UNIVERSIDADE TÉCNICA DE LISBOA

Faculdade de Medicina Veterinária

Contribution to the study of lipid composition and nutritional value of intramuscular fat in ruminant meats

Cristina Maria Riscado Pereira Mateus Alfaia

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TESE DE DOUTORAMENTO EM CIÊNCIA E TECNOLOGIA ANIMAL

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To my father's memory

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ABSTRACT

Contribution to the study of lipid composition and nutritional value of intramuscular fat in ruminant meats

Fatty acid composition and conjugated linoleic acid (CLA) isomers are nowadays subjects of growing interest as they are of particular relevance to meat quality and human health. Ruminant meats have been suffered from a negative health image related to the nature of their lipid fraction, mainly due to the higher content in saturated fatty acids. Even so, ruminant edible fats are the major natural source of CLA, being the bioactive isomers (*c*9,*t*11 and *t*10,*c*12) associated with potential health benefits. In Portugal, the scientific information available to support the quality and reputation claimed for meat from autochthonous bovine breeds reared according to the specifications of Protected Designation of Origin (PDO) remains largely restricted. Moreover, available data doesn't allow to attain a global perception how distinct factors affect meat composition from production to consumption.

In view of this, we focus our investigation in animal feed standards (pasture *vs.* concentrate), post-slaughter meat treatment (irradiation) and cooking processing (boiling, microwaving and grilling), upholding the emphasis on lipid profiles, specially CLA isomers, and nutritional value of ruminant meats. Four traditional PDO meats (Carnalentejana, Mertolenga Barrosã and Arouquesa) were selected to be characterised. The results suggest that PDO beef intramuscular fat, relative to that from PDO veal, presents low nutritional value throughout the year, which can be explained by the semi-extensive production system of Alentejano and Mertolengo young bulls. In addition, Carnalentejana-PDO beef is of greater nutritional quality than that of intensively produced beef from crossbred young bulls. To assess more detailed data on the effect of feeding systems on the nutritional value of beef lipids and their usefulness as chemical discriminators of the production system, a trial under controlled environmental conditions was performed. The data reinforced the evidence that beef from pasture-fed animals has a higher nutritional quality (mainly due to the higher levels of *n*-3 PUFA and CLA) when compared to that from concentrate-fed bulls, as a result of the beneficial effects of grass intake on meat fatty acid profiles. Concerning to changes on intramuscular fat composition after slaughter and before consumption, as an outcome of meat treatments, no significant differences was reported following irradiation, a prospective technology to meat preservation. Nevertheless, thermal treatments frequently used in culinary practices, induced a moderate impact on the fatty acid profile mainly due to moisture loss and, subsequently, fatness increase. In summary, and based on the values of *n*-3 PUFA, total and *c*9,*t*11 CLA isomer and *n*-6/*n*-3 ratio, the lipid composition of intramuscular fat of the studied traditional bovine meats depicts a nutritional added value to the consumers contributing to market differentiation.

KeyWords: ruminant meats; nutritional value; fatty acid composition; conjugated linoleic acid isomers; cooking methods.

RESUMO

Contribuição para o estudo da composição lipídica e do valor nutricional da gordura intramuscular na carne de ruminantes

A composição em ácidos gordos e o ácido linoleico conjugado (CLA) são temas actuais de grande interesse, com particular relevância para a qualidade da carne e saúde humana. As carnes de ruminantes têm sido consideradas alimentos pouco saudáveis, principalmente devido à sua fracção lipídica com elevada concentração em ácidos gordos saturados. Contudo, as gorduras edíveis dos ruminantes são a principal fonte natural de isómeros CLA, sendo os isómeros bioactivos (*c*9,*t*11 e *t*10,*c*12) associados a propriedades potencialmente benéficas para a saúde humana. Em Portugal, a informação científica disponível para apoiar a qualidade e a reputação da carne bovina de raças autóctones criadas segundo as especificações de Denominação de Origem Protegida (DOP) continua restrita. Para além disso, os dados disponíveis não permitem ter uma percepção global de como distintos factores influenciam a composição da carne, desde a sua produção até ao consumo.

Neste contexto, a nossa investigação foi desenvolvida estudando o efeito dos regimes alimentares dos animais (pastagem×concentrado), processamento tecnológico da carne após o abate (irradiação) e tratamentos térmicos (fervura, microondas e grelhagem), no perfil lipídico, especialmente nos isómeros do CLA, e no valor nutricional de carne de ruminantes. Foram seleccionadas e caracterizadas 4 carnes tradicionais DOP (Carnalentejana, Mertolenga, Barrosã e Arouquesa). Os resultados sugerem que a gordura intramuscular das carnes de novilho relativamente às de vitela, apresentam menor valor nutricional ao longo do ano, devido ao sistema de produção semi-extensivo utilizado nos novilhos. Por sua vez, a Carnalentejana-DOP apresenta uma qualidade nutricional superior comparativamente à carne de vaca de produção intensiva. No sentido de avaliar em detalhe o efeito de diferentes regimes alimentares no valor nutricional dos lípidos da carne, bem como a sua utilidade como discriminantes químicos da origem da carne, realizou-se um ensaio controlado. Os dados obtidos reforçam os resultados anteriores que sugeriam que a carne de animais de pastoreio apresenta melhor qualidade nutricional (teores mais elevados de *n*-3 PUFA e CLA) do que a carne de animais alimentados a concentrado. Este facto traduz os efeitos benéficos da pastagem no perfil de ácidos gordos da carne. Relativamente às alterações na composição da gordura intramuscular, resultantes do efeito de processamentos seguintes ao abate, a irradiação, uma tecnologia prospectiva para a conservação das carnes, não promoveu modificações significativas. Os tratamentos térmicos, frequentemente utilizados em culinária, induziram um impacto moderado no perfil dos ácidos gordos, como consequência das perdas de humidade e concentração da gordura. Em resumo, a composição lipídica da gordura intramuscular das carnes de bovino DOP estudadas apresenta um valor nutricional acrescentado para os consumidores, considerando os valores de *n*-3 PUFA, CLA (total e do isómero *c*9,*t*11) e da razão *n*-6/*n*-3, contribuindo para a sua diferenciação no mercado.

Palavras-Chave: carne de ruminantes; valor nutricional; composição em ácidos gordos; isómeros do ácido linoleico conjugado; tratamentos térmicos.

INTERNATIONAL PEER-REVIEWED PAPERS

This PhD thesis was based on the following papers:

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6. Alfaia, C.M.M., Ribeiro, P.J.L.C., Trigo, M.J.P., Alfaia, A.J.I., Castro, M.L.F., Fontes, C.M.G.A., Bessa, R.J.B., Prates, J.A.M. (2007). Irradiation effect on fatty acid composition and conjugated linoleic acid isomers in frozen lamb meat. *Meat Science*, 77, 689-695.

7. Alfaia, C.M.M., Alves, S.P.A., Lopes, A.F., Fernandes, M.J.E., Costa, A.S.H., Castro, M.L.F., Bessa, R.J.B., Prates, J.A.M. (2009). Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat (Submitted).

CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND SYMBOLS

A	Autumn
AA	Arachidonic acid, 20:4*n*-6
ADA	American Dietetic Association
Ag^+-HPLC	Silver-ion high performance liquid chromatography
ALA	α-Linolenic acid, 18:3*n*-
ANOVA	Analysis of variance
ANCRA	Associação Nacional dos Criadores da Raça Arouquesa
AOAC	Association of Official Analytical Chemists
ATR	Attenuated total reflectance
BSE	Bovine spongiform encephalopathy
BVC	Beef and veal consumption
CACI-MS/MS	Covalent adduct chemical ionization tandem mass spectrometry
CAP	Common Agricultural Policy
CAPOLIB	Cooperativa Agrícola de Boticas CRL
CCC	Concentrate or feedlot
CDA	Canonical discriminant analysis
CECF	Centro de Estudos em Ciências Farmacêuticas
CIISA	Centro Interdisciplinar de Investigação em Sanidade Animal
CHD	Coronary heart disease
CHIP	Centro de Higienização por Ionização de Produtos
CHR	Cholesterol
CLA	Conjugated linoleic acid
cm	Centimetre
°C	Degree Celsius
^{60}Co	Cobalt
CoA	Coenzyme A
COPs	Cholesterol oxidation products
d	Day

DAD	Diode array detector
DGADR	Direcção-Geral de Agricultura e Desenvolvimento Rural
DGDG	Digalactosyldiacylglycerol
DHA	Docosahexaenoic acid, 22:6*n*-3
DNA	Desoxiribonucleic acid
DPA	Docosapentaenoic acid, 22:5*n*-3
EC	European Commission / Enzyme Commission
ECC	European Economic Community
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid, 20:5*n*-3
EU	European Union
EZN	Estação Zootécnica Nacional
FAME	Fatty acid methyl esters
FDA	Food and Drug Administration
FID	Flame ionization detector
FTIR	Fourier-transform infrared spectroscopy
g	Gram
G	Group of animals
GC	Gas chromatography
GC-MS/MS	Gas chromatography-tandem mass spectrometry
GLM	General linear model
GPP	Gabinete de Planeamento e Políticas
h	Hypocholesterolaemic fatty acids; Hour
H	Hypercholesterolaemic fatty acids
HDL	High density lipoprotein
hPa	Hectopascal
HPLC	High-performance liquid chromatography
IA	Index of atherogenicity
IMF	Intramuscular fat
INE	Instituto Nacional de Estatística
INIA	Instituto Nacional de Investigação Agrária
INIAP	Instituto Nacional de Investigação Agrária e das Pescas
IOM/NAS	Institute of Medicine/National Academy of Sciences
IP	Intensively produced
INRB	Instituto Nacional de Recursos Biológicos
IT	Index of thrombogenicity
ITN	Instituto Tecnológico Nuclear
IUPAC	International Union of Pure and Applied Chemists
kcal	Kilocalorie
kg	Kilogram
kGy	Kilogray
kJ	Kilojoule
kt	Kilotonnes
LA	Linoleic acid, 18:2*n*-6
LC	Liquid chromatography

LC PUFA	Long-chain polyunsaturated fatty acids
LD	*Longissimus dorsi*
LDL	Low density lipoprotein
LS	Pelleted dehydrated lucerne supplemented with linseed oil
LSD	Least significant difference
LSMEANS	Least squares means
LT	*Longissimus thoracis*
LTB_4	Leukotriene B_4
LTB_5	Leukotriene B_5
m	Month/Metre
M	Muscle type; Molar
MDA	Malondialdehyde
MGDG	Monogalactosyldiacylglycerol
mg	Miligram
mL	Mililitre
µg	Microgram
µL	Microlitre
µm	Micrometre
Mhz	Megahertz
min	Minute
mm	Milimetre
MS	Mass spectrometry
MUFA	Monounsaturated fatty acids
n	Number
NCP_{total}	Total net cattle production
NCP_{trad}	Traditional net cattle production
nm	Nanometre
ns	Not significant
NIRS	Near infrared reflectance spectroscopy
NL	Neutral lipids
NMR	Nuclear magnetic resonance spectroscopy
P	Probability
PDO	Protected Designation of Origin
%	Percent
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
PGI_2	Prostaglandin I_2
PGI_3	Prostaglandin I_3
PGI	Protected Geographical Indication
pH	Potential of hydrogen
PL	Polar lipids
PPARγ	Peroxisome proliferator activated receptor gamma
PCC	Pasture with finishing on concentrate during 4 months
PPC	Pasture with finishing on concentrate during 2 months
PPP	Pasture

PUFA	Polyunsaturated fatty acids
s	Second
S	Spring; Season
SACN/COT	Scientific Advisory Committee on Nutrition / Committee on Toxicity
SAS	Statistical analysis system
SCD	Stearoyl-CoA desaturase
SEM	Standard error of mean
SF	Pelleted dehydrated lucerne supplemented with sunflower oil
SFA	Saturated fatty acids
SFLS	Pelleted dehydrated lucerne supplemented with a blend of sunflower oil and linseed oil
SREBPs	Sterol regulatory element binding proteins
ST	*Semitendinosus*
t	Tonnes
TAG	Triacylglycerols
TBARS	Thiobarbituric acid reactive substances
TFA	*Trans* fatty acids
TNF-α	Tumor necrosis factor alpha
TR	True retention values
TSG	Traditional Specialties Guaranteed
TXA_2	Thromboxane A_2
TXA_3	Thromboxane A_3
UCP-2	Uncoupling protein-2
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
v	Volume
VA	(*trans*) Vaccenic acid, *trans*11-18:1 or 18:1*t*11
WHO	World Health Organization
WHO/FAO	World Health Organization/Food and Agriculture Organization
wt	Weight

1

SCIENTIFIC BACKGROUND AND OBJECTIVES

1.1 INTRODUCTION

Remarkable changes in consumer's attitude towards food and nutrition have been occurring during the last decade and the increase in consumer's demand for high quality products, which represents one of the fastest growing segments of the food industry, continues to drive new opportunities to address their needs. The role of a balanced diet is to provide sufficient nutrients to meet the nutritional requirements of an individual for a normal development and maintenance of a healthy life (Froidmont-Görtz, 2007). Nowadays, the main objective of nutrition is to optimize the quality of diets in order to guarantee daily intakes of energy, nutrients and eventually bioactive compounds, to improve health status and/or reduce the risk associated with some diseases (Serrano et al., 2007). The consumer interest relates to the health enhancing role of specific foods, containing physiologically/biologically active components in addition to the classical nutrients, so-called functional foods[1], designed foods or nutraceuticals, that may have health benefits for humans (*e.g.* Prates & Mateus, 2002; Raes et al., 2004; Dhiman et al., 2005; Froidmont-Görtz, 2007). Nevertheless, each potential functional food should be evaluated on the basis of scientific evidence to ensure appropriate integration into a mixed and balanced diet. According to the American Dietetic Association (ADA, 1999) the knowledge of the role of physiologically active food components, both from plant and animal sources, has changed the role of diet in health. Indeed, consumers are becoming more aware of the relationships between diet and health, particularly in relation to cancer, atherosclerosis, obesity/type 2 diabetes, hyperlipidemia and hypertension, conditions that are considered prevalent in industrialized countries. Lipids are among the bioactive components that have received most attention, dealing with the development of healthier meat products (Jiménez-Colmenero, 2007).

Fat, especially animal fat, has been the subject of much concern and debate because of the association with risks for some chronic diseases when consumed in excess. Current insights and nutritional guidelines suggest the need to consider the quantity as well as the quality of fat in diet. Therefore, the amount and the structure of the fatty acids[2] should be regarded in view of its implications for food quality and human health. In fact, there has been an increased interest to

[1]Functional foods: any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains (IOM/NAS, 1994).

[2]Fatty acids are the basic components of most naturally-occurring lipids in both animals and plants. The fatty acids are essential nutrients for life, being used by organisms as an energy source. The diversity of the chain length, degree of unsaturation, geometry and position of double bonds, as well as the presence of other groups, provide their composition the most definitive characteristic of these lipids and their origin (Lima & Abdalla, 2002).

understand the metabolism of dietary lipids and their role in human health and well-being (Givens, 2005; Bessa et al., 2008). This interest has been acknowledged by technical and analytical advances which improved our ability to identify and quantify specific fatty acids in food products and the intermediates of fatty acid metabolism.

Today's unhealthy diet and lifestyles promote excessive intakes of saturated fats (WHO, 2003). Saturated fatty acids (SFA) and *trans* fatty acids (TFA) have been recognised by the international dietary authorities as primary targets for global reduction. As well, ruminant meats have been suffered from a negative health image related to the nature of their lipid fraction. In fact, beef and lamb meats can be issue of concern since their intramuscular fat is relatively saturated than those of nonruminants, even though they are low in fat (Lee et al., 2006). Ruminant edible fats are particularly rich in SFA and TFA due to the extensive microbial biohydrogenation of dietary polyunsaturated fatty acids (PUFA) in the rumen. Moreover, the consumption of ruminant meats is often associated with an increased incidence of coronary heart disease (CHD) in humans (Barton et al., 2007). Whilst biohydrogenation has a negative effect on the nutritional value of ruminant fats, some derived biohydrogenation intermediates, such as conjugated linoleic acid (CLA) and *trans* vaccenic acid (TVA, *trans*11-octadecenoic acid or 18:1*t*11), could be of particular relevance to human health. Most research has focused on this minor group of fatty acids that are characteristic of ruminant-based products, with isomer-specific biological effects. However, only recently, analytical methods and commercial standards have been developed to unequivocally determine profiles of the geometrical and positional isomers of CLA in ruminant products.

Many efforts have been made to improve the nutritional value and quality of ruminant meats, by controlling the intramuscular fat (IMF) deposition and its fatty acid composition. Since part of the unsaturated fatty acids may escape ruminal biohydrogenation, the diet can thus notably affect the fatty acid composition of ruminant tissues and products. In Portuguese autochthonous cattle breeds, reared in traditional production systems according to the specifications of Protected Designation of Origin (PDO) rules, the magnitude of fatty acid composition, with special emphasis on the contribution of CLA isomers to support the quality and reputation claimed is presently quite limited. Moreover, lipid composition of meat is necessarily connected to the feeding systems of the animals. Considering that some of these animals, raised under traditional production systems usually have a finishing period on concentrate feed (3 to 6 months, according to the product specifications), only few studies concerning the long-term influence of grains and grass and/or forages on fatty acid composition and CLA content of beef have been documented. Moreover, the effect of different technological and heating treatments on the intramuscular fatty acid composition, namely the content and detailed isomeric profile of CLA, has been so far less investigated.

Overall, this work focus on the intramuscular lipids of ruminant meats, aiming to provide findings in an attempt to explain the differences achieved and that surely dictate meat quality, in general, and in Portuguese production systems, in particular. Indeed, it's our goal to contribute to the mass of knowledge on the research of bioactive lipids present on ruminant meats aiming to clarify the added value of Portuguese traditional bovine meats. Beyond its scientific value, this work may provide information to the consumers concerning nutritional composition of meats to enable them to make the best choices, in terms of a more conscious selection of bovine meats based on the quality/price ratio, as well as to enlighten the influence of the common culinary practices on the oxidative stability and nutritional value of beef intramuscular fat. Moreover, autochthonous catle breed farmers may also benefit from the possible genetic valorisation of the breeds, extremely important for the surviving of these breeds with low market growth rate. Meat producers might also benefit from the possible scientific support for the claimed health and quality of cattle PDO meats, from the control of authenticity, willingly expected for the commercial valorisation of these meats.

Thus, in this general introduction, current information concerning beef and veal consumption in Portugal, as well as in European Union (EU), will be briefly reviewed, given special attention to the importance of traditional bovine meats. Subsequently, awareness will be driven on the nutritional value of fatty acids and their implications for human health, as well to the dietary recommendations in relation to fat in the diet. Finally, fat content and fatty acid composition, with emphasis on the content and profile of CLA isomers, of ruminant meats (beef and veal particularly) will be presented in addition to the major factors and strategies that could change the lipid composition of meat. After this brief overview, the specific objectives of this work will be described.

1.2 BEEF AND VEAL CONSUMPTION, HEALTH CONCERNS AND MEAT QUALITY

Meat is a concentrated nutrient source essential for optimal human growth and development. A study of human and pre-human diet history shows that for a period of at least 2 million years the human ancestral line had been consuming increasing quantities of meat (Mann, 2000). During that time, evolutionary selection was in action, adapting our genetic makeup and hence our physiological features to a diet high in lean red meat, which includes beef, veal, pork and lamb (< 5% fat, Food Advisory Committee, 1990). It was concluded that lean meat is a healthy and beneficial component of any well-balanced diet as long as it is fat trimmed and consumed as part of a mixed diet (Mann, 2000). So, beef is considered to be a highly nutritious and valued food as a source of high biological value protein, that contain all the essential amino acids, and important micronutrients, such vitamins (*e.g.* vitamin B_2, B_3, B_6 and B_{12}) and minerals (*e.g.* iron, zinc, selenium and phosphorous) all of

which are known to enhance well-being and health (Williamson et al., 2005; Schönfeldt & Gibson, 2008).

In the last years, changes in consumer demand for beef are related with diverse socio-economical factors as well as with health concerns. The most important ones are products characteristics [sensory attributes, nutritional properties, price, convenience and safety aspects, such as the indiscriminate use of hormones, the use of several chemicals and prophylactic antibiotics as well as the fear of bovine spongiform encephalopathy (BSE)], and environment-related ones (health concerns, educational aspects, economic aspects, climate, legislation) (Jiménez-Colmenero et al., 2001; García-Segovia et al., 2007).

Cattle, like all ruminants, have played, and will continue to play, a valuable role in sustainable agricultural systems (Rodrigues et al., 1998). In Portugal, beef cattle production has a major importance in the regions of Alentejo and the northern interior. In the North and in the Azores dairy breeds predominate, whilst in Alentejo slaughter breeds provide a larger contribution. In the last decades, there was a change in the structure of beef production (see Figure 1). During the period under review, between 1990 and 2007, the annual average net production[3] of beef and veal in Portugal was about 108 ± 12 kt (kilotonnes or 10^3 tonnes) reaching its high value in 1991. The decline observed during the 90's agrees with the downward trend anticipated since the beginning of the 90's, related to a continuing fall in the dairy cow herd. In 1996, with the first BSE outbreak, there was a sharp fall all over Europe. The European Commission (EC) took several measures to improve

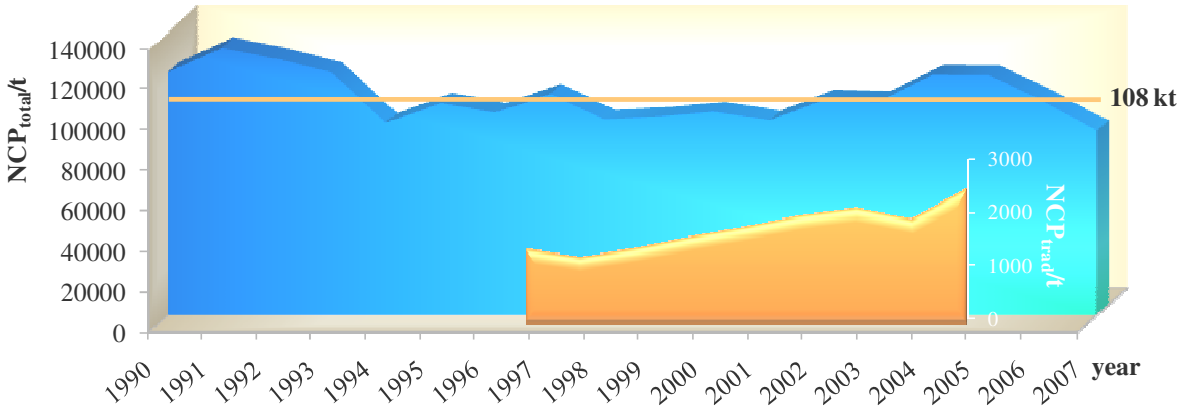

Figure 1. Total and traditional net cattle production (NCP_{total} and NCP_{trad}, respectively) in Portugal (based on data from INE, 2007).

[3]Net production of meat: production corresponding to animal slaughtering performed within a territory and approved for consumption, regardless to the origin of the animals slaughtered, domestically produced or imported (GPP, 2006/2007).

the demand, trade and prices. An exceptional measure to support the beef and veal market was the purchase regime for slaughter and destruction of > 30 month old animals [Commission Regulation (EC) nº 2777/2000]. In 2002, the cattle sector gradually came out of the animal health crises, but after 2005 the data available pointed out to a marked decline until today. Portugal is not self sufficient in beef production (62% in 2005) and it is difficult to envisage market recovery, due to non-nutritional issues such as animal health scares (*e.g.* BSE), although it can be foresee an emergent market for beef originating from Portuguese indigenous cattle breeds (Andrade et al., 1999).

According to the Instituto Nacional de Estatística (INE) and Portuguese food supply balances sheet in the European Union (EU) and Portugal, the *per capita* consumption of beef and veal (Figure 2) increased over the last decades, but in 1996, with the first BSE outbreak, there was a sharp fall (GPP, 2004). Over the following years, between 1997 and 2000, the consumption recovery was considerable reaching 17.3 kg/person/year. In 2001, consumption dropped significantly with decreases in imports both of live animals and particularly of beef and veal. This decline in beef consumption had led to substantial increase in the market share of poultry meat at the expense of beef. In the following years, beef consumption gradually regain its former share of the market and *per capita* consumption recovered in 2002, with increases both in slaughters for consumption and in beef and veal imports. In 2004, *per capita* consumption of beef and veal reached the highest value of the last years (18.8 kg/person/year). Between 2006 and 2007, the beef consumption maintains stable around 18 kg/person/year. The Portuguese *per capita* consumption was inferior to that of the European Union EU15 (19.5 kg/person/year), but higher than the EU 25's (17.5 kg/person/year) (GPP, 2006/2007). A great number of factors may explain this decrease in meat consumption, for instance

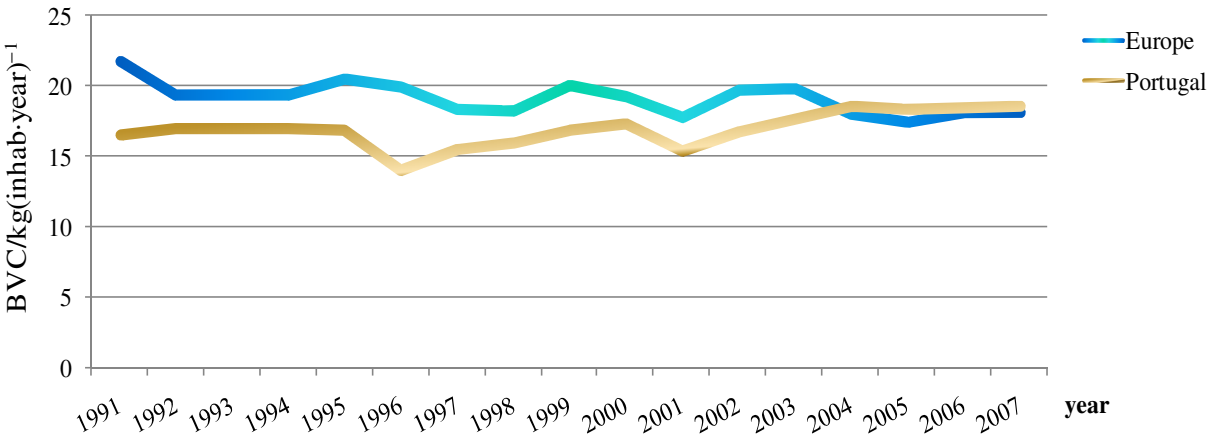

Figure 2. Beef and veal *per capita* consumption (BVC) in Portugal and in the European Union (based on GPP, 2006/2007; INE, 2007).

the commercial competition with white meats, differences in socio-cultural and traditional dietary habits and also media events, such as the illicit trading and use of hormones, the outbreak of BSE,and economic transformations (industrialisation, intensification of agriculture, urbanisation). The tendency in beef consumption in the next years may come as a result of the change in the lifestyles promoted by health education, and the larger choice of meat on offer.

The drop in beef production at national level that occurred in 1998, mostly attributed to the scare caused by BSE in 1996, was also felt in the production of meat with Protected Designation (see Figure 1). Nevertheless, the production of such meat soon regained in the following year. This development is certainly related to a greater consumer' s demand for this type of meat, recognising their genuine qualities, but also with the policies established for the sector compatible with the current direction of change in the Common Agricultural Policy (CAP), with particular reference to support the conservation of native breeds.

As regards to traditional production systems, the consumption approved cattle slaughters almost double from late 90's to 2005. After the slight decline observed during 2004, traditional production gradually regains its former share in the following year. However, it was not possible to confirm if continues to increase or follows the decreased trend observed for the global annual net production after that year (Figure 2). Despite the slow progress, but positive, the importance of meat with Protected Designation in the total production of cattle in Portugal still occupy a niche market, not reaching 3% of national production in 2005. Between 2001 and 2002, the first positive results from the investment made by regional livestock producers in autochthonous breeds (Arouquesa Barrosã and Minhota/Galega) by means of a certified brand begins to show, and in 2003 the production of these bovine PDO meats increased 16% compared to 2001 (GPP, 2004). The extensive animal production systems are characterised by the usually low capital inputs and by structure that tend to assure a high quality product and differentiation. These characteristics are essential for the intended added value, specific preference and consumer's demand as well as for the protection of the environmental quality (Rodrigues et al., 1998; Vaz Portugal, 2002). In fact, these beef production systems based on pasture feeding and traditional practices respect the physiological and behavioural needs of cattle and provide them welfare, without the risk of metabolic and feed-related disorders, such as acidosis, commonly associated with the intensive feeding systems (Nielsen & Thamsborg, 2005). As producers benefited from a higher commercial valorisation of these meats, consumers reacted positively, and this type of meat have become a consumer's favourite. It also offers dietary improvements to the consumers as pasture feeding can lead to increased contents of PUFA, which play a favourable role in human health. In addition, the health concerns mostly linked to BSE, increased the awareness of consumers to the geographic origin and the production system of beef, pressing the food industry for accurate labelling of products (Bessa et al., 2000). The implementation

of the European legislation [Council Regulation (EEC) nº 2081/92 of 14/7, which were repealed by Council Regulation (EC) nº 510/2006 of 20/3] in order to protect and promote the edible products with PDO and Protected Geographical Indication (PGI) is an important contribution to the sustainability of the production chains. Bessa and co-workers (2000) point out that more than 80% of total products protected by Commission Regulation are from Mediterranean countries, including Portugal. According to the Regulation (EC) nº 510/2006 of 20 Mars of 2006 designations of origin means the name of a region, a specific place or in exceptional cases, a country, used to describe an agricultural product or a foodstuff, which was originated in that region, specific place or country, the quality or characteristics of which are essentially or exclusively due to a particular geographical environment with its inherent natural and human factors, and the features of production, processing and preparation which take place in the defined geographical area. Moreover, at least for the PDO products, specifications can be very precise considering the genotype of animals, feeding and management systems, and technological practices, which is a mean of compliance verification and also a way of setting rules that producers should respect and follow (Bessa et al., 2000).

In Portugal, as in other countries within the EU, many local cattle breeds are kept under traditional conditions and their meat is labelled [Commission Regulation (EC) nº 1825/2000 of 25/8] for a quality guarantee (Holló et al., 2006). The knowledge of meat quality of traditional animal breeds is required for the effective product output. Quality is therefore an important social and economic challenge which is amplified by the saturation of food markets due to the high efficiency of modern agriculture. According to Scollan et al. (2006) definition of meat quality is becoming increasingly complex as it encompasses the physical intrinsic qualities (*e.g.* colour, tenderness, juiciness, flavour) and extrinsic qualities (*e.g.* brand, quality mark, origin, healthiness, production environment). There has been an increasing trend towards the certification of products derived from these natural and environmentally favourable production systems and, in the case of beef cattle, is mainly linked to the use of the indigenous breeds. The process of certification also requires control tools in order to guarantee that the specification commitments have been fully met [Council Regulation (EC) nº 882/2004 of 29/4]. Herd book registration allows the identification and the recording of all the registered animals, guaranteeing not only the authenticity of the breed, but also the feed conditions, health control and livestock management (GPP, 2007). Portugal is an important reservoir of genetic resources, being officially recognised 42 autochthonous breeds of which 15 of bovine animals (GPP, 2007). In Portugal, between 2002 and 2004, there were thirteen bovine meats with protected names, of which nine PDO (Barrosã, Mirandesa, Maronesa, Arouquesa, Marinhoa, Carnalentejana, Mertolenga, Cachena da Peneda and Charneca), three PGI (Vitela de Lafões, Açores and Cruzado Lameiros do Barroso) and one Traditional Specialties Guaranteed (TSG) (Bovino Tradicional do Montado). These bovine PDO meats are distributed in limited regions of the north (Barrosã,

Maronesa and Mirandesa) centre (Marinhoa and Arouquesa) and south (Alentejana and Mertolenga) of Portugal. The contribution of each type of PDO meat to the total production of bovine meats protected by Commission Regulation was very variable. In fact, between 2002 and 2004 (Figure 3), corresponding to the years of harvest in this study, the most important commercial Portuguese bovine PDO meats were Carnalentejana-PDO beef (average values of 42%, 833.1 carcass tons), followed by Mirandesa-PDO veal (14.3%, 284.7 carcass tons), Mertolenga-PDO beef (13.5%, 269.8 carcass tons) and Barrosã-PDO veal (12.6%, 250.8 carcass tons) (DGADR, 2005).

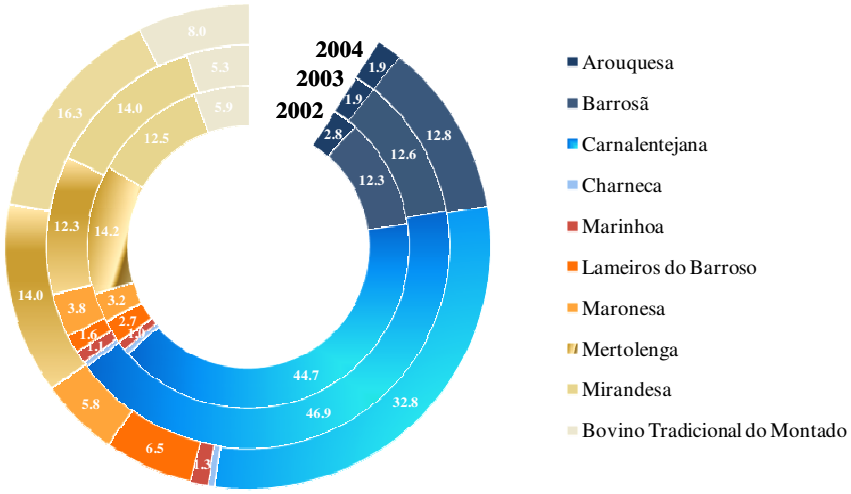

Figure 3. Production of Portuguese bovine meats with Protected Designation between 2002 and 2004 (% based on data from DGADR, 2005).

Recently, the term traceability [Commision Regulation (EC) 178/2002] has grown in importance due to the consumer's increasing attention to food quality matters. This general definition implies both the origin of the entity (animal's identity, breed and also geographical origin) and the mode of production (including feeding regimes, processing and conservation, and also any adulteration of products) along the full production chain to provide safe and high quality meat for the consumer (Prache et al., 2005). Traceability issues were not only mandatory within the general framework of the certification of PDO status to differentiate niche market products, but also used to implement food safety. In fact, it represents an essential tool to safeguard public and animal health and to valorise typical production systems (Dalvit et al., 2007). In order to guarantee the product's authenticity and quality, satisfy consumer protection requirements facilitate monitoring and allow complete traceability of both the product and the procedure, all the meat operations (processing and

packaging) must take place within the specified geographical area, together with the birth, rearing, fattening and slaughtering of the animals and the cutting of the carcasses.

Meanwhile great efforts have been made to develop analytical tools to quantify specific compounds in the product or in the animal tissues that can act as tracers of the animal's feeding system and also give some insights into traceability of geographical origin (Prache et al., 2005; Luykx & van Ruth, 2008). The potential tracers in meat and milk or animal tissues may come directly from the diet (direct markers), such as plant biomarkers (carotenoids, terpenes and phenolic compounds), or may be metabolic markers deriving from animal metabolism (indirect markers), like fatty acid composition of meat and milk. The ratios of the stable isotopes (physic-chemical markers) are also potential tracers of animal diets and of the geographical origin of meat (Müller & Steinhart, 2007). The spectral characterisation of meat using visible and near infrared reflectance spectroscopy (NIRS) and the development of the functional genomics constitute promising approaches that have just emerging and needs experimental evaluation, but already allowed to discriminate animal products obtained in contrasting feeding conditions as well as to establish the geographical origin of different foods (Prache et al., 2005; Luykx & van Ruth, 2008).

1.3 NUTRITIONAL VALUE OF FATTY ACIDS AND DIETARY RECOMMENDATIONS

The importance of fatty acid composition in the healthiness and quality of meat, especially in ruminant meat, contributed to the development of specific guidelines from the World Health Organization in relation to fat in the diet. On this sense, proportion of total fat, saturated fatty acids, TFA and cholesterol (CHR) has been considered key nutritional targets to public health (Kraft et al., 2008). This emergent interest of consumers in fat composition of meat and meat products have increased the relationship between diet and health, as recognised by several health organizations, claiming that composition of fats in the diet has a significant role in modifying the risk of cardiovascular diseases (British Department of Health, 1994; Hu et al., 2001). Initially, it was recommended a reduction in total fat intake in order to reduce the potential adverse effects of fats on obesity and CHD predisposed by excessive fat consumption (Webb & O'Neill, 2008). Recently, nutritional guidelines are clearly changing towards fat quality rather than fat quantity *per se*. It means that these recommendations refer not only to the amount of fat but also to the fatty acid composition and the CHR levels in foods, of which meat contribute with a major part. More specifically, is recommended a lower intake of SFA and TFA as well as a higher PUFA intake (especially of *n*-3 family of PUFA to comply with an appropriate *n*-6/*n*-3 balance) in order to prevent cardiovascular-type diseases (Griffin, 2008; Sierra et al., 2008).

The World Health Organization Study Group (WHO, 1990), together with the Joint WHO/FAO Expert Consultation on diet, nutrition and prevention of chronic diseases (WHO, 2003), recommended the intake goals of specific fatty acids and cholesterol (Nishida et al., 2004). These nutritional recommendations are population intake goals[4] not individual dietary guidelines. According to them, dietary fat intake should ideally be less than 30% of total diet energy and for the intake of specific fatty acids as a proportion of total diet energy no more than 10% of total energy should be from SFA, less than 1% from TFA and around 10-15% from monounsaturated fatty acids (MUFA). Recently, Mosca et al. (2007) recommended an intake of saturated fat less than 7% in order to prevent CVD. Regarding PUFA 5-8% of calorie intake should be from *n*-6 PUFA and 1-2% from *n*-3 PUFA, in particular from eicosapentaenoic acid[5] (EPA, *all-cis*5,8,11,14,17-eicosapentaenoic acid, 20:5*n*-3) and docosahexaenoic acid (DHA, *all-cis*4,7,10,13,16,19-docosahexaenoic acid, 22:6*n*-3). Moreover, since the health implications of fat consumption are determined by the proportions between fatty acids, some recommendations are still made on the basis of specific fatty acid ratios, which are considered to be beneficial for consumer health (Jiménez-Colmenero, 2007). In this regard, within the PUFA, the *n*-6/*n*-3 ratio should ideally be below 4.0 and a PUFA/SFA ratio of 0.45 or higher for the diet as a whole is recommended (British Department of Health, 1994). Recently, Griffin (2008) reviews the practically utility of the dietary ratio of *n*-6 to *n*-3 PUFA in optimising the benefits of *n*-3 fatty acids on cardiovascular health. According to the former author, the ratio of of *n*-6/*n*-3 PUFA has therefore utility in providing information on the physiological effects of linoleic acid (LA, *cis*9,*cis*12-octadecadienoic acid, 18:2*n*-6) and α-linolenic (ALA, *cis*9,*cis*12,*cis*15-octadecatrienoic acid, 18:3*n*-3), and the metabolic conversion of the latter fatty acid to EPA (Burdge & Calder, 2005). The PUFA/SFA ratio, based only on the chemical structure of fatty acids, may not be an adequate index to evaluate the nutritional value of fat because it considers all SFA and ignore the effects of MUFA. A better approach should be the use of another index, the ratio of hypocholesterolaemic/hypercholesterolaemic fatty acids (h/H), based on the functional effects of fatty acids on cholesterol metabolism (Santos-Silva et al., 2002). Furthermore, according to Ulbricht

[4]Population intake goals defined as the population average intake that is judged to be consistent with maintenance of health in a population (Nishida et al., 2004).

[5]The fatty acids are commonly expressed by their trivial names (*e.g.* eicosapentaenoic acid, EPA) while the exact structure is by its systematic name (*cis*5,*cis*8,*cis*11,*cis*14,*cis*17-eicosapentaenoic acid) and by the abbreviation (20:5*n*-3). According to the International Union of Pure and Applied Chemists (IUPAC) terminology, straight-chain fatty acids are named after the parent hydrocarbon. The position of the ethylenic bond(s) is designated by counting from the carboxyl carbon (designation Δ) and/or from methyl carbon (designation *n*), and the geometry of the ethylenic bond is shown by *cis* or *trans* configuration (Seppänen-Laakso et al., 2002).

and Southgate (1991) the PUFA/SFA ratio as a measure of the propensity of the diet to influence the incidence of CHD should be replaced by the indices[6] of atherogenicity (IA) and thrombogenicity (IT) taking into account the diverse effects of different fatty acids on human health. In addition, apart from reducing the SFA content in the diet, nutritional guidelines recommend to limit cholesterol intake to 300 mg/day (WHO, 2003), in order to reduce the risk associated with obesity and hypercholesterolemia (Ganji et al., 2003; Valsta et al., 2005; Jiménez-Colmenero, 2007). Moreover, cholesterol oxidation products in food, exhibiting mutagenic, carcinogenic and cytotoxic properties (Guardiola et al., 1996), are strongly dependent on the initial cholesterol concentrations (Engeseth & Gray, 1994).

Eynard and Lopez (2003) proposed that the reciprocal proportions of SFA plus total cholesterol (CHR) and total CLA [CLA/(SFA+CHR) ratio] might explain the association between the intake of beef fat and colon cancer. Interestingly, lean beef (15% fat, with < 5% of intramuscular fat), showing a high CLA/(SFA+CHR) ratio (0.09), has a protective effect against colon cancer, whereas fatty beef (37% fat), with a low CLA/(SFA+CHR) ratio (0.007), is associated with a higher risk. Thus, the beneficial effects of minor amounts of CLA may be relatively enhanced in lean meat compared with fatty meat.

1.3.1 Fatty acids

Ruminant meat has often been criticised in view of the generally saturated nature of their fatty acids and the potential negative effect this can have on human health. In fact, excessive dietary intake of fat and/or SFA was mostly associated with higher consumption of meat. A survey of European diets revealed that 21% of total fat intake comes from meat and meat products (Prates & Bessa, 2009).

Meat lipids usually contain up to 50% of SFA, of which 25-35% have atherogenic properties (Jiménez-Colmenero et al., 2001). The goal in decreasing the intake of SFA is to help consumers to reduce their risk for coronary heart disease (Ulbricht & Southgate, 1991; Barton et al., 2007). Some SFA, particularly, lauric (dodecanoic, 12:0), myristic (tetradecanoic acid, 14:0), and palmitic (hexadecanoic acid, 16:0) acids are hypercholesterolaemic and their intake should be restricted (Givens, 2005). These SFA are well known to raise total and low density lipoprotein (LDL) cholesterol levels, showing different effects among the fatty acids. Cholesterol-raising effects are

[6]Index of atherogenicity (IA) = [(12:0) + (4 × 14:0) + (16:0)] × [(*n*-6 and *n*-3 PUFA) + MUFA)]$^{-1}$ and index of thrombogenicity (IT) = [(14:0) + (16:0) + (18:0)] × [(0.5 × MUFA) + (0.5 × *n*-6 PUFA) + (3 × *n*-3 PUFA) + (*n*-3 × *n*-6^{-1} PUFA)]$^{-1}$ (Ulbricht & Southgate, 1991).

generally greater for fatty acids with medium-chain lengths, such as 12:0, 14:0 and 16:0 than for those with longer chain lengths. The short chain SFA (4:0 and 6:0) and the long-chain stearic acid (octadecanoic acid, 18:0) are considered neutral in this regard (Givens, 2005; Lee et al., 2006). It has been recognised that 14:0 increase cholesterol levels more potently than 16:0, being the most atherogenic or hypercholesterolaemic, while 18:0 appears to have no effect on cholesterol levels (Williamson et al., 2005). Even so, the dietary recommendations for the intake of saturated fat include also stearic acid along with the other SFA that raise blood cholesterol. Therefore, a diet to lower blood cholesterol should emphasise the decrease of SFA other than stearic acid.

Saturated, monounsaturated and *n*-6 PUFA, mainly LA, make up most of the fatty acids present on the human diet. Most unsaturated fatty acids in the diet exist in the *cis* configuration and the double bonds are usually positioned at the 3rd, 6th or 9th carbon atom from the terminal methyl group. Regarding MUFA, these fatty acids occur mainly as *cis* and *trans* isomers of 18:1, of which the most prominent is oleic acid (*cis*9-octadecenoic acid, 18:1*c*9). From a nutritional perspective, human diets rich in *cis*-MUFA have been related to positive health benefits. A substantial increase in oleic acid content in the diet is desirable as it can be regarded as antiatherogenic (Molketin, 2000). In fact, 18:1*c*9 is known to have hypocholesterolemic properties, reducing LDL-cholesterol levels and increasing high density lipoprotein (HDL)-cholesterol concentrations in blood, which result in lower risk of coronary problems (Katan et al., 1994).

The dietary PUFA, LA and ALA, must be provided with the diet to humans and others mammals as they cannot be synthesized *de novo*. The *n*-3 PUFA, especially α-linolenic, is present in large quantities in certain plant sources and derived vegetable oils (canola oil, soybean oil, walnuts and flaxseed or linseed oil), while the long-chain *n*-3 PUFA (LC *n*-3 PUFA), EPA and DHA are found largely in seafood (seafoods, fish oils and algae of marine origin). Although meat has lower concentrations of *n*-3 PUFA compared to oil fish, it is a very significant source due to general low fish consumption (Scollan et al., 2001; De Henauw et al., 2007). These essential fatty acids (LA and ALA) are of prime importance for health, because they can be converted to long-chain PUFA via a series of stepwise desaturations and elongations. In the body LA is metabolized to arachidonic acid (AA, *all-cis*5,8,11,14-eicosatetraenoic acid, 20:4*n*-6) and ALA is metabolized to EPA and DHA (Simopoulos, 2002). The PUFA composition is strictly controlled by a complex enzymatic system, such as Δ^5 and Δ^6 desaturases and elongases lipogenic enzymes, responsible for the conversion of 18:2*n*-6 and 18:3*n*-3 to their long-chain metabolites (Smith, 2007). Both fatty acids compete for the activity of a rate-limiting Δ^6 desaturase, and although this enzyme shows greater substrate specificity for ALA, the surplus of dietary LA limits the conversion of ALA to EPA *in vivo* (Burdge & Calder, 2005). Indeed, the intake of these essential fatty acids and their PUFA

metabolites, particularly of LC *n*-3 PUFA, has been associated with improved health benefits (Howe et al., 2006). The LC *n*-3 PUFA are considered to be more beneficial to human health than the shorter carbon chain, α-linolenic acid. Both families of PUFA have a major role in eicosanoid metabolism and regulation of gene expression as important cell-signalling molecules, but they are metabolically and functionally distinct with different physiological functions in human health (Nakamura et al., 2004; Simopoulos, 2006). However, the balance between *n*-6 and *n*-3 PUFA in human diets is essential for homeostasis, normal growth and development, because the eicosanoid metabolic products from *n*-6 and *n*-3 fatty acids have opposing biological properties (Prates & Bessa, 2009). In fact, these PUFA form their own series of eicosanoids, which are precursors for hormones-like mediator substances, such as prostaglandins, thromboxanes and leukotrienes (Kelly, 2001; Seppänen-Laakso et al., 2002; Wood et al., 2008), being of extreme importance in the formation of cell membranes, maintaining their function and integrity, and also participate in the regulation of plasma lipids levels. The eicosanoids from AA, the principal membrane of *n*-6 PUFA, are biologically active in very small quantities (specifically prostaglandins - PGG_2, PGH_2, PGI_2, thromboxanes - TXA_2, leukotrienes - LTB_4, hydroxyl fatty acids, and lipoxins). Moreover, if they are formed in large amounts than those formed from *n*-3 fatty acids (in particularly EPA) they contribute to the formation of thrombi and atheromas, the development of allergic and inflammatory disorders, as well as cell proliferation. Thus, a diet rich in *n*-6 PUFA, as in modern diets, shifts the physiological state to one that is prothrombotic, proaggregatory and proinflammatory (Simopoulos, 2006).

Although the consumption of the essential *n*-6 PUFA has been correlated with tumour promotion, the intake of *n*-3 PUFA has been shown to have protective properties. An important feature of *n*-3 fatty acids, which are vital components in the retina and the membrane phospholipids of the brain, is their role in the prevention and modulation of several chronic diseases, particularly coronary heart disease confirmed by clinical investigations (Connor, 2000). Pregnant and lactating women and infants should benefit since their diet is deficient in *n*-3 PUFA, especially for the vegetarians (Simopoulos, 1999). The LC *n*-3 PUFA are central constituents of the phospholipids of practically all animal and plant cell membranes (Raes et al., 2004). Nutritional research has focused on the health benefits of both EPA and DHA, which are synthesized endogenously in the human body but at low levels considered inadequate to meet the requirements of LC *n*-3 PUFA (Lourenço et al., 2008).

The consumption and/or dietary supplementation of EPA and DHA may reduce the risk of cardiovascular disease as well as some inflammatory and neurological diseases (Rymer & Givens, 2005; Scollan et al., 2006; Griffin, 2008). The EPA is released to compete with the AA for enzymatic metabolism inducing the production of less inflammatory and chemotactic derivatives (cytokines as TXA_3, PGI_3 and LTB_5). Imbalance in the *n*-6/*n*-3 PUFA ratio may be related to the increased production of pro-inflammatory cytokines and eicosanoids contributing to the aetiology of numerous

illnesses states, including cardiovascular disease, cancer, osteoporosis, and inflammatory and autoimmune diseases (Simopoulos, 2002). In order to obtain an adequate intake of these fatty acids it will be necessary to consider the enrichment of food supply with *n*-3 PUFA. Food industry have recognised the need to provide LC *n*-3 PUFA to the consumer diets and, in the last ten years, a range of nutritional supplements and functional food enriched in *n*-3 PUFA have been developed and encouraged by the introduction of nutritional and health claims (USA FDA, 2004). A host of health agencies and professional organizations worldwide have issued recommendations on the intake of LC *n*-3 PUFA. The British Department of Health (1994) recommends a daily intake of 0.2 g of LC *n*-3 PUFA. Additional recommendations for PUFA, reviewed by Simopoulos (2000), are 1% of diet energy for ALA and 0.3% for EPA+DHA. Recently, SACN/COT (2004) recommended that the intake of LC *n*-3 PUFA in United Kingdom (UK) should be increased from the current value of around 0.2 g/day to 0.45 g/day. According to Wijendran & Hayes (2004), EPA and DHA intake of 0.2-0.5% of energy (0.4-1 g/d) reduced risk of CHD. De Henauw et al. (2007) reported that feasible and realistic *n*-3 fatty acids enrichments of foods from animal origin can be add substantially to the overall efforts for reducing the *n*-6/*n*-3 ratio intake in the population. Thus, it is widely acknowledged that the return to a balanced fatty acid diet could be achieved by improving the intake of polyunsaturated fats, mainly *n*-3 fatty acids (and low *n*-6/*n*-3 PUFA ratio), which exert suppressive effects of inflammatory cytokines (Evans et al., 2002; Simopoulos, 2006).

Special attention has been given to some unsaturated fatty acids, which have one or more double bonds in the *trans* configuration, instead of the *cis* configuration. *Trans* fatty acids occur naturally in some foods, especially those from ruminant animals. These fatty acids arise as intermediates of the ruminal biohydrogenation of dietary unsaturated fatty acids (Fritsche & Steinhart, 1998a). In addition, they are also intentionally produced in great amounts by partial hydrogenation of unsaturated vegetable oils during the industrial process (Craig-Schmidt & Holzer, 2000).

The interest in the health effects of TFA has centered on potential adverse effects of *trans* fatty acids on lipid risk factors for cardiovascular disease, as well as on metabolism of the essential *n*-6 and *n*-3 fatty acids, particularly in relation to infant growth and development (Willett et al., 1993; Friesen & Innis, 2006). Although the recommendations of public health organizations are to reduce the content of total TFA in foods, growing evidence has been reported that different TFA have distinct biological effects. An association between the intake of *trans* fatty acids (4-6% of diet energy) with CHD and atherosclerosis risk has been reported in prospective cohort studies (Hunter, 2006; Willett, 2006; Prates & Bessa, 2009). However, it is still not clear which TFA isomers are responsible making it difficult to draw conclusions. This association generally do not apply to TFA of ruminants sources (Parodi, 2004; Jakobsen et al., 2006). According to Pfeuffer and Schrezenmeir (2006) *trans* fats not only raise LDL-cholesterol levels in the same way as SFA but also decrease the favourable HDL-

cholesterol. The high levels of dietary TFA have been found to increase the ratio of LDL-cholesterol/HDL-cholesterol, which is unfavourable for human health.

Within Europe, the Mediterranean consumers seem to have a lower TFA intake compared to those from Northern European countries (range from 0.5-2.6% of diet energy). Intakes in Europe range from minimal values in Italy, Portugal, Greece and Spain (1.4-2.1 g/day) to greater values for Germany, Finland, Denmark, Sweden, France, United Kingdom, Belgium, Norway, The Netherlands, and Iceland (2.1-5.4 g/day). The contribution of TFA from ruminant fat ranges 30-80% of total TFA corresponding to 0.3-0.8% of energy (EFSA, 2004; Craig-Schmidt, 2006; Prates & Bessa, 2009). It was estimated that meat and meat products may contribute from 10 to 30% of TFA for human diet (Wolff et al., 1998; Prates & Bessa, 2009). However, nowadays, the TFA intakes have a tendency to decrease mainly due to industry efforts in replacing or reducing TFA contents in foods (Craig-Schmidt, 2006). The main dietary TFA are *trans*-octadecenoic acids (18:1*t*), which contribute to approximately 80-90% of total TFA content in foods, whereas *trans*-polyunsaturated fatty acids appear only in trace amounts (reviewed by Larqué et al., 2001). Within the *trans*-octadecenoic acids, which have a double bond position from Δ6-Δ16, the 18:1*t*9 (elaidic acid or *trans*9-octadecenoic acid) and 18:1*t*10 isomers are more effective in increasing plasma cholesterol ratio than 18:1*t*11. Vaccenic acid is the major TFA in ruminant meats, and the endogenous precursor of *cis*9,*trans*11-18:2 (*c*9,*t*11) isomer, also called rumenic acid (Prates & Bessa, 2009). A number of animal studies indicates that 18:1*t*11, in contrast to the other TFA, is neutral and has been suggested to be health promoting due to its conversion to *c*9,*t*11 isomer (Corl et al., 2003; Valeille et al., 2005). Nonetheless, the effects of ruminant-derived TFA on human health have yet to be fully clarified and increased amounts of these fatty acids in ruminant products should be viewed with caution.

1.3.2 Conjugated linoleic acid isomers

The isomer *c*9,*t*11 belongs to a specific group of unsaturated fatty acids, named conjugated linoleic acid, a multiplicity of positional and geometric isomers of linoleic acid, with two double bonds located on adjacent carbons, from positions 6,8 to 12,14. The geometric configuration of these double bond pairs can be *trans/trans, trans/cis, cis/trans* or *cis/cis*. Thus, many of the CLA isomers are also a specialised form of *trans* fatty acids. The term conjugated octadecadienoic acid is sometimes used synonymously as CLA. These isomers, as TFA, also arise as a metabolic by-product of rumen biohydrogenation of dietary unsaturated fatty acids and appear to be functional in health promoting properties, as pointed out by the increasing research which has been mainly conducted in animal models and *in vitro* studies. Studies with animals clearly demonstrate that dietary CLA, at up to the 1% level, induces a variety of health-related benefits (Parodi, 2002a). The major CLA isomer in

foods from animal origin is *c*9,*t*11 accounting for more than 75-90% of CLA intake in the human diet (Kramer et al., 1998; Fritsche et al., 1999; Bauman, 2007). The remainder consists of several minor CLA isomers of which *t*7,*c*9, in general, is the second more prominent ones in ruminant fat, representing 3-16% of total CLA in milk fat (Yurawecz et al., 1999) and 8-15% of total CLA in beef fat (Fritsche et al., 2000).

CLA isomers are of utmost importance in human health, even if only two are thought to possess significant biological activity. The *c*9,*t*11 isomer, which is the most common natural form and has been regarded as the physiologically most active isomer, and the *t*10,*c*12 isomer, that represents only 3-5% of total CLA (Ha et al., 1990; Ip et al., 1991; Parodi, 2003). Kramer et al. (1998) reported that *c*9,*t*11 generally accumulated in higher amounts in tissue lipids while *t*10,*c*12 only in trace levels, depending on the nature of the diet. These observations partly rely on differences in metabolism among isomers. Murru et al. (2003) showed that *c*9,*t*11 and *t*10,*c*12 isomers are differently metabolized, which may be in part explained by their different biological activities. The physiological effects produced by these CLA isomers result in some cases from the independent actions of a single isomer, in other cases from synergistic interactions involving both isomers with numerous metabolic signaling pathways (Pariza, 2002; Pariza et al., 2003).

Current worldwide scientific interest in CLA was primarily encouraged by its identification as an anticarcinogenic compound present in grilled ground beef (Pariza & Hargraves, 1985) being their structure determined later by Ha et al. (1987). It is now recognised that CLA inhibits initiation and growth of breast, colorectal, prostate and skin cancers in animal models (Jahreis et al., 2000; Wahle et al., 2004; Bhattacharya et al., 2006; Kent & Muga, 2008). Findings from studies of human CLA intake and cancer incidence are inconsistent as long as the only data available on the effects of CLA on cancer in humans come from epidemiological studies, as shown in Table 1. One of the major limitations to achieve conclusive findings from these studies relies in the attempt to discriminate the effects of CLA through dietary surveys when a wide array of other compounds, such as linoleic acid, other *trans*-fatty acids, and saturated fatty acids, found in the same food sources as CLA, may also interfere with cancer development (Kent & Muga, 2008). Until now, the exact mechanism of carcinogenesis modulation by CLA is not entirely understood, although it may be related to its antioxidative properties (Belury, 2002) or even to the induction of apoptotic cell death and cell-cycle regulation (Yamasaki et al., 2006). It was also suggested that CLA may inhibit carcinogenesis by modulating several cellular events that are mediated in part by the plasma membrane, when it is incorporated into cell membrane phospholipids. The replacement of PUFA with CLA in the plasma membrane composition may affect for example oxidative balance, eicosanoid synthesis and signal transduction (Fritsche & Steinhart, 1998a).

Table 1. Biological role of CLA and its isolated isomers (*c*9,*t*11 and *t*10,*c*12) in different models of health-related disorders.

Health-related disorders	*Main biological effects*	*CLA isomer*	*Experimental Design*			*Ref.*
			Animal studies	*In vitro studies*	*Clinical/Epidemiological studies*	
Cancer	↓ skin tumor burden	mixture	rat			1
	protection against mammary tumor induction	mixture	rat			2
	↓ pulmonary metastasis in mammary cancer	mixture; *c*9*t*11; *t*10*c*12	rat			3
	↓ chemically induced breast cancer	mixture		MCF-7 cells		10
	↓ risk of breast cancer development	serum CLA			women	11
	no link between CLA and ↓ risk of breast cancer	dietary CLA			women	12, 13
	↓ gastrointestinal/colon rectal tumor burden	mixture	mouse, rat			4, 5
		*t*10*c*12		HT-29 colon cancer cells line		6
	↓ peritoneal metastasis of colon+ gastric cancer	*t*10*c*12		Caco2 colon cancer cells		7
		mixture		human gastric+colon cancer lines		8
	↓ risk of colorectal cancer	dietary CLA			women	9
	↓ prostate tumor burden	mixture	mouse			14
	↓ prostate cancer proliferation	mixture; *t*10*c*12; *c*9*t*11		PC-3 prostate carcinoma cell lines		15
	↓ angiogenesis	*c*9*t*11; *t*10*c*12	mouse			16
		mixture	mouse	mammary stromal cells		17
Body fat composition	↓ fat depot mass	mixture	pig			18
		mixture; *t*10*c*12; *c*9*t*11	hamster			20
		*t*10*c*12		3T3-L1 adipocytes		19
	↓ mean adipocyte volume	mixture	rat			21
	↑ adipocyte size	*c*9*t*11	rat			36
	↓ body fat mass	mixture			overweight/obese human	22
		mixture			healthy volunteers	23
Diabetes	modulation (hyperinsulinemia, glucose tolerance)	mixture	ZDF rats			24, 25
	induced lipodystrophy and insulin resistance	mixture	C57BL/6 mice			26
	induced hyperinsulinemia and fatty liver	*t*10*c*12	mouse			27
	↓ insulin resistance	80% *c*9*t*11:20% *t*10*c*12	rats			35
	↑ expression of PPARγ	*c*9*t*11		human preadipocytes		28
	↑ insulin resistance	*t*10*c*12			obese human metabolic syndrome	29
	↑lipid peroxidation, ↓ insulin sensitivity	*c*9*t*11			obese human metabolic syndrome	30
Atherosclerosis	regression of aortic lesions	*c*9*t*11; *t*10*c*12	rabbit			31
	↑ HDL:LDL cholesterol ratio	*c*9*t*11	hamster			32
	modulation of vascular homeostasis	mixture		bovine aortic endothelial cells		33
	↑/↓ HDL:LDL ratio	*c*9*t*11; *t*10*c*12			healthy human	34

[1] Ha et al. (1987); [2] Ip et al. (1995); [3] Hubbard et al. (2003); [4] Ha et al. (1990); [5] Kim & Park (2003); [6] Kim et al. (2003); [7] Kim et al. (2002); [8] Kuniyasu et al. (2006); [9] Larsson et al. (2005); [10] Park et al., 2000; [11] Aro et al. (2000); [12] Chajes et al. (2002); [13] Voorrips et al. (2002); [14] Cesano et al. (1998); [15] Ochoa et al. (2004); [16] Masso-Welch et al. (2004); [17] Masso-Welch et al. (2002); [18] Ostrowska et al. (1999); [19] Choi et al. (2000); [20] de Deckere et al. (1999); [21] Poulos et al. (2001); [22] Blankson et al. (2000); [23] Mougios et al. (2001); [24] Houseknecht et al. (1998); [25] Ryder et al. (2001); [26] Tsuboyama-Kasaoka et al. (2003); [27] Clement et al. (2002); [28] Brown et al. (2003); [29] Risérus et al. (2002); [30] Risérus et al. (2004); [31] Kritchevsky et al. (2004); [32] Valeille et al. (2004); [33] Coen et al. (2004); [34] Tricon et al. (2004); [35] Choi et al. (2007); [36] Lopes et al. (2008).

A significant number of reports have provided either direct or indirect evidence suggesting that CLA may interfere with prostaglandin production through a decrease in the supply of arachidonic acid precursor, may improve insulin function with positive implications for diabetes, but in contradiction some animal and human studies have shown unsafe effects (Ryder et al., 2001). Recent molecular assays have also indicated that CLA acts at least partially through its high affinity for ligand of peroxisome proliferator activated receptor gamma (PPARγ), which is a family of transcription factors known to affect gene expression involved in numerous pathways of lipid transport and metabolism (reviewed by Kent & Muga, 2008). Further clinical studies are warranted to determine how pro-insulin and lipid peroxidation levels may relate to CLA-dependent insulin resistance in humans with symptoms of metabolic syndrome (reviewed by Bhattacharya et al., 2006).

Nowadays, most of CLA research has focused on these two isomers due to the circumstance that synthetic available CLA supplements consist of an equimolar mixture of *t*10,*c*12 and *c*9,*t*11 CLA isomers (often in a 1:1 ratio), with variable amounts of other geometrical and positional isomers, as minor components. Although commercial CLA is produced by alkali isomerisation of linoleic acid-rich oils, such as sunflower or safflower oil, and tends to contain the two main isomers, a lot of nutritional studies have been carried out with more complex mixtures (Christie, 2003). Christie et al. (1997) and later Yurawecz et al. (1999) showed that the main CLA isomers present in supplements, in addition to the *c*9,*t*11 and *t*10,*c*12 isomers, were *t*8,*c*10 and *c*11,*t*13 which were produced from *c*9,*t*11 and *t*10,*c*12 by thermal sigmatropic rearrangement (Pariza et al., 2001; Destaillats & Angers, 2003). The majority of the food supplements contain between 60 and 80% of CLA in the form of free fatty acids (Saebø, 2003). With the advent of technology, enriched or purified *c*9,*t*11 and *t*10,*c*12 CLA formulations have become commercially available in recent years, leading to studies with pure single or individual isomers examining the effects in health-related disorders (Bhattacharya et al., 2006). The use of enzymes, seem to be promising tools to purify and separate the *c*9,*t*11 and *t*10,*c*12 CLA isomers (Saebø, 2003). These isomers can be elongated and desaturated in animal tissues by the enzymes involved in the biosynthesis of arachidonic acid to produce analogs that may even be responsible for the biological activity of CLA (Sebédio et al., 1997; 2001).

Most of the evidence in relation to the health effects of CLA isomers have been derived from animals cell culture studies, and the challenge is to define the health effects of CLA in human beings. The studies in several animal models showed that CLA usually reduces fat depot mass, possibly by inhibiting lipoprotein lipase-mediated lipid uptake into adipocytes. Some of the underlying mechanisms suggested to be involved in fat reduction with CLA intake are increased energy expenditure and fat β-oxidation, decreased adipocyte size and energy intake, and also inhibition of enzymes involved in fatty acid metabolism and lipogenesis such as lipoprotein lipase. Increased

apoptosis was also observed in adipocytes of CLA-fed animals, as inferred from levels of apoptotic factors such as tumor necrosis factor alpha (TNF-α) and uncoupling protein-2 (UCP-2) (reviewed by Park & Pariza, 2007).

A recent study in animal models of atherosclerosis demonstrates favourable properties of CLA, although its effects on cholesterol ratios seem to be isomer-dependent (Kent & Muga, 2008). Further work needs to be done to investigate promising putative mechanisms, for instance downregulation of prostaglandin I_2 and ligand-activation of PPARγ, sterol regulatory element binding proteins (SREBPs) and stearoyl-CoA desaturase (SCD) activity as well as to identify other potential routes capable of explaining the isomer-specific effects of CLA on cholesterol ratios.

Some short- and long-term studies in healthy/obese and in sedentary/exercised humans have suggested the beneficial effects of CLA in reducing fat mass without significantly affect body weight (Tricon et al., 2004). However, the noteworthy effects seen in animal studies, especially in mice, have not been reflected in humans. This may be partly due to CLA dosage used in human studies, much lower compared to the doses used in animal studies. Moreover, most animal studies have been done in young growing mice or rats, whereas studies in humans were mostly in mature volunteers (Bhattacharya et al., 2006). So, to date, work in human subjects remains inconclusive to define the potential of CLA for use in the treatment or prevention of obesity, emphasizing the need for controlled clinical trials, short and long-term clinical studies, in the near future. With the growing interest on CLA, epidemiologic studies will be decisive to achieve scientific support on the potential effects of CLA in human health. Conjugated linoleic acid has been found both in human blood and in breast milk and has been suggested the key factor for the inverse relationship between breast cancer risk and milk consumption in humans (Knekt et al., 1996). Low concentrations of CLA are found in human blood and tissues, but blood levels can be increased by dietary sources (Huang et al., 1994).

Risérus et al. (2002) reported that diet supplementation with the *t*10,*c*12 CLA isomer increases oxidative stress and inflammatory biomarkers in obese men. In addition, Poirier et al. (2006) showed that *t*10,*c*12 CLA isomer can induce inflammation of white adipose tissue. In fact, the use of CLA isomers as a weight loss supplement presents a particularly complex safety health concern, since overweight or obese individuals may be predisposed to develop other conditions, such as atherosclerosis and diabetes, in which CLA appears to play a modulator role. However, in most of these studies synthetic CLA supplements were used, surpassing usual dietary intake by far and do not reflect natural isomer composition, making it difficult to judge whether dairy and meat products may exert the same effects (Collomb et al., 2006). Only a few studies have investigated the health effects in humans of naturally occurring CLA from food products and evidence is weak and conflicting at current levels of intake. Moreover, the research in humans has focused mainly on the effects of

*t*10,*c*12 CLA isomer, which is present at residual levels in foodstuffs, rather than on the predominant *c*9,*t*11 isomer (Prates & Bessa, 2009).

To assess the role of CLA in the prevention of disease, using epidemiologic and clinical intervention studies is necessary to have accurate data for CLA levels and composition in synthetic CLA, foodstuff or biological matrices (Parodi, 2003). In the past ten years there have been marked improvements in the analysis of fatty acids and CLA isomers. In animal tissues, natural CLA is in esterified form and is present at low levels. In this regard, sample preparation and extraction methods are critical since they can lead to destruction and/or incomplete extraction of lipids. The two established methods currently applied for total lipid extraction from foodstuffs or biological matrices are based on the Folch method (Folch et al., 1957) and Bligh and Dyer procedure (Bligh & Dyer, 1959 quoted by Cruz-Hernandez et al., 2006) or modifications thereof.

The most common derivatives used for chromatographic analysis of fatty acid composition, including CLA isomers, in meat and dairy products, are methoxi derivatives prepared by reaction with excess of methanol in the presence of catalytic amounts of acid or base. Methoxi derivatives, such as fatty acids methyl esters (FAME), are preferred because of their greater volatility in nature and superior resolution by gas-chromatography (GC) as well as they are chemically simple (Adlof, 2003; Cruz-Hernandez et al., 2006). Different procedures for acid and/or base-catalysed transesterification have been applied in fatty acid analysis. Substantial works confirm that acid-catalysed transterification (HCl/methanol, H_2SO_4 in dry methanol, and BF_3/methanol) is undesirable, in general, for the preparation of FAME, because it can cause geometrical isomerisation with an increase in the relative proportion of *trans*,*trans* isomers and produce methoxy artifacts (Christie et al., 2001; Luna et al., 2007). On the other hand, free fatty acids, *N*-acyl lipids (i.e. sphingolipids and glycosphingolipids) and alk-1-enyl ethers are not methylated by base-catalyts, such as NaOMe, NaOH, KOH and tetramethylguanidine (Kramer et al., 1997; Cruz-Hernandez et al., 2006). In fact, there is no single derivatization procedure that adequately addresses each of these shortcomings. Therefore, it has been recommended consecutive methylations (combine transterification procedure) for the derivatization of meat lipids in particular (Cruz-Hernandez et al., 2006; Nuernberg et al., 2007).

Recent advances in technology have allowed the identification and characterisation of specific isomers of fatty acids present in ruminant-derived products. Capillary gas-chromatography is by far the most widely and available method for fatty acids analysis and the availability of longer columns (100 m or more) allow the optimum resolution of fatty acids (plus a range of fatty acids from 8:0 to long-chain PUFA as 22:6*n*-3) and determination of CLA content. However, a effectively separation of the geometric and positional isomers of octadecadienoic acid (18:2) by GC even when using 100 m capillary columns is incomplete and overestimates the major isomer because of the extensive

overlap of *trans,trans* and *cis/trans* 18:2 isomers (usually *c*9,*t*11 co-elute with *t*7,*c*9 and *t*8,*c*10) (Yurawecz & Morehouse, 2001; Murru et al., 2003). These limitations of GC in CLA isomer resolution are well known (Roach et al., 2002). Detailed information on CLA isomer composition can be better achieved with silver ion high-performance liquid chromatography (Ag^+-HPLC). In fact, silver-ion chromatography has become an important methodology in the fractionation and characterisation of lipids (Dobson, 2003). The complementary of new innovative techniques to GC, such as tandem-column Ag^+-HPLC, Fourier-transform infrared spectroscopy (FTIR) and near infrared transmittance spectroscopy (NIRS), attenuated total reflectance (ATR), mass spectrometry (MS) and carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR), have enabled complete and accurate assessment of all fatty acids and CLA isomers (Kramer et al., 2004; Aldai et al., 2006a; Cruz-Hernandez et al., 2006; Nuernberg et al., 2007; Sierra et al., 2008; Prates & Bessa, 2009).

It is well known that products derived from ruminant animals, including milk (Collomb et al., 2004, 2006; Pestana et al., 2009; Rego et al., 2009), dairy products (Ma et al., 1999; Rickert et al., 1999; Martins et al., 2007) and meat (Fritsche et al., 2001; Dannenberger et al., 2005; De La Torre et al., 2006; Schmid et al., 2006) are the most important sources of CLA in the human diet (Jahreis & Kraft, 2002). Meat and meat products contribute about 25-30% of the total human CLA intake in Western populations whereas only trace amounts of CLA occur in fish and some vegetables (Chin et al., 1992). The levels of total CLA in various foods have been reported to vary from negligible amounts in seafoods and vegetables oils (0.2 mg/g fat) to higher levels in milk fat (30 mg/g fat), of which *c*9,*t*11 accounts up to 90% of the total CLA (Chin et al., 1992; Parodi, 1994). This wide occurrence of CLA in common foods leads to different human dietary intakes, depending on food selection and eating preferences (ruminant *vs.* nonruminant sources and plant *vs.* animal sources) (O'Shea et al., 1998). A variety of factors can potentially influence CLA intake, including the amount of full fat dairy foods and meat consumed, and possibly age, gender, and physiological state (McGuire et al., 1999).

Little or no data exist regarding optimal dietary intake of CLA in humans. The minimal effective dose response is still unknown. Hypotheses based on extrapolations from animal studies and on calculations from epidemiologic findings range between 0.095 and 3.5 g/day (Ha et al., 1989; Enser, 1999). In fact, few estimates of dietary CLA intake are available and information on the availability of CLA contents from foods or supplements is scarce. Moreover, data on the extent of incorporation of dietary CLA into plasma lipids are limited in humans (Fremann et al., 2002). However, on the basis of the anticancer effects of CLA in rats, a daily consumption of 0.8-3.0 g of CLA might provide a significant health to humans (Ip et al., 1994; Parrish et al., 2003). Recent human studies have demonstrated beneficial effects of *c*9,*t*11 CLA isomer on human health, at concentrations >1.2 g of *c*9,*t*11 CLA/day (Tricon et al., 2004), whereas animal studies suggest that as little as 0.8 g of *c*9,*t*11

CLA/day may be sufficient for tumor inhibition (Watkins & Li, 2003). These authors calculated the human equivalent CLA intake, based on 0.1% dietary CLA given to rats, to be 0.72 g of CLA for a 70 kg person, adjusting for the difference in metabolic rate of humans *vs.* rats, for anticarcinogenic protective effects in humans.

It was estimated that human CLA intake range from 0.3 to 1.5 g/person/day and appear to be dependent on gender and on the intake of food from animal and vegetable origin (Fritsche & Steinhart, 1998a). The same authors also reported that the mean dietary intake of CLA in German population was estimated to be 0.36 or 0.44 g/day, for women and men, respectively, according to their habits of consumption (Fritsche & Steinhart, 1998b). Recently, Collomb et al. (2006) reviewed the estimation of average total CLA intake and stated a range between 0.095 and 0.44 g, depending on the country. Australia shows the highest CLA consumption, 0.5 to 1.0 g/day, although the estimation method applied is unknown (Parodi, 1994). Our work group has determined the contents of CLA isomers in the most consumed Portuguese CLA rich foods (milk, butter, yoghurt, cheese, beef and lamb meat) and the contribution of these ruminant-derived foods to the daily intake of CLA isomers based on Portuguese consumption habits. The average estimated total CLA intake for the Portuguese population was 0.074 g/day (Martins et al., 2007). Wolff and Precht (2002), based on milk consumption data, estimated the *c*9,*t*11 ingestion in fifteen European countries, obtaining higher intake values in North Europe and lower in Mediterranean countries. According to them, France and Italy showed consumption average values close to the EU and similar daily intake were observed for Spain (0.14 g), Greece and Portugal (0.15 g). The difference from our values for Portugal might be explained by the distinct estimation methods and statistics sources used. According to these all assumptions, the estimated average total CLA intake by humans from dietary sources is well below to that is thought to be required for exert the potential beneficial biochemical, molecular and physiological effects against cancer, atherosclerosis and obesity based on animal studies.

1.4 FAT CONTENT AND FATTY ACID COMPOSITION OF RUMINANT MEAT

Fat content and fatty acid composition of ruminant meats have been extensively studied. Even so, it still receives a special attention in research since meat contains relatively higher amounts of saturated fat (Webb & O'Neill, 2008; Muchenje et al., 2009). For most consumers, fat is considered an unpopular constituent of meat being labelled as unhealthy. However, fat and long-chain fatty acids, whether in adipose tissue or muscle, contribute significantly to various aspects of meat quality and are fundamental to the nutritional value of meat (Wood et al., 2008). Moreover, fat also provides palatability and flavour to foods, playing a key role in manufacture and cooking processes

(Williamson et al., 2005). Meat fat is an important source of energy (fat provides 37 kJ or 9 kcal versus 4 kcal provided by either protein or carbohydrate), bioactive lipid components, antioxidants, and also serves an important carrier or delivery medium for nutrients, including lipid-soluble vitamins (vitamins A, D, E and K) and essential fatty acids. Therefore, fat is an essential component of the diet even though it should be consumed in moderation (Kelly, 2001).

Meat fats are commonly classified as depot fats (subcutaneous, intermuscular and intramuscular). Scollan et al. (2006) point out that meat fat is presented as membrane fat (as phospholipids), intermuscular fat (between muscles), intramuscular (within muscles) and subcutaneous fat (visible fat). Intramuscular fat deposition, in most cases designated as marbling fat, can be defined as the total fat content of muscle (i.e. neutral lipids plus phospholipids) (Rhee, 2000; Pannier et al., 2006), being irreversibly connected with meat because it cannot be trimmed before or during human consumption, contrarily to visible fat (Chizzolini et al., 1999).

Ruminant fats comprised a wide range of lipid classes with complex structures and with most of the fatty acids bound to chemical structures to form fractions such as triacylglycerols (TAG), phospholipids and galactolipids (Aldai et al., 2006a). Ruminant fats mainly consist of triacylglycerols and phospholipids. The TAG are the main neutral lipids (NL) components of all depot fats, serving as energy stores and deposited in adipocytes, consisting of fatty acids and glycerol. The phospholipids or polar lipids (PL), rich in PUFA, are the building blocks of cell membranes. The IMF content depends on the amount of TAG, while the amount of phospholipids in beef muscle (0.5 to 1% of muscle weight) is relatively constant (Scollan et al., 2006). Hence, there is a strong relationship between IMF and the content of TAG, increasing the deposition of triacylglycerols in adipocytes with increasing intramuscular fat content. According to the former authors, lean beef has a low IMF content, typically between 2 and 5%, which is accepted as being “low in fat”. Intramuscular fat has been reported as the fat depot of most interest in relation to fatty acid composition and human health. In fact, given the more PUFA amounts of this fat compared to removable fat depots, there is a large relevance of this fat for the intake of LC-PUFA (Raes et al., 2004). Moreover, the content and fatty acid composition of intramuscular fat of ruminant meat are major factors affecting its technological and sensory quality, mainly shelf life (lipid and pigment oxidation) and flavour (Wood et al., 2004).

1.4.1 Fatty acid composition of intramuscular fat and cholesterol

Fatty acid composition of ruminant meats, particularly beef and veal, comprises mostly SFA and MUFA (Valsta et al., 2005). Saturated and monounsaturated fatty acids constitute the major component of TAG and are present in similar proportions in lean red meats (Williamson et al., 2005). The main SFA are 16:0 and 18:0 representing the later 30% of total SFA. Both SFA and 18:1*c*9, the

predominant MUFA, account for approximately 80% of total triacylglycerol fatty acids (Scollan et al., 2006), being the most ubiquitous non-essential fatty acids. A substantial proportion of SFA in ruminant meats (beef and lamb) results from the extensive biohydrogenation by the rumen bacteria of dietary unsaturated fatty acids, after lipolysis (reviewed by Jenkins et al., 2008). The rumen microorganisms are also responsible for the presence of odd-number carbon and branched-chain fatty acids (with *iso*- or *anteiso* structure) in beef intramuscular fat, which have been found at low levels. Branched-chain fatty acids make up 15 to 20% of total bacterial fatty acids, and as much as 30% of fatty acids in bacterial phospholipids (Jenkins, 1994). Their main function in membranes may be to increase the fluidity of lipids as an alternative to double bonds, which are more liable to oxidation. The presence of odd- and branched-chain fatty acids in milk can be used to identify shifts in the rumen microbial population, as most are of bacterial origin (Kliem et al., 2008). According to Raes et al. (2004), little attention has been paid to these fatty acids, but their presence in ruminant milk and meat has been related to differences in feeding strategies (Dewhurst et al., 2002).

The proportion of PUFA in phospholipids is much higher (45-55% of the total fatty acids) than in TAG, being 18:2*n*-6 and 18:3*n*-3 the foremost PUFA and representing approximately 2-3% of total triacylglycerol fatty acids (Geay et al., 2001; Scollan et al., 2006). Linoleic acid, which is at high levels in concentrate feedstuffs (grains and oilseeds) is converted into TFA and SFA by microbial biohydrogenation and only a small proportion, around 10% of 18:2*n*-6, is available for incorporation into tissue lipids. In ruminants, 18:2*n*-6 occurs at higher levels in muscle than in adipose tissue whereas in pigs the fatty acid is at higher levels in adipose tissue even if the fatty acid composition of the two tissues is largely similar (Wood et al., 2008). α-Linolenic is abundant in fresh forages (constitutes over 50% of total fatty acids) and is also stored in significant amounts in ruminant tissues (Geay et al., 2001; Wood et al., 2008). The amount and the nature of meat lipids stored in muscle depend on several factors, such as feed conditions, digestion, intestinal absorption, hepatic metabolism and lipid transport systems to muscle (Webb & O'Neill, 2008). Even if meat contains lower concentrations of PUFA compared to fish oil, their contribution can become significant if large amounts of these foods are being consumed. In phospholipids, the proportion of PUFA contains not only the essential fatty acids 18:2*n*-6 and 18:3*n*-3 but also their long derivatives such as the AA, EPA, docosapentaenoic acid (DPA, *all-cis*7,10,13,16,19-docosapentaenoic acid, 22:5*n*-3) and DHA. Generally, intramuscular fat of ruminant animals is composed of approximately 45-55% of SFA, 45-50% of MUFA and relatively minor amounts, up to 5%, of PUFA (Enser et al., 1996; Scollan et al., 2006). The predominant fatty acids include myristic, palmitic and stearic acids as SFA, palmitoleic (*cis*9-hexadecenoic acid, 16:1*c*9) and oleic acids as MUFA and linoleic, α-linolenic and arachidonic acids as PUFA. The TFA occurs naturally in trace amounts, usually 2-4% of total fatty acids in ruminant meats (beef and lamb) while meat from nonruminants such as pork or poultry, present

lower values of TFA (Fritsche & Steinhart, 1998a; Valsta et al., 2005). The TFA content in meat products varied from 0.2% to 3.4% of total fat, which are intermediate values between meat from ruminants and non-ruminants (Fritsche et al., 1999). The major 18:1*t* in ruminant-derived foods is 18:1*t*11, which prevails with around 60% of total 18:1*t* (Valsta et al., 2005; Prates & Bessa, 2009). High concentrations of 18:1*t*10 have been observed in tissues of concentrate-fed ruminants, even without oil inclusion, whereas 18:1*t*11 concentration is consistently associated with forage feeding (Bessa et al., 2006; Dugan et al., 2007).

Ruminant meats usually have a more favourable *n*-6/*n*-3 ratio when compared with meat from monogastric animals, due to the relatively high levels of 18:3*n*-3 in pasture and forage. The *n*-6/*n*-3 ratio in beef from pasture-fed animals is beneficially low, less than 2, reflecting the amounts of *n*-3 PUFA, particularly 18:3*n*-3 and the long-chain fatty acids (EPA and DPA). The predominant LC *n*-3 PUFA is DPA, an intermediate in the production of DHA from EPA, for which functional and nutritional effects are less known (Howe et al., 2006, 2007). In contrast, the PUFA/SFA ratio is typically low, around 0.1, reflecting the ruminal biohydrogenation of unsaturated fatty acids (Moloney et al., 2004; Scollan et al., 2006; Wood et al., 2008). It was shown that the fat level is inversely related to the PUFA/SFA, mainly observed in meat from ruminants (Raes et al., 2004). As fatty acids, cholesterol is also an important nutritional component of foods of animal origin, especially meats, since cholesterol is an essential constituent (sterol) of animal cells, located primarily in the membrane component (Chizzolini et al., 1999) and serves as a precursor for steroid hormones including estrogen, testosterone, aldosterone and bile acids. Cholesterol content in beef depends on IMF content and therefore meat with high levels of phospholipids has higher levels of cholesterol. The average cholesterol contents of the main meat species ranged between 60-81 mg/100 g of muscle (Chizzolini et al., 1999). Although variations can be seen among different species, their magnitude appears to be generally low. In fact, a number of studies have demonstrated that the differences, sometimes detected in cholesterol content among some breeds, or between sexes or in relation with some feeding regimes, are small. The works of Padre et al. (2007) and Muchenje et al. (2009) reported low levels of cholesterol in beef from pasture-based production systems. Significant and interesting variations, instead, have been reported in CHR levels between muscle types (Alasnier et al., 1996). Differences in fibre type might be the presumably reason for some of the differences observed in meat cholesterol content among different muscles of the same species and between the same muscles in different species (Chizzolini et al., 1999).

1.4.2 Conjugated linoleic acid isomeric profile of intramuscular fat

Ruminant meats contain high amounts of SFA and TFA, which can be major risk factors for the development of cardiovascular disease. However the consumption of beef and lamb in human diets

also supply CLA, which have shown a wide range of biological activities. CLA are naturally formed by biosynthesis in ruminants using two major different pathways, which mainly lead to *c*9,*t*11 isomer. As mentioned above the most prevalent isomer in beef is the *c*9,*t*11, found to contribute about 60% of the total CLA (Shanta et al., 1994). Rumenic acid, as well as other minor conjugated and nonconjugated *cis/trans* dienes, are produced directly in the rumen of polygastric animals (Figure 4) by the action of a linoleic acid isomerase (EC 5.2.1.5, Δ^{12}-*cis*, Δ^{11}-*trans* isomerase) of the anaerobic bacteria *Butyrivibrio fibrisolvens* and other rumen fermentative bacteria species (group A and B, species of *Micrococcus* and *Eubacterium*) (Kritchevsky, 2000) on dietary unsaturated fatty acids, linoleic acid mainly, as a first step of the biohydrogenation process (Schmid et al., 2006). At this step no hydrogen addition occurs and the same number of double bonds is maintained within the acyl chain (Teter & Jenkins, 2006). This implies a prior lipolysis of galactolipids, phospholipids and TAG of the diet before isomerisation takes place (Bessa et al., 2000). Then, the hydrogenation produces a multitude of positional and geometric *trans* monoenes, of which 18:1*t*11 is usually found in highest concentration. Further hydrogenation of 18:1*t*11 to stearic acid (18:0), the end product of ruminal biohydrogenation, appears to involve a different group of organisms (group B, two species of *Fusocillus* and a Gram negative) and occurs at a slow rate compared to the initial steps (Harfoot & Hazelwood, 1997). For this reason, 18:1*t*11 typically accumulates in the rumen. Hydrogenation is

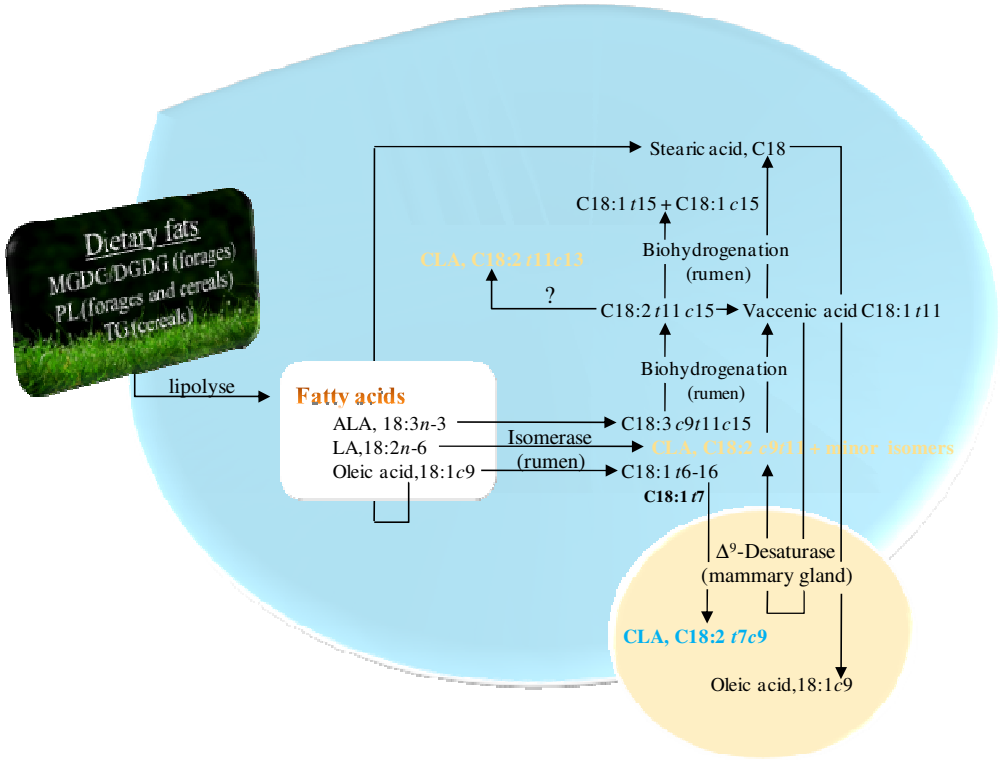

Figure 4. Major metabolic pathways of CLA biosynthesis (adapted from Bauman et al., 2003 and Collomb et al., 2006).

efficiently consummate when ruminal pH is suitable, at 6.0 or above, for the bacteria involved in the isomerisation and hydrogenation of PUFA. Additionally, results *in vitro* support the possibility that 18:1*t* isomers are synthesised from 18:1*c*9 by ruminal microbes (Mosley et al., 2002). However, the biohydrogenation pathway of oleic acid has not been thoroughly explored in review articles.

The predominant PUFA of pasture, the α-linolenic acid cannot be converted directly to *c*9,*t*11 but produce 18:1*t*11 (Parodi, 2003). The pathway for biohydrogenation of 18:3*n*-3 results in a greater diversity of products. In fact, α-linolenic acid is converted initially to the conjugated *cis*9,*trans*11,*cis*15-18:3 by ruminal microorganisms, recently named as rumelenic acid (Destaillats et al., 2005), which is subsequently hydrogenated to the nonconjugated *trans*11,*cis*15-18:2, then to 18:1*t*11 and finally to 18:0 (Teter & Jenkins, 2006; Jenkins et al., 2008). Kraft et al. (2003) hypothesised that α-linolenic acid is the indirect precursor of another CLA, the *t*11,*c*13 isomer. However, the pathway from the nonconjugated *trans*11,*cis*15-18:2 to the *t*11,*c*13 CLA isomer is as yet unclear. Recently, Alves and Bessa (2007) identified a minor polyenoic nonconjugated FAME (*cis*12,*cis*15-18:2*n*-3) present in ruminant fat from lambs fed linseed oil (rich in 18:3*n*-3) by acetonitrile covalent adduct chemical ionization tandem mass spectrometry (CACI-MS/MS), as an intermediate of 18:3*n*-3 biohydrogenation.

The isomerases express their activity by moving the methylene-interrupted double bonds in linoleic and linolenic acids to conjugated bonds. Subsequent action of redutases reduces or eliminates the double bonds. A proportion of *c*9,*t*11 and 18:1*t*11 escape further rumen hydrogenation and after absorption pass via the circulatory system to adipose tissue and the mammary gland (Figure 4). However, most of the *c*9,*t*11 arises from endogenous synthesis (80-90% of the total CLA) by Δ^9 desaturation of 18:1*t*11 via stearoyl-CoA desaturase (EC 1.14.99.5) (Griinari & Bauman, 1999). Enser et al. (1999) suggested a linear relationship between muscle 18:1*t*11 and CLA content. Similarly, the isomer *t*7,*c*9, also arises from Δ^9 desaturation pathway of 18:1*t*7 (Parodi, 2002b). In addition, it has been demonstrated that liver microsomes as well as the mammary gland are capable of desaturating certain *trans* monoenoic isomers, the 18:1*t*7 and 18:1*t*11 to form CLA (Corl et al., 2001). Other rumen isomerases produce small amounts of other CLA isomers (Parodi, 2003). But these other individual CLA isomers found in ruminant fat make up a very small portion of total CLA (< 0.5% of the total CLA) and are derived exclusively from rumen output (Bauman et al., 2003). CLA isomers in ruminant meat are mainly incorporated as triacylglycerols in intramuscular fat and adipose tissue (Shingfield et al., 2007).

Ruminant-derived foods are the primarily source of CLA being the contribution of milk and dairy products greater than beef or lamb (O'Shea et al., 1998; Shingfield et al., 2007). Ruminant meats (beef and lamb) are known to have markedly higher CLA contents compared to those from

nonruminant origin. In general, higher CLA contents in muscles are associated with higher intramuscular fat content (Raes et al., 2004). CLA concentrations in meat and meat products were reviewed by Parodi (2003) and Schmid et al. (2006). The highest CLA contents were found in lamb (4.3-19.0 mg/g fat) whereas in beef were slightly lower (1.2-10.0 mg/g fat), representing 0.5-2% of fatty acids. Lamb meat is usually originated from grass feeding systems and it is well known that the inclusion of grass in the diet improves CLA content in meat. CLA contents in pork, chicken, or meat from horses is usually lower than 2 mg/g fat. The presence of CLA in nonruminant animal fat may result from the inclusion of ruminant-derived products (milk powder, meat meal, or tallow) in the diets. Schmid et al. (2006) reported also data on CLA contents of animals less common in human diets like meat from elk (1.3-2.1 mg/g FAME), bison (2.9-4.8 mg/g FAME) and water buffalo (1.8 mg/g FAME). In fish and marine products, the CLA contents were negligible (Chin et al., 1992; Fritsche & Steinhart, 1998a). Furthermore, CLA contents (mg/g fat) vary substantially not only between species but also from animal to animal and within animal in different tissues. Dufey (1999), quoted by Schmid et al. (2006) showed that CLA concentrations in beef meat from different countries varied from 3.2 to 6.2 mg/g fat, with beef from Argentine, Brazil and Australia displaying the highest values while beef from USA displays the lowest levels. The great variations found in CLA contents were ascribed to differences in feeding regimes between countries (Kraft et al., 2003; Realini et al., 2004).

1.5 FACTORS INFLUENCING FAT CONTENT AND FATTY ACID COMPOSITION OF RUMINANT MEAT

Meat fatty acid composition is highly dependent of both genetic (*e.g.* species, muscle, age or live weight, sex and breed) and environmental factors (*e.g.* nutrition, accommodation, season, climate and animal welfare) (Raes et al., 2001; Aldai et al., 2006b; Okeudo & Moss, 2007). Although the amounts of fatty acids and CLA isomers in beef and lamb are mainly influenced by feeding conditions, the intrinsic factors have also been proposed to modulate these variations (Dannenberger et al., 2007). In addition, post-slaughtering activities, such as the trimming of visible fat, shelf life technologies and cooking methods, can greatly modify the muscle tissue properties and final meat quality (Schönfeldt & Gibson, 2008).

1.5.1 Genetic factors

Fatness is a major animal factor influencing the fatty acid composition of muscle. In fact, fat content of ruminant meat varies widely depending on the animal breed, age, sex and on the part of the carcass used (De Smet et al., 2004).

Deposition of fat in the muscle occurs mainly in the NL fraction (triacylglycerols), with mainly SFA and CLA being deposited in this fraction, while PUFA are mainly presented in the phospholipids (Lourenço, 2007). Rumenic acid distribution is not similar to other PUFA, as it was independent of fat location (Santos-Silva et al., 2003). In ruminant muscles, it is know that CLA is mainly associated to the triacylglycerol fraction, which is linked to the fat content of tissues (Raes et al., 2003b). Variations in metabolic fibre type between muscles are also reflected in differences on fatty acid composition. Due to the higher amount of mitochondria and a high fat turnover, the muscles more oxidative, commonly namely "red", contain a higher proportion of phospholipids than glycolytic ones, being characterised by a high PUFA content (20-50% of total fatty acids) mainly represented by long-chain fatty acids from C18 to C22 and from two to six double bonds (Raes et al., 2004; Wood et al., 2004). In addition, muscles differ in fat concentration based on function and location on the body.

Studies with reference to the effect of age or live weight on intramuscular fat composition have also been related to body fatness. Nürnberg et al. (1998) and Rhee (2000) have shown that increasing age/weight, animals gain a tendency to deposit higher amounts of fat leading to an increase in SFA and a decrease in PUFA levels. In fact, it is accepted that the proportion of PUFA increases with decreasing level of fatness related to higher proportions of phospholipids (Indurain et al., 2006).

Sex and related-hormones may also link to fatness level, being male animals leaner (low IMF) with higher intramuscular PUFA and lower SFA and MUFA amounts than females (Nürnberg et al., 1998). Monteiro et al. (2006) point out that few trials have been conducted to compare fatty acid composition of meat from bulls and steers and the data revealed consistently large differences between the genders in carcass and/or intramuscular fat percentage. De Smet and co-workers (2004) reported that differences in fatty acid composition between the two genders can be explained partially by the degree of fatness and changes associated with triacylglycerol/ phospholipid ratio. The castration procedure also influences the intramuscular fatty acid composition (less PUFA and more SFA) when intact and castrated animals are compared at equal fatness level (Monteiro et al., 2006). Regarding CLA, Fritsche and Fritsche (1998) found no difference in CLA concentration or isomer distribution between fat from bulls and steers, suggesting that CLA content of meat is not influenced by hormonal status.

Meat species are also related with fat deposition presenting ruminant animals, in comparison to monogastrics, a tendency to preferentially incorporate essential fatty acids into muscle lipids, especially phospholipids, rather than storing them in adipose tissue (Wood et al., 2008). In general, IMF content in beef is low but still higher than other animal species, such poultry and pork (Scollan

et al., 2006). The differences between these species may only be partly explained by differences in the digestive process.

The breed (and genotype effect) has been reported by several authors, representing one of the main factors affecting fatty acid composition and carcass conformation because fat deposition differs between breeds and is related to the ratio of triacylglycerols to phospholipids (Raes et al., 2001; Aldai et al., 2006b). Even though breed differences are generally small, they reflect differences in underlying gene expression or enzymes activities regarding fatty acid metabolism, and, therefore, deserve some further consideration (Scollan et al., 2006; Barton et al., 2007). In fact, Δ^9, Δ^6 and Δ^5 desaturases, elongase, SEBP-1c and leptin have been regarded as the most important genetic factors associated with fatty acid deposition in carcass fat. Breed effects could act by enhancing or inhibiting the expression level and activity of the Δ^9 desaturase (Taniguchi et al., 2004; Scollan et al., 2006). The level of expression and activity of the Δ^9 desaturase enzyme is associated with the MUFA content in the muscle of ruminants as well as the levels of CLA as it converts the 18:1*t*11 to *c*9,*t*11, the major CLA isomer in ruminant fats (Lourenço, 2007). Recently, Warren et al. (2008) showed that Holstein-Friesians steers fed grass silage formed more DHA in phospholipids from its precursor supply (18:3*n*-3) than Aberdeen Angus steers. The last authors concluded that Holstein–Friesians have a greater activity or a greater expression of Δ^5 and Δ^6 desaturase enzymes. As point out by De Smet et al. (2004) and Scollan et al. (2006) both enzyme activities as well as the precursor supply will determine the extent of these processes and the outcome proportions of LC-PUFA in intramuscular fat. The breed effects on meat quality based on the presence of mutations within the myostatin gene producing muscular hypertrophy have been less studied (Raes et al., 2001). According to Aldai et al. (2006b, 2007) this effect of muscular hypertrophy on total fatty acid composition has been mainly studied phenotypically, not genotypically. The identification of genes that contribute to continuous variation of meat quality, including intramuscular levels, is a challenge for molecular genetics in this area. So, the DNA variations or DNA polymorphisms may have the potential to serve as marker of lipids in meat and, therefore, constitute a tool to improve the quality of beef (Pannier et al., 2006).

1.5.2 Feeding factors

Diet is an important production factor affecting the composition of meat from cattle and sheep (Dian et al., 2008). Out of the production factors that affect beef production, the feeding system, whether using concentrate, pasture or both, has by far the major influence on nutritional value of meat lipids. Sensory and nutritional properties of meat from pasture-fed animals differ from those of grain-fed animals in flavour, tenderness, colour and fatty acid composition (Aurosseau et al., 2004). Indeed,

meat quality, fatty acid composition and concentration of several nutrients depend largely on the diet fed to ruminants. Intramuscular fatty acid composition is affected by nutritional factors often related to a particular feeding practice and production system (reviewed by De Smet et al., 2004). Several factors have been identified as affecting the fatty acid profile and CLA contents in ruminant edible products, such as the grazing period and the type of forage (French et al., 2000), the season of the year (Jahreis et al., 1999), the forage to concentrate ratio of the diet (Santos-Silva et al., 2002; Lee et al., 2006), the supplementation with unsaturated and unprotected fat (Mir et al., 1999; Bolte et al., 2002) or the sources of the lipid supplements (Duckett et al., 2002).

The major nutritional and/or management tool for increasing the development of marbling is to maximise the availability of net energy (and glucose) for fat synthesis during finishing of animals (Harper & Pethick, 2004). This fact explains the lower IMF content of beef from pasture-fed cattle compared to grain-fed animals. As stated by Alves and Bessa (2007) the fatty acid pattern of ruminant edible fats is very complex and its diversity is mostly determined by the interaction between dietary factors and rumen metabolism. The microbial action in rumen largely determines the pattern of fatty acids available to the animal.

Ruminants naturally consume a diet which is low in fat but high in PUFA whether in fresh or conserved grass. However, a high proportion of PUFA undergoes microbial biohydrogenation leading to predominantly SFA being absorbed in the intestine and deposited in tissues. Therefore, it is more difficult to change fatty acid composition of the intramuscular fat in ruminants through dietary modifications (increase tissue lipid unsaturation) than in monogastric animals (Demeyer, 1999; Rhee, 2000). On the study of Lourenço et al. (2005), the rumen and milk data gave some evidence of a partial inhibition of rumen biohydrogenation when replacing dietary intensive forage by several combinations of grassland silages, resulting in a higher accumulation of biohydrogenation intermediates in the rumen. Additionally, the botanical composition of pastures influences rumen and/or intermediary fatty acid metabolism (Lourenço et al., 2008; Meľuchová et al., 2008). Notwithstanding, the fatty acid profile in ruminants is not a direct consequence of the dietary fatty acid composition due to hydrogenation by rumen microorganisms, some changes in this profile can be related to the diet (Varela et al., 2004). Changes in substrate supply and extent of biohydrogenation will affect the supply of intermediates (in the form of 18:1*trans*, 18:1*cis,* conjugated and nonconjugated 18:2 isomers) and end products of biohydrogenation, influencing the fatty acid composition and CLA contents in meat from ruminants (Dhiman et al., 1999; Bessa et al., 2008).

It was reported that beef from grass-fed cattle has a higher concentration of fatty acids considered to be beneficial to human health (higher proportions of α-linolenic acid, less linoleic acid and overall,

usually higher proportions of PUFA) than beef produced from more intensive production systems (Moloney et al., 2004; Descalzo et al., 2005). The pasture has major effects by decreasing SFA and increasing fatty acids considered as favourable for human health (18:1*c*9, 18:3*n*-3 and *c*9,*t*11 CLA isomer), compared to other diets, especially those based on maize silage and concentrates. Eriksson and Pickova (2007), point out that this higher PUFA percentage in meat from pasture-fed bulls could result from the higher protection of fatty acids in fresh grass from the ruminal biohydrogenation, relative to that of grain or silage. Moreover, this increase in meat PUFA percentage may be also due to the presence of secondary plant metabolites in spontaneous pastures that might inhibit microbial biohydrogenation activity within the rumen (Lourenço et al., 2008). Of particular interest is the use of grass and forages to increase the concentration of ALA in meat since lipids of green forage contain high proportions of 18:3*n*-3 (Razminowicz et al., 2006). According to these authors cattle eating forage-only diets can have a profound impact on the nutritive attributes of beef by altering the lipid composition of meat through lower concentrations of SFA and higher concentrations of LC-PUFA. Previous studies have also addressed that beef from grass fed ruminants contains increased concentrations of β-carotene and α-tocopherol, as well as higher concentrations of *n*-3 fatty acids and conjugated linoleic acid, all substances reported to have favourable effects on human health. In contrast, concentrate diets based on grains and protein supplements have higher amounts of 18:2*n*-6, the precursor of the *n*-6 series of PUFA. These compounds, LA and ALA, can therefore be used as diet markers. However, grass is a variable feedstuff, according to the season of year and whether it is fed fresh or conserved as silage or hay (Wood et al., 2006). Feeding mixtures of forage legume and red clover relative to grass alone results in further enhancements in PUFA content, both 18:2*n*-6 and 18:3*n*-3. These responses in both SFA and *n*-3 PUFA contribute towards beneficial changes in increasing PUFA/SFA and decreasing the *n*-6/*n*-3 ratios (Scollan et al., 2006).

The content and proportions of the individual CLA isomers in ruminant fat can also be clearly affected by diet (Mir et al., 2004; Nuernberg et al., 2007). Finishing ruminants on pasture has been shown to enhance levels of total CLA content (on an mg/g fat basis), and particularly of the *c*9,*t*11 isomer (Santos-Silva et al., 2002; Steen & Porter, 2003; Schroeder et al., 2004; Nuernberg et al., 2005; Lorenzen et al., 2007). In fact, French et al. (2000) and Realini et al. (2004) showed that meat fat from grazing beef had higher CLA contents (10.8 and 5.3 mg/g fat, respectively) than those obtained from concentrate-fed animals (3.7 and 2.5 mg/g fat). Depending on diet, *t*7,*c*9 or *t*11,*c*13 is typically the second most abundant CLA isomer in ruminant meat. According to Dannenberger et al. (2005), the differences found in CLA profiles may be explained by distinct grass intake, since it was shown that pasture feeding, compared to concentrate feeding, increases the proportion of the *t*11,*c*13, *t*11,*t*13 and *t*12,*t*14 CLA isomers (grass intake indicators), and decreases the percentage of the *t*7,*c*9 isomer, in beef lipids. Diets rich in forage favour the growth of fibrolytic microorganisms responsible

for the rumen production of CLA (Madron et al., 2002). On the contrary, lower CLA contents found on beef of concentrate-fed animal's result from the lower PUFA content in concentrates compared with grass pasture and the lower rumen pH due to high concentrate supply (Jahreis & Kraft, 2002). According to Choi et al. (2005) rumen bacteria from cows fed forage diet produced mostly *c*9,*t*11 CLA at pHs higher than 6.2, but those from concentrate-fed cows produced more *t*10,*c*12 CLA than *c*9,*t*11 CLA at lower pHs.

Other studies have suggest that animal products from systems using botanically diverse forages have a healthier fatty acid profile for humans, with a higher PUFA content (*e.g.*, Collomb et al., 2002; Dewhurst et al., 2006; Lourenço et al., 2008). However, climatic environment differs from one year to another and can shorten the availability of grass, so that it becomes necessary to finish some ruminants on concentrate (Aurousseau et al., 2007). Seasonal differences in pasture nutritive value and availability have been reported to have an important influence on the intramuscular fat content and composition as well as underlined the importance of pre-weaning nutrition on the fatty acid composition including CLA isomers of meat (Santos, 2006; Aurousseau et al., 2007).

Despite the positive effects of pasture feeding on the fatty acid composition as well as in the nutritional value of meat, the growth performance of animals are often negatively correlated under these conditions, mainly because of the limited energy density of the diet and differences in feed intake (Webb & O' Neill, 2008). In addition, meats with higher PUFA content, as in pasture feeding, would be more prone to oxidation than those with more saturated ones (Mercier et al., 2004). The increase of the unsaturation degree of meat may promote the development of organoleptic problems due to a higher susceptibility to lipid oxidation and therefore can give rise to rancidity and the formation of undesirable odour and flavours, which affects the sensory and nutritional value of meat (Bou et al., 2001). However, pasture feeding has the advantage over concentrates since offers high antioxidant content especially vitamins from group A and E, carotenoids and flavonoids, which can protect against oxidation (Wood & Enser, 1997; Gatellier et al., 2004). α-, β-, γ- and δ-Tocopherols, together with the corresponding tocotrienols, are the natural diterpenes with vitamin E activity, and they are the primary fat-soluble vitamins with antioxidant properties in biological systems due to its location in membranes and fat (Kerry et al., 2000; Prates et al., 2006). β-Carotene, a precursor of vitamin A, is another important fat-soluble antioxidant that quenches sites localised within the hydrophobic region of biological membranes, contrasting with the scavenging activity of α-tocopherol close to the membrane surface. Although β-carotene is less reactive than α-tocopherol, both antioxidants can exert a synergistic antioxidant activity (Descalzo et al., 2005). Antioxidants delay or prevent lipid oxidation by reducing free radical activities in meat, which are able to initiate or propagate the chain reactions that damage cells. These antioxidants cannot be synthesised by

animals, but green herbage is an excellent source of these natural antioxidants when compared with cereals. Grains also contain antioxidants such as polyphenols, like proanthocyanidins, and phytic acid. These natural compounds also act as a chain breaking in radical production and are considered a second line of protection against reactive oxygen species (Gatellier et al., 2005). Hence, increased levels of dietary antioxidants can improve meat quality and have beneficial effects on human health, performing an important role in prevention of cardiovascular diseases and cancer (Guardiola et al., 1996).

1.5.3 Technological factors

Most studies mainly describe the fatty acid composition of raw/fresh meat from farm animals. However, meat, like other foods, undergo different thermal and non-thermal treatments prior to consumption (*e.g.* processing technologies, storage or culinary practices), which may affect fatty acid profile and meat quality. In fact, accurate estimation of fatty acid composition is crucial to define not only nutrient composition of meat, but also to accurate determination of treatments effects that may modify fatty acid composition, CLA content and isomeric profile in meat (Murrieta et al., 2003).

Different treatments have been devoted to find ways of preserving meat during processing. The methods commonly used are drying, salting or heating. Later innovations in food preservation include the use of chemicals additives, freezing, refrigeration and, more recently, irradiation. Indeed, irradiation has joined to food protection methods recognised as a safe and effective physical technology. In 1997, the Food and Drug Administration (FDA) approved irradiation treatment for poultry and red meats to control foodborne pathogens and to extend product shelf life (USA, FDA, 1997). In the USA, as in other countries worldwide, this technology is currently applied not only for food safety but also for technological purposes, although it is still not permitted in the EU for meat products. One of the major concerns with meat irradiation is its effects on lipid oxidation (Ahn et al., 2000). In fact, heat treatment and radiation processing can lead to undesirable modifications, such as the decrease in nutritional value, mainly due to vitamin and mineral losses (Gerber et al., 2009), and also changes in the fatty acid composition due to increased production of lipid peroxidation products (Rodriguez-Estrada et al., 1997).

Oxidation of lipids is the major cause of quality deterioration of meat and occurs much faster in cooked/processed meat than in fresh meat (Kingston et al., 1998). Oxidative processes are major non-microbiological factors involved in rancidity of meat because induce modifications of muscle lipids and proteins and, therefore, affect significantly the organoleptic and nutritional properties of meat and therefore its acceptability by the consumer. However, intrinsic properties of the muscle and the nutritional background of the animals have a profound effect on the extent of *postmortem*

oxidation of these macromolecules in the muscles (Trout, 2003; Insani et al., 2008). Protein denaturation by cooking can lead to the loss of antioxidant enzyme activity or the release of catalytically-active iron from metallo-proteins (mainly myoglobin) as well as disruption of cell membranes (bring PUFA into contact with pro-oxidants) and thermal decomposition of hydroperoxides to pro-oxidant species leading to an significantly increase in oxidation as reflected by the formation of toxic compounds that compromise meat quality and reduce shelf life, such as the formation of cholesterol oxidation products (COPs) and thiobarbituric acid reactive substances (TBARS) (Grau et al., 2001).

During the cooking process complex interactions that involve heat and mass transfer take place, leading to changes probably due to the induction of water loss, stimulation of thermo-oxidation reactions, change of colour to brown or modification of the fatty acid profile (Ramirez et al., 2004; Saghir et al., 2005). Cooking methods can cause greater alterations in edible fats, although reports on meat fatty acid composition have been inconsistent (Harris et al., 1992; Badiani et al., 2002).

The data available on the influence of heat treatments regarding CLA concentrations in beef, and especially CLA isomeric profile, is sparse. Early studies suggested that CLA content could be increased in foods that are heat processed (Ha et al., 1989). However, later, Shanta et al. (1994) suggested that CLA is not increased by cooking, but remains constant when expressed as mg/g of fat basis. CLA has been described to be more sensitive to oxidation or to isomerisation during processing and heat treatment than nonconjugated fatty acids, because of the chemical structure containing a conjugated double bond system. Therefore, research interests should be focussed to determine the influence of these methods on the CLA content and CLA isomers profile as well as to check the stability of CLA in food products in order to avoid a decrease in CLA concentration by oxidative damage (Gnadig, 2002).

1.6 STRATEGIES FOR IMPROVING THE FATTY ACID COMPOSITION OF RUMINANT MEAT

As a result of the fatty acid imbalance in human diets, different strategies have been used to improve the nutritional and health value of intramuscular fat of ruminant meats. Advances in food processing technologies and breeding programmes as well as modification of animal feeds have all led to a reduction in the fat content of carcass meat with the net effect to meet consumers' demands for leaner meat (Jiménez-Colmenero et al., 2001; Strong, 2006). In fact, crossbreeding has long been recognised as a powerful tool for exploiting genetic differences between breeds as a mean of improving traits of economic importance (Malau-Aduli et al., 2006). In addition, different nutritional strategies have been used whereby meat fatty acid composition of ruminant animals can be modified,

simultaneously with fat reduction or otherwise, such as replacing part of the animal fat present with another more suitable to human needs (less hypercholesterolemic saturated and more unsaturated) or replacement with substitutes that improve the protein/fat/carbohydrate balance.

Manipulation of animal feed has been used as an alternative method to improve the dietary quality of meat (Dhiman et al., 2005). Most of the attempts to change the fatty acid composition of beef have been aimed to increase the ratio PUFA/SFA and to reduce that of the *n*-6/*n*-3 PUFA (Givens et al., 2006). Therefore, manipulating the diet and feedstuffs may improve fatty acid composition of beef, by increasing the content of *n*-3 PUFA, 18:1*t*11 and CLA (Scollan et al., 2006; Bessa et al., 2008; Kraft et al., 2008). Although meat, particularly red meat, is already an important dietary source of LC *n*-3 PUFA, in which DPA predominates, further enrichment of ruminant meat with these PUFA rich lipids may be an effective approach of increasing intake of *n*-3 fatty acids as well as a mean of promoting a reduction in the *n*-6/*n*-3 PUFA ratio (Simopoulos, 2002; Jiménez-Colmenero, 2007).

Dietary lipid supplementation of ruminant diets is the most straightforward way to increase energy intake and modify the fatty acid composition of meat (Raes et al., 2004; Schmid et al., 2006). However, rumen biohidrogenation of dietary PUFA is modulated by several factors, such as the amount and type of lipid supplement resulting in differences in the amount of PUFA that escapes biohydrogenation and in the type of biohydrogenation intermediates (Bessa et al., 2007). It has been clearly demonstrated that the *n*-3 fatty acids content of meat and fat from livestock (beef, pork, chicken) can substantially be enhanced through altered feeding strategies, especially through replacement of *n*-6 PUFA rich grains, oils and oilseeds (such as wheat, maize, soybean, sunflower, safflower seeds) with *n*-3 PUFA rich oils and oilseeds (including colza, linseed or flaxseed) and/or grass in the animal's diet (Raes et al., 2004). As vegetable seeds or oils, protected or not, are no dietary sources of EPA and DHA, diets should be supplemented with fish oil or algae marine (Givens & Gibbs, 2006). Besides supplementation of ruminant diets with lipid sources, exploiting the potential of herbage as an alternative to marine sources of PUFA is an important nutritional strategy for enhancing the content of *n*-3 PUFA in beef. The transfer of 18:3*n*-3 from forage through meat incorporation is dependent on two important processes: (1) increasing the level of 18:3*n*-3 in the forage (and hence into the animal); (2) reducing the extent of ruminal biohydrogenation. Therefore, an increase in dietary PUFA does not necessarily lead to a direct increase in these fatty acids in meat (Padre et al., 2007).

Studies involving diet modulation have documented enhancing CLA levels in lean tissue lipids of swine, poultry and ruminants (Joo et al., 2002; Mir et al., 2004). However, an approach to heightening CLA levels in whole-muscle beef is warranted to provide levels necessary for possible health benefits and potentially to provide added value to beef meat (Baublits et al., 2007). The

contents of intramuscular CLA found in meat are lower relative to the recommended daily intake for health benefits in human, which is 3.5 g/day (Ha et al., 1989; Scollan et al., 2006). Thus, diets containing high levels of 18:3*n*-3, such as fresh grass or grass silage, or adding linoleic and linolenic acid rich sources (vegetable oils), as well as fish oil and marine algae have proved to be efficient strategies to increase the CLA content in muscle lipids (Schmid et al., 2006). However, comparisons between different types of vegetable oils (rich in oleic acid, LA and ALA) suggest that those rich in linoleic acid increase most effectively CLA concentration (Collomb et al., 2004). In fact, it contributes to increase both CLA and 18:1*t*11 in the rumen, whereas biohydrogenation of ALA contributes only to 18:1*t*11 (Harfoot and Hazlewood, 1988; Bessa et al., 2007). In general, studies have shown that equivalent amounts of dietary fish oils are equally or even more effective than plant oils or oil seeds at increasing milk fat CLA content (Chilliard et al., 2001; Collomb et al., 2006). The LC-PUFA from fish oil inhibits the complete biohydrogenation of LA in the rumen by reducing the growth of bacteria responsible for hydrogenating 18:1*t*11 (Griinari & Bauman, 1999). Besides supplementation of ruminant diets with lipid sources, forage feeding has been shown to be a good strategy to increase the proportion of *n*-3 PUFA, 18:1*t*11 and *c*9,*t*11 in ruminant milk and meat, as forage are a major source of 18:3*n*-3 (Dewhurst et al., 2006; Scollan et al., 2006; Lourenço, 2007). Furthermore, the inclusion of high levels of PUFA oils in diets for increasing CLA content in meat is more effective when using forage based diets (Bessa et al., 2005). Within forage based strategies, organic farming systems, with higher dietary forage proportions, have been reported to result in higher PUFA proportions than conventional farming systems (Nielsen & Thamsborg, 2005). Thus, the CLA content of beef and lamb muscle is regulated mainly by the type of diet being fed and related mainly to the concentrations of PUFA in the diet. Although, high forage and high oil diets (CLA-promoting diets) are effective in modifying the fatty acid composition of ruminant products, they often depress dry matter intake with occasional reduction in animal performance (Bessa, 2001). Khanal and Olson (2004) stated that probably is not an extra supply of linoleic or α-linolenic acids but the feed sources that inhibit further hydrogenation of vaccenic acid to stearic acid would enhance CLA content in milk and meat. Moreover, the high concentration of soluble fiber and fermentable sugars in fresh grass may create an environment in the rumen without lowering the ruminal pH that is favourable for the growth of the microbes responsible for CLA and VA production (Dhiman et al., 2005; Meľuchová et al., 2008). However, the capacity to manipulate the fatty acid composition is limited since dietary inclusion of fatty acids must be restricted (6% of dietary dry matter), to avoid impairment of rumen function (Scollan et al., 2006). Increased PUFA in the diet may limit ruminal production of CLA and VA and/or may depress stearoyl-CoA desaturase expression or activity in lean tissues, which in turn limits CLA formation and accretion in tissues (Griswold et al., 2003).

In ruminants, any attempt to improve productive efficiency by modifying the amount or composition of lipid added to the diet must take into account the ruminal metabolism of lipids. In fact, the major limitation to manipulating the fatty acid composition of ruminant products through dietary means is microbial biohydrogenation of dietary unsaturated fatty acids in the rumen, which is largely responsible for the high saturated nature of ruminant products (Lee et al., 2006). Jenkins (1994) reported that the caloric value of dietary fat changes little as it passes through the rumen and, on average, 87% of fatty acids consumed are recovered at the duodenum. This small fatty acid loss is often compensated by *de novo* lipid synthesis by ruminal microbes, causing a net gain of fatty acids across the rumen. Despite small losses of fatty acids across the rumen, adding fat to ruminant diets sometimes increases digestible energy less than expected. This occurs when the added fat is poorly digested, as seen for hydrogenated fats with large particle size, or when the added fat reduces digestibility of the basal diet (Jenkins, 1990). The latter problem is due to inhibition of fiber digestion in the rumen resulting from antimicrobial effects of fatty acids. However, ruminal fermentation problems can be minimized, or even eliminated, by feeding calcium salts of fatty acids, hydrogenated fats, or encapsulated fats (Jenkins 1994). Hence, these strategies of protecting the lipid supplements, can achieve a good ruminal protection of rumen microbial metabolism in intramuscular fat of beef cattle. Thus, cattle industry should enhance a significant beneficial quality of beef adjusting feedlot rations to include a combination of oil feeding and bacteria drenching. Other approach to reduce ruminal biohydrogenation includes the use of increasing amounts of concentrate in the diet, which is considered to be associated with a reduction in rumen pH (Kucuk et al., 2001). Indeed, carbohydrates, such as starch, are rapidly fermented by ruminal microorganisms, often resulting in a decline in ruminal pH. This response appears to be associated with microbial changes in rumen bacteria, reducing cellulolytic bacteria, including *Butyrivibrio fibrisolvens,* which is known to produce 18:1*t*11 the precursor of *c*9,*t*11 in ruminant tissues. The higher microbial population of lactate-producing (*Streptococus bovis*) and lactate-utilizing bacteria (*Selenomonas ruminantium* and *Megasphera elsdenii*) leads to the formation of 18:1*t*10 and *t*10,*c*12 CLA (Cruz-Hernandez et al., 2006; Lee et al., 2006). Previously, Kucuk et al. (2001) have also reported that decreasing the forage proportion of the diet increased the duodenal flow of 18:1*t*11 and resulted in a shift in the ruminal production of CLA from *c*9,*t*11 CLA towards *t*10,*c*12 and its reduction to 18:1*t*10. Efforts have been made to reduce SFA in meats by increasing the MUFA content at the expenses of PUFA content, through modification of animal diets as well as by direct incorporation of a vegetable oil high in MUFA, such as olive, rapeseed and peanut oils (good sources of oleic acid) during processing (Rhee, 2000).

Enrichment of CLA in beef is achieved primarily by supplementing animals with CLA precursor fatty acids compared with the direct feeding of CLA to fish, pigs, and chickens (Watkins & Li,

2003). Supplementation of farm animals with CLA and its subsequent accumulation in tissue lipids may be a mean for improving carcass and meat quality and also for providing animal products with several functional properties (reviewed by Schmid et al., 2006; Martin et al., 2008). Compared to ruminants, in monogastric animals dietary fats are unmodified prior to digestion and absorption (Schmid et al., 2006). So, a diet has to include 18:1*t*11 as substrate for endogenous CLA synthesis or CLA itself designed for elevate the tissue CLA concentration. As point out by Martin et al. (2008) feeding with CLA causes a decrease in the ratio of unsaturated/saturated fatty acids, which may have harmful implications for human health. In fact, studies in pigs indicate that CLA supplementation increased the saturated fat content and a parallel decrease in MUFA (mainly 18:1*c*9) occur in tissues due to an inhibitory effect of CLA isomers by down-regulating the Δ^9 desaturase activity. This improved the firmness of meat but also made it more atherogenic (Ramsay et al., 2001; Kelley & Erikson, 2003). The inclusion of vegetable oil high in MUFA in ruminant diets when supplemented with CLA could be a strategy for counteracting the decrease in MUFA caused by CLA (Rhee, 2000).

The effect of supplementing diet with sources rich oils (unsaturated fats) may increase the TBARS values as a result of higher proportions of PUFA. This is important, as already mentioned, because increasing the amount of unsaturated fatty acids in meat reinforces the occurrence of oxidation, a process that has undesirable sensory and health effects. In fact, the PUFA is often responsible for the reduction of meat oxidative stability. Nevertheless, a number of strategies are being used to enhance antioxidant activity in meat systems and to reduce the formation of oxidation products which have a subsequent impact on ageing, cancer and cardiovascular disease (Decker & Xu quoted by Jiménez-Colmenero et al., 2001). Some of them are based on intervention in raw materials, as for instance, the modification of animal diet to increase the amount of endogenous natural antioxidants (*e.g.* vitamin E, carotenoids, ascorbic acid), to reduce the amount of pro-oxidants and/or to alter the oxidisable substrate (PUFA). Antioxidant supplementation of feed is an efficient method for increasing oxidative stability (Wood et al., 2004; Descalzo et al., 2005). Thus, high levels of vitamin E in diets may increase oxidative stability and hence meat quality, particularly in ruminant meats, were high concentrations resulting from grass feeding prevent fatty acid oxidation and extend shelf life and shelf stability of the fat and meat. In earlier studies, it was suggested that CLA has antioxidant activities *in vitro* (Ha et al., 1990). Ip et al. (1991) observed that dietary CLA decreased the concentrations of TBARS in rat liver and mammary gland tissues. In addition, lower levels of oxidation have been detected in meat from CLA-fed animals (Martin et al., 2007). However, the antioxidant effect of CLA remains unclear and controversial since other studies have even shown a pro-oxidant effect of CLA or no implication at all in the oxidative processes (Hur et al., 2007; Martin et al., 2008).

1.7 OBJECTIVES

In line with the former considerations, we aimed to elucidate some questions concerning the effect of several factors (*e.g.* seasonal variation, muscle type, production practices and processing) on the fatty acid composition, with special regard on CLA isomers, and consequently on the nutritional value and quality of intramuscular fat of ruminant meat.

As being so, the specific objectives of this work were:

- to characterise the lipid composition and nutritional quality of intramuscular fat in four traditional Portuguese bovine meats (Carnalentejana-PDO and Mertolenga-PDO beef, and Barrosã-PDO and Arouquesa-PDO veal), regarding the influence of slaughter season, (Autumn and Spring with the least and the most abundant green pastures, respectively), and muscle type (*longissimus dorsi* and *semitendinosus* muscle) on its lipid composition (Chapters 2, 3, 4 and 5, respectively);

- to assess the effect of feeding systems on the health value of intramuscular fat of beef. Firstly, this objective was focused on the differences between lipid composition of beef from intensively produced crossbred bullocks (Charolais × Alentejana) with beef from autochthonous Alentejana breed (Chapter 2) and, later, a trial under controlled environmental conditions was conducted to evaluate the effect of feeding systems (exclusive pasture, pasture feeding followed by 2 or 4 months of finishing on concentrate, and exclusive concentrate) in the nutritional value of beef lipids, from Alentejano purebred bulls, as well as their usefulness as chemical discriminators of the production system (Chapter 6);

- to investigate the effect of irradiation, a prospective technology for meat preservation, on the nutritional value of intramuscular fatty acids, with special emphasis on the CLA content and detailed CLA isomeric composition of lamb meat, enriched in CLA levels (Chapter 7);

- to study the impact of markedly different heat treatments, frequently used in households (boiling, microwave and grilling) on meat fatty acid composition, including complete CLA isomeric profile and nutritional value of beef from cattle fed two diets (concentrate or pasture), in order to obtain beef with distinct levels of PUFA and CLA. In addition, the influence of these common culinary practices on the oxidative stability of beef intramuscular fat were assessed (Chapter 8).

2

FATTY ACID COMPOSITION, CONJUGATED LINOLEIC ACID ISOMERS AND CHOLESTEROL IN BEEF FROM CROSSBRED BULLOCKS INTENSIVELY PRODUCED AND FROM ALENTEJANA PUREBRED BULLOCKS REARED ACCORDING TO CARNALENTEJANA-PDO SPECIFICATIONS

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Fatty acid composition, conjugated linoleic acid isomers and cholesterol in beef from crossbred bullocks intensively produced and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications

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The purpose of this study was to characterise and compare the nutritional value of Portuguese Carnalentejana-PDO beef, obtained from Alentejana purebred bullocks reared in a semi-extensive system, with the meat from Alentejana × Charolais crossbred bullocks produced in a conventional intensive concentrate-based system. In addition, seasonal changes in Carnalentejana-PDO beef quality were assessed, by analysing meat samples from animals slaughtered in early autumn and late spring. The results showed that PDO beef has different intramuscular fat characteristics in comparison with meat from crossbred bullocks fed intensively with concentrate. However, the finishing period of Alentejana purebred bullocks with concentrate seems to attenuate most of the typical grass-fed characteristics of meat fat. Nevertheless, from a human nutrition perspective, Carnalentejana-PDO beef seems to be healthier than that from intensively reared animals since it has a lower *n*-6/*n*-3 ratio, although always above the recommended guidelines for human diet, and higher proportions of *c*9,*t*11 conjugated linoleic acid isomer and total CLA relative to saturated fatty acids plus total cholesterol [CLA/(SFA+CHR)]. Furthermore, no seasonal variation in the nutritional quality of PDO beef was apparent. Taken together, the data indicate that Carnalentejana-PDO beef is of greater nutritional value than intensively produced beef from crossbred bullocks throughout the year.

Keywords: beef; fatty acids; CLA isomers; cholesterol; meat quality; production systems.

2.1 INTRODUCTION

Fatty acid composition and cholesterol levels in meat have received an increased interest considering their implications for human health and product quality (Wood et al., 2004). The ratios of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) and *n*-6/*n*-3 PUFA are widely used to evaluate the nutritional value of fat. Low ratios of PUFA/SFA and high levels of cholesterol in typical Western diets have been considered as major risk factors of cardiovascular diseases, which are among the most important causes of human mortality in developed countries (Katan, 2000; Ganji et al., 2003). Moreover, typical Western diets display a very high *n*-6/*n*-3 ratio (15-17/1), which favours the development of cardiovascular diseases, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2002). It is well established that the lower PUFA/SFA and higher *n*-6/*n*-3 ratios of most meats are a major cause of the imbalance in the fatty acid intake of today's consumers (Wood et al., 2004). In addition, meat provides from one third to one half (Chizzolini et al., 1999) of the maximum daily-recommended cholesterol intake (300 mg; WHO, 2003).

Recently, research has focused on a minor group of fatty acids that are characteristic of ruminant fat, named conjugated linoleic acid (Ip et al., 1994; Prates & Mateus, 2002). The CLA acronym refers to a heterogeneous group of positional and geometric isomers of linoleic acid (18:2*n*-6), in which the double bonds are conjugated (from positions 6,8 to 13,15). The major CLA isomer, *c*9,*t*11, is produced in the rumen during the microbial biohydrogenation of dietary 18:2*n*-6 and in the tissues through Δ^9 desaturation of 18:1*t*11 (Griinari & Bauman, 1999). Twenty different CLA isomers have been reported as occurring naturally in food, especially in ruminant fat (Sehat et al., 1998). In animal models, CLA exhibits anticarcinogenic, antithrombotic, antiatherogenic and immune modulator properties (Whigham et al., 2000; Belury, 2002). The National Academy of Sciences of USA recognised, in 1996, that CLA is the only fatty acid shown unequivocally to inhibit carcinogenesis in experimental animals (National Research Council, 1996). Specific physiological effects have been linked with individual CLA isomers. The *t*10,*c*12 isomer may play an important role in lipid metabolism, while the *c*9,*t*11 and the *t*10,*c*12 isomers seem to be equally effective in anticarcinogenesis (Pariza et al., 2001; Evans et al., 2002). Since individual CLA isomers have different biological activities, the determination of the CLA isomeric profile in meat is required.

Beef fatty acid composition is influenced by dietary factors and, to a lesser extent, by other environmental and genetic factors (De Smet et al., 2004). Dietary factors are often linked with a particular production system (Geay et al., 2001; Raes et al., 2004). Meat from grazing ruminants is expected to reflect the variability of pasture biomass. In fact, the nutritive value of pasture is highly dependent on cultural practices, season and geographical factors (Moloney et al., 2001). In addition, breed differences in fatty acid composition in several farm animal species have also been reported

(De Smet et al., 2004). Furthermore, variations in beef cholesterol content are highly dependent on oxidative fibre proportions in muscle (Chizzolini et al., 1999). The level of oxidative fibres in muscle depends on environmental conditions and, thus, it is influenced by the management of the production system (Klont et al., 1998).

In Portugal, commercial crossbred bovines produced under intensive regimens provide the main supply of beef at competitive prices (Rodrigues et al., 1998). However, meat from autochthonous bovine breeds, originating from traditional production systems, has been progressively reintroduced in Portuguese diets as a result of its putative highly intrinsic quality (Costa et al., 2003) and of public perception of higher BSE and chemical residues safety (Rodrigues et al., 1998). Meats with Protected Designation of Origin, derived from local production systems and animal breeds, are certified by European Union legislation and are expected to present unique quality and organoleptic characteristics, especially associated with specific properties of its lipid fraction (Council Regulation nº 2081/92 of 14/7, EEC). One such example is Carnalentejana-PDO beef, which is obtained from Alentejana purebred bullocks produced in a traditional production semi-extensive system in Alentejo and some councils within the district of Setúbal (south of Portugal) according to the product specifications (extensive grazing system based on natural pastures of Montado with finishing on concentrate during 3-6 months).

In spite of being the most important commercial Portuguese PDO meat (991 carcass tons in 2003, Instituto do Desenvolvimento Rural e Hidráulica, 2003), there are no detailed reports on the composition of Carnalentejana-PDO beef. Moreover, little work has been conducted to assess seasonal changes in beef CLA profile. Therefore, the goal of this work was to characterise the nutritional value of Portuguese Carnalentejana-PDO beef in two distinct slaughter seasons (early autumn and late spring), and to compare it with the meat obtained from Alentejana × Charolais crossbred bullocks produced in a typical intensive concentrate-based system.

2.2 MATERIALS AND METHODS

2.2.1 Reagents

Analytical grade and liquid chromatographic grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). Sodium methoxide (0.5 M solution in anhydrous methanol) and a cholesterol standard were obtained from Sigma-Aldrich Ltd. (St. Louis, MO, USA). The fatty acid methyl ester (FAME) standard mixtures were acquired from Nu-Chek-Prep Inc. (Elysian, MN, USA) and from Supelco Inc. (Bellefonte, PA, USA). Additional standards of individual CLA isomers

(*c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11) as methyl esters were purchased from Matreya Inc. (Pleasant Gap, PA, USA).

2.2.2 Animals and meat samples

Alentejana purebred bullocks (n = 30) were reared in a semi-extensive system, following the Carnalentejana-PDO beef specifications (Commission Regulation nº1107/96 of 12/06, EC). The animals were raised, in different representative private farms, under an extensive grazing system based on natural pastures under holm and cork oak, which is usually referred to as Montado. Supplementation with cereals and dry forages (hay and straw) was provided during periods of feed scarcity. The bullocks were finished on concentrate, which chemical composition is presented in Table 2, for the last 5 (October sampling) or 3 months (June sampling). Bullocks slaughtered (Regional Abattoir of Alto Alentejo, Sousel) in October 2002 (early autumn sampling) had been exposed to a period of grass abundance (late winter and early-middle spring) and finished on concentrate since May (n = 15; mean ± standard error of age and carcass weight were 21 ± 0.7 months and 347 ± 18 kg). Bullocks slaughtered (Regional Abattoir of Alto Alentejo, Sousel) in June 2003 (late spring sampling) had been raised on late winter grass and finished on concentrate since March (n = 15; 20 ± 0.6 months and 359 ± 8 kg).

Alentejana × Charolais crossbred bullocks (n = 15) from the conventional intensive production system were maintained confined and fed with a commercial pelleted concentrate. The chemical composition of the diet is presented in Table 2. The crossbred bullocks were slaughtered at Regional Abattoir of Baixo Alentejo (Beja), in October 2002, at 20 ± 0.5 months of age and 388 ± 12 kg of carcass weight.

Meat samples were taken from the ribeye portion (T1-T3 *longissimus thoracis*, LT) of *longissimus dorsi* (LD) muscle and from the distal region of *semitendinosus* muscle (ST) of bullocks. All meat samples were collected 2-3 days after slaughter (+1 ºC), ground using a food processor (3 × 5 s), vacuum packed and stored at −80 °C until required for analysis.

2.2.3 Lipid extraction and methylation

Meat samples were lyophilised (−60 °C and 2.0 hPa) until constant weight using a lyophilisator Edwards Modulyo (Edwards High Vacuum International, UK), maintained desiccated at room temperature, and analysed within two weeks. Lyophilised meat samples were weighed approximately

Table 2. Chemical (g/kg dry matter) and fatty acid (% sum of fatty acids) composition of the concentrates from Alentejana × Charolais crossbred bullocks intensively produced (IP) and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications.

	Concentrates	
	PDO Bullocks	IP Bullocks
Chemical composition		
Crude protein	128	150
Total fat	30	29
Crude fibre	65	83
Ashes	90	112
Fatty acids		
14:0	3.8	3.3
16:0	13.4	15.0
18:0	2.2	3.3
18:1	22.5	22.4
18:2*n*-6	52.7	48.5
18:3*n*-3	2.7	3.3

250 mg), in triplicate (two tubes for total lipid determination and the other tube for both FAME and CLA methyl esters profiles), into screw teflon-lined cap tubes. Intramuscular fat was extracted three times with methylene chloride-methanol (4:1 v/v) and a fourth extraction was performed with *n*-hexane, as was described by Fritsche et al. (2000) and Fritsche et al. (2001). Fatty acids were converted to methyl esters by base-catalysed transesterification, in order to avoid isomerisation of CLA isomers, with sodium methoxide (0.5 M solution in anhydrous methanol) during 2 hours at 30 °C, as was proposed by Park et al. (2001) and Kramer et al. (2002). The same FAME solution was used for the analysis of both fatty acid composition and CLA profile, enabling the direct comparison of quantitative data and eliminating differences in sample preparation. Total lipids were measured gravimetrically, in duplicate, by weighing the fatty residue obtained after solvent evaporation.

2.2.4 Determination of fatty acid composition

Gas chromatography analyses of FAME were performed with an Agilent 6890 Series II gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) fitted with a flame ionization detector (FID). The FAME were separated on a SP^{TM}-2560 fused-silica capillary column (100 m × 0.25 mm i.d., 0.2 µm film thickness, Supelco Inc., Bellefonte, PA, USA), coated with cyanopropyl polysiloxane stationary phase, using a split/splitless injection system (split ratio of 1:5) and helium as

carrier gas at a flow rate of 1.5 mL/min. After injection (1 μL), the column temperature was held at 75 °C for 2 min and then increased to 180 °C at 5 °C/min. The temperature was kept at 180 °C for 33 min, followed by an increase of 4 °C/min to 225 °C and, finally, held at 225 °C for 44 min. The detector and injection temperatures were set at 250 °C. Identification was accomplished by comparing the retention time of peaks from samples with those of FAME standard mixtures (Figure 5) and with values published in the literature (Kramer et al., 1998; Fritsche et al., 2001). Quantification of FAME was based on the internal standard technique, using nonadecanoic acid (19:0) as internal standard, and on the conversion of relative peak areas into weight percentages, using the corrected response factor of each fatty acid (ES ISO 5508, 1990). Fatty acids were expressed in gravimetric contents (mg/g muscle) or as a percentage of the sum of identified fatty acids (% w/w).

2.2.5 Determination of individual CLA isomers

The methyl esters of CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using an high performance liquid chromatography system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and diode array detector (DAD) adjusted to 233 nm. The mobile phase was 0.1% acetonitrile in *n*-hexane maintained at a flow rate of 1 mL/min and injection volumes of 20 μL were used (Alfaia et al., 2003). 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, 2001). In addition, the identity of each isomer was controlled by the typical UV spectra of CLA isomers from the DAD in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems rev. A.09.01 (Agilent Technologies, 2001). Total and individual CLA isomer contents in meat were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalization (AOAC 963.22, 2000). The CLA isomers were expressed in gravimetric contents (mg/g muscle and mg/g fat) or as a percentage of the sum of identified CLA isomers (% total CLA).

2.2.6 Determination of total cholesterol

Total cholesterol was extracted from lyophilised meat, after direct saponification with saturated methanolic KOH solution, according to the procedure of Naeemi et al. (1995), except that three extractions with *n*-hexane were used (recoveries were higher than 94%). Cholesterol was separated

by normal-phase HPLC (column Zorbax Rx-Sil, 250 mm × 4.6 mm i.d., 5 μm particle size, Agilent Technologies Inc., Palo Alto, CA, USA), using an HPLC system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and DAD set at 206 nm, with a solvent (3% isopropanol in *n*-hexane) flow rate of 1 mL/min and injection volumes of 30 μL. Total cholesterol content in meat was calculated, in duplicate, based on the external standard technique, from a standard curve for peak area *vs.* cholesterol concentration.

2.2.7 Statistical analysis

The data were analysed using the MIXED procedure of Statistical Analysis System (SAS, 2004), considering the animal within group as subject and the muscle as repeated measures. The model considers as fixed effects the experimental group of animals (PDO beef from early autumn, PDO beef from late spring and intensively produced beef), muscle type (LT and ST) and the interaction between experimental group of animals and muscle type. Orthogonal contrasts were constructed for testing differences between PDO beef from early autumn and that from late spring, as well as for testing differences between PDO beef and intensively produced beef.

2.3 RESULTS AND DISCUSSION

2.3.1 Intramuscular fatty acid composition

Data referring to the fatty acid composition (wt %) of intramuscular fat in Carnalentejana-PDO beef, obtained at early autumn and late spring seasons, and in meat from crossbred bullocks fed intensively with concentrate are presented in Table 3. The predominant fatty acids in intramuscular fat from all types of beef were 16:0 and 18:0 as SFA, 18:1*c*9 as MUFA, and 18:2*n*-6 as PUFA. Similar results were found by many other authors in beef (*e.g.* Raes et al., 2003a; Realini et al., 2004; Varela et al., 2004). The content of *trans* octadecenoic fatty acids was expressed as a single value (18:1*t*) because of its incomplete chromatographic resolution. These *trans* octadecenoic isomers result from the incomplete microbial hydrogenation of unsaturated fatty acids in the rumen (Bessa et al., 2000).

No seasonal variations ($P > 0.05$) in fatty acid composition of Carnalentejana-PDO beef were apparent, except for some minor fatty acids (16:1*t*9, 18:3*n*-6, 20:1*c*11, 22:4*n*-6 and 22:6*n*-3), which showed higher percentages in beef from early autumn. This similarity in meat fatty acid profiles is probably due to the finishing period on concentrate that attenuates the differences in fatty acid

Table 3. Fatty acid composition (% w/w) of intramuscular fat in the *longissimus thoracis* (LT) and *semitendinosus* (ST) muscles of beef from Alentejana × Charolais crossbred bullocks intensively produced (IP) and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications and slaughtered in early autumn (A) and late spring (S).

	Carnalentejana-PDO beef				Intensively produced beef			Significance level			
	Autumn		Spring					Contrasts		Muscle	G × M
Fatty acids	LT	ST	LT	ST	LT	ST	SEM	A *vs.* S	PDO *vs.* IP		
8:0	0.01	0.02	0.02	0.02	0.01	0.01	0.008	ns	ns	ns	ns
10:0	0.18	0.05	0.06	0.05	0.04	0.05	0.057	ns	ns	ns	ns
12:0	0.07	0.05	0.06	0.03	0.07	0.08	0.012	ns	*	ns	ns
14:0	2.30	1.82	1.92	1.61	2.28	1.80	0.144	ns	ns	***	ns
14:1	0.36	0.37	0.33	0.40	0.36	0.35	0.044	ns	ns	ns	ns
15:0	0.30^{a}	0.26^{b}	$0.25^{a,b}$	$0.27^{a,b}$	0.39^{c}	$0.28^{a,b}$	0.019	ns	**	***	***
15:1	0.01^{a}	0.00^{a}	0.00^{a}	0.01^{a}	0.15^{b}	0.10^{c}	0.007	ns	***	**	***
16:0	21.2	21.0	19.8	20.6	20.5	19.4	0.602	ns	ns	ns	ns
16:1*t*9	0.56	0.70	0.39	0.41	0.43	0.87	0.103	*	ns	*	ns
16:1*c*9	3.09	2.92	3.09	3.20	2.55	2.69	0.192	ns	*	ns	ns
17:0	$0.82^{a,c}$	0.70^{b}	$0.77^{a,b}$	$0.77^{a,b}$	0.90^{c}	0.70^{b}	0.038	ns	ns	***	***
17:1*c*9	$0.67^{a,b}$	$0.63^{a,b}$	0.69^{a}	0.74^{b}	$0.63^{a,b}$	0.61^{a}	0.047	ns	ns	ns	*
18:0	$16.8^{a,c}$	13.0^{b}	15.7^{a}	12.8^{b}	17.7^{c}	12.7^{b}	0.567	ns	ns	***	*
18:1*t*	2.88^{a}	2.15^{b}	$2.66^{a,b}$	$2.55^{a,b}$	3.84^{c}	$2.28^{a,b}$	0.256	ns	ns	***	***
18:1*c*9	34.3	30.7	35.4	34.1	30.6	26.6	1.354	ns	**	***	ns
18:1*c*11	2.00	2.14	2.32	2.14	2.28	2.10	0.222	ns	ns	ns	ns
18:2*t*9,*t*12	0.18	0.18	0.15	0.18	0.16	0.11	0.019	ns	*	ns	ns
18:2*n*-6	8.11^{a}	11.4^{b}	$10.0^{a,b}$	$11.3^{a,b}$	$11.0^{a,b}$	17.7^{c}	1.216	ns	**	***	***
18:3*n*-6	0.04	0.05	0.02	0.02	0.02	0.02	0.010	*	ns	ns	ns
18:3*n*-3	0.31	0.37	0.32	0.39	0.30	0.42	0.050	ns	ns	***	ns
20:0	$0.15^{a,c}$	$0.13^{a,c}$	$0.11^{a,b}$	$0.08^{b,d}$	0.16^{c}	0.05^{d}	0.015	**	ns	***	**
20:1*c*11	$0.19^{a,c}$	$0.19^{a,c}$	0.10^{b}	0.11^{b}	0.20^{c}	$0.13^{a,b}$	0.021	**	ns	ns	*
20:2*n*-6	0.14^{a}	0.20^{b}	$0.11^{a,b}$	$0.12^{a,b}$	$0.10^{a,b}$	0.07^{a}	0.037	ns	ns	ns	*
20:3*n*-6	0.59^{a}	1.17^{b}	0.81^{a}	1.06^{b}	0.61^{a}	1.29^{b}	0.108	ns	ns	***	*
20:4*n*-6	2.71^{a}	$6.21^{b,c}$	3.63^{a}	5.28^{b}	3.50^{a}	7.24^{c}	0.588	ns	ns	***	*
20:5*n*-3	0.10	0.36	0.14	0.29	0.07	0.39	0.061	ns	ns	***	ns
22:4*n*-6	0.31^{a}	0.53^{b}	0.05^{c}	0.07^{c}	0.35^{a}	0.60^{b}	0.049	***	***	***	**
22:5*n*-3	0.62	1.04	0.54	0.87	0.40	0.89	0.114	ns	ns	***	ns
22:6*n*-3	0.30	1.04	0.17	0.09	0.03	0.04	0.232	*	ns	ns	ns

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: G × M, interaction between group of animals (G) and muscle type (M).

composition between pastures. In fact, the bullocks exposed to more abundant pastures (late winter and early-middle spring grass) had been finished on concentrate during 5 months (slaughtered in October), although the animals exposed to less abundant pastures (late winter grass) had been

finished on concentrate only during 3 months (slaughtered in June). PDO beef showed greater relative proportions of 16:1*c*9 ($P < 0.05$), 18:1*c*9 ($P < 0.01$) and 18:2*t*9,*t*12 ($P < 0.05$), but lower percentages of 12:0 ($P < 0.05$), relative to meat from the intensive production system. This difference in 18:1*c*9 might be explained by higher gene expression of stearoyl-CoA desaturase in Alentejana purebred animals because pasture is lower in 18:1*c*9 than cereals (Palmquist, 1988) and finishing concentrates used had similar 18:1*c*9 content for all animal groups (see Table 2). In fact, it was shown that the increased oleic acid content of sheep tissues in response to concentrate-rich diets is associated with an increase in stearoyl-CoA desaturase gene expression and not with substrate availability (Daniel et al., 2004). Since there is a consistent positive relationship between the levels of 18:1*c*9 in LT muscle and the scores obtained in taste panels (Dryden & Marchello, 1970), Carnalentejana-PDO is expected to be more acceptable to the consumers. Meat from concentrate-fed bullocks had similar percentages ($P > 0.05$) of 18:3*n*-3 than that from Alentejana purebred bullocks, although 18:2*n*-6 showed significant interaction ($P < 0.001$) between animal group and muscle type. The absence of higher 18:3*n*-3 in meat from Alentejano bullocks, in which grass lipids are rich (Palmquist, 1988), suggests a strong attenuation of the grass effect during the finishing period with cereal-based concentrate feed (Marmer et al., 1984; Demeyer & Doreau, 1999). Furthermore, the concentrate from Alentejano bullocks was richer in 18:2*n*-6 fatty acids and poorer in 18:3*n*-3 than the concentrate from intensively produced bullocks (see Table 2).

The LT muscle, relative to the ST muscle, exhibited greater relative proportions of 14:0 ($P < 0.001$), 18:1*c*9 ($P < 0.001$) and 22:5*n*-3 ($P < 0.001$), and lower values of 16:1*t*9 ($P < 0.05$), 18:3*n*-3 ($P < 0.001$) and 20:5*n*-3 ($P < 0.001$). Significant interactions between the experimental group of animals and muscle type were observed for the percentage of many individual fatty acids (15:0, 15:1, 17:0, 17:1*c*9, 18:0, 18:1*t*, 18:2*n*-6, 20:0, 20:1*c*11, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6 and 22:4*n*-6). These interactions may result from modifications of muscle metabolic types caused by adaptations to the distinct environmental conditions (reared in confinement or in pasture) of the two production systems under analysis (Klont et al., 1998). In fact, it is well known that differences in muscle fibre type between muscles are reflected in differences in fatty acid composition (Wood et al., 2004).

The results concerning the total lipids (mg/g muscle) and partial sums (wt %) of intramuscular fatty acids in Carnalentejana-PDO beef, obtained in two distinct slaughter seasons (early autumn and late spring), and in meat from crossbred bullocks fed intensively with concentrate are presented in Table 4. Total lipid content was higher ($P < 0.01$) in PDO beef from autumn relative to that from spring, which can be explained by the largest finishing period on concentrate of autumn-slaughtered bullocks (5 *vs.* 3 months). In addition, Carnalentejana-PDO beef had greater fat content ($P < 0.05$) than intensively produced beef, possibly due to genetic differences in fat deposition between Alentejana

Table 4. Total cholesterol (mg/g muscle), total lipids (mg/g muscle), total fatty acids (mg/g muscle), partial sums of fatty acids (% w/w) and nutritional ratios of intramuscular fat in the *longissimus thoracis* (LT) and *semitendinosus* (ST) muscles of beef from Alentejana × Charolais crossbred bullocks intensively produced (IP) and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications and slaughtered in early autumn (A) and late spring (S).

	Carnalentejana-PDO beef				Intensively produced beef			Significance level			
	Autumn		Spring					Contrasts		Muscle	G×M
	LT	ST	LT	ST	LT	ST	SEM	A *vs.* S	PDO *vs.* IP		
Total cholesterol	0.48	0.42	0.49	0.43	0.37	0.35	0.010	ns	***	***	ns
Total lipids	21.9	13.1	14.8	11.2	15.2	8.61	1.351	**	*	***	ns
Total fatty acids[+]	13.7	5.78	9.22	5.91	11.6	5.90	1.466	ns	ns	***	ns
Partial sums											
Σ SFA	41.8^{a}	37.1^{b,c}	38.7^{b}	36.3^{c}	42.0^{a}	35.1^{c}	0.942	ns	ns	***	**
Σ MUFA	40.6	36.9	41.9	40.6	36.7	32.6	1.472	ns	**	***	ns
Σ TFA	3.61^{a}	3.02^{b}	3.20^{a,b}	3.13^{a,b}	4.43^{c}	3.25^{a,b}	0.285	ns	ns	***	*
Σ PUFA	13.2^{a}	22.4^{b}	15.8^{a}	19.5^{b,c}	16.4^{a,c}	28.7^{d}	2.058	ns	*	***	**
Σ *n*-6	11.9^{a}	19.6^{b,c}	14.6^{a,b}	17.9^{c}	15.6^{a,b,c}	26.9^{d}	1.865	ns	*	***	**
Σ *n*-3	1.33	2.81	1.16	1.64	0.80	1.74	0.306	ns	ns	***	ns
Σ h	47.5^{a}	53.0^{b,c}	51.1^{b}	53.6^{c}	46.9^{a}	55.3^{c}	1.110	ns	ns	***	**
Σ H	23.6	22.9	21.7	22.2	22.9	21.3	0.708	ns	ns	ns	ns
Ratios											
n-6/*n*-3	11.4	10.0	13.7	11.5	20.2	16.7	1.322	ns	***	**	ns
PUFA/SFA	0.32^{a}	0.63^{b}	0.42^{a}	0.56^{b,c}	0.40^{a,c}	0.84^{d}	0.070	ns	ns	***	*
h/H	2.03^{a}	2.38^{b,c,d}	2.38^{a,c}	2.47^{b,c}	2.09^{a,d}	2.71^{b,c}	0.133	ns	ns	***	*
CLA/(SFA+CHR)	0.016	0.017	0.018	0.024	0.013	0.012	0.0028	ns	*	ns	ns

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 mean as follow: G × M, interaction between group of animals (G) and muscle type (M); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids.

[+] Total fatty acids is the sum of identified fatty acids.

Σ *n*-6 = sum of 18:2*n*-6, 18:3*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6 and 22:4*n*-6.

Σ *n*-3 = sum of 18:3*n*-3, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3.

Σ h = hypocholesterolaemic fatty acids (sum of 18:1*c*9, 18:2*n*-6, 18:3*n*-3, 18:3*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3).

Σ H = hypercholesterolaemic fatty acids (sum of 12:0, 14:0 and 16:0).

n-6/*n*-3 = *n*-6/*n*-3 ratio [(sum of 18:2*n*-6, 18:3*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6 and 22:4*n*-6)/(sum of 18:3*n*-3, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3)].

PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n*-6, 18:3*n*-3, 18:3*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3)/(sum of 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].

h/H = hypocholesterolaemic/hypercholesterolaemic ratio [(sum of 18:1*c*9, 18:2*n*-6, 18:3*n*-3, 18:3*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3)/(sum of 12:0, 14:0 and 16:0)].

CLA/(SFA+CHR) = conjugated linoleic acid and saturated fatty acids plus cholesterol ratio [(total CLA)/(sum of SFA and cholesterol), each expressed in mg/g muscle].

purebred and Alentejana × Charolais crossbred bullocks (De Smet et al., 2004). Although fat content was higher in meat from autochthonous purebred animals than in that from crossbred bullocks, both beef are considered lean according to the Food Advisory Committee (1990) criteria (< 5% fat). In contrast, slaughter season of Alentejana bullocks did not affect ($P > 0.05$) the values of total fatty acids in PDO beef, which were similar to those obtained for beef from intensively produced crossbred meat. Total fatty acids, calculated as the sum of identified fatty acids, represented on average 77% of the sum of detected fatty acids (GC analysis) and 61% of total lipids (gravimetric analysis). Our sum of identified fatty acids relative to the sum of detected fatty acids (77%) is close to the 83% value reported by De Smet et al. (2000).

The LT muscle had greater ($P < 0.001$) contents of total lipids and intramuscular fatty acids compared to the ST muscle, simultaneously with a higher relative proportion of MUFA. In contrast, the percentages of *n*-3 PUFA were lower ($P < 0.001$) in LT muscle than in ST muscle. In addition, no seasonal changes ($P > 0.05$) in the various partial sums of fatty acids were observed for Carnalentejana-PDO beef. Moreover, PDO meat showed higher ($P < 0.01$) relative proportions of MUFA and similar percentages ($P > 0.05$) of *n*-3 PUFA and hypercholesterolaemic fatty acids than intensively produced beef. The higher relative proportions of MUFA in PDO beef can be explained by higher gene expression of stearoyl-CoA desaturase in Alentejana purebred bullocks, as described above for 18:1*c*9. Analogous differences in these families of fatty acids were observed, for concentrate-fed *vs.* pasture-fed animals, by Enser et al. (1998) and Sañudo et al. (2000).

It has been reported that fat from grass-fed ruminants is more saturated, mainly due to higher proportions of 18:0 (Bas & Morand-Fehr, 2000; Rhee et al., 2000), which was explained by the inhibition of rumen biohydrogenation found in concentrate-fed animals (Doreau & Ferlay, 1994) or by *de novo* fatty acid synthesis. In contrast, French et al. (2000) found a linear decrease in the proportion of SFA with the increase of grass intake, which was principally due to a lower content of 16:0 in grass relative to concentrate. Our results do not show any apparent grass effect, as 16:0 was not affected ($P > 0.05$) by the production system and 18:0 and SFA showed significant interactions between animal group and muscle type. This observation may be due to the attenuation of grass effect on SFA in Alentejano bullocks during the finishing period on concentrate. In spite of attenuating the typical grass-fed characteristics of meat fatty acid composition, the finishing period with concentrate improves carcass conformation and meat eating quality of the animals raised on pasture (Vestergaard et al., 2000b). Finally, there was an interaction between the experimental group of animals and muscle type for SFA ($P < 0.01$), *trans* fatty acids (TFA; $P < 0.05$), PUFA ($P < 0.01$), *n*-6 PUFA ($P < 0.01$) and hypocholesterolaemic fatty acids ($P < 0.01$), reflecting the patterns described above for individual fatty acids. Since PUFA are much more abundant in the phospholipid fraction than in triacylglycerol fraction (Wood et al., 2004), this difference in PUFA reflects distinct

triacylglycerol/phospholipid ratios between muscles, as a consequence of the different fat level. Furthermore, it is well established that the phospholipid content is greatest in red oxidative muscle fibres, the *longissimus dorsi* muscle of cattle is relatively white (Enser et al., 1998) and the muscle fibre type is affected by environmental conditions, such as the level of confinement (Klont et al., 1998).

In order to evaluate the nutritional value of intramuscular fat, *n*-6/*n*-3, PUFA/SFA and hypocholesterolaemic/hypercholesterolaemic (h/H) ratios of fatty acids (as defined in Table 4) were calculated (Table 4). Similar to the total fatty acids and fatty acid composition data, there were no seasonal effects ($P > 0.05$) on these nutritional indices, which are related to healthy human nutrition. In contrast, PDO beef had a lower ($P < 0.001$) *n*-6/*n*-3 ratio than intensively produced crossbred beef, although significant interactions in PUFA/SFA and h/H ratios were observed between animal group and muscle type, reflecting the patterns observed for partial sums of fatty acids. The values of *n*-6/*n*-3 ratio for meat from concentrate-fed bullocks (16.7-20.2) are very close to those reported by Enser et al. (1998) for British meat generated from concentrate-fed cattle (15.6-20.1). Interestingly, the figures of *n*-6/*n*-3 ratio achieved by the same authors for meat from grass-fed bullocks (2.0-2.3) are much lower than our values for meat from Alentejana purebred bullocks (10.0-13.7). Our intermediate *n*-6/*n*-3 ratio in Carnalentejana-PDO beef, mainly due to lower *n*-6 PUFA content than in concentrate-fed cattle, may be explained by the mixed-diet of Alentejano bullocks. It is well known that the use of cereals (rich in *n*-6 PUFA) in concentrates shifts the meat fatty acid composition to an increased ratio of *n*-6/*n*-3 when compared with animals produced on pasture (Nuernberg et al., 2002; Yang et al., 2002; Raes et al., 2004). Furthermore, it was also shown that finishing cattle exclusively on pasture enhances the unsaturated fatty acid profile of beef fat, decreasing *n*-6/*n*-3 and increasing PUFA/SFA ratios (Enser et al., 1998; French et al., 2000; Realini et al., 2004). In contrast, Sañudo et al. (2000) observed that fat from ruminants raised on pasture had a lower PUFA/SFA ratio than that obtained from concentrate-fed animals, which were usually below the recommended value for the human diet. In addition, Raes et al. (2004) suggested that the PUFA/SFA ratio is mainly influenced by genetics and much less by nutrition. Our results, which showed significant interaction between animal group and muscle type for PUFA/SFA ratio, do not seem to depict any of these grass or breed effects. The figures of *n*-6/*n*-3 ratio were higher ($P < 0.01$) in LT muscle when compared with the ST muscle.

Current nutritional recommendations are that the PUFA/SFA ratio in human diets should be above 0.45 (British Department of Health, 1994) and, within the PUFA, the *n*-6/*n*-3 ratio should be between 1 and 2 (National Institute of Health of USA, quoted by Simopoulos, 2002). Fats presenting low PUFA/SFA ratio are considered unfavourable, because they may induce an increase in cholesterolaemia. In view of the above recommendations, the PUFA/SFA ratio, although depicting

significant interaction ($P < 0.05$) between animal group and muscle type was higher, and so more favourable, for ST muscle (0.56-0.84) than for LT muscle (0.32-0.42). However, the h/H ratio, based on the functional effects of fatty acids on cholesterol metabolism, provides a better approach to the nutritional evaluation of fat, since some SFA do not increase plasma cholesterol and also considers the beneficial effects of MUFA (Santos-Silva et al., 2002). Similar to the PUFA/SFA ratio, there was a significant interaction ($P < 0.05$) between animal group and muscle type for h/H ratio. The results for this nutritional ratio (2.0-2.7) are close to those obtained in lamb meat by Santos-Silva et al. (2003) (1.8-2.1). For *n*-6/*n*-3 ratio, the values are higher than that recommended for human diets in both beef production systems (10.0-20.2), which results from the relatively greater amounts of *n*-6 PUFA and to the relatively lower contents of *n*-3 fatty acids. However, the data indicate that Carnalentejana-PDO beef had lower *n*-6/*n*-3 values ($P < 0.001$), with no seasonal effects ($P > 0.05$), than that from animals fed with concentrate-based diet.

2.3.2 Intramuscular CLA isomeric profile

The values of total (mg/g muscle) and specific (mg/g fat) CLA contents and its isomeric profile (% total CLA) in intramuscular fat of Carnalentejana-PDO beef and in meat from crossbred bullocks fed intensively with concentrate are displayed in Table 5. Total CLA content was lower ($P < 0.05$) in intensively produced beef than in Carnalentejana-PDO meat, which did not show significant differences ($P > 0.05$) when the slaughter season was compared. Furthermore, LT muscle had a higher ($P < 0.001$) total CLA content relative to ST muscle. In addition, no significant differences ($P > 0.05$) regarding specific CLA content were observed when slaughter season, production system and muscle type were analysed. These variations are explained by differences in intramuscular fat content among meats (see Table 4) because CLA content in intramuscular fat was similar ($P > 0.05$) among meats. A direct linear relationship between grass percentage in cattle diet and meat CLA content has been described by French et al. (2000), although the mechanism remains controversial. French et al. (2000) suggested that grass in the diet enhances the growth of ruminal bacterium *Butyrivibrio fibrisolvens*, which converts 18:2*n*-6 into *c*9,*t*11 CLA isomer through the action of a linoleic acid isomerase. More recently, Daniel et al. (2004) proposed that the increased content of CLA in animals fed forage-based diets is associated with an increase in 18:1*t*11, which is the substrate of stearoyl-CoA desaturase in tissues. Shanta et al. (1997) reported 7.7 and 5.2 mg total CLA/g fat in *semimembranosus* muscle for grass-fed and corn supplemented grass-fed beef, respectively. These findings are consistent with those published very recently by Realini et al. (2004), who reported that LT fat from grazing-based production systems had greater CLA contents (5.3 mg/g fat) than that obtained from concentrate-based production systems (2.5 mg/g fat). As

expected, the values of specific CLA contents described in this work (3.82-5.07 mg/g fat) are within the range reported by those authors.

The season of production did not influence ($P > 0.05$) individual and sums of geometric CLA isomers in Carnalentejana-PDO beef fat, except for the minor *t*11,*t*13 and *t*10,*t*12 isomers, which showed higher percentages ($P < 0.01$) in beef from autumn-slaughtered animals. This similarity in CLA profile of beef from Alentejana purebred bullocks throughout the year may be due to the similar final effect between the grazing on more abundant pastures with a larger finishing period on

Table 5. Total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers (% total CLA) of intramuscular fat in the *longissimus thoracis* (LT) and *semitendinosus* (ST) muscles of beef from Alentejana × Charolais crossbred bullocks intensively produced (IP) and from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications and slaughtered in early autumn (A) and late spring (S).

	Carnalentejana-PDO beef				Intensively produced beef			Significance level			
	Autumn		Spring					Contrasts		Muscle	G×M
	LT	ST	LT	ST	LT	ST	SEM	A *vs.* S	PDO *vs.* IP		
Total CLA content	0.100	0.042	0.066	0.054	0.064	0.024	0.010	ns	*	***	ns
Specific CLA content	5.07	3.82	4.92	5.06	4.45	3.88	0.563	ns	ns	ns	ns
CLA isomers											
*t*12,*t*14	0.70^{a,c}	0.57^{a,b}	0.27^{b}	0.29^{b}	0.55^{a,b}	0.93^{c}	0.111	**	**	ns	*
*t*11,*t*13	1.30	1.29	0.78	0.74	0.57	0.88	0.148	**	*	ns	ns
*t*10,*t*12	0.90	0.92	0.30	0.58	1.04	0.76	0.159	**	ns	ns	ns
*t*9,*t*11	1.77^{a,c}	2.92^{b}	2.48^{a,b}	2.68^{b}	1.16^{c,d}	0.58^{d}	0.291	ns	***	ns	*
*t*8,*t*10	0.50^{a}	0.40^{a,b}	0.26^{a,b}	0.54^{a}	0.37^{a,b}	0.16^{b}	0.098	ns	*	ns	*
*t*7,*t*9	0.43	0.50	1.16	0.75	15.0	20.5	2.256	ns	***	ns	ns
*t*6,*t*8	0.25	0.27	0.22	0.91	0.00	0.00	0.180	ns	**	ns	ns
total *trans,trans*	5.86	6.87	5.46	6.49	18.7	23.8	2.165	ns	***	ns	ns
*c/t*12,14	0.61	1.59	2.03	1.08	1.21	1.05	0.422	ns	ns	ns	ns
*t*11,*c*13	1.34	1.99	1.11	1.32	1.26	3.25	0.672	ns	ns	ns	ns
*c*11,*t*13	0.43	0.51	1.00	1.18	1.10	1.43	0.440	ns	ns	ns	ns
*t*10,*c*12	1.92	1.81	2.30	1.93	3.79	3.43	0.522	ns	**	ns	ns
*c*9,*t*11	79.8	76.7	77.0	75.9	59.9	54.5	2.567	ns	***	*	ns
*t*7,*c*9	9.31^{a}	9.31^{a}	9.05^{a}	10.1^{a,b}	13.0^{b}	10.0^{a}	1.129	ns	ns	ns	**
total *cis/trans*	93.4	92.0	92.5	91.5	80.2	73.6	2.455	ns	***	ns	ns
total *cis,cis*	0.73	1.19	2.05	2.05	1.08	2.51	0.490	ns	ns	ns	ns

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: G × M, interaction between group of animals (G) and muscle type (M).

concentrate and the grazing on less abundant pastures but with a shorter finishing period on concentrate. In fact, as described above, the bullocks with the largest finishing period on concentrate (5 months), slaughtered in October, had been exposed to more abundant pastures (late winter and early-middle spring grass) than the animals with the shortest finishing period (3 months), slaughtered in June (late winter grass). Although little research has been conducted to assess seasonal changes in beef CLA, Lock and Garnsworthy (2003) observed that CLA percentages in milk fat varied throughout the year in UK, with the highest values registered in the summer months (May-July), when cows received fresh grass. The sum of *cis,trans* geometric isomers contributed 91.5-93.4% of total CLA in semi-extensive reared beef and 73.6-80.2% in beef from intensively raised bullocks, while total *trans,trans* isomers contributed only 5.5-6.9% and 18.7-23.9% for meat from semi-extensive and intensively production systems, respectively. In contrast to Carnalentejana-PDO beef, meat from the intensive production concentrate-based system showed a significant increase ($P < 0.001$) in *trans,trans* CLA isomers, due to the *t*7,*t*9 isomer, accompanied by a decrease ($P < 0.001$) in *cis,trans* CLA isomers, due to *c*9,*t*11 isomer. In addition, no significant difference ($P > 0.05$) in total *cis,cis* CLA isomers between production systems were observed, while the average percentages detected, mainly composed by the *c*9,*c*11 isomer, were lower than 2.52%.

The CLA isomeric distribution showed a clear predominance of the bioactive *c*9,*t*11 isomer in both production systems. However, the relative proportion of *c*9,*t*11 to the total CLA in Carnalentejana-PDO beef (75.9-79.8%) was higher ($P < 0.001$) than in intensively reared beef (54.5-59.9%), with the former being much closer to the figures described for beef (Yurawecz et al., 1998; Fritsche et al., 2001; Realini et al., 2004). This variation in the proportion of *c*9,*t*11 CLA isomer between production systems probably reflects either differences in the levels of 18:2*n*-6 in the diet (see Table 2), the levels of linoleic acid isomerase produced by ruminal *Butyrivibrio fibrisolvens* (French et al., 2000), the substrate availability (18:1*t*11) of stearoyl-CoA desaturase in tissues (Daniel et al., 2004) and/or the breed differences in Δ^9 desaturase expression (Taniguchi et al., 2004). However, this effect of grazing is in disagreement with the data of Nuernberg et al. (2002), who observed that the relative *c*9,*t*11 CLA isomer proportion (% FAME) in beef was not significantly increased by grazing in comparison to concentrate feeding. The major effect of pasture on grass obtained by those authors for CLA isomers was a significant sevenfold increase of the mixture *c/t*11,13 isomers, which were not observed in our results ($P > 0.05$). The second or third most prevalent CLA isomer in meat from semi-extensive and intensive production systems, respectively, was *t*7,*c*9 (9.05-13.0%), which showed a significant interaction ($P < 0.01$) between the experimental group of animals and muscle type. This interaction may result from modifications of muscle metabolic types, which are associated with differences in fatty acid composition, likely due to adaptations to the confinement or extensive grazing. In fact, this isomer is mentioned frequently as the second most prevalent CLA isomer

(Yurawecz et al., 1998; Piperova et al., 2002) and, like *c*9,*t*11, its amount in milk and tissues is mostly originated by endogenous synthesis by Δ^9 desaturation of rumen derived *trans* octadecenoate (Corl et al., 2002). In addition, our group found that supplementation of all-forage diets with oils rich in linoleic acid increases the *t*7,*c*9 contents allowing this isomer to be the second most prevalent (Bessa et al., 2005). However, when linseed oil was used, the increase in *t*7,*c*9 proportions was moderated and this isomer falls in the rank to the fourth or fifth position. The *t*7,*t*9 CLA isomer showed higher percentages ($P < 0.001$) in intensively produced meat (15.0-20.5%), where it was the second most abundant isomer, relative to PDO beef (0.43-1.16%). As far as we know, the increase of this CLA isomer percentage in concentrate-fed animals has not yet been reported. However, this difference in the proportion of *t*7,*t*9 isomer in beef can be explained by differences in ruminal biohydrogenation between intensively produced bullocks and Alentejano bullocks. This explanation is based on the supposition that, with the exception of *c*9,*t*11 and *t*7,*c*9 isomers, the origin of all other CLA isomers is ruminal biohydrogenation of dietary unsaturated C18 fatty acids, although the metabolic pathways are not yet elucidated (Kraft et al., 2003; Collomb et al., 2004). The other bioactive CLA isomer, *t*10,*c*12, only presenting residual values (< 3.80%), showed greater percentages ($P < 0.01$) in meat fed intensively than in Carnalentejana-PDO beef but similar contents (mg/g muscle) in both meats. With the exception of *c*9,*t*11 isomer ($P < 0.05$), no significant differences ($P > 0.05$) were observed between LT and ST muscles for individual and sums of geometric CLA isomers. The interaction between experimental group of animals and muscle type was only significant for the percentages of *t*7,*c*9, as described above, and minor *t*12,*t*14, *t*9,*t*11 and *t*8,*t*10 CLA isomers. The differences observed in this study for individual CLA isomers are likely explained by the different production systems (feeding and other management variables) and animal genetics (breeds). Apart from the *c*9,*t*11 isomer, information reporting individual CLA isomers in beef is very scarce and, therefore, the data presented here provides a first step towards the full understanding of CLA isomers in meat.

2.3.3 Content of total cholesterol in beef

Data on the total cholesterol content in different muscles of bovine meats (mg/g muscle), obtained from intensive and semi-extensive production systems, are depicted on Table 4. Slaughter season of Alentejana purebred bullocks did not influence ($P > 0.05$) total cholesterol levels in meat. However, the figures of total cholesterol obtained for Carnalentejana-PDO beef were higher ($P < 0.001$) than those achieved for meat derived from intensively raised cattle. In addition, LT muscle exhibited a greater ($P < 0.001$) content of total cholesterol than ST muscle, while no significant interaction ($P > 0.05$) between the experimental group of animals and muscle type was achieved. In contrast to

intensively produced beef (0.35-0.37 mg/g), Carnalentejana-PDO beef showed values for total cholesterol (0.42-0.49 mg/g) similar to those reviewed by Chizzolini et al. (1999) for beef (0.47-0.57 mg/g). According to those authors, the most likely reason for some of the differences in cholesterol content observed in different muscles might be variations in fibre type composition. This hypothesis results from the observation that oxidative muscles are known to be richer in phospholipids and that there is a direct correlation between the content of phospholipids and cholesterol, which is mainly (60-80%) present in the membrane component of the bovine muscle (Hoelscher et al., 1988). The direct relationship between phospholipids and cholesterol seems to be necessary to maintain membrane fluidity in a narrow range (Alasnier et al., 1996). The results presented in this work, demonstrating a muscle effect on total cholesterol level, are apparently in disagreement with the above hypothesis since LT muscle, which is relatively white in cattle (see above), had higher values of cholesterol relative to ST muscle. However, attending to the fact that fat content was greater ($P < 0.001$) in LT muscle (see Table 4), the reported differences in total cholesterol might result from the contribution of cholesterol from intramuscular adipose tissue.

Recently, Eynard and Lopez (2003) proposed that the reciprocal proportions of SFA plus total cholesterol (CHR) *vs.* total CLA [CLA/(SFA+CHR) ratio; see detailed definition in Table 4] might explain the association between the intake of beef fat and colon cancer. In fact, lean beef (15% fat, with < 5% of invisible intramuscular fat), showing a high CLA/(SFA+CHR) proportion (0.09), has a protective effect against colon cancer, whereas fatty beef derivatives (37% fat), with low CLA/(SFA+CHR) proportions (0.007), is associated with a higher risk (Eynard & Lopez, 2003). Thus, the beneficial effects of minor amounts of CLA may be relatively enhanced in lean meat compared with fatty meat, which contain a substantial amount of SFA and cholesterol, as well as relatively less CLA. Meat derived from autochthonous purebred bullocks showed higher values ($P < 0.05$) of CLA/(SFA+CHR) ratio (0.016-0.024), with no seasonal changes, compared to beef from intensively reared cattle (0.012-0.013). Therefore, attending to the risk of colon cancer, Carnalentejana-PDO beef seems to be healthier than intensively-fed beef. Moreover, although beef from intensively-fed bullocks depicted lower values for total cholesterol, which is more desirable, the overall biological effects of total CLA, total cholesterol and SFA contents seem to be more favourable for PDO beef. Finally, the CLA/(SFA+CHR) proportion did not exhibit significant differences ($P < 0.05$) between LT and ST muscles nor for the interaction between experimental group of animals and muscle type.

2.4 CONCLUSIONS

Meat from Alentejana Portuguese purebred bullocks raised according to Carnalentejana-PDO specifications showed no important seasonal differences in the levels of fatty acids, CLA isomers and total cholesterol. In contrast, there were some different intramuscular fat characteristics when PDO beef was compared with meat from Alentejana × Charolais crossbred bullocks fed intensively with concentrate. This observation might reflect the semi-extensive production system used for raising the Alentejana purebred animals. However, the findings suggest that the finishing period of Alentejana purebred bullocks on concentrate attenuates most of the typical effects on the characteristics of meat fat associated with grass intake. Nevertheless, from a nutritional point of view, Carnalentejana-PDO beef seems to be more healthful than meat obtained from the conventional intensively-fed bullocks because of its lower *n*-6/*n*-3 ratio, although this ratio is always above the recommended values for human diet, and higher proportions of *c*9,*t*11 CLA isomer and CLA/(SFA+CHR). Taken together, the data indicate that Carnalentejana-PDO beef is of greater nutritional quality than intensively produced beef from crossbred bullocks throughout the year.

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3

FATTY ACID COMPOSITION, INCLUDING ISOMERIC PROFILE OF CONJUGATED LINOLEIC ACID, AND CHOLESTEROL IN MERTOLENGA-PDO BEEF

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Fatty acid composition, including isomeric profile of conjugated linoleic acid, and cholesterol in Mertolenga-PDO beef

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This paper describes the fatty acid composition, including isomeric distribution of conjugated linoleic acid, total lipids and cholesterol in *longissimus dorsi* and *semitendinosus* muscles of Mertolenga beef from young bulls reared according to PDO specifications. Mertolenga purebred young bulls (n = 30) were raised in a semi-extensive production system, including a finishing period on concentrate feeds for the last 5 or 3 months (October and June samplings, respectively). Mertolenga-PDO beef showed seasonal changes in the levels of some fatty acids (including the predominant 18:2*n*-6), sums of TFA and *n*-3 PUFA, some CLA isomers (*t*11,*t*13, *t*11,*c*13 and *t*10,*c*12), sums of geometric groups of CLA isomers and total cholesterol. In addition, significant differences were obtained between LD and ST muscles for most of the analysed parameters. From a nutritional perspective, PDO beef from June seems to be more healthful than that from October as a consequence of its lower *n*-6/*n*-3 ratio. Moreover, the results suggest that the PUFA/SFA ratio in the ST muscle is consistently above the recommended guideline for human diets, although the values were below that guideline for the LD muscle. Taken together, the data indicate that, although the finishing period of Mertolengo young bulls on concentrate attenuates most of the beneficial grass effects on meat fat throughout the year, PDO beef from late spring is of greater nutritional value than that from early autumn.

Keywords: Mertolenga-PDO; meat quality; total lipids; total cholesterol; fatty acids; CLA isomers.

3.1 INTRODUCTION

Fatty acid composition and cholesterol levels in meat have received an increased attention in view of their implications for human health and product quality (Wood et al., 2004). The ratios of PUFA/SFA and *n-6*/*n-3* PUFA are widely used to evaluate the nutritional value of fat. Low ratios of PUFA/SFA and high levels of cholesterol in typical Western diets have been considered as major risk factors of cardiovascular diseases, which are among the most important causes of human mortality in developed countries (Katan, 2000; Ganji et al., 2003). Moreover, typical Western diets display a very high *n-6*/*n-3* ratio (15-17/1), which favours the development not only of cardiovascular diseases, but also of cancer, inflammatory and autoimmune diseases (Simopoulos, 2002). In addition, fats presenting low PUFA/SFA ratio are considered unfavourable because they may induce an increase in cholesterolaemia. It is well established that lower PUFA/SFA and higher *n-6*/*n-3* ratios of most meats are a major cause of the imbalance in the fatty acid intake of today's consumers (Enser, 2001; Wood et al., 2004). Finally, meat also provides from one third to one half (Chizzolini et al., 1999) of the maximum daily-recommended intake of cholesterol (300 mg, WHO, 2003).

Conjugated linoleic acid is a generic term used to describe the positional and geometric isomers of linoleic acid (18:2*n*-6) in which the double bonds are conjugated. The major CLA isomer, *c*9,*t*11, is produced in the rumen during the microbial biohydrogenation of dietary 18:2*n-6* and in the tissues (endogenously) through Δ^9 desaturation of 18:1*t*11 (Griinari & Bauman, 1999). It is now accepted that the main contribution to *c*9,*t*11 present in milk (Griinari & Bauman, 1999; Corl et al., 2002) and ruminant meats (Daniel et al., 2004; Palmquist et al., 2004) is from endogenous synthesis. Twenty different CLA isomers have been reported as occurring naturally in food, especially in ruminant fat (Sehat et al., 1998). In animal models, some CLA isomers exhibit anticarcinogenic, antithrombotic, antiatherogenic and immune modulator properties (Belury, 2002; Prates & Mateus, 2002). Specific physiological effects have been linked with individual CLA isomers. The *t*10,*c*12 isomer may play an important role in lipid metabolism, while the *c*9,*t*11 and the *t*10,*c*12 isomers seem to be equally effective in anticarcinogenesis (Pariza et al., 2001; Evans et al., 2002) and antiatherogenesis (Whale et al., 2004). Since individual CLA isomers have different biological activities, the determination of the CLA isomeric profiles in meat is required.

The fatty acid composition of beef is influenced by dietary factors and, to a lesser extent, by non-dietary environmental and genetic factors (De Smet et al., 2004). Dietary factors are often linked with a particular production system (Geay et al., 2001; Raes et al., 2004). Meat from grazing ruminants is expected to reflect the variability of pasture biomass. In fact, the nutritive value of pasture is highly dependent on cultural practices, season and geographical factors (Moloney et al., 2001). In addition, breed differences in fatty acid composition in several farm animal species have also been reported

(De Smet et al., 2004). PDO meats, derived from local production systems and animal breeds, are certified by European Union legislation and are expected to present unique quality and organoleptic characteristics, especially associated with specific properties of their lipid fraction (Council Regulation nº2081/92 of 14/7, EEC). One such example is Mertolenga-PDO beef (Commission Regulation nº1107/96 of 12/06, EC), which is obtained from Mertolengo purebred young bulls raised in a traditional semi-extensive production system in the Alentejo region of southern Portugal according to product specifications.

In spite of being the second most important commercial Portuguese PDO beef (*ca* 260 carcass tonnes in 2003, Instituto do Desenvolvimento Rural e Hidráulica, 2003), there are no detailed reports on the composition of Mertolenga-PDO beef. Moreover, little work has been conducted to assess seasonal changes in beef CLA profile. Therefore, the goal of this study was to characterise the lipid composition and nutritional value of Mertolenga-PDO beef in two distinct and more important slaughter seasons (early autumn and late spring).

3.2 MATERIAL AND METHODS

3.2.1 Reagents

Analytical-grade and liquid chromatographic-grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). Sodium methoxide (0.5 M solution in anhydrous methanol) and a cholesterol standard were obtained from Sigma-Aldrich Ltd. (St. Louis, MO, USA). FAME standard mixtures were acquired from Nu-Chek-Prep Inc. (Elysian, MN, USA) and Supelco Inc. (Bellefonte, PA, USA). Additional standards of individual CLA isomers (*c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11) as methyl esters were purchased from Matreya Inc. (Pleasant Gap, PA, USA).

3.2.2 Animals and meat samples

Mertolengo purebred young bulls (n = 30) were reared in a semi-extensive system, following Mertolenga-PDO beef specifications (Commission Regulation nº1107/96 of 12/06, EC). The animals were raised, on different representative private farms, under an extensive grazing system based on natural pastures under holm and cork oak, which is usually referred as "Montado". Supplementation with cereals and dry forages (hay and straw) was provided during periods of feed scarcity. The young bulls were finished on concentrate (Table 6) in the last 5 or 3 months, before slaughter in October 2002 (early autumn sampling; Regional Abattoir of Baixo Alentejo, Beja) or June 2003 (late spring

sampling; Regional Abattoir of Alto Alentejo, Sousel), respectively. Animals slaughtered in October had been exposed to a period of grass abundance (late winter and early-middle spring) and finished on concentrate since May (n = 15; 17 ± 0.7 months and 239 ± 7.2 kg), while young bulls slaughtered in June had been exposed to the less abundant late winter grass and finished on concentrate since March (n = 15; 24 ± 0.7 months and 231 ± 7.8 kg).

Table 6. Chemical (g/kg dry matter) and fatty acid (% sum of fatty acids, determined in triplicate) composition of the concentrate from Mertolengo young bulls reared according to Mertolenga-PDO beef specifications*.

	Concentrate (n = 4)
Chemical composition	
Crude protein	150
Total fat	32
Crude fibre	67
Ashes	75
Fatty acids	
14:0	1.6
16:0	14.7
18:0	2.5
18:1	21.9
18:2*n-6*	53.8
18:3*n-3*	4.1

* Ingredients: cereals, soybean, soybean meal and a vitamin-mineral premix.

Meat samples were taken from the ribeye portion (T1-T3) of LD and distal region of ST muscles of young bulls. Comparing with ST muscle, LD muscle is relatively red and differently involved in the physical activity imposed by grazing (Vestergaard et al., 2000a). All meat samples were collected 2-3 days after slaughter (+1 ºC), ground using a food processor (3 × 5 s), vacuum packed and stored at −80 °C until required for analysis.

3.2.3 Lipid extraction and methylation

Meat samples were lyophilised (−60 °C and 2.0 hPa) until constant weight using a lyophilisator Edwards Modulyo (Edwards High Vacuum International, UK), maintained exsiccated at room temperature, and analysed within two weeks. Lyophilised meat samples were weighed

(approximately 250 mg), in triplicate (two tubes for total lipid determination and the other tube for both FAME and CLA methyl ester profiles), into screw teflon-lined cap tubes. Intramuscular fat was extracted three times with methylene chloride-methanol (4:1 v/v) and a fourth extraction was performed with *n*-hexane, as described by Fritsche et al. (2000, 2001). Fatty acids were converted to methyl esters by base-catalysed transesterification, in order to avoid isomerisation of CLA isomers, with sodium methoxide during 2 hours at 30 °C, as proposed by Park et al. (2001) and Kramer et al. (2002). However, the free fatty acids and fatty acid in plasmogens are not methylated with base-catalysed transesterification procedures (Cruz-Hernandez et al., 2004). The same FAME solution was used for the analysis of both fatty acid composition and CLA profile, enabling the direct comparison of quantitative data and eliminating differences in sample preparation. Total lipids were measured gravimetrically, in duplicate, by weighing the fatty residue obtained after solvent evaporation.

3.2.4 Determination of fatty acid composition

Gas chromatography analyses of FAME were performed with an Agilent 6890 Series II gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) fitted with a flame ionization detector. The FAME were separated on a SP^{TM}-2560 fused-silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness, Supelco Inc., Bellefonte, PA, USA), coated with cyanopropyl polysiloxane stationary phase, using a split/splitless injection system (split ratio of 1:5) and helium as carrier gas at a flow rate of 1.5 mL/min. After injection (1 μL), the column temperature was held at 75 °C for 2 min and then increased to 180 °C at 5 °C/min. The temperature was kept at 180 °C for 33 min, followed by an increase of 4 °C/min to 225 °C and, finally, held at 225 °C for 44 min. The detector and injection temperatures were set at 250 °C. Identification was accomplished by comparing the retention time of peaks from samples with those of FAME standard mixtures and with values published in the literature (Fritsche et al., 2001; Kramer et al., 1998). Quantification of FAME was based on the internal standard technique, using 19:0 as internal standard, and on the conversion of relative peak areas into weight percentages, using the corrected response factor of each fatty acid (ES ISO 5508, 1990). Fatty acids were expressed in gravimetric contents (mg/g muscle) or as a percentage of the sum of identified fatty acids (% w/w).

3.2.5 Determination of individual CLA isomers

The methyl esters of CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using an HPLC system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA)

equipped with autosampler and DAD adjusted to 233 nm. The mobile phase was 0.1% acetonitrile in *n*-hexane maintained at a flow rate of 1 mL/min and injection volumes of 20 μL were used. 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, 2001). In addition, the identity of each isomer was controlled by the typical UV spectra of CLA isomers from the DAD in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems rev. A.09.01 (Agilent Technologies, 2001). Total and individual CLA isomer contents in meat were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalization (AOAC 963.22, 2000). The CLA isomers were expressed in gravimetric contents (mg/g muscle and mg/g lipids) or as a percentage of the sum of identified CLA isomers (% total CLA).

3.2.6 Determination of total cholesterol

Total cholesterol was extracted from lyophilised meat, after direct saponification with saturated methanolic KOH solution, according to the procedure of Naeemi et al. (1995) except that three extractions with *n*-hexane were used (recoveries were higher than 94%). Cholesterol was separated by normal-phase HPLC (column Zorbax Rx-Sil, 250 mm × 4.6 mm i.d., 5 μm particle size, Agilent Technologies Inc., Palo Alto, CA, USA), using an HPLC system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and DAD set at 206 nm, with a solvent (3% isopropanol in *n*-hexane) flow rate of 1 mL/min and injection volumes of 30 μL. Total cholesterol content in meat was calculated, in duplicate, based on the external standard technique, from a standard curve for peak area *vs.* cholesterol concentration.

3.2.7 Statistical analysis

The data were analysed using the MIXED procedure of SAS (SAS, 2004), considering the animal within slaughter season group as subject and the muscle type as repeated measures. The model considers as fixed effects the slaughter season (PDO beef from early autumn and that from late spring), the muscle type (LD and ST) and the interaction between animal group and muscle type.

3.3 RESULTS AND DISCUSSION

3.3.1 Intramuscular fatty acid composition

Data referring to the fatty acid composition (% w/w) of intramuscular fat in Mertolenga-PDO beef, obtained at early autumn and late spring seasons, are presented in Table 7. In both seasons, the

Table 7. Fatty acid composition (% w/w) in *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Mertolenga-PDO beef from early autumn and late spring.

	Autumn beef		Spring beef			Significance level		
Fatty acids	LD	ST	LD	ST	SEM	Season	Muscle	S × M
10:0	0.06	0.04	0.04	0.03	0.006	*	**	ns
12:0	0.12	0.09	0.06	0.04	0.008	***	***	ns
14:0	2.69	2.08	2.08	1.55	0.138	**	***	ns
14:1	0.30	0.31	0.24	0.28	0.038	ns	ns	ns
15:0	0.37	0.31	0.40	0.30	0.020	ns	***	ns
16:0	22.2	22.2	21.0	21.0	0.567	ns	ns	ns
16:1*t*9	0.42	0.45	0.49	0.52	0.015	**	**	ns
16:1*c*9	3.03	3.16	2.90	3.02	0.172	ns	ns	ns
17:0	0.73^{a}	0.67^{a}	0.89^{b}	0.60^{a}	0.047	ns	***	*
17:1*c*9	0.50	0.49	0.69	0.61	0.029	***	ns	ns
18:0	16.6^{a}	13.7^{b}	18.7^{c}	14.0^{b}	0.542	ns	***	**
18:1t	4.69	3.78	3.38	2.50	0.454	*	**	ns
18:1*c*9	30.2	28.0	31.6	27.5	0.897	ns	***	ns
18:1*c*11	0.37	0.70	1.61	2.06	0.126	***	**	ns
18:2*t*9,*t*12	0.21	0.18	0.13	0.14	0.020	*	ns	ns
18:2*n-6*	11.8	15.4	9.04	13.6	0.932	*	***	ns
18:3*n-6*	0.02^{a}	0.03^{b}	$0.03^{a,b}$	0.07^{c}	0.006	**	***	*
18:3*n-3*	0.53^{a}	0.59^{a}	1.58^{b}	2.36^{c}	0.266	***	***	***
20:0	0.16	0.11	0.15	0.13	0.011	ns	**	ns
20:1*c*11	0.05	0.04	0.09	0.07	0.008	***	ns	ns
20:2*n*-6	0.13	0.16	0.11	0.17	0.014	ns	***	ns
20:3*n*-6	0.57	0.89	0.61	1.20	0.076	ns	***	ns
20:4*n*-6	3.14	4.97	2.57	5.54	0.371	ns	***	ns
20:5*n*-3	0.08	0.25	0.06	0.25	0.058	ns	**	ns
22:4*n*-6	0.11	0.16	0.06	0.13	0.033	ns	*	ns
22:5*n*-3	0.43^{a}	0.75^{b}	1.00^{b}	1.84^{c}	0.184	**	***	**
22:6*n*-3	0.05^{a}	0.09^{a}	0.08^{a}	0.20^{b}	0.017	**	***	***

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; S × M, interaction between slaughter season (S) and muscle type (M).

predominant fatty acids in intramuscular fat were 16:0 and 18:0 as SFA, 18:1*c*9 as MUFA, and 18:2*n-6* as PUFA. Similar results were found by many other authors in beef (Raes et al., 2003a; Realini et al., 2004; Varela et al., 2004). The content of *trans* octadecenoic fatty acids was expressed as a single value (18:1*t*) because of its incomplete chromatographic resolution.

No seasonal variations ($P > 0.05$) in the fatty acid composition of Mertolenga-PDO beef were apparent for the predominant fatty acids, except for 18:0 (LD muscle) and 18:2*n*-6, which showed higher percentages ($P < 0.05$) in beef from late spring and early autumn, respectively. Regarding minor fatty acids, meat from October sampling had higher percentages of 10:0, 12:0, 14:0, 18:1*t* and 18:2*t*9,*t*12, but lower proportions of 16:1*t*9, 17:0 (LD muscle) 17:1*c*9, 18:1*c*11, 18:3*n*-6 (ST muscle), 18:3*n*-3, 20:1*c*11, 22:5*n*-3 and 22:6*n*-3 (ST muscle). The small effect of slaughter season, which includes the animal age effect, on meat fatty acids is probably due to the finishing period on concentrate that attenuates the expected differences resulting from pasture seasonal variations. In fact, the young bulls that were exposed to the more abundant pastures (late winter and early-middle spring grass) were finished on concentrate during 5 months (slaughtered in October), while the animals grazing in the less abundant late winter pastures were finished on concentrate for only 3 months (slaughtered in June). The higher values for 18:2*n*-6 and lower percentages for 18:3*n*-3 in meat from October, in comparison with that from June may be explained by the largest finishing period of young bulls on concentrate (5 *vs.* 3 months), which is richer in *n*-6 PUFA and 18:1*c*9, and poorer in *n*-3 PUFA (see Table 6) than grass (Palmquist, 1988). However, in contrast to *n*-6 and *n*-3 PUFAs, 18:1*c*9 did not show any seasonal variation. This observation may be explained by the fact that oleic acid content of ruminant tissues does not seem to be associated with substrate availability but with stearoyl-CoA desaturase gene expression (Daniel et al., 2004).

The LD muscle, relative to the ST muscle, had greater proportions of 10:0, 12:0, 14:0, 15:0, 17:0 (late spring beef), 18:0, 18:1*t*, 18:1*c*9 and 20:0, and lower values of 16:1*t*9, 18:1*c*11, 18:2*n*-6, 18:3*n*-6, 18:3*n*-3 (late spring beef), 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3 (late spring beef). Significant interactions between slaughter season and muscle type were observed for the percentages of 17:0, 18:0, 18:3*n*-6, 18:3*n*-3, 22:5*n*-3 and 22:6*n*-3. These interactions may result from the different finishing periods on concentrate when comparing autumn-slaughtered and spring-slaughtered young bulls, because the adaptations from the extensive grazing to the more confined finishing period on concentrate may induce changes in muscle metabolic types (Klont et al., 1998). In fact, when different muscles are compared, it is well known that differences in muscle fibre type are reflected in differences in fatty acid composition (Wood et al., 2004) and that the LD muscle (25.5%-31.0% of type I fibres) of young bulls is relatively red in comparison with ST muscle (16.9%-22.1% of type I fibres) (Vestergaard et al., 2000a).

The results for total lipids (mg/g muscle) and partial sums (% w/w) of intramuscular fatty acids in Mertolenga-PDO beef obtained in the two distinct slaughter seasons are presented in Table 8. Slaughter season did not affect ($P > 0.05$) the values of total lipids in meat. In contrast, the LD muscle had higher contents ($P < 0.001$) of total lipids than the ST muscle. PDO beef exhibited values of total lipids (1.7-1.8% and 1.2% for LD and ST muscles, respectively) between those reported for the meat from extensively (lower) and intensively produced young bulls (1.4-2.7% and 1.1-1.8% for LD and ST muscles, respectively) (Vestergaard et al., 2000b). Differences in total lipids between the two muscles likely result from variations in fibre composition. It is well established that the lipid content

Table 8. Total cholesterol (mg/g muscle), total lipids (mg/g muscle), partial sums of fatty acids (% w/w) and nutritional ratios in *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Mertolenga-PDO beef from early autumn and late spring.

	Autumn beef		Spring beef			Significance level		
	LD	ST	LD	ST	SEM	Season	Muscle	S × M
Total cholesterol	0.44	0.40	0.50	0.44	0.011	***	***	ns
Total lipids	17.4	12.0	18.0	11.8	0.934	ns	***	ns
Partial sums								
Σ SFA	43.0	39.1	43.3	37.6	0.824	ns	***	ns
Σ MUFA	34.4	32.7	37.2	33.5	1.011	ns	***	ns
Σ TFA	5.33	4.41	3.99	3.16	0.458	*	**	ns
Σ PUFA	16.9	23.3	15.1	25.4	1.564	ns	***	ns
Σ *n*-6	15.8	21.7	12.4	20.7	1.340	ns	***	ns
Σ *n*-3	1.10[a]	1.68[b]	2.71[b]	4.64[c]	0.440	***	***	***
Ratios								
n-6/*n*-3	14.9	13.6	7.11	6.87	1.012	***	*	ns
PUFA/SFA	0.41	0.61	0.36	0.68	0.047	ns	***	ns
CLA/(SFA+CHR)	0.013	0.013	0.011	0.012	0.0009	ns	ns	ns

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.; S × M, interaction between slaughter season (S) and muscle type (M); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids.

Σ TFA = sum of 16:1*t*9, 18:1*t* and 18:2*t*9,*t*12.

Σ *n*-6 = sum of 18:2*n-6*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6* and 22:4*n-6*.

Σ *n*-3 = sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3* and 22:6*n-3*.

n-6/*n*-3 = *n-6*/*n-3* ratio [(sum of 18:2*n-6*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6* and 22:4*n-6*)/(sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3* and 22:6*n-3*)].

PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n-6*, 18:3*n-3*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6*, 20:5*n-3*, 22:4*n-6*, 22:5*n-3* and 22:6*n-3*)/(sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].

CLA/(SFA+CHR) = conjugated linoleic acid and saturated fatty acids plus cholesterol ratio [(total CLA)/(sum of SFA and cholesterol), each expressed in mg/g muscle].

is higher in red oxidative muscle fibres (Enser et al., 1998) and that, as explained above, the LD muscle of young bulls is relatively red in comparison with the ST muscle. However, according to the Food Advisory Committee (1990) criteria (< 5% fat), Mertolenga-PDO beef may be considered a lean meat.

Meat from October, relative to that from June, had higher proportions ($P < 0.05$) of *trans* fatty acids (TFA), lower proportions ($P < 0.05$) of *n*-3 PUFA and similar percentages ($P > 0.05$) for the other partial sums of fatty acids. The higher percentages of *n*-3 PUFA in meat from June in comparison with that from October may be explained by the shortest finishing period on concentrate, and thus a less attenuated grass effect of the young bulls (3 *vs.* 5 months). Furthermore, a muscle type effect ($P < 0.05$) was obtained for all the partial sums of fatty acids, reflecting the patterns described above for individual fatty acids. Since PUFA are much more abundant in the phospholipid fraction than in the triacylglycerol fraction (Wood et al., 2004), the difference in PUFA reflects distinct triacylglycerol/phospholipid ratios between muscles, as a consequence of the different fat level (see Table 8).

Current nutritional recommendations are that the PUFA/SFA ratio in human diet should be above 0.45 and, within the PUFA, the *n-6*/*n-3* ratio should not exceed 4.0 (British Department of Health, 1994). In order to evaluate the nutritional value of intramuscular fat related to human nutrition, *n*-6/*n*-3 and PUFA/SFA ratios of fatty acids (as defined in Table 8) were calculated (Table 8). A seasonal effect was obtained for the *n-6*/*n-3* ratio ($P < 0.001$) but not for the PUFA/SFA ratio ($P > 0.05$). The values of *n-6*/*n-3* ratio in meat from autumn-slaughtered animals (13.6-14.9) are much higher than those in meat from spring-slaughtered young bulls (6.87-7.11). All the figures are above the recommended values for human diet, although much higher, and thus much more unfavourable, in beef from early autumn than in that from late spring. These values for beef from October are slightly lower than those reported by our group (Alfaia et al., 2006a) for Portuguese meat from crossbred young bulls produced in a conventional intensive concentrate-based system (16.7-20.2), as well as by Enser et al. (1998) for British meat from concentrate-fed cattle (15.6-20.1). However, the figures for Mertolenga-PDO beef from October are much higher than those reported by Enser et al. (1998) for meat from grass-fed young bulls (2.0-2.3), which are within the recommended values for the human diet. The intermediate *n-6*/*n-3* ratio in beef from June, mainly due to higher *n*-3 PUFA content, may be explained, as discussed above, by the shorter finishing period of the young bulls on concentrate feeds (Table 6). It is well known that the *n-6*/*n-3* ratio in meat from cattle fed concentrate-based diets is higher than in meat from pasture-fed cattle (Nuernberg et al., 2002; Raes et al., 2004). The PUFA/SFA ratio is generally increased with pasture feeding (Enser et al., 1998; Nuernberg et al., 2002; Realini et al., 2004), although a contradictory report exists (Sänudo et al., 2000). In addition, Raes et al. (2003a) suggested that the PUFA/SFA ratio is mainly influenced by genetics and much

less by nutrition. The results presented here did not show a seasonal effect for the PUFA/SFA ratio, possibly because of the finishing period of young bulls on concentrate feeds. The values of *n-6/n-3* ratio were higher ($P < 0.05$) in the LD muscle than in the ST muscle. In contrast, the LD muscle had a lower ($P < 0.001$) PUFA/SFA ratio (0.36-0.41), with values below the nutritional guideline recommended for the human diet, and thus unfavourable, than the ST muscle (0.61-0.68), which exhibited values above the nutritional guideline.

3.3.2 Intramuscular CLA isomeric profile

The values of total (mg/g muscle) and specific (mg/g fat) CLA contents and its isomeric profile (% total CLA) in intramuscular fat of Mertolenga-PDO beef are given in Table 9. Total CLA content in Mertolenga-PDO did not show significant differences ($P > 0.05$) when slaughter seasons were compared. Furthermore, the LD muscle had a higher ($P < 0.001$) total CLA content than the ST muscle. In addition, no significant differences ($P > 0.05$) regarding specific CLA content were observed when slaughter season and muscle type were analysed. Therefore, variations in total CLA content are entirely due to the differences in intramuscular fat content among meats (Table 8). This similarity in specific CLA contents in beef throughout the year may be due, as explained above, to the similar final effect of grazing on more abundant pastures with a longer finishing period on concentrate and grazing on less abundant pastures but with a shorter finishing period on concentrate. Although little research has been conducted to assess seasonal changes in beef CLA, Lock and Garnsworthy (2003) observed that CLA percentages in milk fat varied throughout the year, with the highest values registered in the summer months (May-July) when, in the UK, cows receive fresh grass. A direct linear relationship between grass percentage in cattle diets and meat CLA content in LD muscle has been described by French et al. (2000) although the mechanism underlying this variation is not well understood. It has been shown that total CLA content varied from 7.7 and 5.2 mg/g fat in *semimembranosus* muscle of grass-fed and corn-supplemented grass-fed beef, respectively (Shanta et al., 1997). These findings are consistent with those published recently by Realini et al. (2004) who reported that LD fat from grazing-based production systems had a higher CLA content (5.3 mg/g lipids) than that obtained from concentrate-based production systems (2.5 mg/g lipids). The values of specific CLA content found in the present study (2.63-3.65 mg/g fat) are within the range described by those authors, although closer to the figures described for concentrate-fed cattle.

The CLA isomeric distribution in Mertolenga-PDO beef from both seasons showed a clear predominance of the bioactive *c*9,*t*11 isomer (68.7-74.5%), followed by the *t*7,*c*9 isomer (8.47-13.5%), with values close to those reported for beef by several authors (Yurawecz et al., 1998; Fritsche et al., 2001; Realini et al., 2004). However, these percentages for the predominant bioactive *c*9,*t*11 isomer are higher than those obtained by our group for Portuguese meat from crossbred young bulls produced in a conventional intensive concentrate-based system (54.5-59.9%) (Alfaia et al., 2006a). Both predominant CLA isomers showed significant interactions between slaughter season and muscle type, which may result, as explained above, from metabolic adaptations to the different finishing periods on concentrate feeds of autumn- and spring-slaughtered young bulls.

Table 9. Total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers (% total CLA) in *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Mertolenga-PDO beef from early autumn and late spring.

	Autumn beef		Spring beef			Significance level		
	LD	ST	LD	ST	SEM	Season	Muscle	S × M
Total CLA	0.062	0.039	0.066	0.031	0.006	ns	***	ns
Specific CLA	3.65	3.39	3.51	2.63	0.350	ns	ns	ns
CLA isomers								
*t*12,*t*14	0.97^{a}	0.81^{a}	2.05^{b}	1.22^{a}	0.220	*	**	*
*t*11,*t*13	1.47^{a}	0.92^{b}	4.43^{c}	2.51^{a}	0.402	***	***	***
*t*10,*t*12	0.98	0.66	0.92	1.12	0.204	ns	ns	ns
*t*9,*t*11	2.80	2.99	3.36	4.03	0.475	ns	ns	ns
*t*8,*t*10	0.70	0.71	0.67	0.40	0.104	ns	ns	ns
*t*7,*t*9	0.99	1.32	1.02	0.88	0.218	ns	ns	ns
*t*6,*t*8	0.28	0.06	0.32	0.20	0.087	ns	*	ns
total *trans,trans*	8.20	7.46	12.8	10.4	0.871	**	**	ns
*c/t*12,14	1.05^{a}	2.66^{b}	2.29^{b}	1.50^{a,b}	0.386	ns	ns	**
*t*11,*c*13	1.16	2.62	3.55	3.56	0.549	*	ns	ns
*c*11,*t*13	0.51	1.15	0.33	0.34	0.239	ns	ns	ns
*t*10,*c*12	2.44	2.99	1.33	1.48	0.380	*	ns	ns
*c*9,*t*11	74.5^{a}	68.7^{b,c}	69.5^{b}	72.6^{a,c}	1.690	ns	ns	***
*t*7,*c*9	11.1^{a}	13.5^{b}	8.90^{a}	8.47^{a}	1.087	*	ns	*
total *cis/trans*	90.8	91.6	85.9	87.9	0.891	***	*	ns
total *cis,cis*	1.05	0.98	1.32	1.73	0.174	*	ns	ns

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; S × M, interaction between slaughter season (S) and muscle type (M).

Moreover, the *t*7,*c*9 isomer is mentioned frequently as the second most prevalent CLA isomer (Yurawecz et al., 1998; Piperova et al., 2002) and, like *c*9,*t*11, its concentration in milk and tissues is mostly originated by endogenous synthesis by Δ^9 desaturation of rumen-derived *trans* octadecenoate (Corl et al., 2002). Our group found that supplementation of all-forage diets with oils rich in linoleic acid increases the *t*7,*c*9 content and allows this isomer to be the second most prevalent in meat. However, when linseed oil was used, the increase in *t*7,*c*9 proportion was moderated and this isomer fell in ranking to fourth or fifth position. In addition, Dannenberger et al. (2005) reported that *t*11,*c*13 is the second most abundant CLA isomer in the tissue lipids of pasture-fed bulls.

The season of production did not influence ($P > 0.05$) the percentages of individual CLA isomers in Mertolenga-PDO beef fat, except for *t*11,*t*13, *t*11,*c*13 and *t*10,*c*12 isomers. Furthermore, a seasonal effect was obtained for *t*12,*t*14, *c/t*12,14 and *c*9,*t*11 CLA isomers in the LD muscle and for *t*7,*c*9 in the ST muscle. Beef from spring-slaughtered young bulls, relative to that from autumn-slaughtered animals, had higher percentages ($P < 0.05$) of *t*11,*t*13 and *t*11,*c*13 isomers, which likely results from a less attenuated grass effect in these animals due to the shorter finishing period on concentrate feeds. Although only a few publications deal with the CLA isomer distribution in beef, it was recently shown that pasture feeding compared with concentrate feeding mainly increases the proportion of *t*11,*c*13 (up to 18.5% of total CLA in LD muscle), with a decrease in *t*7,*c*9 (down to 4.1% of total CLA in LD muscle), but also increases the percentages of *t*11,*t*13 and *t*12,*t*14 (Nuernberg et al., 2002; Dannenberger et al., 2004). The other bioactive CLA isomer, *t*10,c12, which only presented residual values in the meats (< 3%), showed higher percentages ($P < 0.05$) in meat from autumn-slaughtered young bulls than in meat from spring-slaughtered animals. Moreover, beef from early autumn had higher percentages ($P < 0.001$) of *cis/trans* (*cis,trans* and *trans,cis*) isomers and lower proportions of sums of *trans,trans* ($P < 0.01$) and *cis,cis* ($P < 0.05$) isomers. The sum of *cis/trans* isomers contributed 90.8.-91.6% of total CLA in autumn-slaughtered and 85.9-87.9% in spring-slaughtered young bulls, while total *trans,trans* isomers contributed only 7.46-8.20% and 10.4-12.8% in meat from early autumn and late spring, respectively.

Muscle type affected the proportions of total *trans,trans* ($P < 0.01$) and *cis/trans* ($P < 0.05$) isomers, and individual *t*11,*t*13, *t*6,*t*8 and *c*9,*t*11 isomers ($P < 0.05$) in meat fat from both slaughter seasons. In addition, compared with the ST muscle, the LD muscle had lower proportions of *c/t*12,14 and *t*7,*c*9 isomers in meat from early autumn, and a higher percentage of *t*12,*t*14 in that from late spring. Dannenberger et al. (2004) found that pasture feeding as compared with concentrate feeding results in a variation in the distribution pattern and contents of individual CLA isomers between LD and ST muscles. According to those authors (Dannenberger et al., 2004) although the LD muscle from pasture-fed bulls has higher levels of the sum of CLA isomers and *t*10,*c*12 isomer, with similar

values for *c*9,*t*11 isomer, the ST muscle shows lower contents of *c*9,*t*11 and *t*10,*c*12 isomers, with no variation in the sum of CLA isomers. In contrast to *t*11,*t*13 and *t*11,*c*13 isomers, which seem to be more sensitive grass intake indicators, our data did not show these grass effects on *c*9,*t*11 and *t*10,*c*12 isomers in LD and ST muscles. Apart from the *c*9,*t*11 isomer, information reporting individual CLA isomers in beef is scarce, so the data presented here are valuable for the full understanding of CLA isomers in meat.

3.3.3 Content of total cholesterol in beef

Data on the total cholesterol content in different muscles of bovine meats (mg/g muscle) obtained from Mertolengo young bulls slaughtered in early autumn and late spring are presented in Table 8. Total cholesterol content was higher ($P < 0.001$) in the meat from spring-slaughtered young bulls than in that from autumn-slaughtered animals. In addition, the LD muscle exhibited a higher content ($P < 0.001$) of total cholesterol than the ST muscle, while no significant interaction ($P > 0.05$) between the experimental group of animals and muscle type was achieved. The levels of total cholesterol in Mertolenga-PDO beef (0.40-0.50 mg/g) were similar to those found by Chizzolini et al. (1999) in beef (0.47-0.57 mg/g). According to those authors, variations in fibre type composition might result in differences in cholesterol content in diverse muscles. This hypothesis results from the observation that oxidative fibres are richer in phospholipids than glycolytic fibres and that there is a direct correlation between the content of phospholipids and that of cholesterol, which is mainly (60-80%) present in the membrane component of bovine muscle (Hoelscher et al., 1988). The direct relationship between phospholipids and cholesterol seems to be necessary to maintain membrane fluidity in a narrow range (Alasnier et al., 1996). The results presented in this paper, demonstrating a muscle effect on total cholesterol level, are in agreement with the above hypothesis, since the LD muscle, which is relatively red (see above), had higher levels of cholesterol than the ST muscle. Finally, the lower values of total cholesterol in beef from early autumn may, as explained above, result from the changes in muscle metabolic types induced by the different finishing periods on concentrate of autumn- relative to spring-slaughtered young bulls (5 *vs.* 3 months, respectively).

Eynard and Lopez (2003) proposed that the reciprocal proportions of SFA plus total cholesterol (CHR) and total CLA, i.e. CLA/(SFA+CHR) ratio (see detailed definition in Table 8), might explain the association between the intake of beef fat and colon cancer. Interestingly, lean beef (15% fat, with < 5% of intramuscular fat), showing a high CLA/(SFA+CHR) ratio (0.09), has a protective effect against colon cancer, whereas fatty beef (37% fat), with a low CLA/(SFA+CHR) ratio (0.007), is associated with a higher risk (Eynard & Lopez, 2003). Thus the beneficial effects of minor amounts of CLA may be relatively enhanced in lean meat compared with fatty meat. The

CLA/(SFA+CHR) ratio did not show seasonal changes ($P > 0.05$) between beef from autumn- and spring-slaughtered young bulls (0.011-0.013). Therefore, regarding the risk of colon cancer, no seasonal variations in the health value of Mertolenga-PDO beef were apparent. Furthermore, although beef from autumn-slaughtered young bulls had lower values of total cholesterol than that from spring-slaughtered animals, which is more desirable, the overall biological effects of total CLA, total cholesterol and SFA contents seem to be similar throughout the year. Finally, the CLA/(SFA+CHR) ratio did not show significant differences ($P > 0.05$) between LD and ST muscles.

3.4 CONCLUSIONS

Mertolenga-PDO beef showed seasonal differences in the levels of several fatty acids, some CLA isomers and total cholesterol. In addition, significant differences were obtained between LD (relatively red) and ST (relatively white) muscles for most of the analysed parameters. The data indicate that PDO beef has intermediate levels (between those in meat from grain- and pasture-fed cattle) of several fatty acids, total lipids, *n*-6/*n*-3 ratio, total CLA and some CLA isomers. These data may be explained by the semi-extensive production system of Mertolengo young bulls. However, beef from June, relative to that from October, seems to have a less attenuated grass effect, possibly due to the shorter finishing period of young bulls on concentrate feeds.

From a nutritional point of view, PDO meat from June seems to be more healthful than that from October because of its lower *n*-6/*n*-3 ratio, although this ratio is always above the recommended value for the human diet. In addition, the findings suggest that the PUFA/SFA ratio is consistently above the recommended guideline for the human diet in ST muscle, and thus favourable, but below that guideline in LD muscle. Overall, the data indicate that, although the finishing period of Mertolengo young bulls with cereal-rich concentrate attenuates most of the beneficial grass effects on the characteristics of meat fat throughout the year, PDO beef from late spring is of greater nutritional quality than that from early autumn.

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4

EFFECT OF SLAUGHTER SEASON ON FATTY ACID COMPOSITION, CONJUGATED LINOLEIC ACID ISOMERS AND NUTRITIONAL VALUE OF INTRAMUSCULAR FAT IN BARROSÃ-PDO VEAL

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Effect of slaughter season on fatty acid composition, conjugated linoleic acid isomers and nutritional value of intramuscular fat in Barrosã-PDO veal

This paper describes the influence of slaughter season on lipid content, fatty acid composition, conjugated linoleic acid isomeric profile and nutritional value of fat in Barrosã veal from calves reared according to the specifications of the Protected Designation of Origin. Barrosã purebred calves (n = 27) were raised in a traditional production system and slaughtered in early autumn (October) and late spring (June). Barrosã-PDO veal only presented seasonal differences in the levels of some minor fatty acids and CLA isomers, as well as in the PUFA/SFA ratio. Based on the analysed grass intake indicators, it was shown that PDO veal has similar values to pasture-fed cattle for both slaughter seasons. From a human nutrition perspective, intramuscular fat in Barrosã-PDO veal has a high nutritional value throughout the year, since CLA contents and the percentages of the *c*9,*t*11 isomer are relatively high, and the *n*-6/*n*-3 ratios are within the recommended values for the human diet.

Keywords: veal; fatty acids; CLA isomers; meat quality; production systems.

4.1 INTRODUCTION

Fat content and fatty acid composition of meat have been extensively studied due to its implications for human health (Wood et al., 2004). Health institutions have been recommending reductions in total fat intake (15-30% of calories), particularly for saturated fatty acids (< 10% of caloric intake), due to its association with an increased risk of obesity, hypercholesterolemia and some cancers (WHO, 2003). Meat accounts for 10-20% of the total calories in diets of industrialized countries and it is well documented that ruminant meats are high in SFA (up to 50%) (Chizzolini et al., 1999). In addition, low ratios of polyunsaturated fatty acids to SFA (PUFA/SFA) in typical Western diets have been considered as major risk factors for cardiovascular diseases, which are among the most important causes of human mortality in developed countries (Katan, 2000; Ganji et al., 2003). Moreover, typical Western diets display a very high *n*-6/*n*-3 ratio (15-17/1), which favours the development of cardiovascular diseases, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2002). It is well known that the lower PUFA/SFA and higher *n*-6/*n*-3 ratios of some meats contribute to the imbalance in the fatty acid intake of today's consumers (Wood et al., 2004).

Conjugated linoleic acid is a minor group of fatty acids, composed of positional (from positions 6,8- to 12,14-) and geometric (*trans*,*trans*, *trans*,*cis*, *cis*,*trans* and *cis*,*cis*) isomers of linoleic acid (18:2*n*-6), containing conjugated double bonds, with a multitude of potential health benefits (see *e.g.* Prates & Mateus, 2002; Wahle et al., 2004). Twenty different CLA isomers have been reported as occurring naturally in food, especially in ruminant fat (Sehat et al., 1998). The major CLA isomer, rumenic acid, is produced in the rumen during the microbial biohydrogenation of dietary 18:2*n*-6 and in the tissues through Δ^9 desaturation of 18:1*t*11 (Griinari & Bauman, 1999). It is now accepted that the major contribution to *c*9,*t*11 in ruminant milk (Corl et al., 2002) and meat (Palmquist et al., 2004) is the endogenous synthesis. Some CLA isomers exhibit interesting biological activities that include anticancerinogenic, anti-obesity, antidiabetogenic, anti-atherogenic and immunomodulation and modulation of bone growth (Belury, 2002). The National Academy of Sciences of USA recognised CLA as the only fatty acid that has been shown unequivocally to inhibit carcinogenesis in experimental animals (National Research Council, 1996). The information about CLA isomeric distribution appears to be important as isomer specific biological effects have been reported (Evans et al., 2002).

Meats with Protected Designation of Origin derived from local production systems and animal breeds, are certified by European Union legislation and are expected to present unique quality and organoleptic characteristics, especially associated with specific properties of its lipid fraction (Council Regulation nº 2081/92 of 14/7, EEC). It is well documented that the intramuscular fat content and composition in veal is mainly influenced by the feeding system, age, slaughter weight

and duration of milk consumption (Moreno et al., 2006). Dietary factors are often linked with a particular production system (Geay et al., 2001). Meat from grazing ruminants is expected to reflect the variability of pasture biomass, which is highly dependent on cultural practices, season and geographical factors (Moloney et al., 2001). In Portugal, meat from autochthonous bovine breeds, reared in traditional production systems, has been progressively reintroduced in human diets as a result of its putative highly intrinsic quality (Costa et al., 2003) and of public fears regarding BSE and chemical residue (Rodrigues et al., 1998). One such example is Barrosã-PDO veal, obtained from Barrosã purebred calves, produced in the Minho Highlands and Terras do Barroso (Norwest of Portugal), based on a traditional production pasture-based system according to the product specifications. Specific genetic characteristics have been described for Barrosã purebred bovines (Pereira et al., 2003).

In previous reports we described the lipid composition and nutritional value of two Portuguese traditional meats: Carnalentejana-PDO beef (Alfaia et al., 2006a) and Mertolenga-PDO beef (Alfaia et al., 2006b). Furthermore, cholesterol, tocopherols and β-carotene contents of Barrosã-PDO veal were also published by our group (Prates et al., 2006). This veal is one of the two most important commercial Portuguese PDO veal (266 carcass tons in 2003; Instituto do Desenvolvimento Rural e Hidráulica, 2003) and is mainly produced in the autumn and spring seasons, with an increasing demand by the consumers. Intriguingly, the scientific information available to support the quality and reputation claimed, mainly dependent on its lipid composition, is scarce. Moreover, little work has been conducted to assess seasonal changes in meat CLA profile. Therefore, the aim of this work was to characterise the influence of slaughter season and muscle type on the lipid content, composition (fatty acid composition and CLA isomeric distribution) and nutritional quality of Barrosã-PDO veal.

4.2 MATERIALS AND METHODS

4.2.1 Reagents

Analytical grade and liquid chromatographic grade chemicals were from Merck Biosciences (Darmstadt, Germany). Sodium methoxide (0.5 M solution in anhydrous methanol) was obtained from Sigma-Aldrich Ltd. (St. Louis, MO, USA) and the fatty acid methyl ester (FAME) standard mixtures were from Nu-Chek-Prep Inc. (Elysian, MN, USA) and Supelco Inc. (Bellefonte, PA, USA). Commercial standards of individual CLA isomers (*c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11) as methyl esters were from Matreya Inc. (Pleasant Gap, PA, USA). Additional standards of individual (*t*8,*c*10 and *c*11,*t*13) and mixtures (*cis,trans*, *trans,cis* and *trans,trans* from 7,9 to 12,14) of CLA

isomers were prepared as methyl esters according to the procedure described by Destaillats and Angers (2003).

4.2.2 Animals and meat samples

Barrosã purebred calves (n = 27) were maintained according to the traditional production pasture-based system following the rules established in the Barrosã-PDO product specifications (Commission Regulation nº. 1263/96 of 01/07, EEC). Briefly, the calves and dams were raised under a semi-extensive grazing system based on the natural pastures of mountains and uplands from the Norwest of Portugal, which have great botanic diversity (see Santos (2006) for a detailed description of the flora fed by these grazing bovines). The calves were reared with their dams until weaning at 5.5-6.5 months of age. After weaning, calves were raised on a middle-late summer grass pasture until slaughter (Industrial Abattoir of Penafiel) in October 2002 (early autumn sampling; n = 12; mean ± standard error of age and live body weight were 7.6 ± 0.4 months and 192 ± 10 kg) or were maintained on the early-middle spring grass and slaughtered (Industrial Abattoir of Penafiel) in June 2003 (late spring sampling; n = 15; 7.9 ± 0.5 months and 212 ± 11 kg). Supplementation with cereal grains and forages (hay, straw, ryegrass and maize) was provided to calves and their dams, twice daily, during periods of feed scarcity (mainly in the winter). The carcass yield (carcass weight/live body weight) of the calves used in these experiments was approximately 50%.

Meat samples were taken from the ribeye portion (T1-T3) of *longissimus dorsi* and distal region of *semitendinosus* muscles of calves. Comparing with ST muscle, LD muscle is relatively red as it is involved differently in the physical activity imposed by grazing (Vestergaard et al., 2000a). All meat samples were collected 2-3 days after slaughter (+1 ºC), ground using a food processor (3 × 5 s), vacuum packed and stored at −70 °C until required.

4.2.3 Lipid extraction and methylation

Meat samples were lyophilised (−60 °C and 2.0 hPa) to constant weight using a lyophilisator Edwards Modulyo (Edwards High Vacuum International, UK), maintained exsiccated at room temperature, and analysed within two weeks. Intramuscular fat was extracted from lyophilised meat samples, for total lipid determination and for both FAME and CLA methyl esters profiles, using the procedures described previously by Alfaia et al. (2006a). Fatty acids were converted to methyl esters by base-catalysed transesterification, in order to avoid CLA isomerisation, with sodium methoxide (0.5 M solution in anhydrous methanol) during 2 hours at 30 °C, as proposed by Park et al. (2001) and Kramer et al. (2002). The same FAME solution was used for the analysis of both fatty acid

composition and CLA profile, enabling direct comparison of quantitative data and eliminating differences due to sample preparation. Total lipids were measured gravimetrically, in duplicate, by weighing the fatty residue obtained after solvent evaporation.

4.2.4 Determination of fatty acid composition

Gas chromatography analyses of FAME were performed with an Agilent 6890 gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) fitted with a flame ionization detector. The FAME were separated on a SP^{TM}-2560 fused-silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness, Supelco Inc., Bellefonte, PA, USA), as was mentioned previously in Alfaia et al. (2006a). Identification was accomplished by comparing the retention time of peaks from samples with those of FAME standard mixtures and with values published in the literature (Kramer et al., 1998; Fritsche et al., 2001). The FAME identification was confirmed by gas chromatography and detection by mass spectroscopy (Saturn 2200, Varian, Walnut Creek, CA, USA). Quantification of FAME was based on the internal standard technique, using nonadecanoic acid (19:0) as internal standard, and on the conversion of relative peak areas into weight percentages, using the corrected response factor of each fatty acid (ES ISO 5508, 1990). Fatty acids were expressed in gravimetric contents (mg/g muscle) or as a percentage of the sum of identified fatty acids (wt %).

4.2.5 Determination of individual CLA isomers

The methyl esters of CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using a high performance liquid chromatography system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and diode array detector adjusted to 233 nm, according to Alfaia et al. (2006a). The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial and prepared standards, as well as with values published in the literature (Fritsche et al., 2001). In addition, the identity of each isomer was supported by the typical ultraviolet spectra of CLA isomers from the DAD in the range 190-360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems rev. A.09.01 (Agilent Technologies, 2001). Total and individual CLA isomer contents in meat were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalization (AOAC 963.22, 2000). The CLA isomers were expressed in gravimetric contents (mg/g muscle and mg/g fat) or as a percentage of the sum of identified CLA isomers (% total CLA).

4.2.6 Statistical analysis

The data were analysed using the MIXED procedure of Statistical Analysis Systems Institute (SAS, 2004). The model considers the effects of slaughter season (PDO veal from early autumn and that from late spring), muscle type (LD and ST) and the interaction between animal group and muscle type. Muscle type was treated as repeated measure on animal within slaughter season group as subject. Least square means were determined and compared using the LSD test when an interaction effect was significant ($P < 0.05$).

4.3 RESULTS AND DISCUSSION

4.3.1 Intramuscular fatty acid composition

The effects of slaughter season and muscle type on fatty acid composition (wt %) of intramuscular fat in Barrosã-PDO veal are shown in Table 10. In both seasons, the predominant fatty acids in intramuscular fat were 16:0 (22-24% of total FAME) and 18:0 (13-16%) as SFA, 18:1*c*9 (35-37%) as MUFA, and 18:2*n*-6 (4-5%) as PUFA. Similar results were found by many other authors in cattle (*e.g.* Raes et al., 2003a; Realini et al., 2004). The content of *trans* octadecenoic fatty acids was expressed as a single value (18:1*t*) because of its incomplete chromatographic resolution.

No seasonal variations ($P > 0.05$) in the fatty acid composition of Barrosã-PDO veal were apparent for the predominant fatty acids, except for 16:0 in ST muscle, which showed higher percentages ($P < 0.05$) in meat from late spring than in that from early autumn. Regarding minor fatty acids, meat from October sampling had higher percentages of 8:0, 10:0 (LD muscle), 20:1*c*11 (ST muscle), 20:2*n*-6 (LD muscle), 20:3*n*-6, 20:4*n*-6, 22:5*n*-3 and 22:6*n*-3, but lower proportions of 17:1*c*9 (LD muscle), 18:1*c*11 and 20:2*n*-6 (ST muscle). This small influence of slaughter season on meat fatty acids is surprising if we consider the well known seasonal differences in pasture nutritive value and availability. It appears that the different suckling and grazing periods between autumn-slaughtered and spring-slaughtered calves result in similar final effects. In fact, the animals slaughtered in October were exposed to the less abundant summer pastures but suckled the milk produced during the most rich spring pastures, while the calves slaughtered in June were exposed to the more abundant spring pastures but suckled the milk obtained during the winter pasture scarcity period. However, veal from early autumn had similar percentages ($P > 0.05$) of 18:3*n*-3 (1.02-1.09% of total FAME) when compared with late spring (0.96-1.04%), in which grass lipids are rich (Palmquist, 1988), having both meats values close to those described for pasture-fed cattle (1.34%) (Realini

Table 10. Fatty acid composition (% w/w) of *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Barrosã-PDO veal from early autumn and late spring.

	Autumn		Spring			Significance levels		
Fatty acids	LD	ST	LD	ST	SEM	Season	Muscle	S × M
8:0	0.01	0.01	0.01	0.00	0.003	**	ns	ns
10:0	0.08^{a}	0.06^{b}	0.05^{b}	0.05^{b}	0.005	**	*	*
12:0	0.23	0.16	0.17	0.13	0.020	ns	***	ns
14:0	4.62	3.93	4.35	4.09	0.306	ns	**	ns
14:1	0.61	0.62	0.61	0.58	0.056	ns	ns	ns
15:0	0.65	0.55	0.57	0.50	0.046	ns	*	ns
16:0	23.0^{a,b}	22.2^{a}	22.7^{a,b}	23.6^{b}	0.454	ns	ns	*
16:1*t*9	0.51	0.55	0.59	0.56	0.033	ns	ns	ns
16:1*c*9	3.71	3.77	3.78	3.92	0.150	ns	ns	ns
17:0	0.96	0.93	0.94	0.92	0.029	ns	ns	ns
17:1*c*9	0.73^{a}	0.82^{b,c}	0.85^{b}	0.79^{a,c}	0.038	ns	ns	**
18:0	14.2	13.4	15.9	14.1	0.691	ns	*	ns
18:1*t*	2.51	2.39	2.42	2.24	0.264	ns	ns	ns
18:1*c*9	35.5^{a}	37.0^{b}	35.6^{a,b}	34.9^{a,b}	0.871	ns	ns	*
18:1*c*11	0.44^{a}	0.42^{a}	1.43^{b}	1.75^{c}	0.083	***	*	**
18:2*t*9,*t*12	0.30	0.31	0.35	0.31	0.028	ns	ns	ns
18:2*n*-6	5.06	5.15	4.29	4.97	0.487	ns	ns	ns
18:3*n*-6	0.01	0.02	0.01	0.01	0.005	ns	ns	ns
18:3*n*-3	1.02	1.09	0.96	1.04	0.085	ns	*	ns
20:0	0.14	0.13	0.12	0.12	0.009	ns	ns	ns
20:1*c*11	0.13^{a,b}	0.14^{a}	0.13^{a}	0.11^{b}	0.011	ns	ns	*
20:2*n*-6	0.09^{a,c}	0.07^{a,b}	0.06^{b}	0.11^{c}	0.010	ns	ns	**
20:3*n*-6	0.67	0.62	0.34	0.45	0.089	**	ns	ns
20:4*n*-6	2.18	2.67	1.60	2.17	0.241	*	*	ns
20:5*n*-3	0.42	0.56	0.31	0.62	0.111	ns	**	ns
22:4*n*-6	0.02	0.04	0.03	0.03	0.010	ns	ns	ns
22:5*n*-3	1.04	1.22	0.68	0.85	0.102	**	*	ns
22:6*n*-3	0.21	0.25	0.10	0.17	0.024	**	*	ns

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 mean as follow: S × M, interaction between slaughter season (S) and muscle type (M).

et al., 2004).

The LD muscle, relative to the ST muscle, had greater relative proportions of 10:0 (early autumn veal), 12:0, 14:0, 15:0, 17:1*c*9 (late spring veal), 18:0 and 20:1*c*11 (late spring veal), and lower values of 17:1*c*9 (early autumn veal), 18:1*c*9 (early autumn veal), 18:1*c*11 (late spring veal), 18:3*n*-3, 20:2*n*-6 (late spring veal), 20:4*n*-6, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3. These differences between muscles probably result from distinct percentages in muscle fibre type (Wood et al., 2004), as LD muscle

(25.5%-31.0% of type I fibres) of cattle is relatively red in comparison with ST muscle (16.9%-22.1% of type I fibres) (Vestergaard et al., 2000a). Significant interactions between slaughter season and muscle type were observed for the percentage of some individual fatty acids (10:0, 16:0, 17:1*c*9, 18:1*c*9, 18:1*c*11, 20:1*c*11 and 20:2*n*-6). These interactions may result from modifications of muscle metabolic types caused by adaptations to the distinct grazing periods (summer and spring) (Klont et al., 1998), which, as explained above, are associated with differences in fatty acid composition.

The results concerning the total lipids (mg/g muscle) and the partial sums (wt %) of intramuscular fatty acids in Barrosã-PDO veal, obtained in the two distinct slaughter seasons, are presented in Table 11. Total lipid content was higher ($P < 0.05$) in LD muscle from both slaughter seasons, intermediate in ST muscle from autumn, and lower in ST muscle from spring. These differences in total lipids likely result from variations in muscle fibre composition. In fact, it is well established that lipid content is higher in red oxidative muscle fibres (Enser et al., 1998) and that, as explained above, the LD muscle of cattle is relatively red in comparison with the ST muscle. PDO veal exhibited values of total lipids (1.6-2.3%) closer to those reported for the meat from intensively (1.8-2.7%) than extensively (1.1-1.4%) produced cattle (Vestergaard et al., 2000b), which may result from genetic specificities of fat deposition in Barrosã breed calves (De Smet et al., 2004). In fact, Costa et al. (2006) found even higher total lipid figures in LD muscle (2.7-3.1%) from Barrosã unweaned calves at ages ranging from 6 to 9 months. The difference compared to our values for weaned calves is explained by the high energy content of milk, which increases the amount of fat deposited in suckling calves (Moreno et al., 2006). However, according to the Food Advisory Committee (1990) criteria (< 5 % fat), Barrosã-PDO veal is considered a lean meat.

No seasonal changes ($P > 0.05$) in the various partial sums of fatty acids were observed for Barrosã-PDO veal, reflecting the patterns described above for individual fatty acids. Moreover, the LD muscle, compared with the ST muscle, had higher relative proportions ($P < 0.01$) of SFA, similar percentages ($P > 0.05$) of MUFA (late spring veal), *trans* fatty acids (TFA) and *n*-6 PUFA, but lower percentages ($P < 0.05$) of MUFA (early autumn veal), PUFA and *n*-3 PUFA. Since SFA and PUFA are much more abundant in the triacylglycerol and phospholipid fractions, respectively (Wood et al., 2004), the muscle type effect obtained for these partial sums of fatty acids reflects distinct triacylglycerol/phospholipid ratios between muscles, as a consequence of the different fat level (see Table 11). As fatness increases, increments in the content of SFA are larger than that of PUFA (De Smet et al., 2004). The differences in PUFA levels between LD and ST muscles result from differences in the contents of *n*-3 PUFA but not in that of *n*-6 PUFA. Similar percentages for the various families of fatty acids were obtained by several authors in pasture-fed cattle (*e.g.* Realini et al., 2004; Varela et al., 2004).

Table 11. Total lipids (mg/g muscle), partial sums of fatty acids (% w/w) and nutritional ratios of *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Barrosã-PDO veal from early autumn and late spring.

	Autumn		Spring			Significance levels		
	LD	ST	LD	ST	SEM	Season	Muscle	S × M
Total lipids	23.2[a]	19.6[b]	22.9[a]	15.6[c]	1.136	ns	***	*
Partial sums								
Σ SFA	43.9	41.4	44.9	43.5	0.740	ns	**	ns
Σ MUFA	41.1[a]	42.8[b]	42.5[a,b]	42.1[a,b]	0.959	ns	ns	*
Σ TFA	3.32	3.24	3.37	3.11	0.279	ns	ns	ns
Σ PUFA	10.7	11.7	8.37	10.4	0.897	ns	*	ns
Σ *n*-6	8.03	8.56	6.32	7.73	0.727	ns	ns	ns
Σ *n*-3	2.69	3.12	2.05	2.68	0.255	ns	**	ns
Ratios								
n-6/*n*-3	3.10	2.99	3.05	2.92	0.232	ns	ns	ns
PUFA/SFA	0.25	0.29	0.19	0.24	0.023	*	*	ns

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 mean as follow: S × M, interaction between slaughter season (S) and muscle type (M); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids.
Σ *n*-6 = sum of 18:2*n-6*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6* and 22:4*n-6*.
Σ *n*-3 = sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3* and 22:6*n-3*.
n-6/*n*-3 = *n-6/n-3* ratio [(sum of 18:2*n-6*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6* and 22:4*n-6*)/(sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3* and 22:6*n-3*)].
PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n-6*, 18:3*n-3*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6*, 20:5*n-3*, 22:4*n-6*, 22:5*n-3* and 22:6*n-3*)/(sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].

4.3.2 Nutritional value of intramuscular fat

The ratios of *n*-6/*n*-3 and PUFA/SFA (as defined in Table 11), which are nutritional indices widely used to evaluate the nutritional value of fat for human diet, were calculated and their values are presented in Table 11. Slaughter season and muscle type of Barrosã calves did not affect ($P > 0.05$) *n*-6/*n*-3 ratios of PUFA in Barrosã-PDO veal. In contrast, PUFA/SFA ratios of PDO veal from early autumn had higher ($P < 0.05$) values than that from late spring, although the LD muscle showed lower ($P < 0.05$) values compared with the ST muscle, reflecting the patterns described above for the partial sums of fatty acids. Our values for *n*-6/*n*-3 ratio (2.9-3.1) are close to those reported by Enser et al. (1998) for British meat obtained from grass-fed cattle (2.0-2.3). Moreno et al. (2006) obtained values slightly lower (1.09-1.62) in LD muscle of pasture-fed Spanish Rubia Gallega breed calves than those reported here for the Barrosã breed calves. In contrast, these ratios are considerably lower than the values reported by Enser et al. (1998) for meat from concentrate-fed bullocks (15.6-20.1),

which are in agreement with the values obtained by our group for meat from crossbred bullocks fed intensively on cereal-rich concentrates (16.7-20.2) (Alfaia et al., 2006a). Indeed, it is well established that the use of cereals (rich in *n*-6 PUFA) in concentrates leads to an increased ratio of *n*-6/*n*-3 when compared with animals produced on green pastures (rich in *n*-3 PUFA) (Nuernberg et al., 2002). Our values for the PUFA/SFA ratio (0.19-0.29) are similar to those reported for intramuscular fat from pasture-fed Rubia Gallega breed calves (0.15-0.20) (Moreno et al., 2006). In addition, lower values of PUFA/SFA in LD muscle, relative to ST muscle, were previously described in beef from semi-extensively and intensively reared cattle (Alfaia et al., 2006a). However, in contrast to the *n*-6/*n*-3 ratio, the effect of pasture on meat PUFA/SFA ratio remains controversial.

Current nutritional recommendations are that the PUFA/SFA ratio in the human diet should be above 0.45 (British Department of Health, 1994) and, within the PUFA, the *n*-6/*n*-3 ratio should not exceed 4.0 (British Department of Health, 1994). In view of the above guidelines, *n*-6/*n*-3 ratios in Barrosã-PDO veal are within the recommended values for the human diet, which is favourable, for both slaughter seasons and muscle types (2.9-3.1). These values clearly result from the benefits of grass-fed on ruminant meat, which, as stated by Nuernberg et al. (2005), should be brought to the attention of the public, nutritionists, the medical profession, producers and consumers. In contrast to the *n*-6/*n*-3 index, the values of the PUFA/SFA ratios in PDO veal were below the recommended guideline for the human diet, which is due to the biohydrogenation of feed unsaturated fatty acids in the rumen (see *e.g.* French et al., 2000). Nevertheless, veal from October exhibited higher values of PUFA/SFA ratio (0.25-0.29), and thus was less unfavourable, when compared with that from June (0.19-0.24). In addition, the LD muscle showed lower values for this nutritional index (0.19-0.25), and thus more unfavourable, than the ST muscle (0.24-0.29). However, the balance between *n*-6 and *n*-3 fatty acids seems to be more important for human diet than that between PUFA and SFA (Enser et al., 1996).

4.3.3 Intramuscular CLA isomeric profile

Data on the total (mg/g muscle) and specific (mg/g fat) CLA contents and its isomeric distribution (% total CLA) in the intramuscular fat of Barrosã-PDO veal are shown in Table 12. Total CLA contents in PDO veal did not show significant differences ($P > 0.05$) when the slaughter season was compared. Furthermore, LD muscle had higher ($P < 0.001$) total CLA contents than the ST muscle. However, no significant differences ($P > 0.05$) regarding specific CLA contents were observed when slaughter season and muscle type were analysed. Therefore, variations in total CLA contents are entirely due to the differences in intramuscular fat content among meats (see Table 11). This similarity in specific CLA contents in beef throughout the year may be due, as explained above, to the similar final effects of the different suckling and grazing periods between autumn- and spring-

slaughtered calves. Realini et al. (2004) reported that LD fat from grazing-based production systems had greater CLA contents (5.3 mg/g fat) than that obtained from concentrate-based production systems (2.5 mg/g fat). Our group found specific CLA variations from 3.9 to 4.4 mg/g fat in meat from crossbred bullocks fed intensively with cereal-rich concentrates (Alfaia et al., 2006a). The relatively high values of specific CLA contents described in this work (7.0-8.5 mg/g fat) indicate that Barrosã calves were raised on good quality pastures and suckled milk from mothers fed on those pastures. In addition, since the *c*9,*t*11 isomer is predominantly deposited in the triacylglycerols, higher intramuscular fat levels are usually associated with higher CLA contents (Raes et al., 2003b).

The CLA isomeric distribution in Barrosã-PDO veal from both seasons showed a clear predominance of the bioactive *c*9,*t*11 isomer (83.3-84.7%), followed in decreasing order by the *t*7,*c*9 isomer (4.14-5.91%), which co-eluted with minor amounts of the *t*8,*c*10 isomer, and the *t*11,*c*13 isomer (2.30-3.54%). The sum of the *cis/trans* isomers contributed 92.9-93.5% to the total CLA in Barrosã-PDO veal, while total *trans,trans* and *cis,cis* isomers contributed only 6.00-6.48% and 0.45-0.68%, respectively. The relative proportion of *c*9,*t*11 to total CLA in PDO veal is higher than in semi-extensively reared beef (Carnalentejana-PDO beef, 75.9-79.8%) and much higher than in intensively reared beef (54.5-59.9%) (Alfaia et al., 2006a). The *t*7,*c*9 isomer is mentioned frequently as the second-most prevalent CLA isomer (Yurawecz et al., 1998) and, like the most abundant *c*9,*t*11 isomer, its concentration in milk and tissues mainly results from the endogenous synthesis through the Δ^9 desaturation of the rumen-derived *trans* octadecenoate precursor (Palmquist et al., 2004). With the exception of *c*9,*t*11 and *t*7,*c*9 isomers, the origin of all other CLA isomers is ruminal biohydrogenation of dietary unsaturated C18 fatty acids, although the metabolic pathways producting these compounds are not yet elucidated (Collomb et al., 2004). It was recently shown that pasture feeding compared with concentrate feeding mainly increases the proportion of the *t*11,*c*13 isomer (up to 18.5% of total CLA in LD muscle), with a decrease of *t*7,*c*9 isomer (down to 4.1% of total CLA in LD muscle), while increasing the percentages of *t*11,*t*13 and *t*12,*t*14 isomers (Nuernberg et al., 2002; Dannenberger et al., 2005). However, Dannenberger et al. (2005) also reported that the percentage of *t*11,*c*13 isomer in beef was affected by breed and muscle type. Thus, our slightly lower percentages of *t*11,*c*13 isomer, relative to the *t*7,*c*9 isomer, may be explained by the described breed and muscle effects. In addition, among the *trans*,*trans* isomers of CLA, our values for *t*12,*t*14 (1.04-1.58%) and *t*11,*t*13 (1.17-2.12%) in PDO veal are in agreement with the figures reported for pasture-fed cattle (Dannenberger et al., 2005).

Season of production did not influence ($P > 0.05$) the percentages of individual and sums of geometric CLA isomers in Barrosã-PDO veal fat, except for the minor *t*12,*t*14, *t*11,*t*13, *t*9,*t*11 and *t*10,*c*12 isomers. The bioactive *t*10,*c*12 isomer, which only presented residual values in the meats (< 1%), displayed higher percentages ($P < 0.05$) in meat from autumn-slaughtered calves relative to that

Table 12. Total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers (% total CLA) in *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Barrosã-PDO veal from early autumn and late spring.

	Autumn		Spring			Significance levels		
	LD	ST	LD	ST	SEM	Season	Muscle	S × M
Total CLA	0.199	0.148	0.172	0.111	0.022	ns	***	ns
Specific CLA	8.52	7.59	7.42	7.01	0.795	ns	ns	ns
CLA isomers								
*t*12,*t*14	1.04	1.17	1.45	1.58	0.136	**	ns	ns
*t*11,*t*13	1.66	1.17	2.12	1.81	0.205	*	**	ns
*t*10,*t*12	0.41	0.46	0.44	0.31	0.087	ns	ns	ns
*t*9,*t*11	1.89	2.10	1.46	1.81	0.168	*	ns	ns
*t*8,*t*10	0.31	0.17	0.28	0.18	0.052	ns	*	ns
*t*7,*t*9	0.45^{a}	0.76^{b}	0.46^{a}	0.45^{a}	0.079	ns	*	*
*t*6,*t*8	0.25	0.21	0.28	0.24	0.043	ns	ns	ns
total *trans,trans*	6.00	6.03	6.48	6.38	0.409	ns	ns	ns
*c/t*12,14	0.83	0.95	0.79	1.01	0.143	ns	ns	ns
*t*11,*c*13	3.54^{a}	2.30^{a}	$2.73^{a,b}$	$2.67^{a,b}$	0.419	ns	*	*
*c*11,*t*13	0.18	0.36	0.26	0.24	0.080	ns	ns	ns
*t*10,*c*12	0.49	0.74	0.34	0.35	0.108	*	ns	ns
*c*9,*t*11	84.3	83.3	84.7	83.7	1.095	ns	ns	ns
*t*7,*c*9^{+}	4.14	5.91	4.16	4.93	0.672	ns	**	ns
total *cis/trans*	93.5	93.5	93.0	92.9	0.424	ns	ns	ns
total *cis,cis*	0.48	0.45	0.57	0.68	0.091	ns	ns	ns

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 × M, interaction between slaughter season (S) and muscle type (M). $^{+}$ This CLA isomer co-eluted with minor amounts of the *t*8,*c*10 isomer.

from spring-slaughtered animals. Moreover, ST muscle from late spring veal had higher relative proportions ($P < 0.05$) of *t*7,*t*9 CLA isomer than that from early autumn. This similarity in CLA distribution in beef throughout the year may be explained, as described above, by similar final effects of the different suckling and grazing periods between autumn- and spring-slaughtered calves. Although little research has been conducted to assess seasonal changes in beef CLA, Lock and Garnsworthy (2003) observed that CLA percentages in milk fat varied throughout the year in the UK, with the highest values being in the summer months (May to July), when cows ingested higher proportions of fresh grass.

The LD muscle had lower proportions of *t*7,*t*9 (in early autumn veal) and *t*7,*c*9 CLA isomers and higher percentages of *t*11,*t*13 and *t*8,*t*10, relative to the ST muscle. Significant interactions ($P < 0.05$) between the slaughter season and muscle type were observed only for the *t*7,*t*9 and *t*11,*c*13 CLA

isomers. Dannenberger et al. (2005) found that pasture feeding compared with concentrate feeding resulted in variations in the distribution pattern and contents of individual CLA isomers between LD and ST muscles. According to these authors, although LD muscle from pasture-fed bulls has higher levels of the sum of CLA isomers and *t*10,*c*12 isomer, with similar values for the *c*9,*t*11 isomer, the ST muscle has lower contents of the *c*9,*t*11 and *t*10,*c*12 isomers, with no difference in the sum of CLA isomers. In contrast to the *t*12,*t*14, *t*11,*t*13 and *t*11,*c*13 isomers, which seem to be the most sensitive grass intake indicators, our data did not show effects on the *c*9,*t*11 and *t*10,*c*12 isomers in LD and ST muscles. Apart from the *c*9,*t*11 isomer, information regarding individual CLA isomers in beef is scarce and, therefore, the data presented here are useful to our understanding of CLA isomers in meat.

Finally, and in line with the results presented here, we found that Barrosã-PDO veal had average amounts of total cholesterol and, depending on the muscle, moderate to high contents of α-tocopherol and β-carotene, suggesting good lipid stability and, possibly, high sensorial quality, nutritional value and safety for human health (Prates et al., 2006).

4.4 CONCLUSIONS

Barrosã-PDO veal only showed seasonal differences in the levels of some minor fatty acids and CLA isomers, as well as in the PUFA/SFA ratio. In addition, significant differences were obtained between LD (relatively red) and ST (relatively white) muscles for total lipid and total CLA contents, percentages of several fatty acids and CLA isomers, some partial sums of fatty acids and the PUFA/SFA index. The data indicate that Barrosã-PDO veal has similar values to pasture-fed cattle, in both slaughter seasons, in its content of several fatty acids, some partial sums of fatty acids, *n*-6/*n*-3 ratio, total and specific CLA contents, and some individual CLA isomers (grass intake indicators). This observation might reflect similar final effects of the different suckling and grazing periods between autumn- and spring-slaughtered calves.

From a human nutrition point of view, PDO veal from both slaughter seasons presents health related parameters because the CLA contents and the percentages of *c*9,*t*11 isomer are relatively high, and the *n*-6/*n*-3 ratios are within the recommended values for the human diet. In contrast, the values of the PUFA/SFA index were consistently below the recommended guideline for the human diet. However, the ratio values were less unfavourable in veal from October, compared with that from June, and in ST muscle, relative to LD muscle. Overall, the data indicate that Barrosã-PDO veal intramuscular fat, as a result of the beneficial effects of grass feeding on the characteristics of the meat lipids, has high nutritional quality throughout the year.

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5

INFLUENCE OF SLAUGHTER SEASON AND MUSCLE TYPE ON FATTY ACID COMPOSITION, CONJUGATED LINOLEIC ACID ISOMERIC DISTRIBUTION AND NUTRITIONAL QUALITY OF INTRAMUSCULAR FAT IN AROUQUESA-PDO VEAL

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Influence of slaughter season and muscle type on fatty acid composition, conjugated linoleic acid isomeric distribution and nutritional quality of intramuscular fat in Arouquesa-PDO veal

The effects of the slaughter season and muscle type on lipid and conjugated linoleic acid contents, fatty acid composition and isomeric profile of CLA in Arouquesa veal, from calves reared according to the specifications of the Protected Designation of Origin, were assessed. Arouquesa purebred calves (n = 31) were raised in a traditional production system, slaughtered in early autumn (October) or late spring (June), and the *longissimus dorsi* and *semitendinosus* muscles were sampled for analysis. Arouquesa-PDO veal only showed seasonal differences in the levels of some minor fatty acids (16:1*c*9, 17:1*c*9, 18:1*t*, 18:3*n*-3, 20:0 and 22:4*n*-6) and CLA isomers (*t*12,*t*14, *t*9,t11 and *c*11,*t*13). Furthermore, significant interactions between the slaughter season and muscle type were obtained for several fatty acids and CLA isomers, total lipids and CLA, and the PUFA/SFA ratio. In both seasons, PDO veal depicts values of pasture-fed cattle. From a human nutrition perspective, PDO veal in both slaughter seasons has relatively high CLA contents and percentages of the *c*9,*t*11 CLA isomer, which is favourable, while the *n*-6/*n*-3 ratios are within the recommended values for the human diet. In conclusion, the results suggest that intramuscular fat in Arouquesa-PDO veal has high nutritional value throughout the year.

Keywords: veal; slaughter season; muscle type; fatty acids; CLA isomers; total lipids.

5.1 INTRODUCTION

In most industrialized countries, a high meat intake contributes to higher than recommended total fat (15-30% of calories) and saturated fatty acids (< 10% of calories) intakes (WHO, 2003), which are associated with an increased risk of obesity, hypercholesterolemia and some cancers (Wood et al., 2004). Meat accounts for 10-20% of the total calories in human diets and ruminant meats are high in SFA (up to 50%) (Chizzolini et al., 1999). In addition, it is well known that the lower polyunsaturated fatty acids to SFA (PUFA/SFA) and higher *n*-6/*n*-3 ratios in some meats contribute to the imbalance in the fatty acid intake of today's consumers (Wood et al., 2004). Furthermore, typical western diets display low ratios of PUFA/SFA, which have been considered as major risk factors for cardiovascular diseases, and high *n*-6/*n*-3 ratios (15-17/1), which favour the development of cardiovascular diseases, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2002).

Conjugated linoleic acid comprises a group of positional (from positions 6,8- to 12,14-) and geometric (*trans*,*trans*, *trans*,*cis*, *cis*,*trans* and *cis*,*cis*) isomers of linoleic acid (18:2*n*-6), containing conjugated double bonds, with a multitude of potential health benefits (Prates & Mateus, 2002; Wahle et al., 2004; Tricon & Yagoob, 2006). Twenty different CLA isomers have been reported as occurring naturally in food, especially in ruminant fat (Sehat et al., 1998). The major CLA isomer, rumenic acid, is produced in the rumen during the microbial biohydrogenation of dietary 18:2*n*-6 and in the tissues through Δ^9 desaturation of 18:1*t*11 (Griinari & Bauman, 1999). It is now accepted that the major contribution to *c*9,*t*11 present in ruminant milk (Corl et al., 2002) and meat (Palmquist et al., 2004) is the endogenous synthesis. Some of CLA isomers exhibit interesting biological activities that include anticancerinogenic, anti-obesity, antidiabetogenic, anti-atherogenic, immunomodulation and modulation of bone growth (Belury, 2002).

Lipid content and composition in veal is mainly influenced by the feeding system, age, slaughter weight and duration of milk consumption (Moreno et al., 2006). Meats with Protected Designation of Origin are certified by European Union legislation and are expected to present unique quality and organoleptic characteristics, especially associated with specific properties of its lipid fraction (Council Regulation nº2081/92 of 14/7, EEC). In Portugal, meat from autochthonous bovine breeds, reared in traditional production systems, has been progressively reintroduced in human diets as a result of its highly intrinsic quality (Costa et al., 2003) and of public perception of BSE and chemical residues safety issues (Rodrigues et al., 1998). One such example is Arouquesa-PDO veal, obtained from Arouquesa purebred calves, produced in some councils within the Districts of Aveiro, Viseu, Porto and Vila Real (Centre-North of Portugal), based on a traditional production pasture-based system according to the product specifications.

In previous reports we described the lipid composition and nutritional value of three Portuguese traditional meats: Carnalentejana-PDO beef (Alfaia et al., 2006a), Mertolenga-PDO beef (Alfaia et al., 2006b) and Barrosã-PDO veal (Alfaia et al., 2007a). In addition, we reported cholesterol, tocopherols and β-carotene contents in Barrosã-PDO veal (Prates et al., 2006). Arouquesa-PDO veal is another important commercial Portuguese PDO meat (40 carcass tons in 2003; Instituto do Desenvolvimento Rural e Hidráulica, 2003), in increasing demand by consumers. In spite of this, scientific information available to support the claimed quality and reputation, mainly dependent on its lipid composition, is scarce. Moreover, little work has been conducted to assess the effects of slaughter season and muscle type on meat CLA profiles. Therefore, the aim of this work was to characterise the influence of the slaughter season and muscle type on the content, composition and nutritional value of the intramuscular fat in Arouquesa-PDO veal.

5.2 MATERIALS AND METHODS

5.2.1 Reagents

Analytical grade and liquid chromatographic grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). Sodium methoxide (0.5 M solution in anhydrous methanol) was obtained from Sigma-Aldrich Ltd. (St. Louis, MO, USA) and FAME standard mixtures were acquired from Nu-Chek-Prep Inc. (Elysian, MN, USA) and Supelco Inc. (Bellefonte, PA, USA). Commercial standards of individual CLA isomers (*c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11) as methyl esters were purchased from Matreya Inc. (Pleasant Gap, PA, USA). Additional standards of individual (*t*8,*c*1 and *c*11,*t*13) and mixtures (*cis,trans*, *trans,cis* and *trans,trans* from 7,9 to 12,14) of CLA isomers were prepared as methyl esters as described by Destaillats and Angers (2003).

5.2.2 Animals and meat samples

Arouquesa purebred calves (n = 31) were maintained according to the traditional production pasture-based system under the Arouquesa-PDO product specifications (Commission Regulation nº. 1107/96 of 12/06, EEC). Briefly, the weaned calves and dams were raised under a semi-extensive grazing system based on the natural pastures of Arouca highlands (Centre-North of Portugal), which have considerable floristic diversity. Supplementation with forages (hay, straw, ryegrass and maize) was provided to calves and their dams during periods of feed scarcity (mainly in the winter). During the suckling period, the calves were kept indoors and suckled twice daily until weaning at 5.5-6.5 months

of age. Mothers grazed on pasture during the day and were brought to the pens at evening for suckling and feed supplementation. After weaning, calves were raised on a late summer pasture (less abundant) until slaughter (Industrial Abattoir of Penafiel) in October 2002 (early autumn sampling; n = 15; mean ± standard error of age and live body weight were 7.3 ± 0.2 months and 230 ± 7 kg) or were maintained in the early-middle spring grass (more abundant) and slaughtered (Industrial Abattoir of Penafiel) in June 2003 (late spring sampling; n = 16; 8.6 ± 0.2 months and 226 ± 9 kg). The carcass yield (carcass weight/live body weight) of the autochthonous calves used in these experiments was approximately 50%.

Meat samples were taken from the ribeye portion (T1-T3) of *longissimus dorsi* and distal region of *semitendinosus* muscles of the left side of the carcasses. Compared with ST muscle, LD muscle is relatively red and differently involved in the physical activity imposed by grazing (Vestergaard et al., 2000a). All meat samples were collected 2-3 days after slaughter (+1 ºC), ground using a food processor (3 × 5 s), vacuum packed and stored at −70 °C until required for analysis.

5.2.3 Lipid extraction and methylation

Meat samples were lyophilised (−60 °C and 2.0 hPa) to constant weight using a lyophilisator Edwards Modulyo (Edwards High Vacuum International, UK), kept dry at room temperature, and analysed within two weeks. Intramuscular fat was extracted from lyophilised meat samples (*ca* 250 mg), for total lipid determination and for both FAME and CLA methyl esters profiles, as previously described by Alfaia et al. (2006a). Fatty acids were converted to methyl esters by base-catalysed transesterification, in order to avoid CLA isomerisation, with sodium methoxide (0.5 M solution in anhydrous methanol) during 2 hours at 30 °C, as proposed by Park et al. (2001) and Kramer et al. (2002). The same FAME solution was used for analysis of both fatty acid composition and CLA profile, enabling the direct comparison of quantitative data and eliminating differences in sample preparation. Total lipids were measured gravimetrically, in duplicate, by weighing the fat residue obtained after solvent evaporation.

5.2.4 Determination of fatty acid composition

The FAME were separated on a SP^{TM}-2560 fused-silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness, Supelco Inc., Bellefonte, PA, USA), using a Agilent 6890 gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA), as previously described in Alfaia et al. (2006a). Identification was accomplished by comparing the retention time of peaks with those of FAME standard mixtures and with values published in the literature (Kramer et al., 1998; Fritsche et al.,

2001). The FAME identification was confirmed by gas chromatography with detection by mass spectroscopy (Saturn 2200, Varian, Walnut Creek, CA, USA). Quantification of FAME was based on using nonadecanoic acid (19:0) as internal standard, and on conversion of relative peak areas into weight percentages, using the corrected response factor of each fatty acid (ES ISO 5508, 1990). Fatty acids were expressed in gravimetric contents (mg/g muscle) or as a percentage of the sum of identified fatty acids (wt %).

5.2.5 Determination of individual CLA isomers

The methyl esters of CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using an high performance liquid chromatography system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA), as previously reported by Alfaia et al. (2006a). Briefly, the mobile phase was 0.1% acetonitrile in *n*-hexane, at a flow rate of 1 mL/min, the diode array detector was adjusted to 233 nm and volumes of 20 μL were injected by the autosampler. The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial and prepared standards, as well as with values published in the literature (Fritsche et al., 2001). In addition, the identity of each isomer was controlled by the typical ultraviolet spectra of CLA isomers from the DAD in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems rev. A.09.01 (Agilent Technologies, 2001). Total and individual CLA isomer contents in meat were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalisation, from standard curves for peak area *versus* CLA isomer concentration (AOAC 963.22, 2000). The CLA isomers were expressed in gravimetric contents (mg/g muscle and mg/g fat) or as a percentage of the sum of identified CLA isomers (% total CLA).

5.2.6 Statistical analysis

The data were analysed using the MIXED procedure of Statistical Analysis Systems Institute (SAS, 2004). The model considered the effects of slaughter season (PDO veal from early autumn and that from late spring), muscle type (LD and ST) and the interaction between animal group and muscle type. Since measurements on different muscles from the same animal are not independent observations, muscle type was treated as repeated measure on animal within slaughter season group as subject. Least square means were presented and compared using LSD test when the interaction was significant ($P < 0.05$).

5.3 RESULTS AND DISCUSSION

5.3.1 Intramuscular fatty acid composition

The influence of slaughter season and muscle type on fatty acid composition (wt %) of intramuscular fat in Arouquesa-PDO veal is presented in Table 13. In both seasons, the predominant fatty acids in intramuscular fat were 16:0 (23-24% of total FAME) and 18:0 (14-17%) as SFA, 18:1*c*9 (34-35%) as MUFA, and 18:2*n*-6 (3-5%) as PUFA. Similar results were found by many other authors in cattle (*e.g.* Raes et al., 2003a; Realini et al., 2004; Nuernberg et al., 2005). The content of *trans* octadecenoic fatty acids (1.6-2.4%) was expressed as a single value (18:1*t*) because of its incomplete chromatographic resolution.

No seasonal variations ($P > 0.05$) in the fatty acid composition of Arouquesa-PDO veal were observed for the predominant fatty acids, except for 18:2*n-6* in ST muscle, which showed higher percentages ($P < 0.05$) in meat from late spring than in that from early autumn. Regarding minor fatty acids, meat from October samplings had higher percentages of 12:0 (ST muscle), 18:1*t*, 20:0 and 22:4*n-6*, but lower proportions of 16:1*c*9, 17:1*c*9, 18:3*n-3*, 20:3*n-6* (ST muscle), 20:4*n-6* (ST muscle) and 22:5*n-3* (ST muscle). This small influence of slaughter season, which includes the animal age effect ($P < 0.001$), on meat fatty acids is surprisingly if we consider the well known seasonal differences in pasture nutritive value and availability (Santos, 2006). It appears that the different suckling and grazing periods between autumn- and spring-slaughtered calves results in similar final effects. In fact, the animals slaughtered in October were exposed to the less abundant summer pastures but suckled the milk produced during the most rich spring pastures, while the calves slaughtered in June were exposed to the more abundant spring pastures but suckled the milk obtained during the winter pasture scarcity period. However, veal from early autumn had lower percentages ($P < 0.05$) of 18:3*n-3* (1.0% of total FAME) when compared with late spring (1.4-1.7%), in which grass lipids are rich (Palmquist, 1988), both meats having values close to those previously found for pasture-fed cattle (1.3%) (Realini et al., 2004).

The LD muscle, relative to the ST muscle, had greater relative proportions of 12:0 and 18:0, and lower values of 8:0, 16:1*c*9 and 18:1*c*11. These differences between muscles probably results from different percentages in muscle fibres (Wood et al., 2004), since LD muscle (25.5-31.0% of type I fibres) of cattle is relatively red compared with ST muscle (16.9-22.1% of type I fibres) (Vestergaard et al., 2000a). Significant interactions between slaughter season and muscle type were observed for the percentage of some individual fatty acids (12:0, 14:0, 14:1, 15:0, 17:0, 18:0, 18:2*t*9,*t*12, 18:2*n-6*, 18:3*n-3*, 20:3*n-6*, 20:4*n-6*, 20:5*n-3* and 22:5*n-3*). These interactions may result from modifications of

Table 13. Fatty acid composition (% w/w) in *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Arouquesa-PDO veal from early autumn and late spring.

	Autumn		Spring			Significance levels		
Fatty acids	LD	ST	LD	ST	SEM	Season	Muscle	S × M
8:0	0.01	0.01	0.00	0.01	0.002	ns	*	ns
10:0	0.07	0.06	0.06	0.05	0.006	ns	ns	ns
12:0	0.22^{a}	0.20^{b}	$0.19^{a,b}$	0.13^{c}	0.014	**	***	**
14:0	4.59^{a}	$4.42^{a,b}$	4.64^{a}	3.96^{b}	0.202	ns	***	*
14:1	$0.68^{a,b}$	$0.73^{a,b}$	0.69^{a}	0.62^{b}	0.070	ns	ns	**
15:0	$0.63^{a,b}$	$0.60^{a,b}$	0.67^{a}	0.55^{b}	0.029	ns	***	**
16:0	23.3	23.7	23.6	23.3	0.471	ns	ns	ns
16:1*t*9	0.56	0.54	0.65	0.63	0.051	ns	ns	ns
16:1*c*9	3.11	3.39	3.71	3.76	0.171	*	*	ns
17:0	$1.03^{a,b}$	1.00^{a}	1.10^{b}	0.96^{a}	0.026	ns	***	**
17:1*c*9	0.63	0.64	0.92	0.89	0.044	***	ns	ns
18:0	16.7^{a}	$15.3^{b,c}$	$16.3^{a,c}$	13.9^{b}	0.619	ns	***	*
18:1*t*	2.44	1.94	1.63	1.76	0.215	*	ns	ns
18:1*c*9	34.0	35.1	35.2	34.2	0.748	ns	ns	ns
18:1*c*11	0.90	1.45	1.14	1.57	0.185	ns	***	ns
18:2*t*9,*t*12	$0.33^{a,b}$	$0.34^{a,b}$	0.37^{a}	0.30^{b}	0.029	ns	**	***
18:2*n-6*	3.89^{a}	3.66^{a}	3.27^{a}	4.67^{b}	0.262	ns	**	***
18:3*n-6*	0.01	0.01	0.01	0.01	0.003	ns	ns	ns
18:3*n-3*	1.04^{a}	1.03^{a}	1.41^{b}	1.70^{c}	0.086	***	**	**
20:0	0.15	0.14	0.11	0.10	0.010	**	ns	ns
20:1*c*11	0.15	0.17	0.16	0.14	0.011	ns	ns	ns
20:2*n-6*	0.08	0.08	0.08	0.10	0.015	ns	ns	ns
20:3*n-6*	$0.40^{a,b}$	0.42^{a}	0.29^{b}	0.55^{c}	0.041	ns	***	***
20:4*n-6*	2.01^{a}	2.03^{a}	1.53^{a}	2.88^{b}	0.207	ns	***	***
20:5*n-3*	$0.48^{a,b}$	$0.57^{a,c}$	0.28^{b}	0.73^{c}	0.085	ns	***	**
22:4*n-6*	0.10	0.08	0.04	0.05	0.013	**	ns	ns
22:5*n-3*	$1.21^{a,b}$	1.11^{a}	0.87^{a}	1.48^{b}	0.128	ns	**	***
22:6*n-3*	0.38	0.32	0.12	0.26	0.089	ns	ns	ns

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 × M, interaction between slaughter season (S) and muscle type (M).

muscle metabolic types caused by adaptations to the different grazing periods (summer and spring) (Klont et al., 1998), which are associated with differences in fatty acid composition.

The results concerning total lipids (mg/g muscle) and partial sums (wt %) of intramuscular fatty acids in Arouquesa-PDO veal, obtained in the two distinct slaughter seasons, are shown in Table 14. Total lipid content was higher ($P < 0.05$) in LD muscle from both slaughter seasons and in ST muscle from

autumn than in that from spring. The LD muscle had higher values of total lipids (2.7-3.0%) when compared with the ST muscle (1.7-2.5%), although the difference was not statistically significant ($P > 0.05$) for ST muscle from autumn. These differences in total lipids likely result from variations in muscle fibre composition. In fact, it is well established that lipid content is higher in red oxidative muscle fibres (Enser et al., 1998) and that, as explained above, the LD muscle of cattle is relatively red in comparison with the ST muscle. Arouquesa-PDO veal exhibited values of total lipids (1.7-3.0%) closer to those reported for meat from intensively (1.8-2.7%) rather than extensively (1.1-1.4%) produced cattle (Vestergaard et al., 2000b), which may result from genetic specificities of fat deposition in Arouquesa breed calves (De Smet et al., 2004). Costa et al. (2006) found similar total lipid contents in LD muscle (2.7-3.1%) from Barrosã unweaned calves, with ages ranging from 6 to 9 months. Based on the Food Advisory Committee (1990) criteria (< 5% fat), Arouquesa-PDO veal is a lean meat.

Significant interactions ($P < 0.01$) between slaughter season and muscle type were observed for the various partial sums of fatty acids, with the exception of TFA, that did not show seasonal or muscle type effects. The ST muscle from late spring, compared with the ST muscle from early autumn and the LD muscle from both slaughter seasons, had higher relative proportions ($P < 0.05$) of PUFA, *n-6* PUFA and *n-3* PUFA, similar percentages ($P > 0.05$) of MUFA, but lower percentages ($P < 0.05$) of SFA. Moreover, the relative proportions of MUFA were lower ($P < 0.05$) in the LD muscle from early autumn than in the LD muscle from late spring and the ST muscle from early autumn. The patterns obtained for the different partial sums of fatty acids reflect the values described above for the major individual fatty acids of each group. Since SFA and PUFA are much more abundant in the triacylglycerol and phospholipid fractions, respectively (Wood et al., 2004), the muscle type effect (statistically significant in late spring veal) obtained for these partial sums of fatty acids reflects distinct triacylglycerol/phospholipid ratios between muscles, as a consequence of the different fat level (see Table 14). As fatness increases, increases in the content of SFA are larger than that of PUFA (De Smet et al., 2004). Similar percentages for the various families of fatty acids were obtained by several authors in pasture-fed cattle (*e.g.* Realini et al., 2004; Varela et al., 2004).

5.3.2 Nutritional value of intramuscular fat

The ratios of *n-6/n-3* and PUFA/SFA (as defined in Table 14), which are nutritional indices widely used to evaluate the nutritional value of fat for human consumption, were calculated and are presented in Table 14. Slaughter season and muscle type did not affect ($P > 0.05$) *n-6/n-3* ratios of PUFA in Arouquesa-PDO veal. In contrast, regarding PUFA/SFA ratios, ST muscle from late spring had higher values ($P < 0.05$) than that from early autumn and LD muscle from both slaughter

Table 14. Total lipids (mg/g muscle), partial sums of fatty acids (% w/w) and nutritional ratios in *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Arouquesa-PDO veal from early autumn and late spring.

	Autumn		Spring			Significance levels		
	LD	ST	LD	ST	SEM	Season	Muscle	S × M
Total lipids	26.8[a]	24.6[a]	30.3[a]	16.9[b]	2.563	ns	**	*
Partial sums								
Σ SFA	46.7[a]	45.4[b]	46.7[a,b]	42.9[c]	0.647	ns	***	**
Σ MUFA	39.4[a]	41.5[b]	41.9[b]	41.2 [a,b]	0.862	ns	ns	**
Σ TFA	3.32	2.82	2.65	2.70	0.225	ns	ns	ns
Σ PUFA	9.61[a]	9.31[a]	7.91[a]	12.4[b]	0.897	ns	***	***
Σ *n-6*	6.49[a]	6.29[a]	5.23[a]	8.26[b]	0.499	ns	***	***
Σ *n-3*	3.12[a]	3.02[a]	2.68[a]	4.17[b]	0.257	ns	***	***
Ratios								
n-6/n-3	2.28	2.19	1.95	1.99	0.157	ns	ns	ns
PUFA/SFA	0.21[a]	0.21[a]	0.17[a]	0.29[b]	0.017	ns	***	***

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 × M, interaction between slaughter season (S) and muscle type (M); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids.
Σ *n-6* = sum of 18:2*n-6*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6* and 22:4*n-6*.
Σ *n-3* = sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3* and 22:6*n-3*.
n-6/n-3 = *n-6/n-3* ratio [(sum of 18:2*n-6*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6* and 22:4*n-6*)/(sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3* and 22:6*n-3*)].
PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n-6*, 18:3*n-3*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6*, 20:5*n-3*, 22:4*n-6*, 22:5*n-3* and 22:6*n-3*)/(sum of 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].

seasons, reflecting the patterns described above for the partial sums of fatty acids. The values reported here for *n-6/n-3* ratio (1.9-2.3) are slightly higher than those obtained for LD muscle of pasture-fed Spanish Rubia Gallega breed calves (1.1-1.6) (Moreno et al., 2006). Furthermore, our values for the *n-6/n-3* ratio are very close to those reported by Enser et al. (1998) for British meat from grass-fed cattle (2.0-2.3). In contrast, this ratio is considerably lower than the values reported by these authors for meat from concentrate-fed bullocks (15.6-20.1), which agrees with the values obtained in meat from crossbred bullocks fed intensively with cereal-based concentrates (16.7-20.2) (Alfaia et al., 2006a). Indeed, it is well established that the use of cereals (rich in *n-6* PUFA) in concentrates shifts the meat fatty acid composition to an increased ratio of *n-6/n-3* when compared with animals produced on green pastures (rich in *n-3* PUFA) (Nuernberg et al., 2002). Our values for PUFA/SFA ratio (0.17-0.29) are similar to those reported for intramuscular fat from pasture-fed

Rubia Gallega breed calves (0.15-0.20) (Moreno et al., 2006). In contrast to the *n-6/n-3* ratio, the effect of pasture on meat PUFA/SFA ratios remains controversial.

Current nutritional recommendations are that the PUFA/SFA ratio in human diets should be above 0.45 and, within the PUFA, the *n-6/n-3* ratio should not exceed 4.0 (British Department of Health, 1994). In view of the above guidelines, *n-6/n-3* ratios in Arouquesa-PDO veal are inside the recommended values for the human diet, which is favourable, for both slaughter seasons and muscle types (1.9-2.3). These values clearly result from the benefits of grass-fed on ruminant meat, which, as stated by Nuernberg et al. (2005) should be brought to the attention of the public, nutritionists, the medical profession, producers and consumers. In contrast to the *n-6/n-3* index, the values of PUFA/SFA ratios in PDO veal were below the recommended guideline for the human diet, which is due to the biohydrogenation of feed unsaturated fatty acids in the rumen (French et al., 2000). Nevertheless, ST muscle from late spring calves had higher values of PUFA/SFA ratio (0.29), and thus was more favourable, when compared with that from early autumn and LD muscle from both slaughter seasons (0.17-0.21). However, the balance between *n-6* and *n-3* fatty acids seems to be more important for human diet than that between PUFA and SFA (Enser et al., 1996).

Finally, the nutritional quality of intramuscular fat in Portuguese PDO meats (Carnalentejana-PDO beef, Alfaia et al., 2006a; Mertolenga-PDO beef, Alfaia et al., 2006b; Barrosã-PDO veal, Alfaia et al., 2007a; and Arouquesa-PDO veal, presented here) seems to be the result of the beneficial grass effects on the meat lipids, giving greater nutritional quality than that from intensively produced beef from crossbred young bulls throughout the year. However, the data suggest that PDO beef (Carnalentejana-PDO and Mertolenga-PDO) intramuscular fat, relative to that from PDO veal (Barrosã-PDO and Arouquesa-PDO), has low nutritional quality throughout the year. These differences may be explained by the finishing period of Alentejana and Mertolenga purebred young bulls on concentrate, which diminishes the beneficial effects associated with grass intake.

5.3.3 Intramuscular CLA isomeric profile

Data on the CLA contents and its isomeric distribution in intramuscular fat of Arouquesa-PDO veal are presented in Table 15. A significant interaction ($P < 0.01$) between slaughter season and muscle type was obtained for the contents of total CLA (mg/g muscle). Total CLA contents were higher ($P < 0.05$) in the LD muscle from both slaughter seasons and in the ST muscle from early autumn than in ST muscle from late spring. However, no significant differences ($P > 0.05$) regarding specific CLA contents (mg/g fat) were observed when slaughter season and muscle type were analysed. Therefore, variations in total CLA contents are explained by the differences in intramuscular fat content among meats (Table 14), as shown by the high positive correlations between these two parameters

Table 15. Total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers (% total CLA) in *longissimus dorsi* (LD) and *semitendinosus* (ST) muscles of Arouquesa-PDO veal from early autumn and late spring.

	Autumn		Spring			Significance levels		
	LD	ST	LD	ST	SEM	Season	Muscle	S × M
Total CLA	0.208[a]	0.222[a]	0.257[a]	0.117[b]	0.026	ns	**	**
Specific CLA	8.85	8.86	8.74	6.82	0.623	ns	ns	ns
CLA isomers								
*t*12,*t*14	1.62	1.51	2.15	1.85	0.143	*	*	ns
*t*11,*t*13	2.52[a]	2.68[a]	3.90[b]	3.12[a]	0.338	*	ns	**
*t*10,*t*12	0.44	0.44	0.45	0.43	0.072	ns	ns	ns
*t*9,*t*11	3.29	3.14	2.08	2.05	0.362	*	ns	ns
*t*8,*t*10	0.45	0.43	0.37	0.30	0.101	ns	ns	ns
*t*7,*t*9	0.47[a]	0.48[a]	0.44[a]	0.70[b]	0.066	ns	*	*
*t*6,*t*8	0.36	0.36	0.44	0.35	0.062	ns	ns	ns
total *trans,trans*	9.13[a,b]	9.04[a,b]	9.84[a]	8.80[b]	0.667	ns	*	*
c/*t*12,14	0.97[a]	0.91[a]	1.28[a]	2.36[b]	0.269	**	*	**
*t*11,*c*13	3.81[a,b]	3.80[a,b]	4.32[a]	3.67[b]	0.331	ns	*	*
*c*11,*t*13	0.39	0.46	0.15	0.16	0.105	**	ns	ns
*t*10,*c*12	0.45	0.58	0.53	0.59	0.141	ns	ns	ns
*c*9,*t*11	79.5	80.0	78.6	79.0	1.186	ns	ns	ns
*t*7,*c*9[+]	5.36	4.72	4.67	4.51	0.676	ns	ns	ns
total *cis/trans*	90.5	90.4	89.6	90.3	0.669	ns	ns	ns
total *cis,cis*	0.40	0.52	0.59	0.91	0.089	**	**	ns

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 × M, interaction between slaughter season (S) and muscle type (M). [+] This CLA isomer co-eluted with minor amounts of the *t*8,*c*10 isomer.

(correlation coefficients of 0.761 and 0.873 for LD and ST, respectively, $n = 31$, $P < 0.001$). This similarity in specific CLA contents in beef throughout the year may be due, as explained above, to the similar final effects of the different suckling and grazing periods between autumn- and spring-slaughtered calves. Realini et al. (2004) reported that LD fat from grazing-based production systems had greater CLA contents (5.3 mg/g fat) than that from concentrate-based production systems (2.5 mg/g fat). We found variations in specific CLA values from 3.9 to 4.4 mg/g fat in meat from crossbred bullocks fed intensively with cereal-rich concentrates (Alfaia et al., 2006a). The relatively high values of specific CLA contents (6.8-8.9 mg/g fat) indicate that the Arouquesa calves were raised on abundant green pastures and suckled on milk from mothers fed with those pastures. In addition, since the *c*9,*t*11 isomer is predominantly deposited in the triacylglycerols, higher

intramuscular fat levels are usually associated with higher CLA contents (Fritsche et al., 2001; Raes et al., 2003b).

The CLA isomeric distribution in Arouquesa-PDO veal from both seasons showed a clear predominance of the bioactive *c*9,*t*11 isomer (78.6-80.0%) followed, in decreasing order, by the *t*7,*c*9 isomer (4.5-5.4%), which co-eluted with minor amounts of the *t*8,*c*10 isomer, and the *t*11,*c*13 isomer (3.7-4.3%). The sum of the *cis/trans* isomers contributed 89.6-90.5% of the total CLA in Arouquesa-PDO veal, while total *trans,trans* and *cis,cis* isomers contributed only 8.8-9.8% and 0.4-0.9%, respectively. The relative proportion of *c*9,*t*11 to the total CLA in PDO veal is much higher than in intensively reared beef (54.5-59.9%) (Alfaia et al., 2006a). This variation in the proportion of *c*9,*t*11 CLA isomer between production systems probably reflects either differences in the levels of 18:2*n-6* in the diet, the levels of linoleic acid isomerase produced by ruminal *Butyrivibrio fibrisolvens*, the substrate availability (18:1*t*11) of stearoyl-CoA desaturase in tissues and/or the breed differences in Δ^9 desaturase expression. The *t*7,*c*9 isomer is mentioned frequently as the second-most prevalent CLA isomer (Kramer et al., 1998; Yurawecz et al., 1998) and, like the most abundant *c*9,*t*11 isomer, its concentration in milk and tissues mainly results from endogenous synthesis through the Δ^9 desaturation of the rumen derived *trans* octadecenoate precursor (Palmquist et al., 2004). With the exception of *c*9,*t*11 and *t*7,*c*9 isomers, the origin of all other CLA isomers is ruminal biohydrogenation of dietary unsaturated C18 fatty acids, although the metabolic pathways are not yet elucidated (Collomb et al., 2004). It was recently shown that pasture feeding compared with concentrate feeding mainly increases the proportion of the *t*11,*c*13 isomer (up to 18.5% of total CLA in LD muscle), with a decrease of the *t*7,*c*9 isomer (down to 4.1% of total CLA in LD muscle), while increasing the percentages of *t*11,*t*13 and *t*12,*t*14 isomers (Varela et al., 2004; Dannenberger et al., 2005). However, Dannenberger et al. (2005) also reported that the content of *t*11,*c*13 isomer in beef lipids was affected by breed and muscle type. Thus, the slightly lower percentages of the *t*11,*c*13 isomer, relative to the *t*7,*c*9 isomer, may be explained by breed and muscle effects. In addition, among the *trans*,*trans* isomers of CLA, the values of *t*12,*t*14 (1.5-2.2%) and *t*11,*t*13 (2.5-3.9%) in PDO veal agree with those reported for pasture-fed cattle (Dannenberger et al., 2005).

The production season, which includes the animal age effect ($P < 0.001$), did not influence ($P > 0.05$) the percentages of individual and sums of geometric CLA isomers in Arouquesa-PDO veal fat, except for the minor *t*12,*t*14, *t*11,*t*13 (LD muscle), *t*9,*t*11, *t*7,*t*9 (ST muscle), *c/t*12,14 (ST muscle), *c*11,*t*13 and total *cis,cis* isomers. Moreover, LD muscle from early autumn had lower relative proportions ($P < 0.05$) of the *t*11,*t*13 CLA isomer than that from late spring, while the ST muscle from early autumn depicted lower percentages of the *t*7,*t*9 and *c/t*12,14 isomers relative to that from spring-slaughtered animals. Although little research has been conducted to assess seasonal changes in

beef CLA, Lock and Garnsworthy (2003) observed that CLA percentages in milk fat varied throughout the year in the UK, with the highest values being in the summer months (May-July), when cows ingested higher proportions of fresh grass.

The LD muscle had higher relative proportions ($P < 0.05$) of *t*12,*t*14, *t*11,*t*13 (late spring veal), total *trans,trans* (late spring veal) and *t*11,*c*13 (late spring veal) CLA isomers and lower percentages of *t*7,*t*9 (late spring veal), *c/t*12,14 (late spring veal) and total *cis,cis* isomers, relative to the ST muscle. Significant interactions ($P < 0.05$) between slaughter season and muscle type were observed for *t*11,*t*13, *t*7,*t*9, total *trans,trans*, *c/t*12,14 and *t*11,*c*13 CLA isomers. Dannenberger et al. (2005) found that pasture feeding, compared with concentrate feeding, resulted in variations in the distribution pattern and contents of individual CLA isomers between LD and ST muscles. According to these authors, although LD muscle from pasture-fed bulls had higher levels for total CLA isomers and the *t*10,*c*12 isomer, with similar values for *c*9,*t*11 isomer, ST muscle had lower contents of *c*9,*t*11 and *t*10,*c*12 isomers, with no difference in the sum of CLA isomers. In contrast to *t*12,*t*14, *t*11,*t*13 and *t*11,*c*13 isomers, which seem to be the most sensitive grass intake indicators, the data did not show effects on the *c*9,*t*11 and *t*10,*c*12 (< 0.6%) isomers, in LD and ST muscles.

5.4 CONCLUSIONS

Arouquesa-PDO veal only showed seasonal differences in the levels of some minor fatty acids and CLA isomers. In addition, significant interactions between the slaughter season and muscle type were observed for several fatty acids and CLA isomers, total lipid and CLA, and the PUFA/SFA ratio. The data indicate that Arouquesa-PDO veal has similar values to pasture-fed cattle, in both slaughter seasons, with regard to the content of several fatty acids, some partial sums of fatty acids, *n-6/n-3* ratio, total and specific CLA contents, and some individual CLA isomers (grass intake indicators). This might reflect the similar final effects of the different suckling and grazing periods on autumn- and spring-slaughtered calves.

From a human nutrition point of view, PDO veal in both slaughter seasons has good health related parameters because the CLA contents and the percentages of *c*9,*t*11 CLA isomer are relatively high, and the *n-6/n-3* ratios are within the recommended values for the human diet. In contrast, the values of the PUFA/SFA index were below the recommended guideline for the human diet. However, the ratios were less unfavourable in ST muscle from late spring than in that from early autumn and LD muscle from both slaughter seasons. Overall, the data indicate that Arouquesa-PDO veal intramuscular fat, as a result of the beneficial grass effects, is of high nutritional quality throughout the year.

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6

EFFECT OF THE FEEDING SYSTEM ON INTRAMUSCULAR FATTY ACIDS AND CONJUGATED LINOLEIC ACID ISOMERS OF BEEF CATTLE, WITH EMPHASIS ON THEIR NUTRITIONAL VALUE AND DISCRIMINATORY ABILITY

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Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability

Thirty two Alentejano purebred bulls were used to investigate the effect of four feeding systems (pasture only, pasture feeding followed by 2 or 4 months of finishing on concentrate, and concentrate only) on meat fatty acid composition (GC-FID), including conjugated linoleic acid isomeric distribution (Ag^+–HPLC–DAD). In addition, meat fatty acids and CLA isomers were used to elucidate the impact of the different feeding regimens on the nutritional value of intramuscular fat and their usefulness as chemical discriminators of meat origin. The diet had a major impact on the fatty acid composition of beef (affected 27 of 36 fatty acids and 10 of 14 CLA isomers), which was independent of the fatty acid concentration. Beef fat from pasture-fed animals had a higher nutritional quality relative to that from concentrate-fed bulls. Finally, meat fatty acid composition was an effective parameter to discriminate between ruminant feeding systems, including different finishing periods on concentrate.

Keywords: meat quality; feeding system; finishing period; fatty acids; CLA isomers.

6.1 INTRODUCTION

Scientific evidence and nutritional guidelines recommend a reduction in total fat intake, particularly of SFA (WHO, 2003), which are associated with an increased risk of obesity, hypercholesterolemia and some cancers (Wood et al., 2004). Nutritionist advisers recommend a higher intake of PUFA, especially *n*-3 PUFA at the expense of *n*-6 PUFA (British Department of Health, 1994). Recently, the results of Griffin (2008) reinforced the current recommendations of increasing α-linolenic acid intake and decreasing linoleic acid intake, in order to promote the endogenous synthesis of long-chain (> C18) *n*-3 fatty acids. It is well known that the low PUFA/SFA and high *n*-6/*n*-3 ratios of some meats contribute to the imbalance in the fatty acid intake of todays consumers (Wood et al., 2004). Besides the beneficial effects of *n*-3 fatty acids on human health, CLA isomers have also attracted increased attention as a result of their health promoting biological properties (Aldai et al., 2006a; Prates & Mateus, 2002). In fact, some CLA isomers, at least the *c*9,*t*11 and *t*10,*c*12, exhibit interesting biological activities that include anticarcinogenic, anti-obesity, antidiabetogenic, anti-atherogenic qualities, immunomodulation and modulation of bone growth (Park & Pariza, 2007). Ruminant fats are the richest natural dietary source of *c*9,*t*11, which is the major CLA isomer, commonly known as rumenic acid (Kramer et al., 1998). This isomer is mostly produced in tissues by Δ^9 desaturation of vaccenic acid (18:1*t*11) and by ruminal biohydrogenation of dietary PUFA (Griinari & Bauman, 1999; Nuernberg et al., 2005; Palmquist et al., 2004). Twenty four different CLA isomers have been reported as occurring naturally in food, especially from ruminant origin, including milk, dairy products and meat (Sehat et al., 1998).

As a result of the fatty acid imbalance in human diets, dietary strategies have been used to improve the nutritional and health value of the intramuscular fat of cattle. Thus, manipulation of the fatty acid composition in ruminant meat to reduce SFA content and the *n*-6/*n*-3 ratio whilst, simultaneously increasing the PUFA and CLA contents, is of major importance in meat research. It has been shown that in ruminants grazing have potential beneficial effects on PUFA/SFA and *n*-6/*n*-3 ratios, increasing the PUFA and CLA contents and decreasing the SFA concentration of beef (Enser et al., 1998; French et al., 2000). Although several factors influence the fatty acid composition and the CLA content of beef (*e.g.* seasonal variation, animal genetics and production practices), diet plays the most important role (Schmid et al., 2006). Dietary factors are often linked with particular production systems (Geay et al., 2001).

Meat production systems are particularly important for those legally protected in the European Union, which assume a PDO or a PGI. The added value allowed by legal protection of PDO and PGI products may introduce a strong pressure to the intensification of production systems. In previous

reports we described the lipid composition and nutritional value of some Portuguese PDO meats, including Carnalentejana-PDO beef (Alfaia et al., 2006a) and Mertolenga-PDO beef (Alfaia et al., 2006b). Although these animals are produced under traditional production systems, the product specifications include a finishing period on concentrate feed from 3 to 6 months. The discrimination between meat from ruminants finished on concentrate or pasture has been easily achieved with several chemical and physical markers (Bessa et al., 2006), whereas the differences in fatty acid composition are well known (French et al., 2000). However, a clear discrimination between animals growing on pasture and finished on confinement, for variable time periods, is much more difficult (Bessa et al., 2006). Therefore, the development of methodologies for the estimation of the time on concentrate feed, its effect on meat fatty acid composition and nutritional value is of outmost importance.

The goal of this work was to provide information concerning the meat fatty acid composition and distribution of CLA isomers of cattle allocated to concentrate only, pasture only and pasture feeding followed by 2 or 4 months of finishing on concentrate. In addition, the influence of these four feeding systems on the nutritional value of the intramuscular fat content was assessed. Finally, the usefulness of meat fatty acids and CLA isomers as chemical markers for the discrimination of the finishing period of animal fed on concentrate was also evaluated.

6.2 MATERIALS AND METHODS

6.2.1 Reagents

FAME standard mixtures were acquired from Nu-Chek-Prep Inc. (Elysian, MN, USA) and Supelco Inc. (Bellefonte, PA, USA). Commercial standards of individual CLA isomers (*c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11) as methyl esters were purchased from Matreya Inc. (Pleasant Gap, PA, USA). Additional standards of individual (*t*8,*c*10 and *c*11,*t*13) and mixtures (*cis,trans*, *trans,cis* and *trans,trans* from 7,9 to 12,14) of CLA isomers were prepared as methyl esters according to the procedure described by Destaillats and Angers (2003).

Analytical grade and liquid chromatographic grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). Sodium methoxide (0.5 M solution in anhydrous methanol) was obtained from Sigma–Aldrich Ltd. (St. Louis, MO, USA).

6.2.2 Animals and management

The experiment was conducted at EZN facilities (Vale de Santarém, Portugal). Thirty two young bulls from a Alentejano purebred EZN herd, born between March and July of 2003, were used. The herd was maintained in extensive conditions in alluvium land near the Tagus River (39°07'N; 8°43'W), grazing on spontaneous pastures, summer triticalis and maize stubbles. After weaning, in February 2004, with live body weights ranging between 200 and 300 kg, 8 young bulls were transferred to a feedlot (CCC) and fed at 2.75% live body weights, on a diet consisting of 70% concentrate feed and 30% molasses-fibrous cubes. The ingredient and chemical compositions of the concentrate feed are presented in Table 16.

Table 16. Ingredient (% feed), proximate (mg/g dry matter) and fatty acid (% of sum of fatty acids) compositions of the concentrate feed used in this experiment.

	Concentrate
Ingredients	
Barley	40
Corn	10
Corn gluten	22
Sunflower meal	18
Soybean meal	5
Minerals and premix	5
Proximate composition	
Crude protein	128
Total fat	30
Crude fibre	65
Ashes	90
Nitrogen-free extract	687
Fatty acid composition	
14:0	3.4
16:0	14.5
18:0	2.8
18:1*c*9	19.6
18:2*n*-6	40.8
18:3*n*-3	2.6

The animals were slaughtered with approximately 600 kg of live body weight (September and October 2004). One of the animals failed to grow at normal rates and was removed from the experiment and another animal died. The other young bulls were maintained for 15 months on

pasture lands, until June 2005, when 8 bulls were slaughtered (PPP), and 15 animals were transferred to feedlot facilities and fed the fattening diet described above. Eight bulls were slaughtered after 2 months in feedlot (PPC) and the last 7 animals were slaughtered after 4 months in the feedlot (PCC).

Animals were slaughtered at an EZN experimental abattoir by exsanguination after stunning with a cartridge-fired captive bolt stunner. Carcasses were suspended by the Achilles tendon and chilled at 10 °C for 24 h followed by refrigeration at 2 °C for 5 days. *Longissimus lumborum* muscle samples (ca. 200 g) were collected, trimmed of connective and adipose tissues before being blended in a food processor, vacuum packed and finally stored at −70 °C until lipid extraction and assessment of fatty acid composition and CLA isomeric profile were performed.

6.2.3 Lipid extraction and methylation

Meat samples were lyophilised (−60 °C and 2.0 hPa) to constant weight, using a lyophilisator (Edwards High Vacuum International, UK), kept dry at room temperature, and analysed within two weeks. For total lipid determination, intramuscular fat was extracted from the lyophilised meat samples (ca. 250 mg), using the procedure described previously by Fritsche et al. (2000). Total lipids were measured gravimetrically, in duplicate, by weighing the fatty residue obtained after solvent evaporation.

For both FAME and CLA methyl ester profile analysis, fatty acids and CLA isomers were directly converted to methyl esters by a one-step extraction transterification procedure (Christie et al., 2001), using base catalysis for lyophilised meat (ca. 250 mg) and acid catalysis for concentrate feed (ca. 500 mg). The base-catalysed methylation, used for meat in order to avoid isomerisation of CLA isomers, was performed with sodium methoxide (0.5 M solution in anhydrous methanol), while the acid-catalysed methylation used sulphuric acid (0.04 M in anhydrous methanol), for 2 h at 30 °C, as proposed by Kramer et al. (2002). Nonadecanoic acid (19:0) methyl ester, used as an internal standard, was added to the samples prior to fat extraction and methylation. The same FAME solution was used for the analysis of both fatty acid composition and CLA profile, enabling the direct comparison of quantitative data and eliminating differences in sample preparation.

6.2.4 Analysis of fatty acid methyl esters

Fatty acid composition was analysed by gas chromatography (HP6890A; Hewlett–Packard, Avondale, PA, USA) using a fused-silica capillary column (CP-Sil 88; 100 m × 0.25 mm i.d., 0.2 µm film thickness, Chrompack, Varian Inc., Walnut Creek, CA, USA), equipped with GC-FID as described by Bessa et al. (2007). Briefly, the initial column temperature of 100 °C was held for 15

min, increased to 150 °C at a rate of 10 °C/min and held for 5 min, then increased to 158 °C at 1 °C/min and held for 30 min, and finally increased to 200 °C at a rate of 1 °C/min and maintained for 65 min. Helium was used as carrier gas and the injector and detector temperatures were 250 and 280 °C, respectively. Identification was accomplished by comparison of sample peak retention times with those of FAME standard mixtures and with values published in the literature (Kramer et al., 1998). The identification of some unknown peaks was conducted by GC–MS/MS, using a Varian Saturn 2000 system (Varian Inc., Walnut Creek, CA, USA) equipped with a CP-Sil 88 capillary column (100 m × 0.25 mm i.d., 0.2 µm film thickness). Fatty acids were expressed as g/100 g of total fatty acid content, assuming a direct relationship between peak area and fatty acid methyl ester weight.

6.2.5 Analysis of individual CLA isomer methyl esters

The distribution of methyl esters of CLA isomers were determined using an high performance liquid chromatography system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA), equipped with 100 µL injection loop and DAD operated at 233 nm, according to the procedure described previously by Alfaia et al. (2006a). The CLA isomers were individually separated by triple silver-ion columns in series (ChromSpher 5 Lipids, 250 mm × 4.6 mm i.d., 5 µm particle size, chrompack, Bridgewater, NJ, USA). The mobile phase was 0.1% acetonitrile in *n*-hexane, at a flow rate of 1 mL/min and volumes of 20 µL were injected by the autosampler. Identification of the individual CLA isomers was achieved by comparison of their retention times with commercial and prepared standards, as well as with values published in the literature (Fritsche et al., 2000). In addition, the identity of each isomer was controlled by the typical ultraviolet spectra of CLA isomers from the diode array detector in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3D Systems rev. A.09.01 (Agilent Technologies, 2001). The quantification of total and individual CLA isomer contents were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalisation, from standard curves for peak area *vs*. CLA isomer concentration (AOAC 963.22, 2000). The CLA isomers were expressed as gravimetric contents (mg/g muscle and mg/g fat) or as a percentage of the sum of identified CLA isomers (% total CLA).

6.2.6 Statistical analysis

Fatty acid composition and CLA isomers data were subjected to analysis of variance (ANOVA), considering the treatment of different feeding regimens as a single effect, using the GLM procedure of Statistical Analysis Systems Institute (SAS, 2004). Least squares means were presented and

compared, using the LSD test, when the effect of feeding system was significant ($P < 0.05$). Canonical discriminant analysis (CDA) was applied to fatty acids and CLA isomers data in order to classify and predict the feeding system. Variable selection for CDA was achieved using: (1) the significant variables after ANOVA and (2) an interactive forward stepwise analysis (SAS, Proc STEPDISC) that select the variables with a major discriminant capacity. The CDA and cross-validation were conducted using Proc DISCRIM from SAS.

6.3 RESULTS AND DISCUSSION

6.3.1 Intramuscular fatty acid composition

Level of intramuscular fat (mg/g muscle) and fatty acid composition (expressed as % of total fatty acids) in *L. lumborum* muscle of Alentejano purebred bulls fed on different diets are presented in Table 17. Meat from cattle finished on concentrate feed usually has higher intramuscular fat than that from animals finished on pasture (Wood et al., 2008). Therefore, the former meat has higher proportions of triacylglycerols, relative to phospholipids and, as a result, lowers percentages of PUFA and higher proportions of SFA and MUFA. In the present experiment, the content of intramuscular fat was not directly related with the extent of the finishing period on concentrate. Thus, the changes observed in fatty acid composition reflect mainly dietary influences rather than intramuscular fatness effects. According to the Food Advisory Committee (1990) criteria (< 5% fat), beef produced in this experiment can be classified as a lean meat.

The predominant fatty acids in intramuscular fat were palmitic (16:0, 18-23% of total FAME) and stearic (18:0, 15-18%) acids as SFA, oleic acid (18:1*c*9, 20-29%) as MUFA, and linoleic (10-13%) and arachidonic (20:4*n*-6, 2-4%) acids as PUFA. Similar results were reported previously for beef cattle (*e.g.* Alfaia et al., 2006a; Nuernberg et al., 2005; Realini et al., 2004). The feeding system had a major impact on the fatty acid profile of beef since 27 of the 36 fatty acids analysed were affected ($P < 0.05$) by the production system. Saturated hypercholesterolemic fatty acids (12:0, 14:0 and palmitic acid) and their monounsaturated derivatives (14:1*c*9 and 16:1*c*9) were affected ($P < 0.001$) by the dietary treatment, showing the lowest values in animals fed with pasture only (PPP group). In addition, meat from animals fed on concentrate diets had higher percentages of oleic acid than meat from grazing bulls (CCC > PPC > PPP). Concentrate diets are expected to increase the absorption of oleic acid and linoleic acid, which are major fatty acids in cereal grains (Jenkins, 1994). However,

Table 17. Effect of the four feeding systems on intramuscular fat (mg/g muscle) and fatty acid composition (g/100 g total fatty acids) of *longissimus lumborum* muscle from Alentejano purebred bulls. The feeding regimens were as follows: feedlot (CCC), pasture with finishing on concentrate during 4 (PCC) and 2 (PPC) months, and pasture grazing (PPP).

	Treatments				SEM	P^A
	CCC	PCC	PPC	PPP		
Total lipids	13.03	12.37	11.45	9.76	1.004	ns
Fatty acids						
12:0	0.06^{ab}	0.09^{c}	0.07^{b}	0.05^{a}	0.006	***
14:0	1.84^{b}	2.54^{c}	2.01^{b}	1.24^{a}	0.154	***
14:1*c*9	0.26^{b}	0.31^{b}	0.24^{b}	0.10^{a}	0.027	***
15:0	0.35	0.39	0.42	0.37	0.026	ns
16:0	20.79^{b}	23.37^{c}	22.07^{bc}	18.42^{a}	0.584	***
16:1*c*9	2.22^{bc}	2.44^{c}	1.83^{b}	1.18^{a}	0.166	***
16:1*t*9	0.08^{a}	0.08^{a}	0.08^{a}	0.14^{b}	0.012	**
17:0	1.05	0.98	0.99	0.90	0.046	ns
17:1*c*9	0.69^{c}	0.57^{b}	0.51^{ab}	0.45^{a}	0.033	***
18:0	14.96^{a}	15.56^{a}	16.43^{ab}	17.54^{b}	0.591	*
18:1*c*9	28.64^{c}	27.74^{bc}	24.75^{b}	20.49^{a}	1.270	***
18:1*c*11	2.19^{b}	1.76^{a}	1.78^{a}	1.69^{a}	0.077	***
18:1*c*12	0.26	0.27	0.32	0.26	0.032	ns
18:1*c*13	0.16^{b}	0.18^{b}	0.14^{b}	0.09^{a}	0.017	**
18:1*c*15	0.10^{a}	0.13^{b}	0.15^{b}	0.14^{b}	0.011	*
18:1*t*6+*t*8	0.19^{b}	0.16^{b}	0.17^{b}	0.12^{a}	0.014	*
18:1*t*9	0.26^{b}	0.26^{b}	0.26^{b}	0.15^{a}	0.018	***
18:1*t*10	1.21^{b}	0.81^{b}	0.98^{b}	0.20^{a}	0.152	***
18:1*t*11	0.92	1.10	1.15	1.35	0.132	ns
18:1*t*12	0.23	0.24	0.24	0.25	0.021	ns
18:1*t*16+*c*14	0.19^{a}	0.23^{ab}	0.29^{bc}	0.35^{c}	0.032	**
18:2*n*-6	11.95	10.07	11.38	12.55	1.180	ns
18:2*t*	0.38	0.30	0.35	0.26	0.036	ns
18:2*t*11,*c*15	0.06^{a}	0.18^{ab}	0.29^{bc}	0.35^{c}	0.045	***
18:3*n*-3	0.48^{a}	0.84^{a}	1.96^{b}	5.53^{c}	0.289	***
20:0	0.19^{b}	0.18^{b}	0.08^{a}	0.25^{c}	0.013	***
20:1*c*11	0.12^{c}	0.10^{b}	0.02^{a}	0.10^{b}	0.004	***
20:2*n*-6	0.13	0.11	0.12	0.15	0.011	ns
20:3*n*-6	0.96	0.74	0.76	0.91	0.096	ns
20:4*n*-6	3.80^{b}	2.45^{a}	2.67^{a}	4.04^{b}	0.395	*
20:5*n*-3	0.47^{a}	0.77^{a}	1.28^{b}	2.13^{c}	0.154	***
22:2*n*-6	0.15^{b}	0.12^{b}	0.02^{a}	0.24^{c}	0.017	***
22:4*n*-6	0.08^{b}	0.05^{a}	0.03^{a}	0.09^{b}	0.011	**
22:5*n*-3	0.91^{a}	1.04^{ab}	1.48^{b}	2.56^{c}	0.158	***
22:6*n*-3	0.11^{a}	0.12^{a}	0.14^{a}	0.20^{b}	0.021	*
24:1*c*15	0.34^{b}	0.18^{a}	0.13^{a}	0.20^{a}	0.027	***

[A] Statistical probability of treatment: 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.

other factors might be involved in the increased meat oleic acid concentration associated with concentrate feeding. In fact, it was recently reported that the high level of oleic acid observed in

ruminant fed high grain diets might be explained by an increase in Δ^9 desaturase activity, probably mediated by a higher production of insulin (Daniel et al., 2004; Sinclair, 2007). In this study, no significant differences were observed for the concentration of linoleic acid among all treatments. In contrast, meat from the PPP diet had the highest percentages of ALA (5.53%), followed by the PPC group (1.96%), while meat from CCC and PCC animals presented the lowest proportions of this fatty acid (0.48-0.84%). It is well known that green pastures are a good source of ALA, in contrast to concentrate diets, which explains this inverse relationship between the finishing period on concentrate and the levels of ALA (Wood & Enser, 1997). The percentages of long-chain *n*-3 fatty acids EPA, DPA and DHA acids were significantly higher ($P < 0.05$) in bulls fed with grass when compared to concentrate-fed animals.

Trans octadecenoates are the major intermediates formed during rumen biohydrogenation of C18 PUFA (Bessa et al., 2000). Significantly higher contents of 18:1*t* were found in animals fed on concentrate diets relative to the PPP diet (data not shown). This is mainly due to the *t*6 + *t*8, *t*9 and *t*10 isomers, since the vaccenic acid and 18:1*t*12 remains unaffected by the dietary treatments. High concentrations of 18:1*t*10 have been observed in tissues of concentrate-fed ruminants, whereas vaccenic acid is consistently associated with forage feeding (Bessa et al. 2006; Dugan et al., 2007). Moreover, vaccenic acid is the precursor of rumenic acid, the major CLA isomer in animals and humans (Turpeinen et al., 2002) and, therefore, it might be considered as a fatty acid with beneficial properties. Otherwise, recent studies on rabbits suggested that butter enriched with 18:1*t*10, but not with vaccenic acid, had deleterious effects on plasma lipids and lipoprotein metabolism (Roy et al., 2007). The 18:2*t* represents the octadecadienoic acid isomers (*trans,trans*, *cis,trans* and *trans,cis*) eluting after 18:1*c*15 and before 18:2*t*11,*c*15. The nonconjugated linoleic acid isomer, 18:2*t*11,*c*15 is an intermediate of α-linolenic acid biohydrogenation (Bessa et al. 2007), which explains its lowest concentration in CCC treatment and the highest proportion in PPP diet.

Regarding partial sums of fatty acids (Table 18), the observed patterns reflect the values described above for the major individual fatty acids of each group. The relative proportions of MUFA increased in meat from concentrate-fed animals when compared to animals fed on pasture diets, which is mainly due to an increase in oleic acid. Meat from pasture-fed bulls (PPP group) depicted significantly higher levels of PUFA ($P < 0.05$) than that from animals fed on concentrate feeding (CCC, PCC and PPC animals). Several authors have reported a similar pattern of fatty acid composition in pasture-fed cattle (*e.g.* Realini et al., 2004). Lorenzen et al. (2007) showed that meat from cattle finished on pasture had higher concentrations of PUFA than that from cattle finished on feedlot diets. In addition, the sum of *n*-3 PUFA increased with the inclusion of grass to the diet, whereas the proportion of *n*-6 PUFA was unchanged for all treatments. According to Eriksson and

Pickova (2007), the explanation for this higher PUFA percentage in meat from pasture-fed bulls may be the higher protection of fatty acids in fresh grass from the ruminal biohydrogenation, relative to that of grain or silage. Moreover, this increase in meat PUFA percentage could also result from the presence of secondary plant metabolites in spontaneous pastures that might inhibit microbial biohydrogenation activity within the rumen (Lourenço et al., 2008).

The ratios of PUFA/SFA and *n*-6/*n*-3, which are indices widely used to evaluate the nutritional value of fat for human consumption, are defined and presented in Table 18. According to current nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45 and, within the PUFA, the *n*-6/*n*-3 ratio should not exceed 4.0 (British Department of Health, 1994). In view of

Table 18. Effect of the four feeding systems on partial sums of fatty acids (g/100 g total fatty acids) and nutritional fatty acid ratios of *longissimus lumborum* muscle from Alentejano purebred bulls. The feeding regimens were as follows: feedlot (CCC), pasture with finishing on concentrate during 4 (PCC) and 2 (PPC) months, and pasture grazing (PPP).

	Treatments				SEM	P^A
	CCC	PCC	PPC	PPP		
Partial sums						
∑ SFA	39.27^{ac}	43.11^{b}	42.08^{ab}	38.76^{c}	1.048	*
∑ MUFA	34.99^{c}	33.69^{bc}	29.87^{b}	24.69^{a}	1.430	***
∑ TFA	3.52	3.36	3.82	3.17	0.230	ns
∑ *n*-6 PUFA	17.08	13.54	14.99	17.97	1.654	ns
∑ *n*-3 PUFA	1.97^{a}	2.77^{a}	4.85^{b}	10.41^{c}	0.552	***
∑ PUFA	19.06^{a}	16.31^{a}	19.84^{a}	28.99^{b}	2.014	**
∑ unidentified	3.15^{a}	3.54^{a}	4.39^{b}	4.99^{c}	0.193	***
Ratios						
PUFA/SFA	0.50^{a}	0.38^{a}	0.48^{a}	0.74^{b}	0.063	**
n-6/*n*-3	8.99^{c}	5.26^{b}	3.16^{a}	1.77^{a}	0.573	***

[A] Statistical probability of treatment: 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.
∑ SFA = sum of 12:0, 14:0, 15:0, 16:0, 17:0 18:0 and 20:0.
∑ MUFA = sum of 14:1*c*9, 16:1*c*9, 17:1*c*9, 18:1*c*9, 18:1*c*11, 18:1*c*12, 18:1*c*13, 18:1*c*15, 20:1*c*11 and 24:1*c*15.
∑ TFA = sum of 16:1*t*9, 18:1*t*6+*t*8, 18:1*t*9, 18:1*t*10, 18:1*t*11, 18:1*t*12, 18:1*t*16+*c*14, 18:2*t*11,*c*15 and 18:2*t*.
∑ *n*-6 PUFA = sum of 18:2*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6; 22:2*n*-6 and 22:4*n*-6.
∑ *n*-3 PUFA = sum of 18:3*n*-3, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3.
∑ PUFA =sum of 18:2*n*-6, 18:3*n*-3, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 22:2*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3.
PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n*-6, 18:3*n*-3, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 22:2*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3)/(sum of 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].
n-6/*n*-3 = *n*-6/*n*-3 ratio [(sum of 18:2*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6; 22:2*n*-6 and 22:4*n*-6)/(sum of 18:3*n*-3, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3)].

the above guidelines, the *n*-6/*n*-3 ratio in meat was higher in animals fed with the concentrate treatment (CCC, 8.99), intermediate in PCC animals (5.26) and lower in animals of the PPC and PPP diets (1.77-3.16). Thus, the ratio of *n*-6/*n*-3 fatty acids in meat from the grass-based PPP and PPC groups was within the nutritional recommendations for the human diet. However, this ratio increased moderately in meat for animals fed with concentrates based on cereal grains (rich in linoleic acid) for 4 months (PCC) or during all the experiments (CCC). Other works have reported that high levels of concentrate in the diet increase the *n*-6/*n*-3 ratio of meat (Alfaia et al., 2006a; Eriksson & Pickova, 2007; Nuernberg et al., 2002). Compared to available data, our value for the *n*-6/*n*-3 ratio in meat from pasture feeding (1.77) is very close to those reported by Enser et al. (1998) for British meat from grass-fed cattle (2.0-2.3). In contrast, the same nutritional index for meat from concentrate feeding (8.99) was considerably lower than the values reported by the former authors for concentrate-fed bull meats (15.6-20.1). Furthermore, it was also shown that the unsaturated fatty acid profile of beef fat was enhanced with the pasture finished cattle, decreasing *n*-6/*n*-3 and increasing PUFA/SFA ratios (Enser et al., 1998; French et al., 2000; Realini et al., 2004). In line with this, the PUFA/SFA ratio was significantly higher ($P < 0.05$) in pasture-fed animals. However, our values of the PUFA/SFA ratio in meat from all treatments (0.38-0.74) were within or above the recommended guideline for the human diet.

Our group had previously characterised the fatty acid composition of Carnalentejana-PDO beef (Alfaia et al., 2006a), a traditional meat also obtained from Alentejano purebred bulls, produced in a semi-extensive system with finishing on concentrate during 3 to 6 months, according to Carnalentejana-PDO specifications. As reported in the previous study, Carnalentejana-PDO showed no apparent seasonal variations in the nutritional quality of beef, but different intramuscular fat characteristics, including lower *n*-6/*n*-3 ratios and higher proportions of *c*9,*t*11 CLA isomer, when compared to meat from crossbred bulls fed intensively with concentrate. However, the finishing period of Alentejano purebred bulls with concentrate seems to attenuate most of the beneficial grass-fed characteristics of meat fat. Comparing the fatty acid composition of those traditional meats with the meat obtained in this experiment, in which the Alentejano purebred bulls were kept under fairly controlled conditions, it is suggested that Carnalentejana-PDO beef depicts, in general, a fatty acid profile similar to those presented by animals fed on concentrate-based diets (PCC and CCC groups). This finding might reflect the attenuation of the grazing markers of Alentejano purebred bulls from Carnalentejana-PDO by a finishing period on concentrate during 3-6 months. However, it is well established that meat fatty acid composition is affected by many factors, including muscle type, management conditions, intensity of feeding, quality of pasture and concentrate composition (De Smet et al., 2004). Variations in the fatty acid profile within the same breed have already been reported and reviewed by Schmid et al. (2006).

In summary, meat from pasture-fed animals (PPP group) and from bulls with a 2 month finishing period on concentrate (PPC diet) depicted PUFA/SFA and *n*-6/*n*-3 ratios of intramuscular fat in *L. lumborum* inside the recommended values for the human diet, in contrast to that from animals exposed to longer finishing periods on concentrate (except for PUFA/SFA ratio in CCC bulls), as a result of the beneficial effects of grass on meat lipids profile.

6.3.2 Intramuscular CLA isomeric profile

Detailed data on the contents and isomeric profile of CLA in the intramuscular fat of Alentejano purebred bulls fed different diets are displayed in Table 19. Concerning total CLA contents (mg/g

Table 19. Effect of the four feeding systems on total (mg/g muscle) and specific (mg/g fat) CLA contents, and its isomeric distribution (% of total CLA) of *longissimus lumborum* muscle from Alentejano purebred bulls. The feeding regimens were as follows: feedlot (CCC), pasture with finishing on concentrate during 4 (PCC) and 2 (PPC) months, and pasture grazing (PPP).

	Treatments				SEM	P^A
	CCC	PCC	PPC	PPP		
Total CLA	0.035	0.036	0.034	0.023	0.005	ns
Specific CLA	2.65^{a}	5.76^{b}	5.60^{b}	5.14^{b}	0.639	**
CLA isomers						
*t*12,*t*14	0.90^{a}	2.31^{ab}	3.37^{b}	5.99^{c}	0.629	***
*t*11,*t*13	1.19^{a}	5.57^{b}	8.94^{b}	15.76^{c}	1.414	***
*t*10,*t*12	0.99^{a}	0.87^{a}	0.80^{a}	0.36^{b}	0.104	***
*t*9,*t*11	2.54^{a}	2.82^{ab}	3.12^{bc}	3.31^{c}	0.169	*
*t*8,*t*10	0.24	0.25	0.25	0.18	0.036	ns
*t*7,*t*9	0.57^{a}	0.56^{a}	0.61^{a}	0.38^{b}	0.054	*
*t*6,*t*8	0.03	0.08	0.12	0.15	0.034	ns
total *trans,trans*	6.45^{a}	12.46^{b}	17.21^{b}	26.13^{c}	2.011	***
c/*t*12,14	0.63	0.57	1.00	0.89	0.136	ns
*t*11,*c*13	1.76^{a}	4.13^{ab}	6.28^{bc}	7.37^{c}	0.861	***
*c*11,*t*13	0.09^{a}	0.12^{a}	0.13^{a}	0.39^{b}	0.054	***
*t*10,*c*12	0.22	0.15	0.16	0.04	0.058	ns
*c*9,*t*11	81.34^{a}	76.39^{a}	68.31^{b}	61.87^{b}	2.392	***
*t*7,*c*9^{+}	8.37	5.47	6.22	2.79	1.278	*
total *cis/trans*	92.41^{a}	86.82^{ab}	82.10^{b}	73.36^{c}	1.988	***
total *cis,cis* (*c*9,*c*11)	1.14^{a}	0.71^{b}	0.68^{b}	0.52^{b}	0.143	*

[A] Statistical probability of treatment: 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.
[+] This CLA isomer co-eluted with minor amounts of the *t*8,*c*10 isomer.

muscle), no significant differences ($P > 0.05$) were observed for the four diets, while specific CLA contents (mg/g fat) were higher ($P < 0.05$) in pasture-based treatments (5.14, 5.60 and 5.76 mg/g fat for PPP, PPC and PCC groups, respectively), when compared to the concentrate-fed animals (2.65 mg/g fat for CCC diet). Our results are in agreement with those reported by French et al. (2000) and Realini et al. (2004), who showed that meat fat from grazing beef had higher CLA contents (10.8 and 5.3 mg/g fat, respectively) than those obtained from concentrate-fed animals (3.7 and 2.5 mg/g fat). Similarly, Lorenzen et al. (2007) reported higher CLA contents, on an mg/g fat basis, for fat from pasture cattle than for grain-fed cattle. However, as far as we know, only a few studies have reported the effect of pasture *vs*. concentrate feeding on the detailed CLA isomeric profile of beef fat, which is only achieved by high performance liquid chromatography using three silver-ion columns in series (Fritsche et al., 2000; Nuernberger et al., 2002; Dannenberger et al., 2005).

The feeding system had a major impact on the CLA isomeric distribution of beef (affected 10 of 14 CLA isomers). The CLA isomeric profile showed a clear predominance of the bioactive *c*9,*t*11 isomer for all diets (61.9-81.3%). Concentrate feeding significantly decreased ($P < 0.05$) the concentration of *c*9,*t*11 CLA isomer compared to the PPP group (2.16 *vs*. 3.18 mg/g fat) in L. *lumborum* muscle. Several authors reported that diets containing proportionally high levels of ALA, such as fresh grass, grass silage and concentrates containing linseed, resulted in increased deposition of the *c*9,*t*11 CLA isomer in muscle (Dannenberger et al., 2005; French et al., 2000). Although the lipids in pasture-based systems are rich in ALA, the biohydrogenation by rumen microorganisms does not include the *c*9,*t*11 CLA isomer as an intermediate. The vaccenic acid is the common intermediate during the biohydrogenation of dietary linoleic acid and ALA to stearic acid (Harfoot & Hazelwood, 1997). Since only a relatively small percentage of the *c*9,*t*11 CLA isomer, formed in the rumen, is available for deposition in the muscles, the major source of this isomer in muscle results from the endogenous synthesis involving Δ^9 desaturase and vaccenic acid (Griinari & Bauman, 1999).

Our results reinforce those of Dannenberger et al. (2005), which suggest that *t*11,*c*13, *t*11,*t*13 and *t*12,*t*14 CLA isomers are increased in meat from pasture-fed animals. However, in our experiment the *t*11,*t*13 isomer was the second-most predominant CLA isomer in meat from pasture-fed animals, instead of the *t*11,*c*13 isomer as found by Dannenberger et al. (2005). This discrepancy may be explained by differences in pasture type and composition. In fact, as mentioned above, animals of the experiment reported here grazed on alluvium pastures, summer triticalis and maize stubbles. The proportion of *t*11,*t*13 CLA isomer increased from 1.19% in the muscle of concentrate-fed bulls to 15.76% in meat from bulls fed on pasture. Moreover, the *t*11,*c*13 CLA isomer was the third most prevalent CLA isomer in PPC and PPP diets, with 6.28% and 7.37%, respectively. In contrast, the *t*7,*c*9 CLA isomer, which co-eluted with minor amounts of the *t*8,*c*10 isomer, was the second-most

abundant CLA isomer in the concentrate diet (CCC group). This isomer is usually identified as the second-most prevalent CLA isomer in commercial milk fat and beef muscle (Dannenberger et al., 2005; Fritsche et al., 2000). The *t*7,*c*9 CLA isomer, like the most abundant *c*9,*t*11 isomer, mainly result from the endogenous synthesis through Δ^9 desaturation of the rumen derived trans octadecenoate precursor (Palmquist et al., 2004). With the exception of *c*9,*t*11 and *t*7,*c*9 isomers, the origin of all CLA isomers, including the *t*11,*t*13 isomer, is supposed to be the ruminal biohydrogenation of C18 PUFA. However, the metabolic pathways leading to the formation of these compounds have not yet been elucidated (Nuernberger et al., 2002). Recently, results obtained with two new *Butyrivibrio fibrisolvens* strains indicate that the *t*11,*c*13 CLA isomer can be formed by isomerisation of 18:2*t*11,*c*15 (Hino & Fukuda, 2006). Moreover, Bessa et al. (2007) suggested that the origin of some minor CLA isomers may not be related with ruminal biohydrogenation of dietary unsaturated C18 fatty acids, but with bacterial *de novo* synthesis in the rumen.

The other bioactive CLA isomer, *t*10,*c*12, which apparently affects lipid metabolism, was not influenced by dietary treatments ($P > 0.05$). The content of this isomer in meat, which was residual in all treatments, ranged from between 0.04% and 0.22% of total CLA content. In addition, the diet had a significant effect ($P < 0.05$) on the total *cis/trans* (*cis,trans* and *trans,cis*), and *cis,cis* isomers, as well as in total *trans,trans* CLA isomers. Regarding the sum of *trans,trans* isomers, pasture feeding resulted in higher percentages of *t*11,*t*13 and *t*12,*t*14 CLA isomers in meat than in CCC animals. This distribution pattern of CLA isomers is consistent with other experiments, in which animals were fed pasture *vs*. concentrate (Nuernberger et al., 2002; Dannenberger et al., 2005). According to Dannenberger et al. (2005), the differences found in CLA profiles may be explained by distinct grass intake, since it was shown that pasture feeding, compared to concentrate feeding, increases the proportion of the *t*11,*c*13, *t*11,*t*13 and *t*12,*t*14 CLA isomers, and decreases the percentage of the *t*7,*c*9 isomer, in beef lipids. As suggested by the same authors, our results confirmed that *t*11,*t*13, *t*11,*c*13 and *t*12,*t*14 CLA isomers are sensitive grass intake indicators.

The CLA isomeric profile of intramuscular fat in Carnalentejana-PDO, a traditional meat previously characterised by our group (Alfaia et al., 2006a), is similar to the profile described here for meat from concentrate-fed Alentejano bulls (CCC diet). As mentioned above, depending on several factors, such as diet composition, slaughter season and muscle type, different profiles of CLA isomers are expected. Nevertheless, the specific CLA contents of Carnalentejana-PDO beef (4.92-5.07 mg/g fat) are in agreement with those found in this experiment for pasture-fed bulls (PPP treatment).

6.3.3 Discriminatory ability of intramuscular fatty acid pattern

Canonical discriminant analysis was applied to a fatty acid profile in order to classify and discriminate the feeding systems used in this experiment. A stepwise forward discriminant analysis was previously applied in order to select the most relevant variables for classification. In this procedure, variables that contribute with the most discriminatory power were selected. The application of canonical discriminant analysis to selected variables produced three canonical discriminant functions, which maximise the ratio between class variance and minimises the ratio within class variance. The coefficients obtained for each variable are presented in Table 20. A larger coefficient corresponds to a greater contribution of the respective variable to the discrimination between groups. For treatment differentiation, the first two canonical discriminant functions were selected (Figure 5). The recognition ability of the discriminant model was evaluated by the correct

Table 20. Results of canonical discriminant analysis: loadings of correlation matrix between predictor variables (standardized canonical coefficients) and discriminant functions (roots 1, 2 and 3), and some statistics for each function.

	Root 1	Root 2	Root 3
Fatty acids			
12:0	-2.554	-0.395	2.140
14:0	3.540	-0.525	-1.878
16:1*t*9	1.020	-1.795	-1.640
17:1*c*9	10.073	1.062	-3.969
18:0	1.124	-0.290	-1.181
18:1*c*9	-1.647	-2.345	-0.157
18:1*c*13	-3.256	-0.387	1.610
18:1*t*10	-2.942	-0.740	0.010
18:1*t*16+*c*14	2.306	0.763	-1.648
18:3*n*-3	0.712	-4.588	0.289
20:0	2.940	-1.600	1.484
20:1*c*11	1.397	1.707	3.770
22:4*n*-6	1.929	0.099	-1.657
24:1*c*15	5.222	0.562	-2.624
*t*11,*t*13 CLA isomer	1.422	-1.302	-0.638
Statistics			
Canonical R	0.996	0.983	0.965
Eigenvalue	139.4	28.22	13.70
Cumulative proportion	0.769	0.924	1.000
Probability	$P < 0.001$	$P < 0.001$	$P < 0.001$

classifications of 100% during the modelling step, allowing the differentiation of the four feeding systems. Afterwards, the prediction ability was carried out with a cross-validation method, in which one sample at a time was removed from the training set and considered as a test set. A correct classification of 100% obtained for all treatments showed a high sensitivity and specificity of the class model (Table 21).

Considering the data presented in Figure 5, and the discriminant root 1, feeding on grass and on concentrate (PPP and CCC treatments, respectively) were located on the right side of the plot, whereas the treatments fed on grass with finishing period on concentrate (PCC and PPC groups) were located on the left side. Moreover, according to discriminant root 2, the CCC and PCC treatments had positive scores, while the PPP treatment had a negative score and the PPC diet is located close to the origin. Nevertheless, better separation was achieved with the PPP and CCC diets.

Figure 5. Plot of the discriminant functions (root 1 *vs.* root 2) for classification of bulls according to their feeding system. The feeding regimens were as follows: feedlot (CCC), pasture with finishing on concentrate during 4 (PCC) and 2 (PPC) months, and pasture grazing (PPP).

The meat fatty acids with the highest discriminant power were the 17:1*c*9, 24:1*c*15, 14:0 and 18:1*c*13, on the root 1, and the ALA, oleic acid, 16:1*t*9 and 20:1*c*11, on the root 2. In addition, the only CLA isomer in meat that entered into the discriminant function was the *t*11,*t*13 isomer, which was the most sensitive grass intake indicator.

This methodology has been previously applied by Santos-Silva et al. (2002) to the discrimination of lamb production systems: pasture, pasture supplemented with concentrate and concentrate-fed in confinement. The results indicated that the meat fatty acid profile enabled the lambs to be allocated to one of the three feeding systems with good accuracy, while both α-linolenic acid and CLA (GC peak consisting mainly on *c*9,*t*11 isomer) were the most discriminant fatty acids. Moreno et al. (2006) applied this method to discriminate veal from weaned and unweaned calves. The SFA lower than C11, followed by the heptadecanoic acid (17:0) and the *n*-6/*n*-3 ratio, was the most discriminant variables for separating the two weaning classes, regardless of the gender. Moreover, Dias et al. (2008) used the fatty acid profile in order to differentiate the meat production sub-system (traditional and organic farming) of two autochthonous bovine cattle breeds (Mirandesa and Barrosã). The authors conclude that the fatty acid composition, together with the PUFA/SFA and *n*-6/*n*-3 ratios, could be used as an effective tool to differentiate the breed and the production sub-system used. However, the authors suggested a larger number of meat samples should be analysed in order to support a better understanding of the role of breed and production system on the fatty acid profile.

In summary, the results indicate that prediction of the feeding system using intramuscular fatty acids as tracers, including the duration of the finishing period of animals on concentrate, is highly accurate.

Table 21. Classification matrix of cross validation results for Alentejano purebred bulls in the four feeding systems, using canonical discriminant analysis. The treatments were as follows: feedlot (CCC), pasture with finishing on concentrate during 4 (PCC) and 2 (PPC) months, and pasture grazing (PPP).

	Treatments			
	CCC	PCC	PPC	PPP
Classified as "CCC"	7	0	0	0
Classified as "PCC"	0	7	0	0
Classified as "PPC"	0	0	8	0
Classified as "PPP"	0	0	0	8
Total	7	7	8	8
% correct classification	100	100	100	100

However, caution must be taken in the interpretations of the results since the number of animals used here was small and the number of variables analysed is large. Thus, this approach must be extended to larger databases.

6.4 CONCLUSIONS

Data reported here indicate that feeding systems have a major impact on the fatty acid profile, including CLA isomers, of Alentejano beef, which is independent of the degree of intramuscular fat deposition. α-Linolenic acid, LC *n*-3 PUFA, and *t*11,*t*13, *t*11,*c*13 and *t*12,*t*14 CLA isomers in meat were the most sensitive grass intake indicators. In addition, the data reinforced the evidence that beef from pasture-fed animals has a higher nutritional quality (mainly due to the higher levels of *n*-3 PUFA and CLA) when compared to that from concentrate-fed bulls, as a result of the beneficial effects of grass on meat fatty acid profiles. Meat from bulls fed with pasture only and from animals with 2 months of finishing on concentrate showed PUFA/SFA and *n*-6/*n*-3 ratios of intramuscular fat inside the recommended values for the human diet, in contrast to that from animals exposed to longer finishing periods on concentrate (except for PUFA/SFA ratio in CCC diet). Finally, meat fatty acid profiles seems to be an efficient chemical marker to discriminate between the four feeding systems analysed, including the different finishing periods of animals on concentrate, suggesting that this approach might be useful for the development of a practical discrimination tool for Portuguese PDO beef industry.

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IRRADIATION EFFECT ON FATTY ACID COMPOSITION AND CONJUGATED LINOLEIC ACID ISOMERS IN FROZEN LAMB MEAT

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Irradiation effect on fatty acid composition and conjugated linoleic acid isomers in frozen lamb meat

The effect of gamma radiation processing on the lipid content, fatty acid composition and conjugated linoleic acid profile in frozen lamb meat was investigated. Samples of *longissimus thoracis* muscle from lambs fed lucerne basal diets either unsupplemented or supplemented with polyunsaturated vegetable oils were irradiated (7 kGy) and analysed. CLA contents in lamb meat did not affect ($P > 0.05$) the levels of lipid oxidation induced by the irradiation. No significant differences ($P > 0.05$) were observed for fatty acid composition, related nutritional indices (*n*-6/*n*-3 and PUFA/SFA), as well as for total lipid and CLA contents, between non-irradiated (control) and irradiated meat samples. In contrast, meat irradiation affected the relative proportions of total *trans, trans* and *cis/trans* CLA isomers ($P < 0.001$), in addition to the percentage of some minor individual CLA isomers (*t*11,*t*13 and *t*9,*t*11, with $P < 0.05$ and $P < 0.001$, respectively). The percentage of total *cis*/*trans* CLA isomers slightly decreased in irradiated samples, while the relative proportion of total *trans,trans* isomers slightly increased. This observation may be explained by the higher susceptibility to autoxidation of the cis double bond relative to the *trans* configuration.

Keywords: fatty acid composition; CLA isomers; irradiation; lamb meat.

7.1 INTRODUCTION

Meat irradiation is recognised as a safe and effective method among the existing technologies to attain meat preservation (Kanatt et al., 2006). The use of high energy gamma rays or accelerated electrons to irradiate fresh meat extends shelf life and protects proliferation of pathogenic bacteria. The Food and Drug Administration approved irradiation for poultry meat and red meats (USA FDA, 1997) to control foodborne pathogens and to extend product shelf life (Ross & Engeljohn, 2000). In the European Union, the opinions on the use of irradiation differ in member countries. The greatest suppliers of irradiated foodstuffs are Belgium (*e.g.* meat, fish, eggs and cheese), France (*e.g.* mechanically recovered poultry meat and frozen frog legs) and the Netherlands (*e.g.* frozen poultry meat, spices and dehydrated vegetables) (Grolichová et al., 2004; European Union, 2006). In the USA, this technology is more common and there are also attempts to enforce irradiation not only for food safety but also for technological purposes.

Irradiation is a prospective technology and its application causes physical-chemical and biochemical changes that may affect the nutritional value and sensory characteristics of irradiated food (Dogbevi et al., 1999; Grolichová et al., 2004). However, according to the same authors, irradiation at low doses (up to 10 kGy) may have either non-measurable or non-significant impacts on meat properties. One of the major concerns with meat irradiation is its effects on lipid oxidation, meat colour and off-odor production (Ahn et al., 2000). Fats are among the least stable food components being very susceptible to ionizing radiation (Hammer & Wills, 1979), which may induce autoxidation. Radiation processing generates free radicals and accelerates oxidation of unsaturated fatty acids that may induce some biochemical changes in meat and influence its quality, such as the nutritional value (Du et al., 2000). Polyunsaturated fatty acids of the phospholipid fraction, which represent 0.5–1% of the total lipids in meat, are the major contributors for the development of rancidity during meat storage (Giroux & Lacroix, 1998) and so, the most susceptible during irradiation.

Conjugated linoleic acid is a minor group of fatty acids, composed of positional (from positions 6,8- to 12,14-) and geometric (*trans,trans*, *trans,cis*, *cis,trans* and *cis,cis*) isomers of linoleic acid (18:2*n*-6) containing conjugated double bonds with a multitude of potential health benefits (see *e.g.* Prates & Mateus, 2002; Wahle et al., 2004). Twenty different CLA isomers have been reported as occurring naturally in food, especially in ruminant fat (Sehat et al., 1998). The major CLA isomer, rumenic acid, is produced in the rumen during the microbial biohydrogenation of dietary 18:2*n*-6 and in the tissues through Δ^9 desaturation of 18:1*t*11 (Griinari & Bauman, 1999). It is now accepted that the major contribution to *c*9,*t*11 in ruminant milk (Corl et al., 2002) and meat (Palmquist et al., 2004) is the endogenous synthesis. Recent interest in some CLA isomers was sparked off by biological activities that include anticancerinogenic, antiobesity, antidiabetogenic, anti-atherogenic and

immunomodulation and modulation of bone growth (Cook & Pariza, 1998; Whigham et al., 2000; Belury, 2002; Parodi, 2002a). The information about CLA isomeric distribution appears to be important as isomer specific biological effects have been reported (Evans et al., 2002).

Although many publications have evaluated the quality of irradiated bovine, ovine, swine and poultry meat (see *e.g.* Ahn et al., 2000; Du et al., 2000), the information on changes in fatty acid composition is scarce (see *e.g.* Brito et al., 2002; Kanatt et al., 2006). In addition, as far as is known, there is no report on the effect of radiation processing on CLA isomeric distribution in meat. Moreover, it is still unknown if CLA enriched meat is more susceptible to irradiation induced lipid oxidation. Therefore, the objective of this study was to analyse the influence of gamma radiation processing, at the maximum doses allowed commercially (7 kGy), on the fatty acid composition, including the CLA isomeric profile, of vacuum-packaged frozen lamb meat samples. The samples were collected from an experiment where lambs were fed lucerne basal diets, either unsupplemented or supplemented with polyunsaturated vegetable oils, in order to produce meat with different levels of CLA.

7.2 MATERIALS AND METHODS

7.2.1 Reagents

Analytical grade and liquid chromatographic grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). Sodium methoxide (0.5 M solution in anhydrous methanol) was obtained from Sigma-Aldrich Ltd. (St. Louis, MO, USA) and the fatty acid methyl ester (FAME) standard mixtures were acquired from Nu-Chek-Prep Inc. (Elysian, MN, USA) and Supelco Inc. (Bellefonte, PA, USA). Commercial standards of individual CLA isomers (*c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11) as methyl esters were purchased from Matreya Inc. (Pleasant Gap, PA, USA). Additional standards of individual (*t*8,*c*10 and *c*11,*t*13) and mixtures (*cis,trans*, *trans,cis* and *trans,trans* from positions 7.9 to 12.14) of CLA isomers were prepared as methyl esters according to the procedure described by Destaillats and Angers (2003).

7.2.2 Animals and preparation of meat samples

Samples used in the present study were originated from an experiment where 32 Merino Branco lambs were randomly allocated to 4 groups that were fed, *ad libitum*, with one of the four pelleted

diets: Control – control diet consisting of 100% pelleted dehydrated lucerne (*Medicago sativa* L.); SF – pelleted dehydrated lucerne supplemented with sunflower oil; SFLS – pelleted dehydrated Lucerne supplemented with a blend of sunflower oil and linseed oil (2:1 v/v); LS – pelleted dehydrated lucerne supplemented with linseed oil. The level of oil inclusion in SF, SFLS and LS diets was 7.4% in a dry matter basis. The animals stayed on trial for 6 weeks and the *longissimus thoracis* muscle was collected from the carcasses (kept under refrigeration at +1 °C) 72 h after slaughter, minced, vacuum-packaged in polyethylene bags and stored at −70 °C. Details on animal husbandry and on the effects of lipid supplementation on fatty acid composition of meat are published elsewhere (Bessa et al., 2007). Samples from five lambs of each treatment were submitted to irradiation according to the procedure described below.

7.2.3 Meat irradiation

Irradiation was conducted at the CHIP – Centro de Higienização por Ionização de Produtos, installed in ITN – Instituto Tecnológico e Nuclear (Sacavém, Portugal), with a ^{60}Co source at a dose rate of 4.7 kGy/h. Frozen packed samples with approximately 5 g of homogenised meat were gamma-irradiated at room temperature (+19 °C) and received a dose of 7.0 kGy, which is the maximum dose permitted for frozen meat (USA FDA, 1997). To confirm the target dose, six Red Perspex 4034 dosimeters per cart, calibrated against a ceric-cerous standard dosimeter, were attached to the top and bottom surfaces of the sample. The dosimeter was read using a Shimadzu UV-260 spectrophotometer. Non-irradiated samples were used as controls.

7.2.4 Lipid extraction and methylation

Intramuscular fat was extracted from meat samples (approximately 250 mg), after lyophilisation (−60 °C and 2.0 hPa) using an Edwards Modulyo (Edwards High Vacuum International, UK), for total lipid determination (two tubes) and for both FAME and CLA methyl esters profiles (third tube), as was previously described by Alfaia et al. (2006a). Fatty acids were converted to FAME by base-catalysed transesterification, with sodium methoxide (0.5 M solution in anhydrous methanol) during 2 h at 30 °C, as was proposed by Park et al., (2001), in order to avoid CLA isomerisation. The same FAME solution was used for the analysis of both fatty acid composition and CLA profile, enabling the direct comparison of quantitative data and eliminating differences in sample preparation. Total lipids were measured gravimetrically, in duplicate, by weighing the fatty residue obtained after solvent evaporation.

7.2.5 Determination of fatty acid composition by GC-FID

The gas chromatograph (Agilent 6890, Agilent Technologies Inc., Palo Alto, CA, USA) was equipped with FID. The FAME were separated on a SPTM-2560 fused-silica capillary column (100 m × 0.25 mm i.d. 0.2 μm film thickness, Supelco Inc., Bellefonte, PA, USA), using helium as carrier gas at a flow rate of 1.5 mL/min as was mentioned previously in Alfaia et al. (2006a). Identification was accomplished by comparing the retention time of the peaks from samples with those of FAME standard mixtures analysed at the same time and with values published in the literature (Fritsche et al., 2001). The FAME identification of some unknown peaks was confirmed by gas chromatography with detection by GC–MS using a Varian Saturn 2000 system (Varian Inc., Walnut Creek, CA, USA). Quantification of FAME was based on the internal standard technique, using nonadecanoic acid (19:0) as internal standard, and on the conversion of relative peak areas into weight percentages, using the corrected response factor of each fatty acid (ES ISO 5508, 1990). Fatty acids were expressed in gravimetric contents (mg/g muscle) or as a percentage of the sum of identified fatty acids (wt %).

7.2.6 Determination of CLA isomers by Ag^+-HPLC

The separation of methyl esters of CLA isomers was carried out by three ChromSpher 5 Lipids analytical silver impregnated Ag^+-HPLC columns in series (250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using an Agilent 1100 Series chromatographic system (Agilent Technologies Inc., Palo Alto, CA, USA). The system was equipped with an autosampler and a DAD adjusted to 233 nm, according to the procedure reported previously by Alfaia et al. (2006a). The identification of the individual CLA isomers was achieved by comparison of their retention times with commercial and prepared standards (see section 7.2.1) analysed at the same time as the samples, as well as with values published in the literature (Fritsche et al., 2001). In addition, the identity of each isomer was controlled by the typical ultraviolet spectra of CLA isomers from the DAD in the range from 190 to 360 nm, using the spectral analysis of Agilent Chemstation for LC 3 D Systems rev. A.09.01 (Agilent Technologies, 2001). Total and individual CLA isomer contents in meat were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalisation (AOAC 963.22, 2000). The CLA isomers were expressed in gravimetric contents (mg/g muscle and mg/g fat) or as a percentage of the sum of identified CLA isomers (% total CLA).

7.2.7 Statistical analysis

The data were analysed using the MIXED procedure of Statistical Analysis Systems Institute (SAS, 2004). The model included the fixed effects of diet and irradiation, as well as the interaction between diet and irradiation. Because the diet × irradiation interaction was not significant ($P > 0.05$), it was removed from the model. Irradiation effect was evaluated as repeated measure on the animal within diet. The compound symmetry covariance structure was used according to finite sample corrected Akaike information criterion and Schwarz's Bayesian information criterion (Wang & Goonewardene, 2004).

7.3 RESULTS AND DISCUSSION

Since no significant interactions between diet and irradiation were found for the analysed variables ($P > 0.05$), only the irradiation effects (effective dose of 7.5 kGy) on meat properties were studied and discussed here. Data on the effects of diet on fatty acid composition and CLA isomeric profile are reported elsewhere (Bessa et al., 2007). The absence of interactions between diet and irradiation effects suggests that lamb meat enriched in CLA was not more susceptible to irradiation induced changes than that of non-enriched meat. This is particularly notable because CLA enriched meat, when compared to the control, is also enriched with either linoleic acid (16 *vs*.10%) or linolenic acid (4 *vs*. 2%) whether the lipid supplement was sunflower oil (SF group) or linseed oil (LS group). It is possible that either the high contents of CLA isomers (Yu et al., 2002) or the high levels of α-tocopherol (data not shown), or both, provide antioxidant protection of these PUFA in meat.

7.3.1 Intramuscular fatty acid composition

Fatty acid composition (wt %) of the control (non-irradiated) and irradiated samples of frozen LT muscle from Merino Branco lambs is depicted in Table 22. The major fatty acids in lamb muscle lipids were the SFA, palmitic (16:0; 17–18% of total FAME) and stearic (18:0; 15%) acids, the MUFA oleic acid (18:1*c*9; 28%), and the PUFA linoleic acid (18:2*n*-6; 12%). These percentages of fatty acids in lamb meat result from the polyunsaturated oil supplementation of the lamb diets (see detailed discussion in Bessa et al., 2007). Radiation processing of frozen lamb meat did not affect ($P > 0.05$) the percentage of any of the analysed fatty acids. In addition, no novel fatty acid residues or other artefacts due to irradiation of vacuum-packaged samples were detected by GC.

According to the studies of Raddy et al. (1988), fewer alterations in PUFA composition occur in meat when irradiation is carried out in the frozen state (−20 °C) rather than at 2-5 °C.

Table 22. Fatty acid composition (% w/w) in non-irradiated (as control) and irradiated samples of *longissimus thoracis* muscle from lambs (n = 20).

Fatty acids	Non-irradiated	Irradiated	SEM	P^A
12:0	0.18	0.19	0.019	0.585
14:0	2.25	2.28	0.159	0.829
14:1	0.11	0.11	0.008	0.855
15:0	0.32	0.34	0.029	0.599
16:0	17.55	17.42	0.564	0.743
16:1*t*9	0.84	0.83	0.066	0.721
16:1c9	1.08	1.09	0.061	0.892
17:0	0.67	0.67	0.052	0.943
17:1*c*9	0.41	0.37	0.034	0.145
18:0	14.78	14.85	0.236	0.678
18:1*t*	5.43	5.06	0.618	0.372
18:1*c*9	28.11	28.43	0.862	0.554
18:1*c*11	1.95	2.05	0.175	0.673
18:2*t*9,*t*12	0.27	0.31	0.051	0.497
18:2*n-6*	11.95	11.64	0.561	0.596
18:3*n-3*	2.63	2.66	0.369	0.704
20:0	0.15	0.17	0.015	0.336
20:2*n-6*	0.11	0.11	0.009	0.851
20:3*n-6*	0.11	0.16	0.048	0.145
20:4*n-6*	6.67	6.53	0.564	0.667
20:5*n-3*	0.89	0.91	0.101	0.770
22:5*n-3*	1.57	1.70	0.170	0.280
22:6*n-3*	0.48	0.52	0.048	0.392

[A]Statistical probability of treatment; SEM, standard error of mean.

The results concerning the total lipids (mg/g muscle), the total (mg/g muscle) and the partial sums (wt %) of intramuscular fatty acids in frozen lamb meat, in both control and irradiated samples, are presented in Table 23. Total fatty acids, calculated as the sum of identified fatty acids, represented, on average, 81% of the sum of detected fatty acids (GC analysis) and 78% of total lipids (gravimetric analysis). The sum of identified fatty acids obtained relative to the sum of detected fatty acids (81%) is close to the value (83%) reported by De Smet et al. (2000). No significant changes ($P > 0.05$) in total lipids, total fatty acids and in the various partial sums of fatty acids were observed between the irradiated and control samples, reflecting the patterns described above for the individual fatty acids.

Also, the data suggests that there were no significant differences ($P > 0.05$) for the ratios *n*-6/*n*-3 and PUFA/SFA (as defined in Table 23), which are nutritional indices widely used to evaluate the nutritional value of fat for the human diet. Current nutritional recommendations are that the PUFA/SFA ratio in human diet should be above 0.45 and, within the PUFA, the *n*-6/*n*-3 ratio should not exceed 4.0 (British Department of Health, 1994). In view of the above guidelines, the PUFA/SFA (0.69 for non-irradiated and irradiated samples) and *n*-6/*n*-3 (4.06 and 3.78 for non-irradiated and irradiated samples, respectively) ratios of lamb meat are within the recommended values for the human diet.

Table 23. Total lipids (mg/g muscle), total fatty acids (mg/g muscle), partial sums of fatty acids (% w/w) and nutritional ratios in non-irradiated (as control) and irradiated samples of *longissimus thoracis* muscle from lambs ($n = 20$).

	Non-irradiated	Irradiated	SEM	P^A
Total lipids	14.21	14.06	0.534	0.751
Total fatty acids$^+$	10.68	11.38	0.812	0.419
Partial sums				
Σ SFA	35.98	36.01	0.699	0.970
Σ MUFA	31.67	32.06	0.890	0.509
Σ TFA	6.54	6.20	0.670	0.414
Σ PUFA	24.42	24.24	1.236	0.869
Σ *n-6*	18.84	18.44	0.975	0.654
Σ *n-3*	5.57	5.79	0.569	0.401
Ratios				
n-6/n-3	4.06	3.78	0.425	0.095
PUFA/SFA	0.69	0.69	0.047	0.948

[A]Statistical probability of treatment; [+]Total fatty acids is the sum of identified fatty acids; The symbols mean as follow: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; TFA, *trans* fatty acids; PUFA, polyunsaturated fatty acids; SEM, standard error of mean.

Σ *n-6* = sum of 18:2*n-6*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6* and 22:4*n-6*.

Σ *n-3* = sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3* and 22:6*n-3*.

n-6/n-3 = *n-6/n-3* ratio [(sum of 18:2*n-6*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6* and 22:4*n-6*)/(sum of 18:3*n-3*, 20:5*n-3*, 22:5*n-3* and 22:6*n-3*)].

PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n-6*, 18:3*n-3*, 18:3*n-6*, 20:2*n-6*, 20:3*n-6*, 20:4*n-6*, 20:5*n-3*, 22:4*n-6*, 22:5*n-3* and 22:6*n-3*)/(sum of 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].

7.3.2 Intramuscular CLA isomeric profile

Data on the total (mg/g muscle) and specific (mg/g fat) CLA contents and its isomeric distribution (% of total CLA) in non-irradiated and irradiated samples of frozen LT muscle from Merino Branco lambs are presented in Table 24. Interestingly, total and specific CLA contents were not significantly different ($P > 0.05$) between irradiated and non-irradiated meats.

The CLA isomeric distribution showed a clear predominance of the bioactive *c*9,*t*11 isomer in both treatments (77.0-78.0% of total CLA), followed, in decreasing order, by the isomers *t*11,*c*13 (10.1-10.3%) and *t*7,*c*9 (2.91–2.94%), which was co-eluted with a residual content of the *t*8,*c*10 isomer. The *t*11,*c*13 isomer is derived from the biohydrogenation of dietary linolenic acid (Collomb et al., 2004) and its relative high concentration results from diet supplementation with linseed oil in the two

Table 24. Total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers (% total CLA) in non-irradiated (as control) and irradiated samples of *longissimus thoracis* muscle from lambs ($n = 20$).

	Non-Irradiated	Irradiated	SEM	P^A
Total CLA	0.20	0.21	0.26	0.513
Specific CLA	14.4	14.7	1.57	0.657
CLA isomers				
*t*12,*t*14	1.23	1.39	0.183	0.152
*t*11,*t*13	2.41	2.82	0.352	0.017
*t*10,*t*12	0.47	0.43	0.056	0.310
*t*9,*t*11	2.17	2.92	0.084	0.0001
*t*8,*t*10	0.18	0.20	0.037	0.560
*t*7,*t*9	0.35	0.38	0.028	0.188
*t*6,*t*8	0.05	0.04	0.020	0.494
total *trans,trans*	6.87	8.18	0.449	0.0001
*c/t*12,14	1.05	1.05	0.192	0.978
*t*11,*c*13	10.3	10.1	2.023	0.583
*c*11,*t*13	0.10	0.08	0.033	0.622
*t*10,*c*12	0.35	0.31	0.079	0.601
*c*9,*t*11	78.0	77.0	2.49	0.133
*t*7,*c*9[+]	2.91	2.94	0.187	0.892
total *cis/trans*	92.8	91.4	0.429	0.0001
total *cis,cis* (*c*9,*c11*)	0.36	0.37	0.072	0.843

[A]Statistical probability of treatment; [+]This CLA isomer co-eluted with minor amounts of the *t*8,*c*10 isomer; SEM, standard error of mean.

experimental groups (Bessa et al., 2007). Meat irradiation had no significant effects ($P > 0.05$) on the percentages of most individual CLA isomers, especially for the two known bioactive isomers (*c*9,*t*11 and *t*10,*c*12), although differences were detected for some minor *trans,trans* isomers (*t*11,*t*13 and *t*9,*t*11). However, the relative proportion of the sum of *trans,trans* isomers is higher ($P < 0.001$) in irradiated meat (8.18%), relative to the non-irradiated meat (6.87%), in contrast to the percentages of total *cis/trans* isomers (*cis,trans* and *trans,cis*), which were higher ($P < 0.001$) in non-irradiated meat (92.8% *vs*. 91.4%). This pattern reflects the tendency of the percentages of individual *cis/trans* CLA isomers to decrease (−1.4%), in contrast to the *trans,trans* isomers (+1.3%), in meat subjected to irradiation. In addition, total *cis,cis* CLA isomers only showed residual percentages of the *c*9,*c*11 isomer (0.36–0.37%), with no significant differences ($P > 0.05$) between meat samples.

These differences in the isomer percentages may be due to the distinct oxidative stability of the various geometric groups of CLA isomers. In fact, it was previously showed that the stability of CLA isomers is determined by its *cis* or *trans* configuration but not by the position of their double bonds (Yang et al., 2000). According to the same authors, *cis/trans* CLA isomers are relatively more susceptible to autoxidation than *trans,trans* isomers, which was explained by the higher instability and vulnerability to oxygen attack of the *cis* double bond relative to the *trans* configuration. In addition, Hamalainen et al. (2001) showed that hydroperoxides are one type of primary oxidation products during the autoxidation of CLA isomers. These results agree well with those reported by several authors, which showed that meat irradiation increases lipid peroxidation (Luchsinger et al., 1996). Furthermore, a dose dependent increase in TBA number relative to radiation processing (2.5 and 5 kGy) was reported for lamb meat (Kanatt et al., 2006). In fact, when compared to the non-irradiated samples, irradiation at 2.5 and 5 kGy resulted in a 34% and an 89% increase in TBA values, respectively. The higher lipid peroxidation of irradiated meat is explained by the accelerated autoxidation led by the free radicals produced during irradiation to form hydroperoxides, which break down into various decomposition products.

Finally, it was previously shown that CLA isomers, both in the form of free fatty acids and triacylglycerols, oxidize considerably faster than other PUFA, including the linoleic, α-linolenic and arachidonic acids (Yang et al., 2000; Zhang & Chen, 1997). This observation suggests that a conjugated double bond is more vulnerable to autoxidation than a nonconjugated double bond. The absence of the effect of gamma irradiation on the proportions of fatty acids and the slight influence on the percentages of CLA isomers reported here, are in agreement with the relative oxidative instability reported for these conjugated isomers when compared with nonconjugated fatty acids.

7.4 CONCLUSIONS

The results presented here indicate that lamb meat enriched with CLA is not more susceptible to irradiation induced oxidation of fatty acids than that of non-enriched meat. The gamma irradiation of vacuum-packaged frozen lamb meat, with the maximum doses allowed commercially (7 kGy), does not seem to change its total lipid content, fatty acid profile and CLA contents. In addition, the data also suggest that the nutritional value of meat fatty acids for human diet is not significantly lost. However, the sums of *trans,trans* and *cis/trans* CLA isomers in meat, as well as some minor individual CLA isomers, seem to be affected by the irradiation processing, although the bioactive *c*9,*t*11 and *t*10,*c*12 CLA isomers were not affected by the treatment. Meat irradiation appears to slightly decrease the percentage of total *cis/trans* CLA isomers and slightly increase the relative proportion of total *trans,trans* isomers, which is likely due to the higher susceptibility of *cis/trans* isomers to peroxidation.

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8

EFFECT OF COOKING METHODS ON FATTY ACIDS, CONJUGATED ISOMERS OF LINOLEIC ACID AND NUTRITIONAL QUALITY OF BEEF INTRAMUSCULAR FAT

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Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat

The effect of boiling, microwaving and grilling on the composition and nutritional quality of beef intramuscular fat from cattle fed with two diets was investigated. *Longissimus lumborum* muscle from fifteen Alentejano young bulls fed on concentrate or pasture were analysed. Cooking losses and, consequently, total lipids, increased directly with the cooking time and internal temperature reached by meat (microwaving > boiling > grilling). The major changes on fatty acid composition, which implicated 16 out of 34 FA, resulted in higher percentages of cooked beef in SFA and MUFA and lower proportions in PUFA, relative to raw meat, while CLA isomers revealed a great stability to thermal processes. Heating decreased the PUFA/SFA ratio of meat but did not change its *n-6*/*n-3* index. Thermal procedures induced only slight oxidative changes in meat immediately after treatments but affected hardly the true retention values of its individual fatty acids (72-168%), including CLA isomers (81-128%).

Keywords: fatty acid composition; CLA isomers; cooking methods; beef.

8.1 INTRODUCTION

Fat content and fatty acid composition of meat are of major importance for consumers due to their importance for meat quality and nutritional value (Wood et al., 2004). Saturated fatty acids and *trans* fats have been recognised by the international dietary authorities as primary targets for global reduction (WHO, 2003). Marked reductions in these specific nutrients, as well as an increase in the PUFA content, especially *n-3* PUFA at the expense of *n-6* PUFA, may have a noticeable knock-on effect on public health improvement (British Department of Health, 1994). Ruminant meat provides a valuable amount of PUFA, namely *n-3* fatty acids, for the human diet (Scollan et al., 2001). In contrast, it is well known that the low PUFA/SFA and high *n-6*/*n-3* ratios of some meats contribute to the imbalance in the fatty acid intake of today's consumers (Wood et al., 2004).

Meat from ruminants represents the major source of natural CLA isomers for human diet. Twenty four CLA isomers occur naturally in foods, especially in meat, milk and dairy products (Sehat et al., 1998). Complete information about CLA isomeric composition is of utmost importance in nutritional/biochemical studies since some CLA isomers, at least the *c*9,*t*11 and *t*10,*c*12, have been shown to present different biological effects (Park & Pariza, 2007). Animal and *in vitro* studies allowed identifying CLA as an important dietary component, with a multitude of potential health benefits on cancer, cardiovascular diseases, diabetes, body composition, immune system or modulation of bone growth (Wahle et al., 2004). Ruminant fats are the richest natural dietary source of *c*9,*t*11, which is the major CLA isomer, commonly known as rumenic acid (Kramer et al., 1998). This isomer is mainly produced by *de novo* endogenous synthesis in different tissues via Δ^9 desaturation of 18:1*t*11 (Griinari & Bauman, 1999). The origin of all other CLA isomers is supposed to arise from ruminal biohydrogenation of dietary unsaturated C18 PUFA (Bessa et al., 2007).

As a result of the fatty acid imbalance in human diets, dietary strategies have been used to improve the nutritional and health value of cattle intramuscular fat. Thus, manipulation of fatty acid composition in ruminant meat to reduce SFA content and *n*-6/*n*-3 ratio and, simultaneously, increase the PUFA and CLA contents, is of major importance in meat research. It has been suggested that in ruminants, grazing has potential beneficial effects on PUFA/SFA and *n*-6/*n*-3 ratios, increasing the PUFA and CLA contents while decreasing the SFA concentration in beef (French et al., 2000). Although several factors may influence fatty acid composition and CLA content of beef (*e.g.* seasonal variation, animal genetics and production practices), diet plays the most important role (Schmid et al., 2006). In addition, dietary factors are often linked with particular production systems (Geay et al., 2001).

Meat composition, as well as its physicochemical properties, undergoes significant changes during heat treatment. It is well known that meat composition, especially its fat content, combined with a specific cooking methodology are among the factors most affect the final quality of meat products (Serrano et al., 2007). Several authors pointed out that cooking process can affect the lipid compounds of meat, especially the fatty acid content, by changing the nutritional value of cooked products in relation to raw samples (Badiani et al., 2002). Moreover, Rodriguez-Estrada et al. (1997) reported that heat treatment can lead to undesirable changes, such as loss of essential fatty acids, reducing the nutritional value of meat, mainly due to lipid oxidation. It has been shown that a higher unsaturation index in meat may affect its oxidative stability, since the unsaturated fatty acids are more prone to oxidation (Bou et al., 2001). However, there is a great variability in changes concerning individual fatty acids in response to different cooking methods (Harris et al., 1992; Badiani et al., 2002).

Despite the various works focusing the effect of cooking on fatty acid composition (see *e.g.* Badiani et al., 2004; Maranesi et al., 2005; Sarriés et al., 2009), as far as we know, no data has been reported regarding the effect of cooking on CLA isomeric distribution in beef fat, which is only achieved by HPLC using three silver-ion columns in series (Prates & Bessa, 2009). Moreover, it is not well known if CLA enriched meat is more susceptible to the thermal processes of household cooking methods. Thus, the aim of this work was to investigate the effect of common culinary practices (boiling, microwaving and grilling) on meat fatty acid composition, with special emphasis on the detailed isomeric distribution of CLA, in meat from cattle allocated to two feeding regimens (concentrate or pasture), in order to obtain beef with distinct levels of PUFA and CLA. In addition, true retention (TR) values of these food components for the studied cooking processes were calculated and compared. Finally, the influence of these household cooking methods on the oxidative stability and nutritional value of beef intramuscular fats was also assessed.

8.2 MATERIALS AND METHODS

8.2.1 Reagents

Analytical and liquid chromatographic grade chemicals were purchased from Merck Biosciences (Darmstadt, Germany). Sodium methoxide (0.5 M solution in anhydrous methanol) was obtained from Sigma-Aldrich Ltd. (St. Louis, MO, USA).

FAME standard mixtures were acquired from Nu-Chek-Prep Inc. (Elysian, MN, USA) and Supelco

Inc. (Bellefonte, PA, USA). Commercial standards of individual CLA isomers (*c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11) as methyl esters were purchased from Matreya Inc. (Pleasant Gap, PA, USA). Additional standards of individual (*t*8,*c*10 and *c*11,*t*13) and mixtures (*cis*,*trans*, *trans*,*cis* and *trans*,*trans* from 7,9 to 12,14) of CLA isomers were prepared as methyl esters according to the procedure described by Destaillats and Angers (2003).

8.2.2 Animals and feeding

The animal experiment was conducted at EZN facilities (Vale de Santarém, Portugal). Sixteen young bulls from Alentejano purebred EZN herd, born between March and July of 2003, were used. After weaning, in February 2004, 8 young bulls were transferred to a feedlot and fed at 2.75% live body weights, on a diet consisting of 70% of concentrate feed and 30% of molasses-fibrous cubes. The ingredient, proximate and fatty acid compositions of the concentrate feed was reported previously by Alfaia et al. (2009a). The animals were slaughtered with approximately 600 kg of live body weight (September and October 2004). One of the animals failed to growth at normal rates and was removed from the experiment. The other 8 bulls were maintained 15 months at extensive management on pasture lands [alluvium land nearby Tagus river (39º07'N; 8º43'W) grazing on spontaneous pastures and summer triticalis and maize stubbles] until slaughtered in June 2005.

Animals were slaughtered at EZN experimental abattoir by exsanguination after stunning with cartridge-fired captive bolt stunner. Carcasses were chilled at 10 ºC/24 h and kept refrigerated at 2 ºC/5 days. *Longissimus lumborum* muscle samples (*ca.* 200 g) were collected, vacuum packed and stored frozen at −70 ºC until analysis.

8.2.3 Cooking treatments and preparation of samples

Frozen muscle samples were thawed overnight at 4 ± 2 ºC, trimmed of connective and adipose tissues, and sliced into cuts with 2.5 cm thickness. The cuts were subjected to each of the cooking treatments used (boiling, microwaving and grilling), while the raw cuts were sampled directly as the uncooked control. Internal temperatures were monitored continuously using thermocouples (type K), inserted into the approximate geometric centre of each cut, and connected to a digital temperature recorder (Lufft C100 Series Digital Instruments, USA). Preliminary cooking trials were conducted to determine cooking times and temperatures required to achieve a constant degree of doneness (medium or well done) for the various cooking methods. Boiling was conducted at 80 ºC during 60 min in a water bath (well degree of doneness). For microwave cooking, the samples were placed on a ceramic container in a Pyrex® pan in the centre of the carousel of a microwave oven (2450 MHz, 900 W variable power, Mod. AVM 559, Whirpool®, USA), set at 750 W. Two heating cycles of 1 min

45 s were used and the cut was turned over between cycles (well degree of doneness). Grilling was performed at 225 ºC, using a large preheated electric grill. The cuts were placed on a turnable rack, arranged approximately 4 cm above the heating elements and turned every 2 min during 30 min (medium degree of doneness). Final internal temperature was the maximum temperature reached by each cut upon removal from the heat source. After cooking and cooling (30 min at 20-22 ºC), the cuts were manually wiped with a paper towel to remove visible exudates. All cuts were weighed before and after cooking in order to determine the percentage of cooking loss.

8.2.4 Analysis of fatty acids and CLA isomers

8.2.4.1 Lipid extraction and methylation of fatty acids

Intramuscular fat was extracted from lyophilised meat samples (–60 °C and 2.0 hPa), using a lyophilisator Edwards Modulyo (Edwards High Vacuum International, UK), for total lipid determination according to Fritsche et al. (2000). Total lipids were measured gravimetrically, in duplicate, by weighting the fatty residue obtained after solvent evaporation.

Fatty acids were directly extracted and methylated from meat samples by a one-step procedure (adapted from Christie et al., 2001). Fatty acids were converted to FAME by a combined transterification procedure with NaOH in anhydrous methanol (0.5 M) followed by HCl/methanol (1/1 v/v) at 50 °C during 30 and 10 min, respectively, according to Raes et al. (2001).

8.2.4.2 Determination of fatty acid composition

FAME were analysed using a HP6890A gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with GC-FID and a fused-silica capillary column (CP–Sil 88; 100 m × 0.25 mm i.d., 0.2 µm film thickness, Chrompack, Varian Inc., Walnut Creek, CA, USA), as described by Bessa et al. (2007). Identification was accomplished by comparison of sample peak retention times with those of FAME standard mixtures and with values published in the literature (Fritsche et al., 2000). The FAME identification of some unknown peaks was conducted by gas chromatography–tandem mass spectrometry (GC–MS/MS), using a Varian Saturn 2000 system (Varian Inc., Walnut Creek, CA, USA) equipped with a CP-Sil 88 capillary column (100 m × 0.25 mm i.d., 0.2 µm film thickness). Fatty acids were expressed as a percentage of the sum of detected fatty acids (g/100 g FAME),

assuming direct proportionality between peak area and fatty acid methyl ester weight, or in gravimetric contents (mg/g muscle), using the conversion factor for lean beef (0.920) for calculation of total fatty acids from total lipids (Weihrauch et al., 1977).

8.2.4.3 Determination of CLA isomers

The methyl esters of CLA isomers were individually separated by triple column silver-ion in series (ChromSpher 5 Lipids analytical, 250 mm × 4.6 mm i.d., 5 μm particle size, Chrompack, Bridgewater, NJ, USA), using an HPLC system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) equipped with autosampler and diode array detector adjusted to 233 nm, according to the procedure reported previously by Alfaia et al. (2006a). Identification of the individual CLA isomers was achieved by comparison of their retention times with commercial and prepared standards, as well as with values published in the literature (Fritsche et al., 2000). Total and individual CLA isomer contents in meat were determined based on the external standard technique (using *c*9,*t*11, *t*10,*c*12, *c*9,*c*11 and *t*9,*t*11 as representatives of each of the geometric groups of CLA isomers) and on the method of area normalization (AOAC 963.22, 2000). CLA isomers were expressed in gravimetric contents (mg/g muscle and mg/g fat) or as a percentage of the sum of identified CLA isomers (% total CLA).

8.2.5 Nutrient retention values

TR values for nutrients were calculated using the following formula (Murphy et al., 1975): TR (%) = [(nutrient content per g of cooked food × g of food after cooking) / (nutrient content per g of raw food × g of food before cooking)] × 100.

8.2.6 Lipid oxidation

Lipid stability of both raw and cooked cuts was immediately evaluated after cooling (30 min at 20-22°C), by measuring the TBARS, based on the method of Grau et al. (2000), with the resulting colour measured at 532 nm in a UV/VIS Spectrophotometer (Pharmacia LKB Biochrom Ltd., UK). A standard curve was constructed with 1,1,3,3–tetraethoxypropane (Fluka Neu Ulm, Germany). The results were expressed as mg of malondialdehyde (MDA) equivalents per kg of meat.

8.2.7 Statistical analysis

The data were analysed using the MIXED procedure of Statistical Analysis Systems Institute (SAS, 2004). The model included the fixed effects of animal diet and cooking treatment, as well as the interaction between diet and treatment. Because no significant diet × treatment interactions ($P > 0.05$) were observed, it was removed from the model. Treatment effect was evaluated as repeated measure on the animal within diet. Least squares means were generated using the LSMEANS option and separated, when significant ($P < 0.05$), using the probability difference procedure (PDIFF option).

8.3 RESULTS AND DISCUSSION

Detailed data on the level of intramuscular fat and fatty acid composition, including CLA isomeric profile, in L. lumborum muscle of Alentejano purebred bulls fed on different diets are reported in Alfaia et al. (2009a). Since no significant interactions ($P > 0.05$) between animal diets and cooking treatments were found, only the cooking methods effects (boiling, microwaving and grilling) on beef properties are presented and discussed here. Data on the effects of diet on fatty acid composition, including CLA isomeric profile, are described elsewhere (Alfaia et al., 2009a). The absence of interactions indicates that meat from bulls fed on concentrate was not more susceptible to changes induced by heating than that from animals grazing on pasture. Thus, the meat samples used in this study, from both concentrate (higher content of MUFA, mainly oleic acid − 18:1*c*9) and pasture (higher contents of *n*-3 PUFA, mainly α–linolenic acid − 18:3*n*-3, and CLA, on an mg/g fat basis) fed cattle (Alfaia et al., 2009a), were analyzed together. However, it is interesting to note that the PUFA enriched meat is not more sensitive to thermal processes when compared with that enriched in MUFA. It is possible that either the high contents of CLA isomers (Yu et al., 2002) or the high levels of α–tocopherol (data not shown), or both, provide antioxidant protection for PUFA in this meat.

8.3.1 Heat-processing parameters, cooking loss and selected nutrients

The three different meat cooking methods used in this study differed in the processing parameters used (cooking time and temperature). These conditions were chosen in order to enable the meat to attain a medium (final internal temperature of 71.2 °C for grilling) to a well (79.0 °C and 92.1 °C for boiling and microwaving, respectively) degree of doneness (Table 25). Lorenzen et al. (1999) observed that consumers preferred medium and well done meat to rare done. Similar observations were made by Savell et al. (1999), who also reported that consumers most frequently cook steaks to the well done stage. In our study, the final internal temperature reached were significantly different ($P < 0.05$) among the cooking processes. In addition, the cooking losses were also affected ($P < 0.05$)

by the cooking method used. It is well known that losses depend on the mass transfer process during thermal treatment, which is directly related to the cooking procedure (*e.g.* heating rate, final cooking temperature, time) and to the properties of raw meat *(e.g.* moisture, fat, and protein composition, size, pH) (Serrano et al., 2007; Gerber et al., 2009). García-Segovia et al. (2007) observed that changes in cooking losses tended to be linear with time, with an increase with temperature. Indeed, we observed that increasing final internal temperature (grilling < boiling < microwaving) resulted in greater cooking losses, as more moisture have been lost by evaporation, during processing. In addition to final internal temperature, it has been suggested that water losses tend to be higher after microwave heating and lower after grilling due to the absence of crust formation during microwave cooking (Serrano et al., 2007). The mean values obtained here for cooking losses are in agreement with those reported in similar works (range 15-40%) (Sheard et al., 1998).

Table 25. Effect of cooking methods on heat-processing parameters, cooking loss and selected nutrients of *L. lumborum* muscle from Alentejano purebred bulls (n = 15).

	Treatments				SEM	P^A
	Raw	Boiling	Microwaving	Grilling		
Final internal temperature (°C)	–	79.0[a]	92.1[b]	71.2[c]	0.330	***
Cooking loss (%)	–	39.9[a]	42.7[b]	32.6[c]	0.551	***
Selected nutrients						
Moisture (%)	74.8[a]	61.2[b]	54.5[c]	62.3[b]	0.461	***
Total lipids (%)	1.25[a]	2.17[b]	2.61[c]	2.21[b]	0.137	***

[A] Statistical probability of treatment: 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 percentages of moisture and total lipids for raw and cooked *L. lumborum* muscle of Alentejano purebred bulls are also presented in Table 25. Compared with the raw meat control, cooking led to a significant loss of moisture and, consequently, to a significantly higher intramuscular fat content, with significant differences ($P < 0.05$) among treatments (microwaving > boiling = grilling). Badiani et al. (2002) reported that most nutrients increased their concentration as a consequence of moisture loss through cooking. Significant losses were more evident in microwave heating, as explained above, which induced a notable decrease in moisture level (20.3%). The values of total lipids obtained in this study (1.2% for raw meat and 2.2 to 2.6% for cooked meat) were generally lower than those reported in the literature for trimmed beef (Wahrmund-Wyle et al., 2000). However, our results support the fact generally accepted that total moisture content decreases as fat content

increases (Woolsey & Paul, 1969). Finally, according to the Food Advisory Committee (1990) criteria (< 5% fat), both raw and cooked beef are considered lean meat.

8.3.2 Intramuscular fatty acid composition

Fatty acid composition (% of total fatty acids) in raw and cooked samples of *L. lumborum* muscle from Alentejano purebred bulls is presented in Table 26. In decreasing order of percentage, the major fatty acids in intramuscular fat of raw and cooked meat, were 18:1*c*9 (23–26%), palmitic (16:0, 20–21%), stearic (18:0, 17–19%) and linoleic (18:2*n*-6, 10–13%) acids. All the cooking methods had a moderate impact on the fatty acid profile of beef, being the content of 16 of the 34 fatty acids analysed affected ($P < 0.05$), with the exception of 12:0, 16:1*c*9 (only affected by grilling) and 20:1*c*11 (affected by boiling and microwaving), by the thermal treatments. In addition, no novel fatty acid residues or other artefacts due to cooking of samples were detected. Some SFA, namely 14:0, 16:0, 17:0 and 18:0, as well as 18:1*c*9 as MUFA, were significantly higher ($P < 0.05$) in cooked meat samples than in the uncooked meat control. In contrast, the percentages of 18:2*n*-6 and almost all long-chain *n-6* fatty acids decreased significantly ($P < 0.05$) in cooked beef compared to raw meat. Within the *n-3* PUFA, no apparent cooking effects were observed, except for DPA (22:5*n-3*), which had a lower content in cooked beef relative to raw meat. Variations in the fatty acid composition of raw and cooked samples have already been reported by Echarte et al. (2003), who observed significant differences in the fatty acid profile for both chicken and beef patties. According to these authors, with microwave heating only 8 of 18 fatty acids did not change, including oleic and eicosapentaenoic acids, while linoleic, α–linolenic and docosahexaenoic acids decrease. Moreover, Scheeder et al. (2001) found slightly changes in fatty acid composition during grilling of beef patties. In contrast, minor variations induced by heating in fatty acid composition of beef lipids were reported, among others, by Harris et al. (1992). However, Duckett and Wagner (1998) reported great differences in fatty acid composition between lipid fractions (neutral and polar lipids), with changes most evident in the polar lipid fraction, where the PUFA are primarily located. In fact, several mechanisms occurring during cooking, such as water loss and lipid oxidation, diffusion and exchange, can lead to relative changes in some fatty acids (Dal Bosco et al., 2001; Rodriguez-Estrada et al., 1997). However, in our experimental conditions, the changes obtained in fatty acids are likely due to the higher susceptibility of PUFA to oxidative degradation, relative to the other fatty acids, since our meat was relatively lean (1.2% of total lipids) and the cooking conditions were relatively high (medium-well done). Finally, the percentages of individual TFA remained unaffected ($P > 0.05$) by the cooking treatment, except for 18:1*t*11, which was higher ($P < 0.05$) in cooked beef than in raw

meat. *Trans* octadecenoic acids are the major intermediates formed during rumen biohydrogenation of C18 PUFA (Bessa et al., 2000). In this study, the most abundant octadecenoic acid found was

Table 26. Effect of cooking methods on fatty acid composition (g/100 g FAME) of *L. lumborum* muscle from Alentejano purebred bulls (n = 15).

Fatty acids	Treatments				SEM	P^A
	Raw	Boiling	Microwaving	Grilling		
10:0	0.10[a]	0.08[b]	0.08[b]	0.08[b]	0.006	**
12:0	0.05[ab]	0.05[b]	0.06[a]	0.06[a]	0.004	*
14:0	1.52[a]	1.82[b]	1.81[b]	1.93[b]	0.132	**
14:1*c*9	0.15	0.17	0.16	0.19	0.024	ns
15:0	0.37	0.42	0.41	0.42	0.022	ns
16:0	19.52[a]	20.96[b]	20.70[b]	21.35[b]	0.603	**
16:1*c*9	2.01[a]	2.20[ab]	2.09[a]	2.36[b]	0.177	*
17:0	0.94[a]	1.07[b]	1.08[b]	1.08[b]	0.042	*
17:1*c*9	0.67	0.63	0.64	0.67	0.044	ns
18:0	16.66[a]	18.20[b]	18.69[b]	17.65[b]	0.645	**
18:1*t*6+*t*8	0.12	0.13	0.14	0.14	0.013	ns
18:1*t*9	0.17	0.18	0.18	0.19	0.013	ns
18:1*t*10	0.62	0.60	0.66	0.68	0.169	ns
18:1*t*11	1.08[a]	1.29[b]	1.33[b]	1.37[b]	0.099	*
18:1*t*12	0.30	0.31	0.32	0.31	0.020	ns
18:1*c*9	23.38[a]	25.78[b]	25.37[b]	26.44[b]	1.281	**
18:1*c*11	2.19	2.08	2.10	2.10	0.104	ns
18:1*c*12	0.40	0.38	0.37	0.38	0.022	ns
18:1*c*13	0.16	0.17	0.16	0.18	0.020	ns
18:1*c*15	0.07	0.08	0.08	0.08	0.012	ns
18:2*t,t+c/t* [a]	0.36	0.30	0.31	0.32	0.023	ns
18:2*t*11,*c*15	0.19	0.25	0.25	0.28	0.056	ns
18:2*n*−6	13.25[a]	10.36[b]	10.24[b]	9.50[b]	1.009	**
18:3*n*−6	0.09[a]	0.07[b]	0.07[b]	0.07[b]	0.007	*
18:3*n*−3	3.14	2.34	2.54	2.42	0.596	ns
20:0	0.08	0.08	0.09	0.08	0.005	ns
20:1*c*11	0.14[a]	0.15[b]	0.16[b]	0.15[ab]	0.011	*
20:2*n*−6	0.13[a]	0.10[b]	0.10[b]	0.09[b]	0.010	**
20:3*n*−6	0.94[a]	0.73[b]	0.70[b]	0.63[b]	0.086	**
20:4*n*−6	3.94[a]	2.94[b]	2.86[b]	2.65[b]	0.378	**
20:5*n*−3	1.17	0.76	0.83	0.83	0.204	ns
22:4*n*−6	0.26	0.23	0.22	0.19	0.034	ns
22:5*n*−3	1.66[a]	1.13[b]	1.18[b]	1.12[b]	0.215	*
22:6*n*−3	0.12	0.09	0.10	0.09	0.012	ns

[A] Statistical probability of treatment: 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; [a] This peak may include several 18:2 *t,t*, *c,t* and *t,c* isomers.

18:1*t*11, followed by the 18:1*t*10. Recent studies on rabbits suggested that butter enriched with 18:1*t*10, but not with 18:1*t*11, had deleterious effects on plasma lipids and lipoprotein metabolism (Roy et al., 2007).

Data on partial sums (wt %) of intramuscular fatty acids, in both raw and cooked samples, are shown in Table 27. The patterns reflect the values described above for the major individual fatty acids of each group. A significant increase ($P < 0.05$) in the relative proportion of SFA (+3.4-3.7%), as well as of MUFA (+1.9-3.4%), occurred after cooking, which is mainly due to an increase in 16:0, 18:0

Table 27. Effect of cooking methods on partial sums of fatty acids (g/100 g FAME), nutritional fatty acid ratios and values of TBARS (mg of malonaldehyde equivalents/kg of meat) of *L. lumborum* muscle from Alentejano purebred bulls ($n = 15$).

	Treatments				SEM	P^A
	Raw	Boiling	Microwaving	Grilling		
Partial sums						
∑ SFA	39.30^a	42.76^b	43.00^b	42.73^b	1.018	**
∑ MUFA	29.11^a	31.57^b	31.05^b	32.48^b	1.569	**
∑ TFA	2.85	3.08	3.20	3.30	0.195	ns
∑ PUFA	24.69^a	18.77^b	18.84^b	17.60^b	1.981	**
∑ *n*–6 PUFA	18.60^a	14.43^b	14.19^b	13.13^b	1.486	**
∑ *n*–3 PUFA	6.09^a	4.34^b	4.65^b	4.47^b	0.994	*
∑ unidentified	4.04^a	3.82^b	3.91^{ab}	3.89^{ab}	0.277	*
Ratios						
PUFA/SFA	0.65^a	0.46^b	0.45^b	0.42^b	0.060	**
n–6/*n*–3	6.41	6.47	6.14	5.91	1.320	ns
Lipid oxidation						
TBARS	0.06^a	0.10^b	0.07^a	0.06^a	0.010	*

[A] Statistical probability of treatment: 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.

Σ SFA = sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0,18:0 and 20:0.

Σ MUFA = sum of *cis*-MUFA = sum of 14:1*c*9, 16:1*c*9, 17:1*c*9, 18:1*c*9, 18:1*c*11, 18:1*c*12, 18:1*c*13, 18:1*c*15 and 20:1*c*11.

Σ TFA = sum of 18:1*t*6+*t*8, 18:1*t*9, 18:1*t*10, 18:1*t*11, 18:1*t*12, 18:2*t*9*t*12 and 18:2*t*11*c*15.

Σ PUFA = sum of 18:2*n*–6, 18:3*n*–6, 18:3*n*–3, 20:2*n*–6, 20:3*n*–6, 20:4*n*–6, 20:5*n*–3, 22:4*n*–6, 22:5*n*–3 and 22:6*n*–3.

Σ *n*–6 PUFA = sum of 18:2*n*–6, 18:3*n*–6, 20:2*n*–6, 20:3*n*–6, 20:4*n*–6 and 22:4*n*–6.

Σ *n*–3 PUFA = sum of 18:3*n*–3, 20:5*n*–3, 22:5*n*–3 and 22:6*n*–3.

PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n*–6, 18:3*n*–6, 18:3*n*–3, 20:2*n*–6, 20:3*n*–6, 20:4*n*–6, 20:5*n*–3, 22:4*n*–6, 22:5*n*–3 and 22:6*n*–3)/(sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)].

n–6/*n*–3 = *n*–6/*n*–3 ratio [(sum of 18:2*n*–6, 18:3*n*–6, 20:2*n*–6, 20:3*n*–6, 20:4*n*–6 and 22:4*n*–6)/(sum of 18:3*n*–3, 20:5*n*–3, 22:5*n*–3 and 22:6*n*–3)].

and 18:1*c*9, respectively. Cooked beef had lower concentrations of PUFA (-5.9-7.1%) than raw meat, due to a significant loss ($P < 0.05$) of some *n-6* and *n-3* PUFA. This is consistent with the results of Maranesi et al. (2005) that compared cooked and uncooked lamb rib-loins. The changes observed in the partial sums of fatty acids in the work reported here are, as explained above for individual fatty acids, likely due to the higher susceptibility of PUFA to oxidative degradation, relative to the other fatty acids. From a nutritional perspective, the quantitative fatty acid composition (expressed as mg/100 g muscle) was determined in both raw and cooked beef (data not shown). As expected, cooking produced significant increases ($P < 0.05$) in fatty acids contents, with the exception of 20:5*n*-3. In general, the microwave cooking provided higher contents of fatty acids than boiling or grilling beef, which likely resulted from the higher moisture loss. The mean content of the health promoting *n*-3 PUFA in cooked cuts was 84.5 mg/100 g muscle (77.5, 77.7 and 98.4 mg/100 g muscle for boiling, grilling and microwave, respectively), which represents a valuable contribution (from 38.8 to 49.2%) to the coverage of daily requirements in *n-3* fatty acids (200 mg, for both sexes), according to the British Department of Health (1994). The ratios of PUFA/SFA and *n-6*/*n-3*, which are indices widely used to evaluate the nutritional value of fat for human consumption, are presented in Table 27. According to some nutritional recommendations (British Department of Health, 1994), the PUFA/SFA ratio in human diets should be above 0.45 and, within the PUFA, the *n-6*/*n-3* ratio should not exceed 4.0. In the present experiment, cooked samples showed significantly lower ($P < 0.05$) PUFA/SFA ratios, with values close to the lower recommended limit. In addition, the cooking method did not change the values of the *n-6*/*n-3* ratio in meat. The value for the *n-6*/*n-3* ratio reported here is, in fact, the average for meat from concentrate-fed (11.6) and pasture-fed (1.89) animals.

In order to evaluate the correct increase or loss/degradation of food components during cooking, the TR values of nutrients were calculated (Table 28). The mean value for TR figures of moisture increased ($P < 0.05$) from microwaving (41.8%) to boiling (49.2%) and grilling (56.1%). In contrast to moisture, most of the intramuscular fat was retained after cooking. The TR values of total lipids were higher ($P < 0.05$) for both microwave and grilled meat when compared with boiled meat. As acknowledge by several authors, TR values of beef lipids can vary widely, namely from 91-160% for boiling and 71-125% for roasting (Harris et al, 1992; Bragagnolo & Rodriguez-Amaya, 2003). This variability has been explained by the presence of unpredictable levels of subcutaneous and intermuscular fats, which liquefying during cooking and absorption by the lean tissue leads to TR values higher than 100% (Smith et al., 1989; Bragagnolo & Rodriguez-Amaya, 2003). However, when only intramuscular fat is present, which was the case in this study, a 100% TR is expected, unless fat is partially degraded or lost to the cooking medium (TR values lower than 100%). In

Table 28. True selected nutrients and fatty acid retention values (%) for cooked samples of *L. lumborum* muscle from Alentejano purebred bulls (n = 15).

	Treatments			SEM	P^A
	Boiling	Microwaving	Grilling		
Selected nutrients					
Moisture	49.21[a]	41.79[b]	56.13[c]	0.706	***
Total lipids	106.60[a]	121.69[b]	120.89[b]	3.987	***
Fatty acids					
10:0	93.54[a]	108.38[b]	99.60[ab]	6.193	**
12:0	95.88[a]	133.85[b]	136.28[b]	10.764	***
14:0	129.33	150.87	157.46	14.116	ns
14:1*c*9	125.50	141.36	167.99	18.814	ns
15:0	124.06	144.74	146.27	12.658	ns
16:0	116.15[a]	133.36[b]	135.22[b]	6.940	*
16:1*c*9	124.95	143.59	152.33	13.348	ns
17:0	128.35	151.97	148.73	11.887	ns
17:1*c*9	101.31[a]	113.14[b]	114.90[b]	5.784	*
18:0	118.10[a]	140.34[b]	129.91[ab]	7.428	**
18:1*t*6+*t*8	118.91	143.06	140.77	12.284	ns
18:1*t*9	114.79	137.76	135.16	12.392	ns
18:1*t*10	126.82	146.44	151.75	15.088	ns
18:1*t*11	124.36	147.04	144.92	11.910	ns
18:1*t*12	109.64	131.48	127.76	9.776	ns
18:1*c*9	121.50[a]	140.02[b]	142.71[b]	9.076	*
18:1*c*11	102.65[a]	99.91[a]	115.92[b]	6.640	**
18:1*c*12	106.37[a]	124.30[b]	124.41[b]	10.185	*
18:1*c*13	120.33	139.95	150.49	18.535	ns
18:1*c*15	111.98	140.53	132.60	18.215	ns
18:2*t*9*t*12	91.68	106.63	107.86	8.910	ns
18:2*t*11*c*15	132.87	162.48	149.11	18.352	ns
18:2*n*−6	85.71	99.41	90.48	6.746	ns
18:3*n*−6	87.14[a]	109.25[b]	94.61[ab]	7.878	*
18:3*n*−3	89.83[a]	109.13[b]	99.90[ab]	4.908	**
20:0	117.61[a]	139.77[b]	128.73[ab]	7.858	*
20:1*c*11	105.28[a]	125.96[b]	120.64[b]	7.623	**
20:2*n*−6	86.53	97.76	90.07	7.078	ns
20:3*n*−6	84.38	91.38	85.37	8.964	ns
20:4*n*−6	85.19	90.24	83.39	9.731	ns
20:5*n*−3	72.24	95.00	98.29	10.606	ns
22:4*n*−6	92.73	108.62	95.36	10.132	ns
22:5*n*−3	78.83	90.89	88.09	9.174	ns
22:6*n*−3	90.76	104.55	94.77	9.688	ns
Partial sums					
∑ SFA	117.90[a]	137.69[b]	134.38[b]	7.347	*
∑ MUFA	119.26[a]	137.39[b]	140.50[b]	8.707	*
∑ TFA	118.52	141.03	142.68	11.307	ns
∑ PUFA	86.04	99.71	90.61	7.605	ns
∑ *n−6* PUFA	85.33	95.17	89.57	7.286	ns
∑ *n−3* PUFA	86.80	102.46	95.89	7.794	ns

[A] Statistical probability of treatment: 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.

contrast, lipid TR values higher than 100% suggest a higher extractability of lipids bound to lipoproteins from the cooked tissues, since heating can denature the muscle lipoproteins and release the bound lipids (Woolsey & Paul, 1969). Moderate differences in TR values for individual (in 12 out of 34 fatty acids) and partial sums (SFA and MUFA) of fatty acids were observed in the different cooking procedures. Moreover, the mean TR values found for individual fatty acids, which varied widely from 72.2 to 168.0%, tended to be lower in boiling than in grilling or microwaving. Since microwave cooking and grilling proceed in the absence of water, these cooking methods probably allowed for a high retention of fatty acids. The TR values obtained for the general individual SFA, MUFA and TFA, as well as for their partial sums, were higher than 100%, whereas for individual and for the sum of PUFA, the values were found to be generally lower (from 72.2 to 109.2%). As explained above, the higher values of TR for SFA, MUFA and TFA are likely due to the great extractability of lipids from the cooked tissues. Meanwhile, within some individual *n*-6 and *n*-3 PUFA, the TR values were below or around 100%, suggesting a little loss or degradation of these fatty acids during cooking. Gandemer (1992), quoted by Maranesi et al. (2005), also reported TR values lower than 100% for C20 and C22 PUFA, which was explained by the oxidative degradation of fatty acids.

8.3.3 Intramuscular CLA isomeric profile

Detailed data of CLA contents and its isomeric distribution in intramuscular fat of raw and cooked beef are displayed in Table 29. Total CLA content (mg/g muscle) were significantly higher ($P < 0.001$) in cooked beef than in raw cuts as a result of moisture loss. In fact, the mean values of total CLA in raw beef amounted to 0.05 mg/g muscle. This value was increased after cooking up to 0.08 mg/g for grilling or 0.09 mg/g for boiling and microwave heating. Previous studies also reported higher values of CLA in cooked beef when compared with uncooked ground beef (Shanta et al., 1994). Regardless of the cooking method employed, the heating methods with higher internal temperatures presented the highest CLA concentrations, probably due to the higher cooking losses. However, if CLA contents are expressed on a basis of mg/g of fat, no significant differences ($P > 0.05$) were observed between raw and cooked meats.

The CLA isomeric profile showed a clear predominance of the bioactive *c*9,*t*11 isomer in all treatments (67.2-67.4% of total CLA), followed by the *t*11,*t*13 (7.5-8.5%) and *t*7,*c*9 (6.3-6.9%) isomers. The *t*11,*t*13 isomer was the second most predominant CLA isomer, instead of the *t*11,*c*13 isomer in meat from pasture-fed animals, or the *t*7,*c*9 isomer in meat from concentrate-fed bulls (Dannenberger et al., 2005). This CLA isomeric pattern may result from the fact that an heterogeneous group of animals was used in this trial. We showed before that pasture feeding

increases the proportion of the *t*11,*c*13, *t*11,*t*13 and *t*12,*t*14 CLA isomers and decreases the percentage of the *t*7,*c*9 isomer in beef lipids, when compared with concentrate feeding (Alfaia et al., 2009a). Although CLA has been described to be more sensitive than 18:2*n*-6 to oxidation and even to isomerisation during heat treatments (Yang et al., 2000), minor changes in the CLA isomeric profile of beef were observed as a result of cooking. In fact, only 3 (*t*7,*t*9, $P < 0.001$; *t*9,*t*11 and *c*11,*t*13, $P < 0.05$) of the 15 CLA isomers identified in beef were changed ($P < 0.05$), when subjected to the three cooking methods. No changes were identified after cooking in the relative proportions of the bioactive *c*9,*t*11 CLA isomer, in contrast to its precursor in muscle, the 18:1*t*11. Likewise, the other known bioactive CLA isomer, *t*10,*c*12, was not influenced by heating treatments ($P > 0.05$). The

Table 29. Effect of cooking methods on total (mg/g muscle) and specific (mg/g fat) CLA contents and its individual isomers (% total CLA) of *L. lumborum* muscle from Alentejana purebred bulls ($n = 15$).

	Treatments				SEM	P^A
	Raw	Boiling	Microwaving	Grilling		
Total CLA	0.05[a]	0.09[b]	0.09[b]	0.08[c]	0.007	***
Specific CLA	3.59	3.95	3.53	3.45	0.254	ns
CLA isomers						
*t*12,*t*14	2.90	3.36	3.58	3.53	0.775	ns
*t*11,*t*13	7.45	8.19	8.48	8.03	1.694	ns
*t*10,*t*12	0.63	0.61	0.59	0.64	0.074	ns
*t*9,*t*11	4.31[a]	3.78[b]	3.44[b]	3.73[b]	0.213	*
*t*8,*t*10	0.26	0.26	0.27	0.25	0.019	ns
*t*7,*t*9	0.92[a]	0.73[b]	0.66[b]	0.71[b]	0.044	***
*t*6,*t*8	0.21	0.19	0.23	0.19	0.022	ns
total *trans,trans*	16.69	17.11	17.25	17.08	2.454	ns
c/*t*12,14	0.94	0.90	0.97	0.97	0.099	ns
*t*11,*c*13	4.48	4.90	4.87	4.51	0.836	ns
*c*11,*t*13	0.75[a]	0.52[b]	0.55[b]	0.52[b]	0.130	*
*t*10,*c*12	1.12	1.08	1.19	1.13	0.154	ns
*c*9,*t*11	67.35	67.34	67.22	67.23	2.359	ns
*t*8,*c*10	1.18	1.47	1.46	1.18	0.135	ns
*t*7,*c*9	6.91	6.26	6.29	6.88	1.003	ns
total *cis/trans*	82.72	82.38	82.55	82.49	2.406	ns
total *cis,cis* (*c*9,*c*11)	0.42	0.49	0.45	0.47	0.059	ns

[A] Statistical probability of treatment: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; means in the same row with different superscripts are significantly different ($P < 0.05$).

percentage of this isomer in meat, which was residual in all treatments, ranged between 1.1 and 1.2% of total CLA. In addition, no significant effect ($P > 0.05$) in total *cis/trans* (*cis,trans* and *trans,cis*), total *cis,cis*, as well as in total *trans*,*trans* CLA isomers, were obtained.

After boiling, the CLA isomeric distribution expressed as mg/g fat (data not shown) varied significantly ($P < 0.05$) for total *trans*,*trans*, *cis/trans and cis,cis* isomers. The individual percentages of *t*12,*t*14 and *c*9,*t*11 CLA isomers increased significantly ($P < 0.05$) after boiling but had no changes with microwaving or grilling. For *t*7,*t*9 and *c*11,*t*13 CLA isomers, the content decreased from raw to cooked cuts, independently to the treatment used. Moreover, the *t*9,*t*11 CLA isomer amounts also decreased significantly ($P < 0.05$) after microwaving and grilling. From a nutritional point of view, CLA content, expressed as mg/100 g muscle, has the greatest importance. As expected, significant increases ($P < 0.05$) in all CLA isomers were observed, thanks to the higher average lipid contents of cooked meat. In order to discriminate if these increases are due to a simple concentration effect, the

Table 30. True CLA retention values (%) for cooked samples of *L. lumborum* muscle from Alentejana purebred bulls ($n = 15$).

	Treatments			SEM	P^A
	Boiling	Microwaving	Grilling		
Total CLA	116.86	112.11	117.48	4.255	ns
CLA isomers					
*t*12,*t*14	119.15	126.59	124.15	7.845	ns
*t*11,*t*13	121.67	122.01	119.34	5.506	ns
*t*10,*t*12	114.98	109.27	117.77	7.594	ns
*t*9,*t*11	101.95	89.77	102.46	5.395	ns
*t*8,*t*10	109.14	106.35	103.74	7.016	ns
*t*7,*t*9	92.74	81.13	85.37	6.808	ns
*t*6,*t*8	92.92	106.19	88.86	9.508	ns
total *trans,trans*	113.88	109.18	114.55	4.942	ns
c/*t*12,14	112.25	116.26	122.77	7.194	ns
*t*11,*c*13	120.80	122.44	111.42	7.918	ns
*c*11,*t*13	105.35	90.84	87.47	7.004	ns
*t*10,*c*12	102.14	113.13	115.44	7.891	ns
*c*9,*t*11	116.75	112.01	117.50	5.598	ns
*t*8,*c*10	124.37	116.41	114.72	6.759	ns
*t*7,*c*9	118.19	110.48	120.07	7.267	ns
total *cis/trans*	115.86	111.12	116.65	4.781	ns
total *cis,cis* (*c*9,*c*11)	128.36[a]	115.73[b]	126.65[a]	7.003	*

[A] Statistical probability of treatment: 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.

TR values for the CLA isomers were calculated (Table 30). The TR values obtained for total CLA in this study, which were in the range 112.1−117.5%, are in line with the TR values obtained for total lipids (see Table 28). This observation is confirmed by the similar specific CLA contents, expressed as mg/g fat, observed between raw and cooked meats.

Regarding the individual CLA isomers, no significant differences in TR values were observed among treatments, with the exception of *c*9,*c*11 isomer, which represented the only *cis,cis* CLA isomer detected. The *c*9,*c*11 TR values obtained with microwaving cooking, with higher internal temperature, was lower ($P < 0.05$) when compared with the values observed for boiling and grilling, that presented lower internal temperatures. The differences of TR values found in this isomer could be attributed to the distinct oxidative stability of the various geometric groups of CLA isomers. It was previously shown that the stability of CLA isomers is determined by its *cis* or *trans* configuration but not by the position of their double bonds (Yang et al., 2000). According to the same authors, *cis,cis* CLA isomers are relatively more susceptible to oxidative degradation, than *cis/trans* or *trans,trans* isomers, as a result of the higher vulnerability to oxygen attack of the *cis* double bond relative to the *trans* configuration.

8.3.4 Lipid oxidation

The oxidation of lipids is one of the most important changes during food storage and processing. It depends on the PUFA content, as well on the balance between anti- and pro-oxidant compounds (Nuernberger et al., 2006). The determination of TBARS is widely used as an index of lipid oxidation. The effect of the cooking methods used in this study on lipid oxidation is also showed in Table 27. TBARS values in raw and cooked beef cuts immediately after treatments were very low, ranging from 0.06 to 0.10 mg MDA/kg meat, which is much below than 1 mg/kg meat, the threshold for off-flavour development (Tim & Watts, 1958, quoted by Jahan et al., 2004). In addition, the increase of TBARS in beef was only significant ($P < 0.05$) for boiling, when compared with the other cooking methods and raw meat. Similar results were found by Dal Bosco et al. (2001), who reported higher TBARS values for boiled rabbit meat relative to fried and roasted samples. Although meat cooking causes the disorganization of cell structures, leading to PUFA and pro-oxidant interactions with the development of lipid oxidation (Rhee, 1988), the results presented here showed that the cooking methods used induce only slight oxidative changes. Comparison with literature data is difficult due to the paucity of trials conducted in the same experimental conditions. However, our results agree with the TBARS values (0 min after stimulation) in raw and grilled pork, found by Nuernberg et al. (2006), also with high levels of PUFA. Guillevic and co-workers (2009) reported similar values of TBARS at 0 min (< 0.10 mg MDA/kg of chop) in chops of pigs fed a control diet

and with linseed diet (with high content of PUFA). It is well known that factors associated with thermal treatment (cooking method, rate and final temperature), as well as with meat composition, such as the amount and type of lipids or antioxidants, should be taking into account for the cooking effects on the rate and extent of lipid oxidation (Dal Bosco et al., 2001). In addition, it is well established that the dietary fat source affects fatty acid composition and influences the oxidative stability of meat (*e.g.* Bou et al., 2001).

8.4 CONCLUSIONS

Taken together the results presented here indicate that beef from pasture-fed bulls, enriched with PUFA and CLA, is not more susceptible to cooking induced changes of fatty acids than that from concentrate-fed bulls. Cooking losses seem to increase directly with the cooking time and internal temperature reached by meat, having the microwaving processing higher moisture lost and, consequently, a great intramuscular fat content. However, all the household cooking methods studied (boiling, microwaving and grilling) seem to increase the percentages of SFA (mainly 16:0 and 18:0) and MUFA (mainly 18:1*c*9), and decrease the relative proportions of PUFA (due to some *n*-6 and *n*-3 PUFA) in meat, in a similar way (16 of 34 fatty acids seem to be affected). Regarding the nutritional fatty acid ratios, the data suggest that heating decreases the beef PUFA/SFA ratio but does not change its *n-6*/*n-3* ratio, relative to raw meat. Total CLA contents seem to be higher in cooked beef than in raw meat, as a result of the moisture loss and, thus, fatness increase. However, minor changes in CLA profile of beef seem to occur as a result of cooking (only 3 of 15 isomers seem to be affected), with no variation of the relative proportions of the bioactive *c*9,*t*11 and *t*10,*c*12 isomers. In addition, the data suggest that the TR values of individual fatty acids (72.2-168.0%), including CLA isomers (81.1-128.4%), vary widely with all the cooking procedures studied. Finally, our results indicate that no significant oxidative changes occur immediately after treatments for microwaved and grilled meat, but a slight oxidative increase occurs for boiled meat.

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9

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

As reported in this work, we have considered two PDO veals from the north and Centre (Barrosã-PDO and Arouquesa-PDO, pasture-based system) and two PDO beef from the south (Carnalentejana-PDO and Mertolenga-PDO, semi-extensive production system) of Portugal (Figure 6) to carry out our studies. These PDO meats were primarily selected by their commercial relevance in national market and the years of harvest were 2002 and 2003. According to the specifications of PDO meats, the diets of the animals are based on natural resources or seeded forages. The application of semi-extensive and extensive systems means a correct and appropriate land use taking into account the specific climate of each region. Therefore, edapho-climatic conditions have an indirect influence on the production and quality of meat as long as they affect the quantity and quality of available forage.

The climate in Portugal is generally classified as Mediterranean; however, in the north and in the centre, there is a significant Atlantic influence, especially near the coast, in contrast to Continental influence that occurs in the south (Alentejo) (Rodrigues et al., 1998). In terms of average ambient temperature, Figures 7A and 7B, corresponding to 2002 and 2003 respectively, depict the differences between the north, the centre and the south farms, home to the studied autochthonous cattle breeds. In the last decade, were observed the 5 warmest years (1997, 1998, 2001, 2002 and 2003) from the past 150 years (Nóbrega, 2006). The year of 2003 in mainland Portugal was characterised by a very warm summer, the hottest since 1931.

Rainfall (Figures 8A and 8B) is usually higher in the north and lower in the south but the evolution of precipitation is characterised by a large interannual variability, with no clear trend in its annual values. Between 2001 and 2002, average precipitation was superior to the mean values calculated for the period of 1961-1990 (Nóbrega, 2006). This data, together with the results from the analysis concerning to the second year of sample collection (between 2003 and 2004) from the same four PDO meats, here studied and discussed, is in progress and will able us to evaluate the interannual variability.

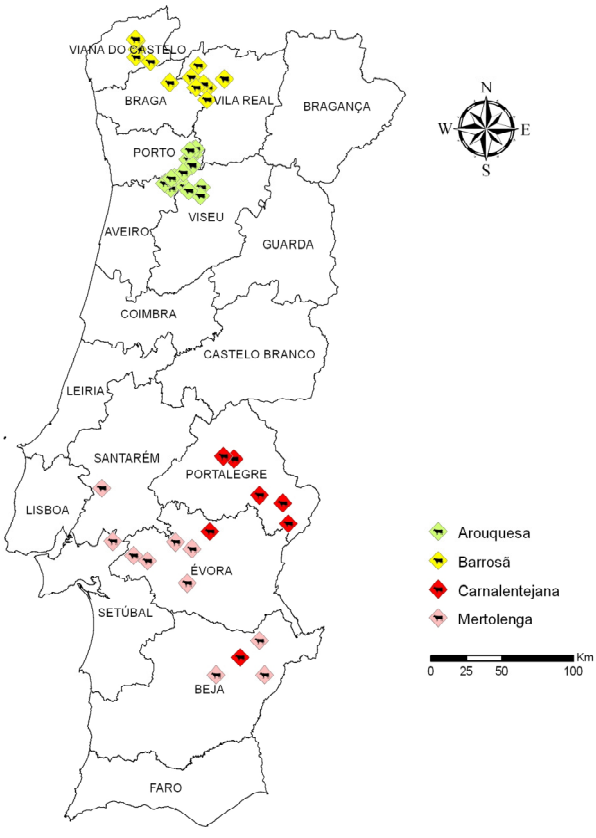

Figure 6. Geographic distribution of the PDO meats under analysis (Program ArcGIS, 2004).

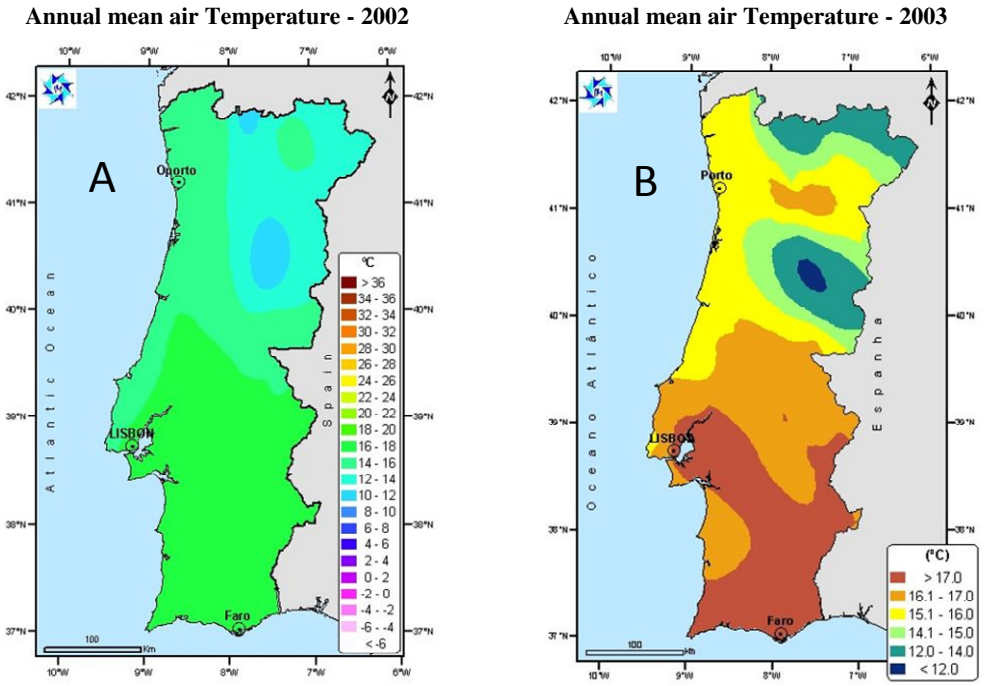

Figure 7. Geographic distribution of mean air temperature in Portugal during 2002 (A) and 2003 (B) (data from Instituto de Meterologia, http://www.meteo.pt/en/oclima/acompanhamento/).

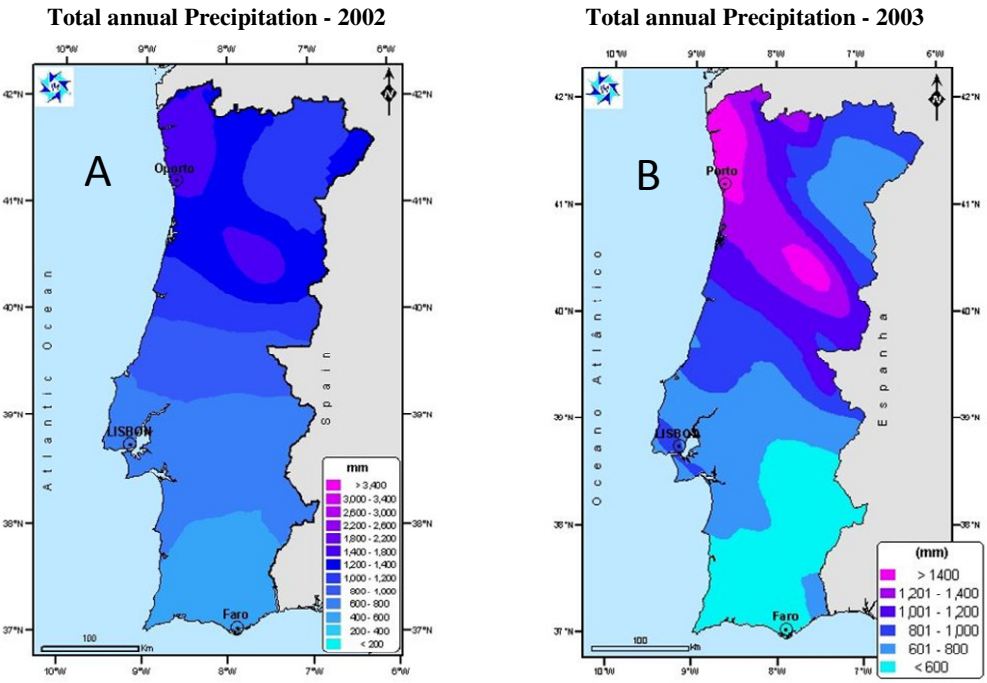

Figure 8. Geographic distribution of precipitation in Portugal during 2002 (A) and 2003 (B) (data from Instituto de Meterologia, http://www.meteo.pt/en/oclima/acompanhamento/).

As specified in previous Chapters (2 to 5), there are marked differences on animal diets within PDO meats considered in this study. In fact, while in the north and centre it is possible to produce green forage on non-irrigated land during a significant part of the year, and in "lameiros" during almost all year, in the south the grazing period for high quality grass is very short. In the seeded or spontaneous non-irrigated pastures of the south, pasture vegetative growth decreases and even ceases during the coldest part of the year (Rodrigues et al., 1998). As temperature rises at the end of the winter, more water is available in the soil, creating ideal conditions for fast vegetative growth. Later, at the end of the spring, the vegetative cycle completes with the higher temperatures and less soil water contents. Generally, feeding is based on the use of natural or improved pasture, hay, cereal straw, crop residues and cereal grain. In the north, livestock are also fed *ferrejos*, turnips and the surplus from other crops, such as potatoes and fruit. In the south, acorns are used. As a result of diets restrictions, the products of these extensive production systems are always limited in terms of beef production. In fact, the animal production system is limited to the capacity of the land to produce feed and, consequently, the production levels cannot be predetermined (Rodrigues et al., 1998). To overcome the limited natural feed resources of south producers, animal diets of purebred young bulls, Alentejana and Mertolenga, comprise a finishing period (3 to 5 months) on cereal-rich concentrate (semi-extensive system productions). So, we hope that new data help us to consolidate our findings and contribute to elucidate the influence of the quantity and quality of available forage on the production and nutritional value of meat fats.

In Chapters 2, 3, 4 and 5, lipid composition (lipid and cholesterol contents, fatty acid composition and conjugated linoleic acid isomers) and nutritional value (PUFA/SFA and *n*-6/*n*-3 ratios) of intramuscular fat in four Portuguese traditional bovine meats, Carnalentejana-PDO and Mertolenga-PDO beef, and Barrosã-PDO and Arouquesa-PDO veal, in two distinct and more important slaughter seasons (early autumn and late spring) were evaluated, as a first part of this work. Additionally, intramuscular fat characteristics of meat obtained from Alentejana × Charolais crossbred young bulls produced in a typical intensive concentrate-based system was also characterised and compared with Carnalentejana-PDO beef (Chapter 2). As reported previously, fatty acid composition of beef lipids from a specific production system represents the effects of breed, genotype, sex, age, nutrition and management (Marmer et al., 1984). Table 31 provides an integrated review of the nutritional value reported for those meats based on lipid composition and related health indices comprising both slaughter seasons and muscles studied. The results concerning to the effect of feeding system (pasture only, pasture feeding followed by 2 or 4 months of finishing on concentrate, and concentrate only) on the nutritional value of the intramuscular fatty acid profile of beef from Alentejano purebred bulls are also presented in Table 31.

The data show that PDO beef (Carnalentejana-PDO and Mertolenga-PDO) has intermediate values, between meat from grain-fed and pasture-fed cattle, in the content of total lipids, some fatty acids, *n*-6/*n*-3 ratio, total CLA and some CLA isomers, which can be explained by the semi-extensive production system of Alentejano and Mertolengo young bulls. In fact, the use of cereals (rich in *n*-6 PUFA) in concentrates shifts the meat fatty acid composition to an increased ratio of *n*-6/*n*-3 and more *t*10,*c*12 CLA when compared to animals raised on pasture. Carnalentejana-PDO and Mertolenga-PDO beef depict, in general, a fatty acid profile similar to those presented by animals fed on concentrate-based diets (PCC and CCC groups). This finding might reflect the attenuation of the grazing markers (*n*-3 PUFA, and *t*11,*t*13, *t*11,*c*13 and *t*12,*t*14 CLA isomers) of Alentejano purebred bulls from Carnalentejana-PDO by a finishing period on concentrate during 2-4 months (see Table 19, Chapter 6). In contrast, PDO veal (Arouquesa-PDO and Barrosã-PDO) seems to show typical pasture-fed characteristics (based on values of *n*-3 PUFA, total and *c*9,*t*11 CLA isomer and *n*-6/*n*-3 ratio), as presented by PPC and PPP groups, and therefore, higher nutritive quality and health added value. In the trial with Alentejano purebred bulls fed with different diets (pasture only, pasture feeding followed by 2 or 4 months of finishing on concentrate, and concentrate only), the values for CLA contents in muscle (mg/g muscle) seem to be lower for pasture-fed cattle. This fact can be explained by the lower fat content of muscle in all Alentejano purebred bulls but more prominent in grass-based animals. Nevertheless, and as reported in Chapter 6 (Table 19), no significant differences on total CLA contents (mg/g muscle) were observed for the four diets (PPP, PPC, PCC and CCC), while specific CLA contents (mg/g fat) were higher in pasture-based treatments (5.14, 5.60 and 5.76 mg/g fat for PPP, PPC and PCC groups, respectively), when compared to the concentrate-fed animals (2.65 mg/g fat for CCC diet).

Overall, Carnalentejana-PDO and Mertolenga-PDO beef intramuscular fat, relative to that from Barrosã-PDO and Arouquesa-PDO veal, depicts a low nutritional quality throughout the year. Yet, the data indicate that intramuscular fat of PDO meats is of greater nutritional quality than that of intensively produced beef from crossbred young bulls (Table 31), as a result of the beneficial grass effects on the characteristics of meat lipids. In fact, due to the lower *n*-6/*n*-3 ratio (even this ratio for Carnalentejana-PDO and Mertolenga-PDO beef is above the recommended values for human diet) and higher contents of total and *c*9,*t*11 CLA isomers, PDO meats seem to be more healthful than meat obtained from the conventional intensively-fed young bulls. These results confirm that pasture intake is a major factor affecting the content and distribution of fatty acids and CLA isomers in ruminant meat fat.

Table 31. Selected lipids and nutritional ratios of intramuscular fat of PDO meats, intensively produced beef and meat from Alentejano purebred bulls allocated to four feeding systems under controlled environmental conditions, pasture grazing (PPP), pasture with finishing on concentrate during 2 (PPC) and 4 (PCC) months, and feedlot (CCC).

	PDO meats				Intensively produced beef	Meat from Alentejano purebred bulls			
	Carnalentejana beef	Mertolenga beef	Barrosã veal	Arouquesa veal		PPP	PPC	PCC	CCC
Total lipids (g/100 g)	1.1-2.2	1.2-1.8	1.6-2.3	1.7-2.7	0.9-1.5	1.0	1.1	1.2	1.3
Total CHR (mg/100 g)	42-49	40-50	42-56	49-53	35-37	38	40	40	41
Partial sum of fatty acids (mg/100 g)									
Σ SFA	372-838	406-714	622-942	664-1296	277-585	346	441	488	469
Σ MUFA	416-814	362-613	602-891	638-1163	257-511	221	313	382	418
Σ TFA	32-72	34-85	44-71	42-81	26-62	28	40	38	42
Σ PUFA	200-269	249-274	149-227	192-236	226-228	259	208	185	227
Σ *n*-6 PUFA	184-239	204-252	110-171	128-159	212-217	161	157	153	204
Σ *n*-3 PUFA	16-34	17-50	38-57	64-76	11-14	93	51	31	24
CLA isomers (mg/100 g)									
Total CLA	4-10	3-7	11-20	12-26	2-6	2	3	4	4
*c*9,*t*11	4-8	2-5	9-17	9-20	1-4	1	2	3	3
*t*10,*c*12	0.08-0.19	0.05-0.15	0.04-0.11	0.07-0.14	0.08-0.24	0.001	0.005	0.005	0.008
Fat-soluble vitamins (μg/100 g)									
α-Tocopherol	138-226	320-462	279-392	460-681	-	660	480	280	180
γ-Tocopherol	2-17	12-14	13-16	7-15	-	7	8	8	9
β-Carotene	2-9	2-10	6-11	20-31	-	7	3	1	1
Nutritional ratios									
PUFA/SFA	0.3-0.7	0.4-0.7	0.2-0.3	0.2-0.3	0.4-0.8	0.7	0.5	0.4	0.5
n-6/*n*-3	10-14	7-15	3	2	17-20	2	3	5	9

Σ SFA = sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0,18:0 and 20:0; Σ MUFA = sum of *cis*-MUFA = sum of 14:1*c*9, 16:1*c*9, 17:1*c*9, 18:1*c*9, 18:1*c*11, 18:1*c*12, 18:1*c*13, 18:1*c*15 and 20:1*c*11; Σ TFA = sum of 18:1*t*6+*t*8, 18:1*t*9, 18:1*t*10, 18:1*t*11, 18:1*t*12, 18:2*t*9*t*12 and 18:2*t*11*c*15; Σ PUFA = sum of 18:2*n*–6, 18:3*n*–6, 18:3*n*–3, 20:2*n*–6, 20:3*n*–6, 20:4*n*–6, 20:5*n*–3, 22:4*n*–6, 22:5*n*–3 and 22:6*n*–3; Σ *n*–6 PUFA = sum of 18:2*n*–6, 18:3*n*–6, 20:2*n*–6, 20:3*n*–6, 20:4*n*–6 and 22:4*n*–6; Σ *n*–3 PUFA = sum of 18:3*n*–3, 20:5*n*–3, 22:5*n*–3 and 22:6*n*–3; PUFA/SFA = polyunsaturated/saturated ratio [(sum of 18:2*n*-6, 18:3*n*-3, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 22:2*n*-6, 20:5*n*-3, 22:4*n*-6, 22:5*n*-3 and 22:6*n*-3)/(sum of 12:0, 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0)]; *n*-6/*n*-3 = *n*-6/*n*-3 ratio [(sum of 18:2*n*-6, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6; 22:2*n*-6 and 22:4*n*-6)/(sum of 18:3*n*-3, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3)].

The consumption of 100 g of PDO meats would provide 3-10 mg of total CLA for PDO beef and 11-26 mg for PDO veal, which represents 4 to 14% and 15 to 35%, respectively, of the estimated average total CLA intake for the Portuguese population (74 mg/day; Martins et al., 2007). Concerning total CLA contents (mg/100 g muscle) in meat obtained from Alentejana × Charolais crossbred young bulls produced in a typical intensive concentrate-based system, as well as in meat from Alentejano purebred bulls fed with different diets (pasture only, pasture feeding followed by 2 or 4 months of finishing on concentrate, and concentrate only), the values were generally lower as result of the lower fat content of these meats. Overall, meat from pasture-based systems presents higher standards of nutritional quality than intensively produced beef from crossbred bullocks.

Regarding to cholesterol, all studied meats showed low average levels. Indeed, the consumption of 100 g of meat represent a cholesterol intake from 35 to 56 mg, which corresponds to less than 20% of the recommended maximum daily intake (300 mg/day, WHO, 2003).

In addition, our research group has also determined the content of fat-soluble vitamins in the PDO meats as well as in meat from Alentejano purebred bulls kept under fairly controlled conditions (preliminary data from project AGRO/512/2003, not published). The quantification of total cholesterol and lipid-soluble antioxidant vitamins could, thus, provide valuable information relating to meat quality and safety. Values for α- and γ-tocopherols in the Alentejano purebred bulls fed on concentrate-based diets (180 and 280 μg/100 g for PCC and CCC groups, respectively) were lower than those on pasture-fed cattle (480 and 660 μg/100 g for PPC and PPP groups, respectively). Yang et al. (2002) reported similar values of α-tocopherol for meat derived from grain-fed cattle (180-240 μg/100 g) and for meat originated on pasture-fed cattle (440-580 μg/100 g). The α-tocopherol values in Barrosã-PDO veal (Prates et al., 2006) and Mertolenga-PDO beef (Quaresma et al., 2007) seem closer to the values reported for meats originated on pasture feeding followed by 2 or 4 months of finishing on concentrate (PPC and PCC, respectively), while Arouquesa-PDO veal tend to have higher mean concentrations of α-tocopherol similar to those on pasture-fed cattle (PPP group) and those on grain-fed cattle receiving supra-nutritional doses of vitamin E (430-600 μg/100 g), as reported by Yang et al. (2002). These authors point out that the supplementation of pasture-fed cattle with vitamin E did not increase the levels of α-tocopherol in meat (430-610 μg/100 g). It appears that exist an upper limit for the accumulation of α-tocopherol in muscle tissues (Arnold et al., 1993; Yang et al., 2002), which in the *longissimus dorsi* seems to be around 700 μg/100 g (Arnold et al., 1993). Regarding β-carotene, in Carnalentejana-PDO and Mertolenga-PDO beef the values are between those described for β-carotene in meat from grain-fed cattle (1-3 μg/100 g) and those from cattle grazed on a good green pasture (9-22 μg/100 g) (Yang et al., 2002), reflecting the finishing period based on concentrate feeds of the production system. Barrosã-PDO and Arouquesa- PDO veal, in

particular, comprises the highest contents of β-carotene. However, the levels of β-carotene described in these traditional meats are much higher than those reached by meat from Alentejano purebred bulls fed on different diets. The differences obtained in the levels of the lipid-soluble antioxidant vitamins suggest that there may be a difference in the α-tocopherol and β-carotene levels found in pastures with different biomass compositions.

Taken together, the data reported here indicate that traditional meats under analysis depict an added nutritional value to the consumers compared to the meat from the conventional intensively-fed young bulls, supporting the fact that its production should be effectively encouraged and promoted. Moreover, these traditional meats produced according to the specifications of PDO, assure the welfare conditions to the animals, respects biodiversity and environment.

As discussed before, the diet (*e.g.* intensity of feeding, quality of pasture and concentrate composition) has a major impact on the fatty acid composition of beef. The results (Chapter 6) indicate that meat fatty acid composition was an effective parameter to discriminate between bovine feeding systems, including different finishing periods on concentrate. The same statistical methodology was applied taking together the experimental data obtained for meats from four autochthonous cattle breeds (Alentejana, Mertolenga, Barrosã and Arouquesa) and from intensive produced beef, presented previously in Chapters 2, 3, 4 and 5. We should note that some aspects were not taken into account to perform this analysis, namely the animal sex and age. The results plotted in Figure 9 clearly differentiate these meats production system under analysis (traditional and intensive). A good separation was achieved between the PDO and intensive produced meats, independently of the slaughter season (Figures 9 A, B and A+B). Nevertheless, within traditional PDO meats, differential discriminant profiles outcome as an effect of slaughter season. In fact, in Autumn (Figure 9A), the meat fatty acids with the highest discriminant power were the 15:1, on the discriminant function 1, and the 12:0, 14:0, and the *c*9,*t*11 and *t*7,*t*9 CLA isomers on the discriminant function 2 (Table 32). These selected discriminant fatty acids allow a broad separation between the Barrosã-PDO and Arouquesa-PDO veal (from the north and centre of Portugal, respectively) and the Carnalentejana-PDO and Mertolenga-PDO beef (from the south). Considering the discriminant function 1, meat obtained from Alentejana × Charolais crossbred young bulls produced in a typical intensive concentrate-based system was located on the right side of the plot, whereas the traditional PDO meats were located on the left side. Moreover, according to discriminant function 2, the Barrosã-PDO and Arouquesa-PDO veal had positive scores, while Mertolenga-PDO and Carnalentejana-PDO beef had negative scores.

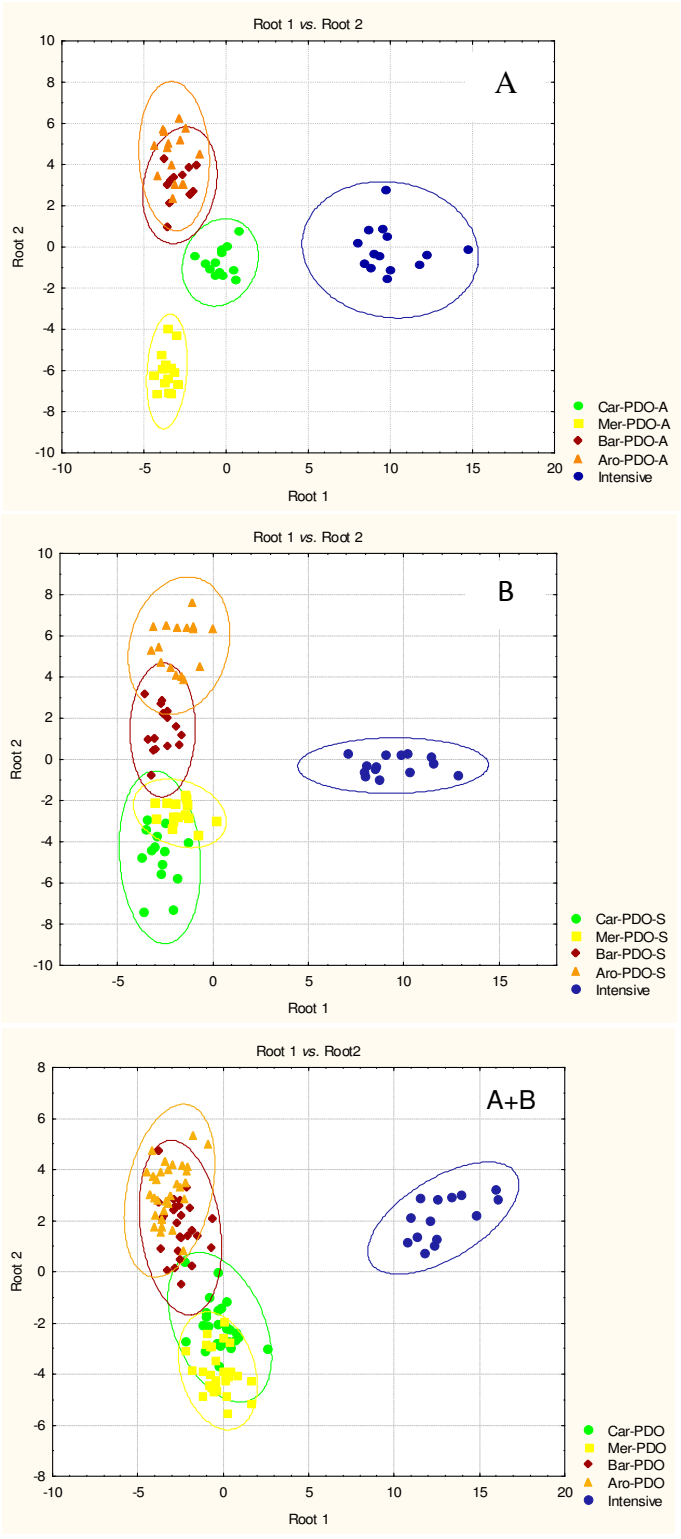

Figure 9. Plot of the discriminant functions (root 1 *vs.* root 2) for classification of traditional PDO (Car - Carnalentejana, Mer - Mertolenga, Bar - Barrosã, Aro - Arouquesa) and intensive meats according to their production system: (A) Autumn, (B) Spring and (A+B) Autumn and Spring.

Intensive produced meat remains located close to the origin. In Autumn (Figure 9A) there is a best separation between Carnalentejana-PDO and Mertolenga-PDO beef from Barrosã-PDO and Arouquesa-PDO veal, probably because the young bulls from Carnalentejana and Mertolenga breeds were finished on concentrate during 5 months (slaughtered in October), while in Spring were finished only during 3 months (slaughtered in June). In Spring (Figure 9B), meat fatty acids with the highest discriminant power was only the 15:1, on the discriminant function 1, and the 14:0, 17:0 and 20:4*n*-6 on the discriminant function 2 (Table 32). Taking data of Autumn and Spring together (Figure 9C), the most discriminant variables for separating the two types of meat was 15:1 on the discriminant function 1, and 18:1*c*9 and 18:2*n*-6, on the discriminant function 2. As previously hypothesized, data from intensive produced meats undoubtedly set part of from data of PDO meats. Nonetheless, it was also our goal to improve our knowledge on changes in intramuscular fat composition of meat after slaughter and before consumption. In fact, we could not forget that meat composition, as well as its physicochemical properties in general, could undergoes significant impact over technological and heat treatments.

Table 32. Results of canonical discriminant analysis for the PDO and intensive produced meats in Autumn and Spring: loadings of correlation matrix between predictor variables (standardized canonical coefficients) and discriminant functions (roots 1 and 2), and some statistics for each function.

	Autumn		**Spring**		**Autumn and Spring**	
	Root 1	**Root 2**	**Root 1**	**Root 2**	**Root 1**	**Root 2**
Fatty acids						
10:0			-0.209	-0.054		
12:0	-0.744	**-2.133**			0.409	-0.418
14:0	0.481	**3.702**	0.168	**2.632**	-1.141	1.306
15:0			-0.977	-1.409	-0.126	-0.980
16:0			0.042	0.350	0.745	-1.811
17:0			0.850	**2.070**		
18:0			-0.904	0.356	0.283	-1.903
20:0			0.660	0.097		
14:1	0.385	0.705				
15:1	**3.628**	0.436	**3.952**	1.327	**3.563**	1.644
16:1*c*9	-1.689	-1.242	-0.096	0.928	0.002	-1.008
17:1*c*9					-0.072	0.370
18:1*c*9	0.153	1.126	-0.532	0.058	0.894	**-3.042**
18:1*c*11	1.223	0.175	0.209	-0.204	0.587	-0.803
18:1*t*					0.493	-1.545
18:2*t*9*t*12			-0.335	0.572		
18:2*n*-6					1.192	**-4.134**
18:3*n*-6					-0.619	-0.214
18:3*n*-3			0.916	0.401	0.754	-1.144
20:2*n*-6			-0.021	0.920	-0.085	0.406
20:3*n*-6			-0.467	-1.388	0.033	-0.622
20:4*n*-6	0.439	0.731	0.154	**2.020**		
20:5*n*-3	-0.107	0.741			-0.086	0.324
22:4*n*-6	-0.071	0.123			0.430	-0.348
22:5*n*-3	-0.567	0.148	-0.532	0.929		
CLA isomers						
*t*12,*t*14	-0.158	0.445			-0.657	0.206
*t*9,*t*11	-0.237	0.796			-0.174	-0.030
*t*8,*t*10					0.286	-0.422
*t*7,*t*9	0.284	**2.502**	0.538	0.228		
*t*11,*c*13	0.027	1.002			-0.072	0.474
*c*11,*t*13			-0.108	-0.326	-0.101	-0.128
*c*9,*t11*	-0.027	**3.306**			-0.725	-0.208
*t*7,*c*9	0.457	**1.920**	0.496	-0.457		
Statistics						
Canonical R	0.983	0.968	0.978	0.965	0.979	0.945
Eigenvalue	28.74	14.93	22.50	13.62	22.64	8.32
Cumulative proportion	0.588	0.893	0.541	0.868	0.658	0.900
Probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

As reported before in Chapter 7, the effects of irradiation on the lipid composition and nutritional quality of meat were evaluated. The use of lamb meat samples was appropriate to better assess

irradiation effects, as long as it depicted higher amounts of CLA than bovine meat samples. The results obtained indicate that lamb meat enriched with CLA is not more susceptible to irradiation induced oxidation of fatty acids than that of non-enriched meat. In fact, the gamma irradiation of vacuum-packaged frozen lamb meat, with the maximum doses allowed by FDA to foods (7 kGy), did not seem to change its total lipid content, fatty acid profile and CLA contents, namely in levels of the bioactive *c*9,*t*11 and *t*10,*c*12 CLA isomers. Overall, the data suggest that the nutritional value of meat fatty acids for human diet is not significantly lost.

Contrary to irradiation, thermal treatments resulted in moderate changes in lipid fraction, mainly due to severe cooking losses (Chapter 8). The absence of interactions indicates that meat from bulls fed on concentrate was not more susceptible to changes induced by heating than that from animals grazing on pasture (Alfaia et al., 2009b). To determine how much of the respective compounds were gained or lost, it is important to compare the contents in absolute terms, based on an initial 100 g of raw beef, to take into account the concentrating effect caused by water loss during cooking. In Table 33 are displayed the contents (mg/100 g muscle) and the TR values (%) of selected lipids compounds related to nutritional value and moisture in raw and cooked samples of *longissimus lumborum* muscle from Alentejano purebred bulls fed on concentrate (CCC) and on pasture (PPP). Compared with the raw meat control, cooking led to a significant loss of moisture and, consequently, to a significantly higher intramuscular fat content. As expected, cooking produced significant increases in fatty acids and CLA isomers contents. In general, the microwave cooking provided higher contents of fatty acids than boiling or grilling beef, which likely resulted from the higher moisture loss. Cooked beef cuts from purebred bulls grass-fed presented the highest contribution to the intake of *n*-3 fatty acids (but lower SFA, MUFA and *n*-6 PUFA intake) than cooked beef produced in concentrate-based system. The mean content of the health promoting *n*-3 PUFA in cooked cuts from pasture is 131.3 mg/100 g muscle compared to 84.5 mg/100 g muscle from concentrate cuts, which represents a valuable contribution (microwaving > boiling = grilling) to the coverage of daily requirements in LC *n-3* PUFA (200 mg/day), according to the British Department of Health (1994). Therefore, cooked beef of animals from pasture resulted in a dietary source of LC *n*-3 fatty acids that meets about 65.6% of human recommended daily intake. It was suggested that increased consumption of *n*-3 fatty acids protects from CHD, whereas excessive consumption of *n*-6 PUFA at the expense of *n*-3 PUFA may promote CHD and other chronic diseases (Griffin, 2008). In Table 33, the mean values for TR of moisture increased significantly from microwave cooking to grilling (microwaving < boiling < grilling) in both types of beef. In contrast to moisture, most of the intramuscular fat was retained after cooking. The TR values of total lipids were higher for both microwave and grilled meat when compared with boiled meat. The TR values found for the partial sums of SFA, TFA and MUFA,

Table 33. Moisture, selected lipids and their true retention values (TR) in raw and cooked samples of *longissimus lumborum* muscle from Alentejano purebred bulls fed on two extreme diets (concentrate - CCC and on pasture - PPP).

	Concentrate (*n* = 7)							Pasture (*n* = 8)						
	Raw	Boiling		Microwave		Grilling		Raw	Boiling		Microwave		Grilling	
		Contents	TR (%)	Contents	TR (%)	Contents	TR (%)		Contents	TR (%)	Contents	TR (%)	Contents	TR (%)
Moisture (%)	75.2	61.2	47.7	53.7	40.0	61.1	54.4	74.4	61.2	50.5	55.3	43.3	63.3	57.7
Total lipids (%)	1.47	2.44	98.2	3.06	118.4	2.65	122.0	1.06	1.94	113.9	2.22	124.6	1.82	119.9
Partial sums of fatty acids (mg/100 g of meat)														
Σ SFA	542.5	926.9	101.9	1198.2	126.1	1027.8	128.5	379.6	790.3	131.9	898.5	147.9	724.3	135.9
Σ MUFA	471.4	809.9	103.4	1008.5	123.6	918.1	135.2	245.9	502.8	133.1	565.9	149.5	473.4	145.1
Σ TFA	42.6	69.1	104.3	96.2	130.7	79.3	135.3	26.5	55.0	132.7	64.2	151.3	55.1	150.1
Σ PUFA	247.5	363.8	90.8	420.0	100.1	335.0	94.7	260.3	342.1	81.9	432.9	99.4	331.3	87.0
Σ *n*-6 PUFA	227.5	335.1	90.9	384.8	86.9	305.6	94.0	168.8	222.0	80.5	279.2	98.3	211.2	85.7
Σ *n*-3 PUFA	58.2	77.5	89.4	98.4	103.8	77.7	103.4	91.5	120.1	84.5	153.7	101.3	120.1	89.3
CLA isomers (mg/100 g of meat)														
Total CLA	5.29	10.0	115.4	10.4	114.6	9.47	121.5	3.88	7.44	118.3	7.21	111.3	6.38	114.1
*c*9,*t*11	3.12	5.97	120.0	6.01	118.1	5.41	123.6	2.40	4.40	115.0	4.33	107.6	3.86	113.2
*t*10,*c*12	0.05	0.09	118.7	0.11	138.6	0.09	132.6	0.03	0.04	88.3	0.05	91.6	0.05	101.5

Σ SFA = sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0,18:0 and 20:0.
Σ MUFA = sum of *cis*-MUFA = sum of 14:1*c*9, 16:1*c*9, 17:1*c*9, 18:1*c*9, 18:1*c*11, 18:1*c*12, 18:1*c*13, 18:1*c*15 and 20:1*c*11.
Σ TFA = sum of 18:1*t*6+*t*8, 18:1*t*9, 18:1*t*10, 18:1*t*11, 18:1*t*12, 18:2*t*9*t*12 and 18:2*t*11*c*15.
Σ PUFA = sum of 18:2*n*–6, 18:3*n*–6, 18:3*n*–3, 20:2*n*–6, 20:3*n*–6, 20:4*n*–6, 20:5*n*–3, 22:4*n*–6, 22:5*n*–3 and 22:6*n*–3.
Σ *n*–6 PUFA = sum of 18:2*n*–6, 18:3*n*–6, 20:2*n*–6, 20:3*n*–6, 20:4*n*–6 and 22:4*n*–6.
Σ *n*–3 PUFA = sum of 18:3*n*–3, 20:5*n*–3, 22:5*n*–3 and 22:6*n*–3.

generally higher in cooked beef from pasture, tended to be lower in boiling than in grilling or microwaving. Since microwave cooking and grilling proceed in the absence of water, these cooking methods probably allowed for a high retention of fatty acids. The TR values obtained for SFA, MUFA, TFA and CLA were higher than 100% due, probably, to the great extractability of lipids from the cooked tissues, whereas for the sum of PUFA, the values were found to be generally lower (80.5 to 103.8). These TR values for *n*-6 and *n*-3 PUFA below or around 100% suggest a small loss or oxidative degradation of these fatty acids during cooking. Taken together, the results indicate that the household cooking methods studied (boiling, microwaving and grilling) seem to increase the SFA and MUFA and decrease PUFA in meat. Additionally, CLA contents seem to be higher in cooked beef than in raw meat, as a result of the moisture loss and, thus, as fatness increase.

In summary, and based on the previous discussion, the data from literature suggest that the production of meats enriched in *n*-3 PUFA should be encouraged. Besides, the results of Griffin (2008) reinforce the recommendations of increasing 18:3*n*-3 intake, and decreasing that of 18:2*n*-6, in order to promote the endogenous synthesis of LC *n*-3 PUFA, contributing this way for improve the balance in the fatty acid intake of today's consumers. Manipulation of fatty acid composition, including CLA, of ruminant meats through nutrition, increasing the content of CLA and *n*-3 PUFA, especially EPA and DHA, and reducing the SFA content with the net effect of increasing PUFA/SFA and reducing *n*-6/*n*-3 ratios, are important priorities and promising health approaches in ruminant meat. In fact, the design of diets enriched with CLA aid to enhance these potential health-promoting fatty acids in ruminant fats. In this sense, the development of novel production strategies to improve nutritional quality is eagerly awaited by the meat industry. Additional efforts should be directed towards increasing pasture intake by ruminants, investing on improved pastures, since product differentiation between pasture and pasture with finishing on concentrate production systems seems to be important to address consumer preferences and meat quality attributes. Nonetheless, further research is required in order to encourage the complete analysis of other meat fats (extend the characterisation to others commercial important Portuguese meats-PDO as well as other types of meat) to provide an accurate and comprehensive data on the fatty acids in ruminant fats for health assessments. Furthermore, the improved ability we have already accomplished in the complete profile analysis of fatty acids and CLA isomers, will be useful in future research to assess biological functionalities of other individual CLA isomers. This constitutes a driving challenge in the coming years. Following, biochemical and molecular genetic studies should bring further impetus for a more targeted control of fat deposition in different tissues. The influence of genetic effects upon fatty acid deposition is also required in order to clarify the genetic/breed effects on the levels of gene expression and enzymes activities related with fatty acid elongation-desaturation pathways, as so, in the metabolism and incorporation of specific fatty acids.

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ANNEX

DETERMINATION OF CONJUGATED LINOLEIC ACID (CLA) ISOMERS IN RUMINANT FOODS BY HPLC-DAD

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Determination of conjugated linoleic acid (CLA) isomers in ruminant foods by HPLC-DAD

Conjugated linoleic acid (CLA) is a mixture of conjugated positional and geometric isomers of linoleic acid (C18:2 n-6) formed by microbial hydrogenation in the gut of ruminant animals. There is an increasing interest in CLA research because some studies have suggested that it may have an important role to play in human health. Fourteen different isomers of CLA have been reported as occurring naturally in the fat of ruminant-based products. Whereas some information on the total CLA content in foods are available, only few reports exist on the distribution of individual CLA isomers in CLA-rich products. Moreover, there is a need to determine CLA isomeric profile in foods because it was very recently shown that specific physiological effects are linked with individual CLA isomers. In this work, it is presented the separation and quantification (external standard) of individual CLA isomers by triple-column silver-ion high-performance liquid chromatography (Ag^+-HPLC). After extraction of lipids from foods of ruminant origin and alkaline transesterification with sodium methoxide, the methyl esters of CLA isomers are completely separated by Ag^+-HPLC with DAD detection at 233 nm. Repeatability, intermediate precision and standard curves of the method are presented for *t*9,*t*11, *t*10,*c*12, *c*9,*t*11 and *c*9,*c*11 CLA isomers. The percentages of recovery from meat and milk are also indicated for the same CLA isomers.

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1. INTRODUCTION

Conjugated linoleic acid (CLA) is a mixture of conjugated positional and geometric isomers of linoleic acid (C18:2*n*-6) (Figure 10) formed by microbial hydrogenation in the gut of ruminant animals. There is an increasing interest in CLA research because some studies have suggested that it may have an important role to play in human health (Prates & Mateus, 2002). Fourteen different isomers of CLA have been reported as occurring naturally in the fat of ruminant-based products. Whereas some information on the total CLA content in foods are available, only few reports exist on the distribution of individual CLA isomers in CLA-rich products. Moreover, there is a need to determine CLA isomeric profile in foods because it was very recently shown that specific physiological effects are linked with individual CLA isomers.

Gas chromatography performed with high polar capillary columns (*e.g.* CP Sil 88 or SP 2560) or high-performance liquid chromatography with silver-ion columns in tandem (Ag^+-HPLC) have been used to analyse CLA in biological matrices and foods (Fritsche et al., 2000). However, the most advanced method available to assess the CLA isomeric distribution in foods, with unequivocal separation between *trans,trans*, *cis/trans* and *cic/cis* isomers and detection specificity, is Ag^+-HPLC. In this work, it is presented and validated the separation and quantification (external standard technique) of individual CLA isomers in ruminant foods (meat and milk) by triple-column Ag^+-HPLC.

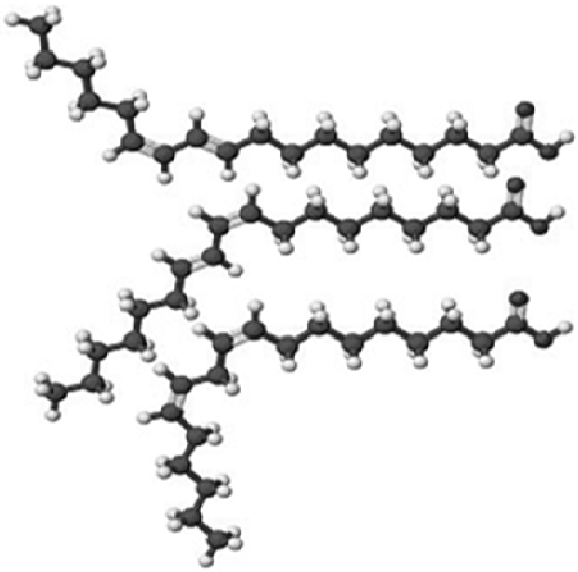

Figure 10. Chemical structure of *t*10,*c*12 CLA, *c*9,*t*11CLA isomers and linoleic acid.

2. MATERIALS AND METHODS

Meat samples were taken from the *longissimus dorsi* of bulls (20 ± 4 months, 350 ± 90 kg), 2-3 days after slaughter (+1 ºC), and stored at −80 °C until analysis. 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 by Fritsche et al. (2000). Milk samples were taken from commercial UHT half-skimmed milk (1.6% fat). For milk fat extraction isopropanol (1×) and *n*-hexane (3×) were used, as described by Jiang et al. (1996). Lipid contents of the meat and milk samples were calculated by weighting the residues obtained after solvents 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.

3. RESULTS AND DISCUSSION

The CLA isomers identification was based on the published literature (Fritsche et al., 2000) and on standards of individual CLA isomers (*t*9,*t*11, *t*10,*c*12, *c*9,*t*11 and *c*9,*c*11 CLA isomers) acquired from Matreya Inc. (Pleasant Gap, PA, USA) and Sigma-Aldrich Ltd. (O5632, St. Louis, MO, USA). Additional standards of individual (*t*8,*c*10 and *c*11,*t*13) and mixtures (*cis,trans*, *trans,cis* and *trans,trans* from 7,9 to 12,14) of CLA isomers were prepared as methyl esters according to the procedure described by Destaillats and Angers (2003) (Figure 11). The UV absorption observed at 233 nm is characteristic for molecules with a conjugated double bond system. Full scan UV spectra between 200 and 400 nm were collected for each sample in order to verify that HPLC peaks were exclusively conjugated fatty acids (Figure 12). All seven *trans,trans* isomers were separated from each other and no interference between *cis/trans* isomers and *cis/cis* isomers occurred. In the *cis/trans* region, not every pair of geometrical isomers could be separated and *t*8,*c*10 isomer co-eluted with *c*9,*t*11 isomer. A partial resolution of the Δ9,11 *cis/trans* isomers can be achieved with six silver-ion columns in series (Sehat et al., 1999).

CLA isomers quantification was performed using the external standard technique. Four calibration plots of area *versus* concentration of *t*9,*t*11, *t*10,*c*12, *c*9,*t*11 and *c*9,*c*11 CLA isomers (0-1 mg/mL) were obtained (Figure 13). Each calibration plot was used for the other positional CLA isomers with

similar geometrical configuration (*trans,trans, trans,cis cis,trans* or *cis/cis*). The coefficients of correlation were higher than 0.9994 for all isomers studied.

The validation of the method was performed as reported in Council Directive 75/318/EEC (1996). The percentages of recoveries for the CLA isomers analysed from meat and milk were, respectively, higher than 78% and 85% (0.01-0.2 mg/mL). Repeatability (3 concentrations with 3 replicates each) and intermediate precision (3 concentrations with 3 replicates each done in 3 different days) were higher than 2.2% and 5.3% (0.01-1 mg/mL), respectively, for all the CLA isomers.

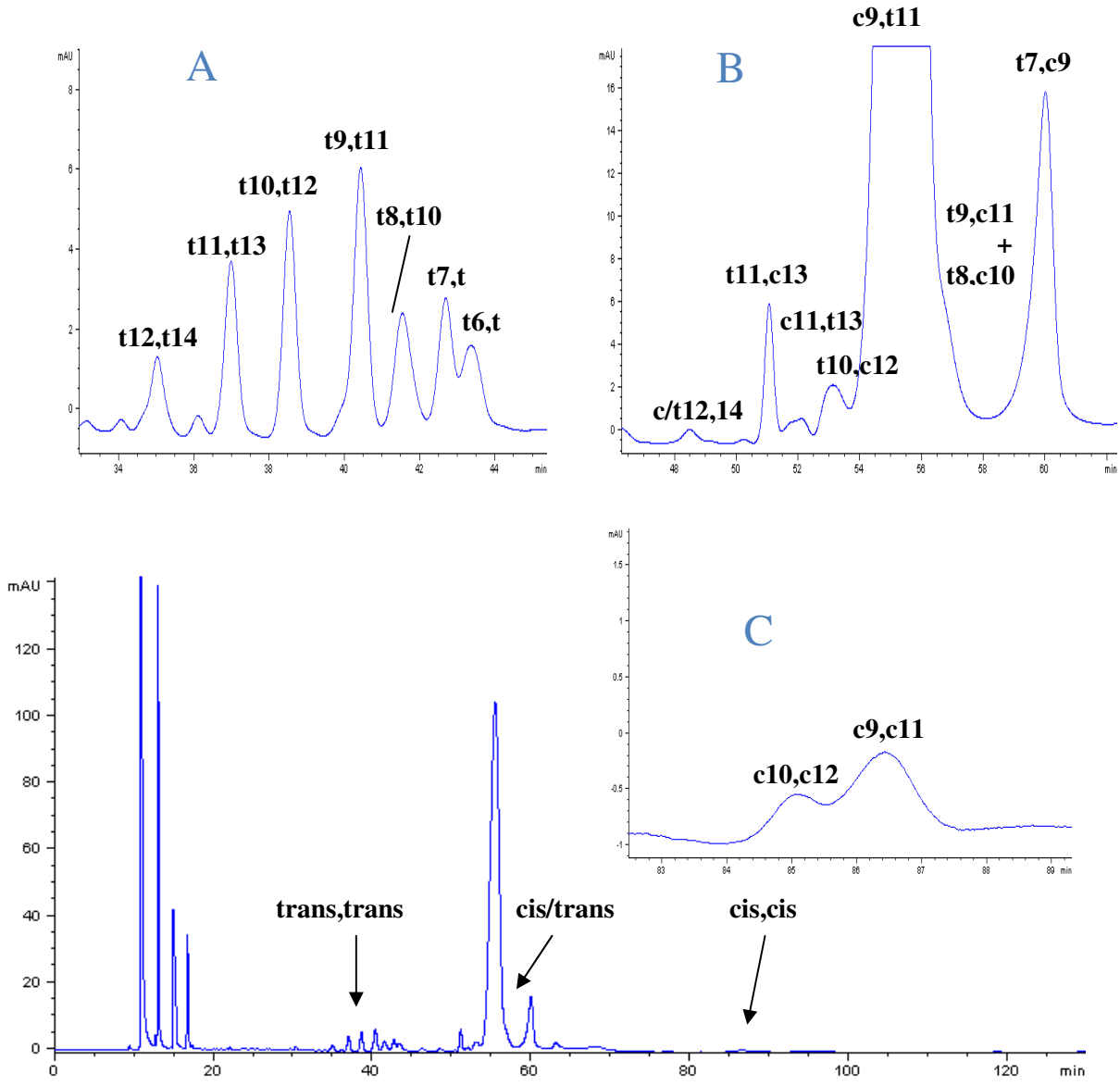

Figure 11. Triple column silver-ion high-performance liquid chromatogram of a typical milk sample (conjugated linoleic acid methyl ester) detected at 233 nm. The regions of *trans,trans* (A), *cis/trans* (B) and *cis,cis* (C) are enlarged.

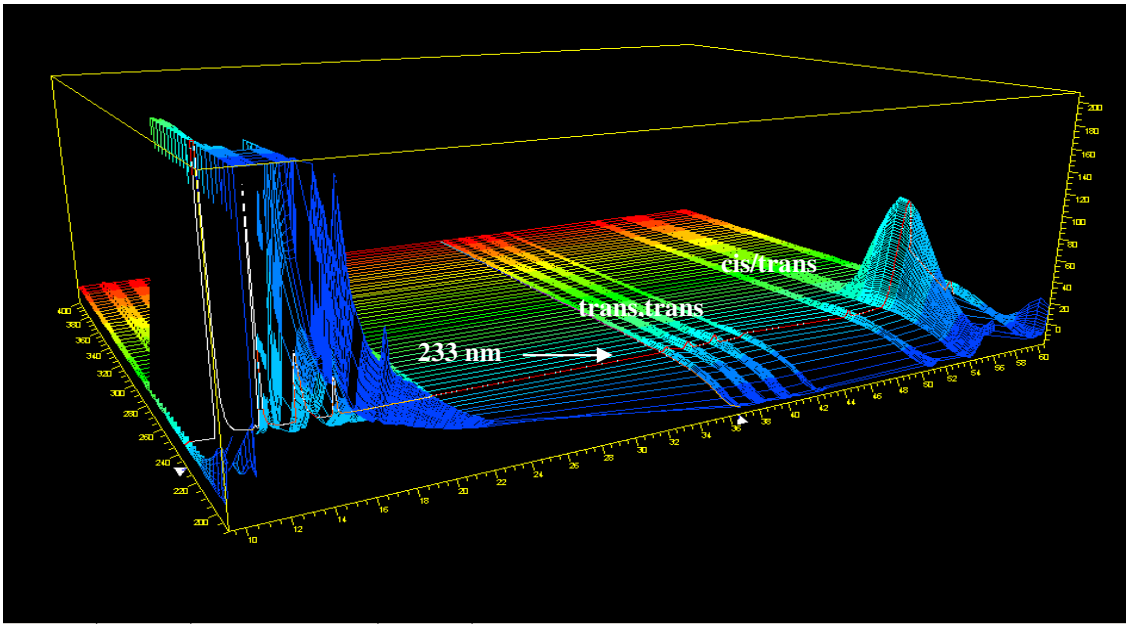

Figure 12. 3D plot of a triple column silver-ion high-performance liquid chromatogram, with full scan UV spectra between 200 and 400 nm, from a typical milk sample (conjugated linoleic acid methyl ester).

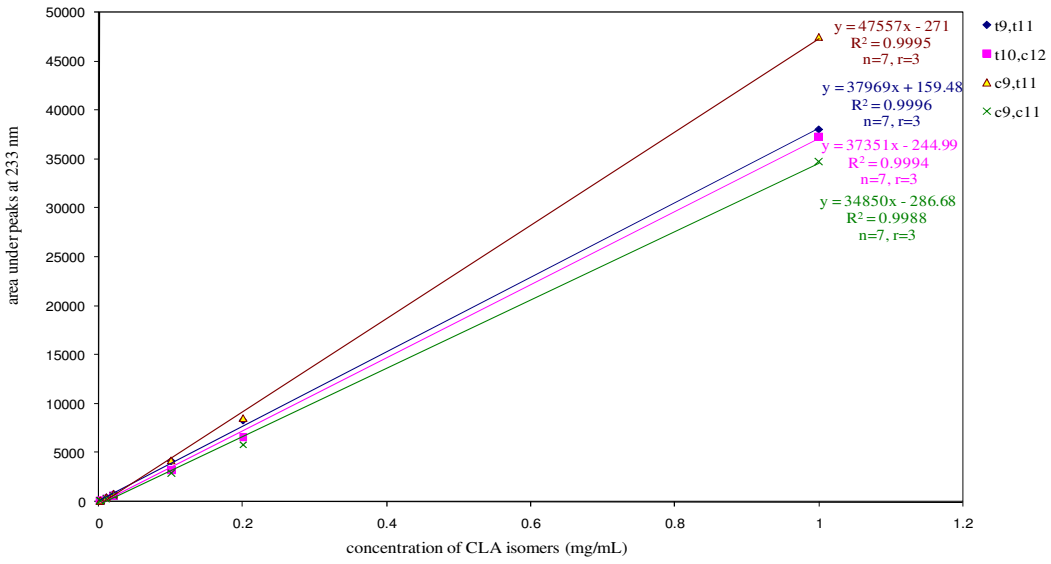

Figure 13. Calibration plots and linear regression parameters for *t*9,*t*11, *t*10,*c*12, *c*9,*t*11 and *c*9,*c*11 CLA isomers.

In conclusion, triple column Ag^{+}-HPLC is a suitable technique for the quantitative determination of individual CLA isomers in ruminant foods (meat and milk).

ACKNOWLEDGMENTS

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