 ± 0.70	1.88 ± 0.73	2.65 ± 2.38	**	ns	ns
t8,t10	0.45 ± 0.37	0.26 ± 0.19	0.44 ± 0.66	0.36 ± 0.31	0.34 ± 0.28	0.18 ± 0.13	ns	ns	ns
t7,t9	0.52 ± 0.20	0.47 ± 0.31	0.48 ± 0.26	0.52 ± 0.27	0.66 ± 0.40	0.77 ± 0.48	*	ns	ns
t6,t8	0.37 ± 0.20	0.29 ± 0.25	0.36 ± 0.31	0.24 ± 0.17	0.29 ± 0.12	0.21 ± 0.13	ns	ns	ns
total t,t	***8.85 ± 3.41***	***9.06 ± 2.58***	***9.07 ± 2.38***	***6.18 ± 1.52***	***6.39 ± 1.42***	***6.74 ± 2.46***	***	**ns**	**ns**
c/t12,14	1.92 ± 1.78 [a]	0.75 ± 0.55 [b,c]	1.98 ± 1.56 [a]	1.35 ± 0.91 [a,c]	2.30 ± 2.08 [a]	1.73 ± 0.85 [a,c]	ns	ns	*
t11,c13	2.73 ± 2.28 [a,c]	3.89 ± 1.35 [a]	2.47 ± 2.28 [c,d]	2.68 ± 2.56 [a,c]	1.08 ± 1.22 [b,d]	0.75 ± 0.87 [b]	***	ns	*
c11,t13	0.41 ± 0.44	0.40 ± 0.54	0.46 ± 0.67	0.28 ± 0.28	0.33 ± 0.36	0.45 ± 0.35	ns	ns	ns
t10,c12	0.45 ± 0.46	0.55 ± 0.49	0.58 ± 0.62	0.38 ± 0.45	0.51 ± 0.73	0.36 ± 0.49	ns	ns	ns
c9,t11	80.62 ± 5.37	79.26 ± 4.47	80.19 ± 4.55	84.71 ± 4.51	85.48 ± 2.93	85.31 ± 3.39	***	ns	ns
t7,c9	4.62 ± 2.36	5.61 ± 2.56	4.73 ± 2.44	3.79 ± 1.44	3.46 ± 1.67	4.20 ± 2.31	*	ns	ns
total c/t	***90.74 ± 3.49***	***90.46 ± 2.60***	***90.41 ± 2.51***	***93.18 ± 1.94***	***93.17 ± 1.34***	***92.81 ± 2.37***	***	**ns**	**ns**
total c,c	***0.41 ± 0.24***	***0.48 ± 0.23***	***0.52 ± 0.37***	***0.64 ± 0.54***	***0.44 ± 0.26***	***0.45 ± 0.35***	**ns**	**ns**	**ns**

B, overall effect of breed and local production system; M, muscle type; ns not significant; means in the same row with different superscripts are different ($P<0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$; [#] (LT=LL, LL=ST and LT>ST).

Conclusions

These preliminary results indicate that when evaluating the CLA isomeric profile, and more specifically the content of the CLA in configuration *cis*-9,*trans*-11 in meat, one must take into account the overall effect of breed and local production system. From the nutritional point of view, the fat from the Barrosã veal may be more healthful because of its higher proportion of *cis*-9,*trans*-11 CLA isomer. Although no significant differences were found for specific CLA contents, the total CLA contents and the sum of *trans,trans* and *cis,trans* isomers were significantly influenced by the traditional production system. The results also showed that in general no significant differences occur within CLA isomeric profile in LT, LL and ST muscles for Arouquesa and Barrosã veal.

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