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CONJUGATED ISOMERS OF LINOLEIC ACID IN MERTOLENGA-PDO BEEF AS AFFECTED BY SLAUGHTER SEASON AND MUSCLE TYPE

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Introduction

Meat from autochthonous bovine breeds based on traditional production systems has been progressively reintroduced in Portuguese market as a result of its putative highly intrinsic quality (Costa *et al*., 2003). One of such example is Mertolenga beef with Protected Designation of Origin (PDO) (Commission Regulation nº1107/96 of 12/06, EC), obtained from Mertolenga breed bullocks raised on a semi-extensive production system, in the south of Portugal (Alentejo), according to the product specifications.

Foods from ruminants, such as milk and meat, are the most important dietary sources of conjugated isomers of linoleic acid (CLA), a minor group of fatty acids formed in the rumen as intermediates during the biohydrogenation of long-chain unsaturated fatty acids or through endogenous Δ^9 desaturation of *trans*-11 octadecenoic acid (Poulson *et al.*, 2004). In recent years, much research has been focused on CLA, particularly in 9*c*11*t*, the main CLA occurring naturally in foods, together with 10*t*12*c*, due to its health-promoting biological activities in animals, including anticarcinogenic, antithrombotic, antiatherogenic, antidiabetogenic and immunomodulatory properties (Belury, 2002).

Meat fatty acid composition is influenced by dietary factors, and in lower extent, by genetic factors (De Smet *et al*., 2004). The dietary factors are often linked with a particular feeding strategy and production system (Raes *et al*., 2004). It is well documented that, including grass in the diet of steers, caused a desirable increase in the CLA concentration (French *et al*., 2000). Thus, meat from grazing ruminants is expected to reflect the variability of pasture biomass and the nutritive value of pasture is highly dependent on cultural practices, season and geographical factors (Moloney *et al*., 2001).

Objectives

The aim of this research was to evaluate seasonal changes (based on early autumn and late spring sampling) and the influence of muscle type (*longissimus thoracis*, *longissimus* *lumborum* and *semitendinosus* muscles) on the profile of conjugated isomers of linoleic acid in Mertolenga-PDO beef.

Methodology

Mertolenga breed bullocks were raised on pasture and finished on concentrate in the last 3 to 6 months. Bullocks were slaughtered in early autumn (n=15; mean ± SE of age and carcass weight were 17±0.7 months and 239±7 kg) and in late spring (n=15; 24±0.7 months and 231±8 kg). Meat samples were taken from *longissimus thoracis* (LT), *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles of bullocks.

Intramuscular fat was extracted with methylene chloride-methanol (4:1 v/v) and *n*-hexane, as described by Fritsche *et al.* (2000). CLA isomers were converted to methyl esters by base-catalysed transesterification with sodium methoxide 0.5 M solution in anhydrous methanol, in order to avoid isomerisation of isomers. 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), using an HPLC system (HP 1100 Series) equipped with autosampler and diode array detector (DAD) adjusted at 233 nm, with a solvent (0.1 % acetonitrile in *n*-hexane) flow rate of 1 ml/min and injection volumes of 20 μl. The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial standards and with values published in the literature (Fritsche *et al*., 2000). Total and individual CLA isomers contents in meat were determined based on the external standard technique (using 9*c*,11*t*, 10*t*,12*c*, 9*c*,11*c* and 9*t*,11*t* as representatives of each of the geometric groups of CLA isomers) and on the method of area normalization (AOAC 963.22, 2000).

The data were analysed using the GLM procedure of SAS (1989). Total and specific CLA contents and the proportion of each CLA isomer were studied by analysis of variance, including the effects of animal inside slaughter season (A(S)), slaughter season (S), muscle type (M) and interaction between S and M. The effect of slaughter season was tested using A(S) as error term. When the *F*-test was significant, the least-squares means were compared.

Results & Discussion

Total CLA (mg/g muscle) and specific CLA (mg/g fat) contents were not affected ($p>0.05$) by slaughter season. Total and specific CLA contents were higher ($p<0.001$) in LT and LL muscles than in ST muscle. The values of specific CLA contents ranged between 2.6 and 4.5 mg/g fat. These values are in accordance with those reported by Realini *et al*. (2004), which found average values of 2.5 mg/g fat for concentrate-fed beef and 5.3 mg/g fat for grass-fed beef.

The slaughter season affected several some individual and the sums of CLA isomers in Mertolenga-PDO beef fat. The isomers 12*t*,14*t* ($p<0.01$), 11*t*,13*t* ($p<0.001$), 11*t*,13*c* ($p<0.01$), 9*c*,11*c* ($p<0.05$) and the sum of total *t*,*t* ($p<0.01$) showed higher percentages in spring slaughtered animals than in autumn slaughtered animals. In contrast, the isomers 10*t*,12*c* ($p<0.05$), 7*t*,9*c* and total *c*/*t* CLA ($p<0.001$) showed higher percentage in autumn slaughtered animals than in spring slaughtered animals. Although little information is currently available to assess seasonal changes in beef CLA, Lock and Garnsworthy

(2003) observed that CLA percentages on milk fat varied throughout the year, with the highest values registered in the summer months (May to July), when cows received fresh grass. The CLA isomeric distribution showed a clear predominance of the bioactive 9*c*,11*t* isomer (rumenic acid) in both seasons. However, the relative proportion of 9*c*,11*t* to the total CLA in Mertolenga-PDO beef (68.5-74.5%), which did not show neither seasonal nor muscle location effects, was lower than the values presented by Fritsche *et al*. (2000). The major difference between muscles was observed for 11*t*,13*t* CLA isomer ($p<0.001$). The interaction between slaughter season and muscle type was only significant for the percentages of the bioactive isomers (10*t*,12*c* and 9*c*,11*t*) and for 12,14*c*/*t* and 11*t*,13*t* CLA isomers.

Conclusions

The results of the present study indicate that total and specific CLA contents of Mertolenga-PDO beef are not significantly affected by slaughter season, although important seasonal variations were observed in CLA isomer distribution. Additionally, the CLA profile is also affected by muscle type, resulting lower amounts of CLA in ST muscle. The rumenic acid (9*c*11*t*) was not affected by slaughter season and muscle type although their significant interaction. The characterization of the Mertolenga-PDO beef based on conjugated isomers of linoleic acid content throughout the year provide added value to the consumers (selection based on the quality/prize ratio) and to the producers (health claims, with higher prices in the market).

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Tables and Figures

Table 1. Total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers of intramuscular fat in *longissimus thoracis* (LT), *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles of beef from Mertolenga bullocks reared according Mertolenga-PDO specifications and slaughtered in early autumn and late spring.

	Mertolenga-PDO beef									
	Autumn			**Spring**				**Effects[1]**		
	LT	**LL**	**ST**	**LT**	**LL**	**ST**	**SEM**	**S**	**M**	**S×M**
total CLA	0.062	0.072	0.039	0.066	0.061	0.031	0.007	ns	***	ns
specific CLA	3.65	4.53	3.39	3.51	4.12	2.63	0.314	ns	***	ns
CLA isomers (% total CLA)										
12*t*,14*t*	0.97	0.96	0.81	2.05	1.75	1.22	0.151	**	**	ns
11*t*,13*t*	1.47[a]	1.37[a,b]	0.92[b]	4.43[c]	3.68[d]	2.51[e]	0.187	***	***	**
10*t*,12*t*	0.98	0.90	0.66	0.92	0.91	1.12	0.191	ns	ns	ns
9*t*,11*t*	2.80	2.88	2.99	3.36	3.70	4.03	0.260	ns	ns	ns
8*t*,10*t*	0.70	0.65	0.71	0.67	0.77	0.40	0.092	ns	ns	ns
7*t*,9*t*	0.99	0.83	1.32	1.02	1.01	0.88	0.176	ns	ns	ns
6*t*,8*t*	0.28	0.31	0.06	0.32	0.44	0.20	0.074	ns	**	ns
12,14*c*/*t*	1.05[a]	1.25[a]	2.66[b]	2.29[b,c]	2.32[b,c]	1.50[a,c]	0.341	ns	ns	**
11*t*,13*c*	1.16	1.40	2.62	3.55	3.41	3.56	0.312	**	*	ns
11*c*,13*t*	0.51	0.68	1.15	0.33	0.42	0.34	0.175	ns	ns	ns
10*t*,12*c*	2.44[a,b]	2.13[a,d]	2.99[b]	1.33[c]	1.69[c,d]	1.48[c]	0.209	*	ns	*
9*c*,11*t*	74.50[a]	71.70[b,d]	68.68[c]	69.51[b,c]	68.52[c]	72.56[a,d]	0.906	ns	ns	***
7*t*,9*c*	2.44	2.13	2.99	8.90	9.96	8.47	0.479	***	ns	ns
9*c*,11*c*	1.05	1.05	0.98	1.32	1.42	1.73	0.159	*	ns	ns
total t,t	8.20	7.89	7.46	12.76	12.26	10.36	0.506	**	**	ns
total c/t	90.75	91.06	91.56	85.91	86.32	87.91	0.544	***	*	ns

[1]Levels of significance: ns, $p>0.05$; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; means in the same row with different superscripts are significantly different ($p<0.05$); SEM, standard error of mean. The symbols used mean as follow: S, season; M, muscle type.