Meat Science 111 (2016) 1–8

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Antioxidant status, lipid and color stability of aged beef from grazing steers supplemented with corn grain and increasing levels of flaxseed

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ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form 28 July 2015
Accepted 29 July 2015
Available online 1 August 2015

Keywords:
Argentine beef
Aging
Retail display
Oxidative stability
Antioxidant
Pasture
Oil seeds

ABSTRACT

Angus steers were grazed on unsupplemented pasture (CNTRL), pasture supplemented with 0.7% BW cracked corn (FLAX-0), FLAX-0 with 0.125% and 0.250% BW of whole flaxseed (FLAX-1 and FLAX-2). Six steers were grazed per treatment for 70 days, with start and finish weights of 458 and 508 kg. At 24 h post slaughter, *longissimus thoracis* were harvested, and steaks assigned to treatments of postmortem aging time under vacuum (PM; 3, 14 and 56 days) with or without five days of aerobic exposure (AE). Meat antioxidant status was higher ($P < 0.05$) when feeding CNTRL and FLAX-1 than FLAX-0 and FLAX-2. Under AE, lipid oxidation was highest for FLAX-2 ($P < 0.05$), and lowest for FLAX-1. Greatest TBARs and lowest antioxidant capacity and redness values were obtained with AE and the longer PM ($P < 0.05$). Beef oxidative stability through AE improved by adding a low flaxseed level to supplemented corn grain, but deteriorated by adding a high flaxseed level or by extending PM.

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1. Introduction

Current dietary recommendations suggest to reduce total fat intake, particularly saturated fatty acids (SFA) and increase the proportion of polyunsaturated fatty acids (PUFA), especially the n − 3 series at the expense of the n − 6 (WHO, 2003). Argentine beef has been traditionally produced on pasture and several studies have shown that meat from animals finished with diets based on forages presents not only lower total fat content but it also has a healthier fatty acid profile than meat from cattle finished on a high concentrate diet (Daley, Abbott, Doyle, Nader, & Larson, 2010; Pavan, 2006; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004). In order to increase energy intake, the supplementation of pasture-based diets with cereal grains at 0.5 to 1.0% animal body weight (BW) is becoming more common among producers. This practice could have a negative impact on n − 6:n − 3 PUFA ratio and CLA *cis-9*, *trans-11* proportion, hence reducing the nutraceutical properties of beef from grazing systems (Garcia et al., 2008; Schor et al., 2007). New strategies of supplementation such as use of flaxseed, has been proposed to increase the concentration of beneficial fatty acids, especially highly unsaturated n − 3 fatty acids in muscle from different ruminant species. Noci, French, Monahan, and Moloney (2007) observed a higher concentration of n − 3 PUFA and CLA *cis-9*, *trans-11* in intramuscular lipids from grazing cattle supplemented with a concentrate containing 15% of linseed oil. Similar results were obtained by Raes, De Smet, Balcaen, Claeys, and Demeyer (2003) in grazing beef cattle and by Jerónimo, Alves, Prates, Santos-Silva, and Bessa (2009) supplementing lambs on concentrate based diets. However, while increasing the content of beneficial fatty acids in meat is commendable from a human health perspective, such changes in fatty acid profile may have deleterious effects on the appearance and shelf-life of meat.

Oxidative damage is the major non-microbial factor responsible for quality deterioration in muscle foods and is one of the main reasons in meat remaining un-sold due to loss of quality during storage and retail display (Faustman, Sun, Mancini, & Suman, 2010; Hur, Park, & Joo, 2007). The oxidative stability of meat lipids depends on the balance between antioxidant and pro-oxidant components in the muscle. Muscle antioxidants comprise endogenous antioxidant systems as well as molecules of dietary origin, such as tocopherols and carotenoids (main fat-soluble antioxidants from plants) among others (Descalzo & Sancho, 2008). Conversely, polyunsaturated fatty acids are highly oxidizable substrates and may act as pro-oxidants (Morrissey, Sheehy,

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http://dx.doi.org/10.1016/j.meatsci.2015.07.026
0309-1740/Published by Elsevier Ltd.

Galvin, Kerry, & Buckley, 1998). The oxidative status of meat is particularly indicated by its color stability and susceptibility to rancidity. The diet of animals can significantly affect the inherent susceptibility of meat lipids to oxidative deterioration, by modifying both the antioxidant and the pro-oxidant components of the muscle. Pasture fodders consumed by cattle supply vitamin E requirements in addition to other natural antioxidants (Gatellier, Mercier, & Renerre, 2004) but also increase the proportion (g/100 g total fatty acids) of highly unsaturated fatty acids (Yang, Lanari, Brewster, & Tume, 2002). Flaxseed oil (mainly fed as seeds) is one of the richest sources of α-linolenic acid (45–52%), but also is naturally high in anti-oxidants nutrients such as lignans, phenolic compounds, flavonoids and tocopherols (α-, β-, γ-, δ) (Singh, Mridula, Rehal, & Barnwal, 2011; Touré & Xueming, 2010).

We therefore hypothesized that the incorporation of natural antioxidants from pasture or supplements with flaxseed could generate in the muscle an adequate antioxidant capacity to protect the oxidation given by the highest dietary PUFA content in beef.

The objective of the study was to evaluate antioxidant vitamin status, lipid oxidation, and color stability of beef aged under vacuum and subsequently exposed to retail display conditions, from grazing steers supplemented with corn grain or corn grain plus flaxseed.

2. Materials and methods

2.1. Animals and diets

The study was carried out in accordance with Argentinian national recommendations for animal handling and those of the National Institute for Agricultural Technology (INTA, Instituto Nacional de Tecnología Agropecuaria) at EEA-Balcarce, Province of Buenos Aires, for the use of experimental animals including animal welfare.

Details of animals, diets, FA intake, carcass weight and fat measurements were presented in full previously (Pouzo, Fanego, Santini, Descalzo, & Pavan, 2015). Briefly, twenty four Angus steers from the same herd were grown on a rotational grazing system without supplementation until they reach 458 ± 42.8 kg LW and were randomly assigned to four dietary treatments of finishing (no-supplement, *CNTRL*; supplemented: 0.7% LW of cracked corn grain plus no flaxseed, *FLAX-0*; plus 0.125% LW of whole flaxseed, *FLAX-1*, or plus 0.250% LW of whole-flaxseed, *FLAX-2*). Throughout the study, steers from the four dietary treatments grazed as one group, but individually received 0.5 kg (as-fed) of wheat bran in addition to their individual dietary treatment (supplement), so that each animal was considered an experimental unit (n = 6).

Fourteen days before starting the study, steers were trained to use gates for individual access to the supplement using wheat brans and in the last 5 days steers were adapted to the assigned supplements. During adaptation steers were allowed to graze on the same pasture that was subsequently used for the trial. After adaptation, steers received their supplement (0.5 kg of wheat bran plus the dietary treatment) on a daily basis for 70 days at a fixed time each day (10:00 am). Individual BW was determined every 21 days at 08:30 am, and the level of supplement to be offered subsequently was adjusted using the average treatment BW. During the study the steers rotationally grazed on annual ryegrass (*Lolium multiflorum* cv Billy Max and cv Jack). Animals were removed from grazed paddocks when pasture height was reduced to approximately 5 cm (visually estimated by trained personnel).

2.2. Antioxidant intake determinations

Forage DM intake and in vivo apparent total DM digestibility were estimated in a previously mentioned study (Pouzo et al., 2015). Briefly, chromium sesquioxide was used as an external marker and indigestible NDF (INDF) was used as an internal marker of the digesta (Lippke, Ellis, & Jacobs, 1986). Forage DMI and dietary DM and NDF in vivo apparent digestibility were calculated based on fecal output and indigestibility. Forage, corn grain, flaxseed and wheat-middling were collected during trial and stored at −25 °C until required for analysis of antioxidant vitamins. The individual antioxidant intake was calculated from DM intake of each feed component (forage, corn grain, flaxseed and wheat bran) and their respective antioxidant concentration obtained by high performance liquid chromatography (see Section 2.4).

2.3. Sample collection and storage treatments

Animals were harvested at a commercial slaughter house after 70 days on trial with an average of 508 kg BW. Plasma samples were collected during exsanguination and stored at −25 °C until subsequent analysis in order to evaluate the relationship between antioxidant intake and its absorption in the blood at harvest.

Sections containing 6–8 ribs were collected from the left carcass sides after 24 h postmortem (PM). The *longissimus thoracis* muscle was removed and cut into six 2.5 cm thick steaks. Steaks obtained from each section were individually vacuum-packaged and randomly distributed among six treatments, generated by the combination of three PM aging periods at 2 °C (PM; 3, 14 and 56 days) and two aerobic exposure periods (AE; 0 and 5 days). For aerobic exposure (simulating retail display) steaks were placed on Polyfoam trays, overwrapped with an oxygen-permeable polyvinylchloride film and stored under simulated retail display conditions of illumination (2000 lx) and temperature (2 °C). After completing their assigned aging and aerobic exposure periods, steaks were vacuum-packed and stored at −25 °C until later analysis.

2.4. Tocopherol (α and γ), β-carotene, retinol and lutein content

α-Tocopherol, γ-tocopherol, β-carotene, retinol and lutein from meat samples from each of the six treatments (aged for 3, 14 and 56 days on vacuum and retail displayed for 0 and 5 days), from feedstuffs and from plasma samples were extracted as described by Buttriss and Diplock (1984). For meat samples, the extraction procedure was adapted from Descalzo et al. (2005). Briefly, 5 g of lean tissue was placed in a plastic conical tube containing 10 mL of phosphate buffer (0.05 M; pH 7.7), and homogenized for 30 s at 3000 rpm with an Ultraturrax T25 (IKA, Germany). Aliquots of 1 g homogenate were placed into a screw cap test tube with 2 mL of ethanol with 1% pyrogallol to prevent oxidation during the extraction. Thereafter, 0.9 mL of 10 N KOH in water was added to each tube for saponification. The tube contents were mixed by vortexing for 10 s, and placed in a stirred water bath for 30 min at 70 °C. After cooling, 1 mL water was added to each tube. Following the addition of 5 mL n-hexane, the samples were mixed by vortexing for 2 min; the upper hexane layer was then transferred into a new screw cap tube and the aqueous phase was re-extracted with 5 mL of hexane. The combined extracts were taken to dryness under a dry nitrogen gas stream, and the residue was dissolved in 500 μL of absolute ethanol (J.T. Baker, Mexico, HPLC grade) and filtered through a 0.45 μm pore nylon membrane before injection of samples.

All samples and standards (external standards for each vitamin) were analyzed by reverse phase high performance liquid chromatography (HPLC) using a quaternary pump (P4000) with a membrane vacuum degasser connected to an auto sampler AS2000 (Thermo Separation Products) with an injection loop (10 to 100 μL) and a C18 column (250 × 4.6 mm i.d., Alltima, 5 μm particle size; Alltech, Argentina) fitted with a guard column (Security GuardAlltima C18, Alltech Argentina) and a mobile phase of ethanol: methanol (60:40, v/v) at a flow rate of 1 mL/min. The technique was optimized to determine tocopherols, carotenoids and retinol within the same elution time of 25 min. For tocopherols, a fluorescent detector (FL3000; Thermo Separation Products, USA) was set at 296–330 nm, k_{exc} and k_{em}, respectively. A diode array detector (UV6000; Thermo Separation Products, USA) was set at