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Concentration of antioxidants in two muscles of mature dairy cows from Azores

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ABSTRACT

This study evaluated the concentrations of α-tocopherol, β-carotene, creatine, carnosine, anserine and coenzyme Q10 in *Longissimus dorsi* (Ld) and *Gluteus medius* (Gm) muscles of culled dairy cows and the impact of age, production status before slaughter (dry-off vs lactating) and carcass weight on them. The effects of applying a finishing feeding regimen before slaughter were also examined. Gm muscle presented higher levels ($P < 0.001$) of α-tocopherol (5.14 vs 3.61 $\mu g \cdot g^{-1}$) β-carotene (0.36 vs 0.27 $\mu g \cdot g^{-1}$), anserine (59.24 vs 43.25 $mg \cdot 100\ g^{-1}$) and coenzyme Q_{10} (3.33 vs 1.73 $mg \cdot 100\ g^{-1}$), and by contrast lower ($P < 0.05$) creatine concentration (502.40 vs 527.28 $mg \cdot 100\ g^{-1}$) than Ld. Dry-off and lactating cows differed significantly in α-tocopherol level ($P < 0.001$) but not in the concentrations of the other compounds ($P > 0.05$). The finishing feeding promoted higher mean concentrations of anserine and creatine but lower carnosine values ($P > 0.05$) than directly slaughtered dry-off cows. The variation between muscles and from animal-to-animal makes it difficult to exactly define the antioxidant status of the dairy cow's meat.

1. Introduction

World-wide, lean beef is recognized as an important dietary source of protein and other nitrogenous components of excellent quality, as well as highly bioavailable B vitamins and minerals, mainly iron, selenium and zinc (Biesalski, 2005; Purchas, Rutherfurd, Pearce, Vather, & Wilkinson, 2004), with a number of nutritional differences existing between meat from grain-fed feedlot-raised animals and that obtained from fresh grass/silage-fed counterparts (Alfaia et al., 2009; DeSmet, Raes, & Demeyer, 2004; Garcia et al., 2008; Purchas & Busboom, 2005). Such differences are often related to fat content, lipid composition (Garcia et al., 2008; Latimori et al., 2008; Pordomingo, Garcia, & Volpi Lagreca, 2012; Realini, Duckett, Brito, Dalla Riza, & de Mattos, 2004; Wood et al., 2003, 2008) and antioxidant "status" (Daley, Abbott, Doyle, Nader, & Larson, 2010), with this latter condition being determined by the presence of various endogenous and exogenous compounds (*e.g.* α-tocopherol, β-carotene, carnosine, coenzyme Q10 and creatine). They are important to improve commercial shelf life by minimizing losses in color and fat quality, slowing down the respective oxidation processes (Insani et al., 2008; Yang, Lanari, Brewster, & Tume, 2002) and, concomitantly, to diminish degenerative processes associated to cardiovascular diseases, aging, diabetes, Alzheimer's diseases and some forms of cancer (Gunter et al., 2007; Johnson & Lund, 2007; Lonn & Yusuf, 1997). Increased levels of those compounds in meat of grass-fed cattle are often reported (Descalzo et al., 2005; Pordomingo, Grigioni, Carduza, & Volpi Lagreca, 2011; Yang et al., 2002). Among internal oxidative stressors, the release of endogenous iron along the processing and storage phases of meat production seems to play a key role in the catalysis of myoglobin and lipid oxidation (Descalzo et al., 2005), promoting metmyoglobin and rancid/off flavor formation, respectively (Gorelik & Kanner, 2001).

Due to various zootechnic and medical reasons, a considerable number of adult dairy cows (Holstein-Friesian) are culled every year from this production sector in S. Miguel, Azores, accounting to about 15 thousand tones of dressed carcasses in 2011 and 2012. Representing a quite heterogeneous population in terms of age and body condition, the meat obtained is usually not considered by stakeholders to have commercial quality traits for trading, either in traditional (small butchers with most cutting service made by consumers demand at the moment) or in modern (self service of packaged whole pieces or respective cuts) regional or national retail traders. Due to this lack of commercial interest, any proposal for finishing feeding cull cows or processing their carcasses/meat by procedures currently implemented for improving meat quality has not been taken into consideration so far, with almost all boned meat obtained being minced for hamburgers or other similar meat product

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manufacture. Since the feeding system of dairy cows in Azores is also based on daily pasture grazing on natural/improved forages or green silages, a good profile of compounds with antioxidant action should be expected. In order to increase the consumer's acceptability for this meat kind, a new labeling paradigm oriented to those compounds was thought to increase commercial value and to represent an important step for that targeted consumer demand. Thus, the aim of this work was to determine the variations in the antioxidant "status" of meat obtained from *Longissimus dorsi* (Ld) and *Gluteus medius* (Gm) muscles and related to α-tocopherol, β-carotene, carnosine, anserine, creatine and coenzyme Q10 concentrations, from adult dairy cull cows differing in age, live weight and production status (lactating vs dry-off) before slaughter. The influence of a flushing feeding period based on supplemented concentrate ration for one month before slaughter in meat quality of dry-off cows was also investigated.

2. Materials and methods

2.1. Animals and diets

Thirty-two Holstein-Friesian cows, reared at different farms and fed under a mixed pasture/concentrate based system, with ages and carcass weight ranging from 25 to 140 months and 180 to 430 kg respectively, were used in this study. Animals were allocated in three groups based on production status (PS): lactating cows (n = 10), dry-off cows (n = 10) and dry-off finishing fed cows (n = 12) (Table 1). This late regimen used a commercial concentrate supplemented with vitamin A (6000 IU), vitamin D_3 (2000 IU) and vitamin E (10 mg kg^{-1}) (3 kg day^{-1} during the first 2 weeks, followed by 5 kg day^{-1} up to the end) and silage "*at libitum*". In order to evaluate the influence of age, two subgroups of <7.5 and >7.5 years old within each production status groups were created, averaging in about 2.9 years and 10.3 years for the dry-off population directly slaughtered, 3.8 years and 8.8 years for the lactating cows and in 6.5 years and 9.3 years for the dry-off finished fed group, respectively.

2.2. Sampling

The Ld between the 11th rib and the 5th lumbar vertebrae and the whole Gm muscles were removed from the left hemi carcass 48 h *post-mortem* and cleaned of superficial fat and connective tissue sheath. After cutting these muscles into portions of about 6 cm width to be randomly used for different aging times, they were afterwards divided into 2 pieces of about 2.5 cm, for measuring color, water holding capacity, Warner-Blatzer maximum shear force and sensory attributes detection (complementary approach to the present work). The resting part of each initial muscle portion was pooled together (about 300 g), minced twice through a 3 mm hole plate grinder and then carefully mixed and packed under vacuum in COEX PA/PE — 20/70 plastic bags (Plásticos Macar, S. Tirso, Portugal — permeability to oxygen: 50 cm^3/m^2/day/bar at 23 °C and 0% of relative humidity). Samples were stored at −75 °C until required for analysis (approximate composition and those analysis undertaken for the present work).

Table 1
Number of animals within each production status group, according to their age and carcass weight.

	2–7.5 years		7.5–12 years		Total
	180–300 kg	301430 kg	180–300 kg	301–430 kg	
Lactating	3	2	2	3	10
Dry-off	3	2	3	2	10
Dry-off finishing	3	3	3	3	12

2.3. Analytical procedures

2.3.1. α-Tocopherol and β-carotene

The quantification of α-tocopherol and β-carotene was determined by HPLC and based in the method described by Prates, Quaresma, Bessa, Fontes, and Alfaia (2006), with minor modifications.

The saponification of 5 g homogenized samples was carried out in a water bath at 80 °C during 15 min with 20 mL of 11% KOH solution in a mixture of ethanol and water (55:45, v/v). After saponification, samples were cooled in tap water for 1 min and 6 mL of water and 12 mL of 25 μg/mL BHT solution in n-hexane were added. The samples were vigorously mixed for 2 min and centrifuged at 1500 ×*g* for 5 min, in order to accelerate the separation of phases. The upper layer (n-hexane) was then dried over anhydrous Na_2SO_4 and filtered through a 0.45 μm hydrophobic membrane (GVS SpA, NY). The chromatographic separation was performed using a normal-phase silica column from Waters (Milford, MA), with fluorescence detection for α-tocopherol (excitation wavelength of 295 nm and emission wavelength of 325 nm) and UV detection for β-carotene (450 nm) in series, using 1% v/v isopropanol in n-hexane as solvent at a flow rate of 1 mL min^{-1}. The α-tocopherol and β-carotene values were calculated in duplicate for each sample, based on the external standard technique.

2.3.2. Coenzyme Q10

For coenzyme Q10, the method described by Matilla and Kumpulainen (2001) was used, with some modifications. Duplicate 4 g samples from frozen comminuted muscle, added with 10 mL of 0.15 M NaCl were homogenized (Polytron — PT3000, Kinematca Ag, Suisse) and remixed with a vortex mixer after adding 10 mL of ethanol. The resulting mixture was then extracted in 15 mL of n-hexane, which was mixed with the same vortex mixer for 1 min and centrifuged at 3000 rpm for 5 min (Sorvall Instruments, Wilmington, Delaware, USA). The upper hexane layer was removed and the extraction procedure repeated twice. The combined hexane extract was dried in a rotating evaporator, taken up in 3 mL of isopropyl alcohol, and the coenzyme Q10 was separated by HPLC using isocratic RP-HPLC separation Atlantis dC18 column (4.6 × 150 mm, 3 mm) from Waters (Milford, MA) at room temperature. The mobile phase, comprising of acetonitrile: tetrahydrofurane:water (55:40:5 v/v/v), was used at a flow rate 1.5 mL min^{-1} with detection being performed at 275 nm with a UV detector (Waters 2487 Dual λ Absorbance detector, Waters, Milford, MA).

2.3.3. Carnosine, anserine and creatine

The three compounds were determined by HPLC according to Mora, Sentandreu, and Toldrá (2007). Eight gram samples were homogenized with 15 mL of 0.01 N HCl (Polytron — PT3000, Kinematca Ag, Suisse) for 8 min and centrifuged at 10,000 rpm for 20 min. Supernatant was filtered through glass fiber filter (Sartorius, Barcelona, Spain), and 250 μL of this solution was deproteinized by adding 750 μL of acetonitrile, standing thereafter at 4 °C for 20 min. The sample was then centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant filtered through Acrodisc 0.45 μm before HPLC analysis. The chromatographic separation was carried out in Atlantis HILIC silica column (4.6 × 150 mm, 3 μm) from Waters (Milford, MA) at room temperature. A linear gradient elution program was performed with a mixture of 0.65 mM ammonium acetate (pH 5.5) in water/acetonitrile (25:75) as solvent A and 4.55 mM ammonium acetate (pH 5.5) in water/acetonitrile (70:30) as solvent B. The gradient changed from 0 to 100% of solvent B in 13 min at a flow rate of 1.4 mL min^{-1}. The separation was monitored using an UV/VIS detector (Waters 2487 Dual λ Absorbance detector, Waters, Milford, MA) at 214 nm.

2.4. Statistical analysis

To determine the effect of carcass weight (W), animal age (A) and production status (PS) before slaughter as well as of the muscle type