Nutritional quality of intramuscular fat in Carnalentejana-PDO beef

Qualidade nutricional da gordura intramuscular da Carnalentejana-DOP

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Abstract: The nutritional quality of intramuscular fat of Carnalentejana-PDO beef, evaluated in longissimus dorsi (LD, relatively red) and semitendinosus (ST, relatively white) muscles, from young bulls slaughtered in early autumn and late spring was assessed in order to determine seasonal variations. The results showed that no important seasonal differences were apparent for Carnalentejana-PDO beef, except for total lipids and total cholesterol. In contrast, LD and ST muscles had significant differences for most of the analysed parameters. The data suggest that beef-PDO has intermediate values (between meat from grain-fed and pasture-fed cattle) for total lipids, total cholesterol, β-carotene and n-6/n-3 ratio, which can be explained by the mixed feeding system of Alentejano young bulls. From a nutritional point of view, Carnalentejana-PDO meat presents similar health value throughout the year, although the n-6/n-3 index seems to be always above the recommended values for human diet (1-2). In addition, the PUFA/SFA ratio seems to be above the recommended guideline for human diet (0.45) in ST muscle (favourable) but below that guideline in LD muscle.

Keywords: Carnalentejana-PDO, meat quality, fatty acids, CLA, cholesterol, tocopherols, β-carotene

Introduction

Fatty acid composition (Wood et al., 2004), cholesterol and antioxidant levels (Prates et al., 2006) in meat have received an increased interest considering their implications for human health and product quality. It is well established that the lower polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA) and higher n-6/n-3 ratios presented by most meats are a major cause for the imbalance on fatty acid intake of today’s consumers (Wood et al., 2004). Recently, research was focused on a minor group of fatty acids that are characteristic of ruminant fat, named conjugated linoleic acid (CLA) (Prates and Mateus, 2002).

The CLA acronym refers to a heterogeneous group of positional and geometric isomers of linoleic acid (18:2n-6), in which the double bonds are conjugated (from positions 6-8 to 13-15). Total cholesterol and lipid-soluble antioxidant vitamins (tocopherols and β-carotene) could also provide valuable information related to meat quality and safety. In fact, meat supplies from one third to one half (Chizzolini et al.,
of the maximum daily-recommended cholesterol intake (300 mg, World Health Organization), which high levels in typical Western diets have been considered as major risk factors of cardiovascular diseases (Ganjí et al., 2003). In addition, it is generally accepted that, apart from microbial spoilage, lipid oxidation is the primary cause of quality deterioration in muscle foods (Monahan, 2000). D-α-, D-β-, D-λ-, and D-δ-Tocopherols, are the natural compounds with vitamin E activity, which is the primary lipid-soluble antioxidant in biological systems (Kerry et al., 2000). β-Carotene, a pro-vitamin A compound, is the predominant carotenoid in meat and meat products (Mortensen and Skibsted, 2000).

In Portugal, meat from autochthonous bovine breeds, reared in traditional production systems, has been progressively reintroduced in Portuguese diets as a result of its putative high intrinsic quality (Costa et al., 2003) and of public perception of BSE and chemical residue safety (Rodrigues et al., 1998). Meats with Protected Designation of Origin (PDO), 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 the specific properties of its lipid fraction (Council Regulation nº2081/92 of 14/7, EEC). One such example is Carnalentejana-PDO beef, which is obtained from Alentejana purebred young bulls produced in a traditional production semi-extensive system in Alentejo region (south of Portugal) according to the product specifications (extensive grazing system based on natural pastures of Montado with finishing on concentrate feeds for 3 to 6 months).

In spite of being the most important commercial Portuguese meat-PDO (991 carcass tons in 2003) (Instituto do Desenvolvimento Rural e Hidráulica, 2003), there are no detailed reports on the lipid composition of Carnalentejana-PDO beef. Moreover, little work has been carried out to assess seasonal changes in beef CLA. Thus, the goal of this work was to evaluate the nutritional quality of intramuscular fat in Portuguese Carnalentejana-PDO beef, in two distinct slaughter seasons (early autumn and late spring).

Material and methods

Animals and meat samples

Alentejana purebred young bulls (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 as Montado. The young bulls were finished on concentrate feeds (Table 1) in the last 5 or 3 months, before slaughter in Regional Abattoir of Alto Alentejo (Sousel) in October 2002 (early autumn sampling) or June 2003 (late spring sampling), 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; mean ± standard error of age and carcass weight were 21±0.7 months and 347±18 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; 20±0.6 months and 359±8 kg).

Meat samples were taken from the ribeye portion (T1-T3) of *longissimus dorsi* (LD) and distal region of *semitendinosus* (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 °C), minced on a food processor (3 x 5 s), vacuum packed and stored at -80 °C until required for analysis.

| Table 1 - Chemical (g/kg dry matter) and fatty acid (% sum of fatty acids) composition of the concentrate from Alentejana purebred bullocks reared according to Carnalentejana-PDO specifications. |
|---------------------------------|-----------------|------------|
| Chemical composition           | Fatty acids     |
| Crude protein                  | 128             | 14:0       | 3.8        |
| Total fat                      | 30              | 16:0       | 13.4       |
| Crude fibre                    | 65              | 18:0       | 2.2        |
| Ashes                           | 90              | 18:1       | 22.5       |
|                                |                 | 18:2n-6    | 52.7       |
|                                |                 | 18:3n-3    | 2.7        |

Lipid extraction and methylation

Intramuscular fat was extracted from lyophilised meat, three times with methylene chloride-methanol (4:1 v/v) and a fourth extraction performed with n-hexane, as described by Fritsche et al. (2000). Fatty acids were converted to methyl esters (FAME) 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 proposed by Kramer et al. (2002). The same FAME solution was analysed for both fatty acid composition and CLA content, 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.

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™-2560 fused-silica
Determination of total CLA

The methyl esters of CLA isomers were determined by triple column silver-ion 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 diode array detector (DAD) adjusted at 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 (Fritsche et al., 2000). Total CLA (sum of individual CLA isomers) content in meat was determined based on the external standard technique and on the method of area normalization (AOAC 963.22, 2000).

Determination of cholesterol, tocopherols and β-carotene

Meat samples used for total cholesterol and lipid-soluble antioxidant vitamins (tocopherols and β-carotene) analysis were submitted to a direct saponification and to an extraction in a single step with n-hexane, according to the procedure described by Prates et al. (2006). The simultaneous analysis of cholesterol, tocopherols and β-carotene in meat was performed using a normal-phase silica column (Zorbax RX-Sil with the corresponding 12.5 mm analytical guard column, 4.6 mm ID × 250 mm, 5 µm particle size, Agilent Technologies Inc., Palo Alto, CA, U.S.A.), with fluorescence detection for tocopherols (excitation wavelength of 295 nm and emission wavelength of 325 nm) and UV-visible DAD detection for cholesterol (202 nm) and β-carotene (450 nm) in series. The solvent (1% v/v isopropanol in n-hexane) flow rate was 1 ml/min, the run lasted for 17 min and the temperature of the column oven was adjusted at +20 °C. The volumes injected varied between 20 and 100 µl in order to obtain values compatible with the linearity range of the standard curves. The contents of total cholesterol, tocopherols and β-carotene in meat were calculated, in duplicate, based on the external standard technique.

Statistical analysis

The data were analysed using the MIXED procedure of 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 (beef-PDO from early autumn and beef-PDO from late spring), the muscle type (LD and ST) and the interaction between animal group and muscle type.

Results and discussion

Intramuscular fatty acid composition and total CLA content

Data referring to the total lipids (mg/g muscle) and partial sums of intramuscular fatty acids (weight %) (detailed information on fatty acid composition published in Alfaia et al., 2006), including total CLA (mg/g muscle), in Carnalentejana-PDO beef, obtained at early autumn and late spring seasons, are presented in Table 2. Total lipid content was higher (P<0.01) in beef-PDO from autumn relative to that from spring, which can be explained by the longest finishing period on concentrate of the autumn-slaughtered young bulls (5 vs. 3 months). In addition, the LD muscle had greater (P<0.001) contents of total lipids relative to the ST muscle. Beef-PDO exhibited values of total lipids (1.5-2.2% and 1.1-1.3% for LD and ST muscles, respectively) between those reported for the meat from extensively (lower) and intensively (1.4-2.7% and 1.1-1.8% for LD and ST muscles, respectively) produced bulls (Vestergaard et al., 2000b). The difference in total lipids in the two muscles likely results from variations in fibre type composition. It is well known that the lipid content is higher in red oxidative muscle fibres (Ensér et al., 1998) and that the LD muscle (25.5%-31.0% of type I fibres) of cattle is relatively red in comparison with the ST muscle (16.9%-22.1% of type I fibres) (Vestergaard et al., 2000a). However, according to the Food Advisory Committee (1990) criteria (<5% fat), Carnalentejana-PDO beef may be considered a lean meat.

As expected, the predominant partial sums of fatty acids in fat from both muscle types were SFA (36.3-41.8%) and monounsaturated fatty acids (MUFA; 36.9%-41.9%). Similar results were reported by many other authors in beef (e.g. Raes et al., 2004; Realini et al., 2004; Varela et al., 2004). In addition, no seasonal changes (P>0.05) in Carnalentejana-PDO beef were observed for SFA, MUFA, trans fatty acids (TFA) and n-3 PUFA. The small effect of slaughter season on meat fatty acids probably results from the relatively long finishing period that may attenuate the expected
differences resulting from pasture seasonal variations. In fact, young bulls grazing on the more abundant pastures (late winter and early-middle spring grass) were finished on concentrate during 5 months (slaughtered in October), while the animals exposed to the less abundant pastures (late winter grass) were finished on concentrate only during 3 months (slaughtered in June).

Muscle type had a significant effect in SFA ($P<0.001$), MUFA ($P<0.001$), n-6 PUFA ($P<0.001$) and n-3 PUFA ($P<0.01$). The LD muscle, relative to ST muscle, presented higher relative proportions of SFA and MUFA but lower percentages of PUFA, n-6 PUFA and n-3 PUFA. Since PUFA are much more abundant in the phospholipid fraction than in triacylglycerol fraction (Wood et al., 2004), these differences in the partial sums of fatty acids may reflect distinct triacylglycerol/phospholipid ratios between muscles, as a consequence of the different fat levels. Interactions ($P<0.05$) between the slaughter season and muscle type were observed for the percentages of PUFA, n-6 PUFA and total CLA. These interactions may result from the different finishing periods on concentrate in autumn and spring-slaughtered young bulls, since the adaptation from the extensive grazing to the more confined finishing period on concentrate may induce changes in muscle metabolism (Klont et al., 1998). Vestergaard et al. (2000b) reported that type I fibres were more represented in extensively than intensively reared bulls (31.0% vs. 25.5% and 22.1% vs. 16.9% for LD and ST muscles, respectively).

Total CLA in LD muscle from autumn (0.100 mg/g muscle) was higher than in LD muscle from spring and ST muscle from both seasons, which presented similar values (0.042-0.066 mg/g muscle). It was recently reported, by Realini et al. (2004), 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). The values of specific CLA contents observed in this work (3.2-4.8 mg/g fat) are within the range reported by those authors.

**Table 2** - Total lipids (mg/g muscle), partial sums of fatty acids (% w/w), including total CLA (mg/g muscle), and nutritional ratios of fat in different muscles (longissimus dorsi, LD, and semitendinosus, ST) of Carnalentejana-PDO beef from bullocks slaughtered in early autumn and late spring.

<table>
<thead>
<tr>
<th></th>
<th>Autumn</th>
<th>Spring</th>
<th>SEM</th>
<th>Significance level</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LD</td>
<td>ST</td>
<td>LD</td>
<td>ST</td>
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<tr>
<td>Total lipids</td>
<td>21.9</td>
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<td>Partial sums</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ SFA</td>
<td>41.8</td>
<td>37.1</td>
<td>38.7</td>
<td>36.3</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>40.6</td>
<td>36.9</td>
<td>41.9</td>
<td>40.6</td>
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<tr>
<td>Σ TFA</td>
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<td>3.02</td>
<td>3.20</td>
<td>3.13</td>
</tr>
<tr>
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<td>22.4b</td>
<td>15.8a</td>
<td>19.5b</td>
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<tr>
<td>Σ n-6</td>
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<td>14.6a</td>
<td>17.9a</td>
</tr>
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<td>Σ n-3</td>
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<tr>
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<td>0.066</td>
<td>0.054b</td>
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<td>PUFA/SFA</td>
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<td>0.63b</td>
<td>0.42a</td>
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<td>0.016</td>
<td>0.017</td>
<td>0.019</td>
<td>0.024</td>
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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, monoenoic fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; and cholesterol ratio [(total CLA)/(sum of SFA and cholesterol), each expressed in mg/g muscle].

**Table 3** - Total cholesterol (mg/g muscle), and lipid-soluble antioxidant vitamins (µg/g muscle) in different muscles (longissimus dorsi, LD, and semitendinosus, ST) of Carnalentejana-PDO beef from bullocks slaughtered in early autumn and late spring.

<table>
<thead>
<tr>
<th></th>
<th>Autumn</th>
<th>Spring</th>
<th>SEM</th>
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</tr>
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<tr>
<td></td>
<td>LD</td>
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<td>LD</td>
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<td>Total cholesterol</td>
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<td>γ-Tocopherol</td>
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<td>0.17</td>
<td>0.13</td>
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<tr>
<td>β-Carotene</td>
<td>0.04a</td>
<td>0.02b</td>
<td>0.09b</td>
<td>0.05abc</td>
</tr>
</tbody>
</table>

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).
Contents of cholesterol and lipid-soluble antioxidant vitamins in beef

Data on total cholesterol (mg/g muscle), tocopherols (µg/g muscle) and β-carotene (µg/g muscle) in different muscles of Carnalentejana-PDO beef, obtained at early autumn and late spring seasons, are shown on Table 3. Carnalentejana-PDO beef from autumn had higher contents \( (P<0.001) \) of total cholesterol than that from spring. In addition, LD muscle exhibited a greater content \( (P<0.001) \) of total cholesterol relative to the ST muscle. We found lower values of total cholesterol \( (0.35-0.45 \text{ mg/g}) \) than those reviewed by Chizzolini \( \text{et al.} \) (1999) for beef \( (0.47-0.57 \text{ mg/g}) \). According to those authors, variations in fibre type composition might result in differences in cholesterol content in different 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 cholesterol, which is mainly \( (60-80\%) \) present in the membrane component of the bovine muscle \( \text{Hoelscher et al.} \) (1988). Our results are in agreement with this hypothesis since LD muscle, which is relatively red (see above), had higher values of cholesterol when compared with the ST muscle.

Values for α- and γ-tocopherols, the last one being present in small amounts, did not show significant differences \( (P>0.05) \) when the slaughter season was compared. The LD muscle had higher \( (P<0.001) \) α- and γ-tocopherols contents relative to the ST muscle. The prevalence of α-tocopherol in meat is well known and is due to the more than tenfold preference of the tocopherol-binding protein for α-tocopherol, relatively to γ-tocopherol, which is the most common vitamin E homologue in plant foods \( \text{Decker et al.} \) (2000). The levels of α-tocopherol in Carnalentejana-PDO beef \( (1.38-2.26 \text{ µg/g}) \) were similar to the values reported for meat derived from grain-fed cattle \( (1.8-2.4 \text{ µg/g}) \) and lower than those reported for meats originated on pasture-fed cattle \( (4.4-5.8 \text{ µg/g}) \) \( \text{Yang et al.} \) (2002).

A significant interaction \( (P<0.05) \) between slaughter season and muscle type was observed for α-carotene. As described above, this interaction may result from modifications of muscle metabolic types, which are associated with differences in lipid composition that might result from adaptations to the confinement or extensive grazing. The values of β-carotene in the Carnalentejana-PDO beef \( (0.02-0.09 \text{ µg/g}) \) are between those described for β-carotene in meat from grain-fed cattle \( (0.01-0.03 \text{ µg/g}) \) and those from cattle grazed on a good green pasture \( (0.09-0.22 \text{ µg/g}) \) \( \text{Yang et al.} \) (2002), reflecting the finishing period based on concentrate feeds of the production system.

Nutritional value of beef fat

In order to evaluate the nutritional value of intramuscular fat of Carnalentejana-PDO beef, n-6/n-3, PUFA/SFA and the reciprocal proportions of SFA plus total cholesterol (CHR) vs. total CLA (CLA/SFA+CHR ratio; see detailed definition in Table 2) were calculated for the different muscle types and slaughter seasons (Table 2). Current nutritional recommendations are that the n-6/n-3 ratio should be between 1 and 2 (National Institute of Health of USA, quoted by Simopoulos, 2002) and the PUFA/SFA ratio in human diet should be above 0.45 (British Department of Health, 1994). No seasonal changes \( (P>0.05) \) in beef-PDO were observed for n-6/n-3 ratio, although LD muscle had higher values \( (P<0.05) \) than the ST muscle. Notwithstanding the fact that n-6/n-3 ratios \( (10.0-13.7) \) are higher than the recommended values for a healthy human diet, these values are lower, and so more favourable, than those obtained by our group \( \text{Alfaia et al.} \) (2006) for Portuguese beef from concentrate-fed young bulls \( (16.7-20.2) \), as well as by Enser \( \text{et al.} \) (1998) for British meat from concentrate-fed cattle \( (15.6-20.1) \). In addition, n-6/n-3 ratios obtained by Enser \( \text{et al.} \) (1998) for meat from grass-fed bulls \( (2.0-2.3) \), which are very close to the guidelines for human nutrition, are lower than our values for meat from Alentejano purebred young bulls. Our intermediate n-6/n-3 ratio in Carnalentejana-PDO beef may be explained by the mixed feeding system of Alentejano young bulls. In fact, it is well known that the use of cereals (rich in n-6 PUFA; see Table 1) in concentrates shifts the meat fatty acid composition to an increased ratio of n-6/n-3 when compared with animals produced on pasture \( \text{Nuenberg et al.} \) (2002; Raes \( \text{et al.} \) (2004).

A significant interaction \( (P<0.05) \) between the slaughter season and muscle type was observed for the PUFA/SFA ratio, reflecting the patterns observed for the partial sums of fatty acids. This ratio was higher in ST muscle \( (0.63 \text{ for autumn and 0.56 for spring}) \), values that are above the guideline recommended for the human diet, and so favourable, than the LD muscle \( (0.32 \text{ for autumn and 0.42 for spring}) \), which exhibited figures below that guideline.

It was proposed that the CLA/SFA+CHR ratio might explain the association between the intake of beef fat and colon cancer \( \text{Eynard and Lopez, 2003} \). Interestingly, lean beef \( (<5\%) \) of intramuscular fat), showing a high CLA/SFA+CHR proportion \( (0.09) \), has a protective effect against colon cancer, whereas fatty beef derivatives \( (37\%) \), with low CLA/SFA+CHR proportions \( (0.007) \), is associated with a higher cancer risk. There were no seasonal or muscle type effects \( (P>0.05) \) on CLA/SFA+CHR proportion \( (0.016-0.024) \). Therefore, the data suggest that, attending to the risk of colon cancer, Carnalentejana-PDO beef display the same risk when
 originated in both slaughter seasons. Moreover, although beef-PDO from spring 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 similar throughout the year.

Conclusions

No important seasonal variations in Carnalentejana-PDO beef were apparent for most of the analysed parameters, although total lipids, total cholesterol and total CLA (only for LD muscle) were higher in beef from early autumn. In contrast, a muscle type effect was observed for most of the analysed parameters. The data indicate that beef-PDO has intermediate values (between meat from grain-fed and pasture-fed cattle) for total lipids, total CLA, -carotene and n-6/n-3 ratio, which can be explained by the semi-extensive production system of Alentejano young bulls. From a nutritional point of view, Carnalentejana-PDO seems to display a similar health value throughout the year. However, the n-6/n-3 index seems to be always above the recommended values for the human diet. In addition, the results suggest that the PUFA/SFA ratio is consistently above the recommended guideline for human diets in ST muscle (relatively white), and so favourable, but below that guideline in LD muscle (relatively red). Taken together, the data indicate that the finishing period of Alentejana purebred young bulls with cereal-rich concentrate (3-5 months) attenuates most of the beneficial grass effects on the nutritional characteristics of meat fat.

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