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α-Tocopherol stereoisomers in beef as an indicator of vitamin E supplementation in cattle diets

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ABSTRACT

These studies investigated the potential application of analysis of stereoisomers of α-tocopherol to discriminate between beef from animals raised at pasture or fed concentrates containing synthetic vitamin E. Muscle α-tocopherol levels were affected ($P < 0.05$) by diet with mean values of 2.63, 1.14, 2.43 and 1.77 μg g^{-1} muscle for beef from animals receiving pasture only (P), a barley-based concentrate with synthetic vitamin E (C), winter silage followed by summer pasture (SiP) and winter silage followed by summer pasture with concentrate (SiPC), respectively. Stereoisomeric analysis of α-tocopherol permitted discrimination between beef from the P/SiP, C and SiPC animals. In a comparison of Irish and non-Irish beef, Brazilian beef had higher ($P < 0.05$) α-tocopherol (8.13 μg g^{-1}) than beef from Austria, England, France, Germany, the U.S. and Ireland (mean 2.51 μg g^{-1}). Stereoisomeric analysis of α-tocopherol in non-Irish beef revealed supplementation with synthetic vitamin E in all samples, including the samples marketed as pasture-fed beef.

1. Introduction

In the aftermath of recent food scandals and associated diseases or toxins in food-producing animals in Europe, consumers have become concerned about the link between their health and the food they consume (Andersen, Oksbjerg, & Therkildsen, 2005; Anon, 2008). They increasingly demand more detailed information about their foods and, particularly, about animal-derived foods and meat products (Prache, 2007; Prache, Cornu, Berdagué, & Priolo, 2005). Many consumers also value organic, grass-fed animal products, which are free of pesticides and medication, and which they consider to be healthier (O'Donovan & McCarthy, 2002; Pirog, 2004). Moreover, in light of the food scares that have arisen from consumption of contaminated feed by animals, characterising the feed which was fed to the animals is also becoming important to consumers. Analytical methods are required which can guarantee that the commitments to the chosen method of production, for example, have been completely adhered to.

At present, the search for suitable methods is in progress (Kelly, Heaton, & Hoogewerff, 2005). A range of potential tracers for the provenance of meat products has been studied up to now (Prache, 2007). Several plant-derived compounds such as tocopherol, carotenoids, terpenes and phenolic compounds have been investigated as possible markers for milk or meat authentication (Prache et al., 2005).

Beef from pasture-fed animals has been shown to have a higher vitamin E content than beef from animals fed cereal-based concentrates (Descalzo et al., 2005; Mercier, Gatellier, & Renerre, 2004; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004; Yang, Brewster, Lanari, & Tume, 2002) and, thus, vitamin E may be considered as one indicator of meat production system, e.g. low intensity pasture-based production versus intensive (indoor) concentrate-based production. Assignment of production system on the basis of muscle vitamin E levels alone is not possible, however, and is open to manipulation, since the vitamin E content of the diet can be altered through vitamin E supplementation of animal diets (Yang et al., 2002). Differences in stereoisomeric forms of vitamin E have the potential to yield useful information about the source of vitamin E (natural versus synthetic supplements) used in beef production systems.

'Vitamin E' is the generic name for a group of eight natural compounds: α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol, which differ in the identity and location of groups on their chromanol ring structure (Scherf, Machlin, Frye, Krautmann, & Williams, 1996). Of the tocopherols, α-tocopherol is the form most commonly found and associated with vitamin E activity in animal tissues. Eight isomeric forms of α-tocopherol exist. In the case of α-tocopherol derived naturally from plant sources the predominant isomer is the RRR-α-tocopherol (Riss, Kormann, Glinz, Walther, &

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Ranalder, 1994) in which the R configuration exists at positions two on the chromanol ring and 4′ and 8′ of the phytyl side-chain (Pongracz, Weiser, & Matzinger, 1995). Completely synthetic α-tocopherol consists of equal fractions of the eight stereoisomers because of the three asymmetrical centres (Kayden & Traber, 1993).

We compared the α-tocopherol content of Irish beef produced under four grass or concentrate-based production systems and investigated the potential for differences in the stereoisomeric forms of α-tocopherol to discriminate between beef from animals fed grass, which contains predominantly RRR-α-tocopherol, and beef from animals fed a concentrate supplemented with synthetic α-tocopherol. Using stereoisomeric analysis of α-tocopherol we then obtained information about the likelihood of unknown Irish and non-Irish beef being derived from production systems in which vitamin E was from natural sources or production systems in which synthetic vitamin E supplementation was used.

2. Material and methods

2.1. Reagents

α-Tocopherol, dimethyl sulphate and 1,2-dimethoxyethane, methanol, *n*-hexane and all other reagents were obtained from Sigma–Aldrich, Ireland Ltd.

2.2. Animals and diets

This study was carried out under licence from the Irish Government Department of Health and Children and with the approval of Teagasc, the Agricultural and Food Development Authority. All procedures used complied with national regulations concerning experimentation on farm animals. Charolais × Limousin crossbred weanling heifers (born between February and March 2006) were sourced from Irish farms through a livestock market and brought to Teagasc Grange Beef Research Centre, Dunsany, County Meath, Ireland. The heifers were weighed, blocked in groups of four on a descending weight basis and within block assigned at random to one of following four treatments, with 25 heifers per treatment: grazed pasture from November 2006 to October 2007 (P); grass silage offered *ad libitum* indoors from November 2006 to April 2007, then grazed pasture from April to October 2007 (SiP); grass silage offered *ad libitum* indoors from November 2006 to April 2007, then grazed pasture plus 50% of the diet dry matter (DM) as supplementary concentrates from April to October 2007 (SiPC); concentrates and straw indoors from November 2006 to October 2007 (C). At the beginning of the experiment the mean (±SD) live weight was 275 ± 27.0 kg and the mean age was 252 ± 28 days.

2.3. Composition of the diets

The P group heifers grazed together on pasture (mainly *Lolium perenne* L., *Poa spp.* and *Trifolium repens* L.) with the herbage mass being rationed daily to maintain sufficient pasture for the 25 heifers to graze from 28 November 2006 to 24 April 2007 on approximately 25 ha of available grassland. The daily ration was a target herbage DM intake of 2% of live weight per heifer above a residual or post-grazing herbage mass of 900 kg DM ha^{-1}. The heifers were offered the daily ration by grazing within 1.5–2.5 ha paddocks subdivided sequentially into daily "breaks" using portable electric fences. Grass silage (predominantly *Lolium perenne* L.) was offered *ad libitum* once daily to the heifers in treatments SiP and SiPC which were housed in groups of five in a slatted floor shed. The concentrates with straw were offered once daily at a restricted level to groups of five heifers accommodated in the same shed. The allowance was adjusted periodically to maintain a similar rate of growth to that the heifers at pasture (P). From April 25 onwards, heifers on treatments P and SiP were set stocked and the area of the paddock adjusted to ensure the required allowance of grass DM was available. Heifers on treatments SiPC were offered a restricted allowance of pasture and, once daily, an increasing amount of concentrate until the allowance reached 0.5 of estimated total daily DM intake. Heifers on treatment C continued to be offered straw and concentrates. Allowances for heifers in treatments SiPC and C were such as to match the expected growth of the P animals when grazing spring grass (AFRC, 1993). The overall strategy was to ensure similar mean body and carcass weights for all treatment groups at slaughter. The composition of the concentrate, which was typical of commercial formulations used in Ireland, was 430 g kg^{-1} rolled barley, 430 g kg^{-1} molassed beet pulp, 80 g kg^{-1} soybean meal, 35 g kg^{-1} molasses, 20 g kg^{-1} mineral/vitamin premix containing 2.5 g kg^{-1} α-tocopheryl acetate (Lutavit E50, BASF) and 5 g kg^{-1} lime. Grass, grass silage and concentrate samples were collected weekly and stored at −20 °C for subsequent analysis.

2.4. Slaughter procedures

One animal from the P group and one animal from the SiP group died during the study from causes unrelated to the dietary treatments. Animals were slaughtered at Meadow Meats Limited, Rathdowney, Co. Laois following EU animal welfare guidelines on the 23 October (23 cattle) and 7 (28 cattle), 13 (24 cattle) and 21 (23 cattle) November 2007. Following overnight chilling of carcasses, the complete *Longissimus dorsi* (LD) muscle was excised from the right side of each carcass. Samples were vacuum packaged and transferred to Teagasc Ashtown Food Research Centre, Dunsinea, Ashtown, Dublin 15 and stored overnight at 4 °C after which a 2.5 cm thick subsample (LD between the 9th and 10th rib) was taken for analysis, vacuum packaged and stored at −20 °C prior to analysis.

2.5. Collection of commercial beef samples of known and unknown dietary background

Beef samples from Austria (n = 6), England (n = 6), France (n = 4) and Germany (n = 8) were obtained frozen from personal contacts of the authors. Beef samples from the U.S. (n = 12), some of which were marketed as pasture-based (n = 6), were obtained through IdentiGEN Inc. (IdentiGEN North America, Inc. Lawrence, KS). Samples from Brazil were sourced through a commercial importer (Dawn Farm Foods Ltd., Naas, Co. Kildare). Three batches of six Brazilian beef samples were obtained at different times: I (Jan 2007), II (February 2007), III (July 2007). Irish beef samples (n = 8) were obtained from a local supermarket (Superquinn, Ballinteer, Dublin 16) and from a producer of pasture-based beef (n = 6) (Omegabeef-direct, Ballymacarbry, Clonmel, Co. Tipperary, Ireland). As far as possible we obtained striploin (LD) samples but samples varied from country to country; all could be classified as either striploin, sirloin or round.

2.6. Measurement of α-tocopherol in feed and muscle

Total α-tocopherol in feedstuffs (grass, grass silage and concentrates) was extracted following the method of Brubacher, Muller-Mulot, and Southgate (1985) while muscle α-tocopherol was extracted using the method of Buttriss and Diplock (1984) as modified by Dunne, Monahan, O'Mara and Moloney (2005).

α-Tocopherol was determined by HPLC using an Agilent 1200 series (Agilent Technologies Inc. Santa Clara, California, USA) equipped with a variable loop injector and a Synergi Hydro – RP