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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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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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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

445 nm and 325 nm for the detection of carotenoids and retinol, respectively.

Calibration curves were performed with DL-α-tocopherol (Merck, Darmstadt, Germany), γ-tocopherol, β-carotene, lutein and retinol standards (Sigma-Aldrich, St. Louis, USA) diluted in ethanol.

Chromatograms were recorded using a Chromquest 4.0 Software platform.

For feedstuff samples, the methodology was identical, except that the samples were diluted 1 to 20 or 1 to 100 with ethanol before injection, depending on the matter. For plasma samples the saponification step was avoided.

2.5. Plasma and muscle ferric reducing-antioxidant power (FRAP)

The FRAP assay was originally developed to measure the total reducing power of biological fluids, and traditionally has been applied to plasma, beverages, fruit and vegetable extracts (Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). Antioxidant compounds such as alpha-tocopherol, trolox, vitamin C, uric acid and bilirubin, among others, are able to reduce ferric to ferrous-tripyridyltriazine which develops a blue color (Benzie & Strain, 1999). At low pH, a ferryl tripyridyltriazine (Fe^{III}–TPTZ) complex is reduced to ferrous (Fe^{II}) form that develops an intense blue color with an adsorption maximum at 593 nm.

For meat samples, this assay was modified to measure endogenous ions that could react with TPTZ and develop blue color (i.e. endogenous Fe^{II}). Following the procedure described by Descalzo, Rossetti, Grigioni, et al. (2007), 5 g of chopped meat samples was disrupted for 2 min at 3000 rpm with an Ultraturrax (IKA, Germany) homogenizer in 10 mL potassium phosphate buffer pH 7.2. Homogenates were centrifuged at 10,000 ×*g* for 30 min and the supernatant collected. 83 μL aliquots of supernatant were added to 2.5 mL of FRAP buffer containing 10 mM TPTZ (Sigma-Aldrich, Argentina, SA), 40 mM HCl and 20 mM Fe_2Cl_3 (Sigma-Aldrich, Argentina, SA) added to a 300 mM acetate buffer. The reaction mixture was incubated for 30 min at 37 °C in water bath, cooled in an ice water bath and immediately measured at 593 nm (Spectrometer UV–vis-BIO Lambda 20, Perkin Elmer). The incubation step at 37 °C was added to the procedure in order to obtain a full color development and a better resolution of the differences between treatments. The plasma samples were added directly to reagents and measured without previous processing. For meat samples, FRAP activity was measured in each PM period at the beginning AE-0 and the end AE-5 of display (days 0 and 5 respectively).

The FRAP activity of the samples was measured against a calibration curve made with ferrous sulphate ($Fe_2SO_4 \cdot 7H2O$, Sigma-Aldrich, Argentina, SA) within the range from 100 to 1000 μM and results were expressed as Fe^{II} equivalent in μM.

2.6. Determination of the oxidative stability: meat color and lipid oxidation

Meat color was measured daily during the five days of retail display (at days 0, 1, 2, 3, 4 and 5 of AE) using a Minolta CR 310 Chroma meter (Minolta Corp., Ramsey, NJ). The instrumental conditions were: large area aperture (5 cm diameter), D65-artificial and 10° standard observation angle. The instrument was calibrated against a white plate. After opening, the vacuum-packed samples were allowed to bloom for 30 min prior to color determination. The values from three scans were averaged for each color determination.

The lipid stability of muscle samples was measured in each PM period (3, 14 and 56 days) at the beginning (AE-0) and the end (AE-5) of display (days 0 and 5 respectively), on the same samples analyzed for color. To assess the amount of lipid peroxidation, TBARS assays were performed according to Descalzo et al. (2005). Briefly, triplicate aliquots (10 g) of meat were chopped and processed in a stomacher-type homogenizer for 180 s in bags containing 50 mL trichloroacetic acid (Merck, Darmstadt, Germany) solution (10% w/v). Slurries were filtered, an equal volume (10 mL) of 0.02 M 2-thiobarbituric acid (Sigma-Aldrich, St. Louis, Mo. USA) was added, and samples were incubated at 25 °C overnight to yield a pink color development. Color intensity was determined at maximum absorption (530 nm) and TBARS concentrations were calculated form a calibration curve using 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, St. Louis, MO, USA) as standard within the range from 0 to 0.5 μM. Results were expressed as mg of malonaldehyde (MDA) /kg meat.

2.7. Peroxidation index calculation

Muscle (*longissimus thoracis*) total FA content and FA composition presented in current study were calculated and analyzed from part of fatty acid data obtained by Pouzo et al. (2015). The peroxidation index (PI) was calculated from muscle FA composition according to the equation suggested by Hu, Frankel, Leibovitz, and Tappel (1989): PI = (% dienoic) + (% trienoic × 2) + (% tetraenoic × 3) + (% pentaenoic × 4) + (% hexaenoic × 5). This index estimates the concentration of bis-allyl hydrogen atoms present in PUFA and therefore, their overall susceptibility to peroxidation.

2.8. Statistical analysis

Data were analyzed using the Proc Mixed procedure (SAS Inst. Inc., Cary, NC), with the main effects of DIETS, PM and AE, as well as their interactions, included in the statistical model; each individual animal was used as the experimental unit (n = 6). Least squares means were computed for main and interactive effects and were separated statistically using F-protected ($P < 0.05$) t-tests (PDIFF option). Meat redness, as denoted by the a^* value, was subjected to the Mixed Procedure of SAS (SAS, 2012) as a design with repeated measures. Fatty acid data and peroxidation index were subjected to the GLM Procedure of SAS and means were separated statistically using Duncan test ($P < 0.05$).

3. Results and discussion

3.1. Antioxidant intake and its relationship with plasma antioxidant status

Dry matter content (DM) and chemical composition of feedstuffs are presented in Table 1.

Alpha-tocopherol and β-carotene are the main antioxidants provided by the grass in extensive beef production systems. It was reported that α-tocopherol concentration ranged from 21 to 85 mg/kg DM and β-carotene from 26 to 61 mg/kg DM depending on forage specie considered (Elgersma, Søegaard, & Krogh Jensen, 2013). The levels of α-tocopherol observed in the forage of the present work were relatively low (10.46; Table 1). Sikel, Bilger, and Ohlson (2012) found that the mean concentration of α-tocopherol in grasses from the Norwegian Alps region ranged from approximately 5 ± 2 (*Poa alpine*) to 649 ± 91 (*Viola biflora*) mg/kg DM of sample tissue. Hartikainen, Xue, and Piironen (2000) reported a mean value of 16.5 ± 2.3 mg/kg of fresh

Table 1
Dry matter content and chemical composition of feedstuffs.

Item	Pasture	Corn grain	Flaxseed	Wheat bran
Dry matter, g/100 g	22.1	92.3	90.9	92.9
Chemical composition, % DM[a]				
Crude protein	8.67	7.35	21.2	14.7
Neutral detergent fiber	55.0	15.3	40.8	35.5
Acid detergent fiber	31.0	3.2	26.6	11.3
Total fatty acids, FA	1.17	3.44	32.3	3.13
Linoleic acid, g/100 g FA	9.45	52.2	14.6	55.7
Linolenic acid, g/100 g FA	40.4	1.38	47.4	4.78
α-Tocopherol, mg/kg	10.5	1.39	1.58	10.9
γ-Tocopherol, mg/kg	10.0	14.1	42.6	9.81
β-Carotene, mg/kg	86.2	1.47	0.23	0.07
Lutein, mg/kg	955	162	6.54	7.78

[a] DM, dry matter.

ryegrass. Whereas the level found in the present experiment, resembles those reported by Lynch, Kerry, Buckley, Morrissey, and Lopez-Bote (2001), of 14.48 ± 0.36 mg/kg for pasture from Ireland. The grass in the finishing period of the present trial (late spring) was withered and in advanced stages of physiological maturity which, in accordance with the findings of other authors (Tejerina, García-Torres, Cabeza de vaca, Vázquez, & Cava, 2011), could render lower levels of tocopherols than in fresh grasses. In contrast, β-carotene concentrations were higher than the 56.6 mg/kg DM reported by Lindqvist, Nadeau, and Jensen (2012) for fresh grasses, thus indicating that losses of vitamins were not due to the handling of the samples (as beta carotene is extremely labile to air and light exposure).

The antioxidant intake in the feeds used was calculated from the content of each individual component and results are similar for all dietary treatments. As it is shown in Table 2, only γ-tocopherol was significantly higher ($P < 0.05$) in the three diets that contained grain. However, there was no effect of diet on this isomer for plasma levels. The levels of α-tocopherol were almost 70-times higher than the levels of the γ-isomer. Despite the relative abundance of both tocopherol isomers within the diet, α-tocopherol is preferentially retained in mammals by the action of the α-tocopherol transfer protein (α-TTP) in the liver, which preferentially incorporates α-tocopherol into blood lipoproteins (Traber, 1999). Nevertheless, levels of α-tocopherol in the plasma were in general lower than those reported by other studies for animals reared on pasture (Descalzo et al., 2005; Yang, Brewster, Lanari, & Tume, 2002). It is important to note that the consumption of α-tocopherol estimated by Yang, Brewster, et al. (2002), exceeded the consumption observed in the present study by almost 20 times; Yang, Brewster, et al. (2002) underlined the high quality of the pasture consumed by animals, but did not report the concentration of α-tocopherol on pasture in their study.

Plasma α-tocopherol was the only plasma antioxidant affected by dietary treatments in present study. Indeed, plasma α-tocopherol concentration was greater ($P < 0.05$) in FLAX-1 than in either FLAX-0 or FLAX-2; CNTRL plasma α-tocopherol concentration was intermediate, not differing from any of the other three dietary treatments ($P > 0.05$). The increase observed in plasma α-tocopherol with FLAX-1 is in agreement with the results obtained by Weiss and Wyatt (2003) when supplemented dairy cattle with increasing levels of fat. Intestinal absorption of α-tocopherol is facilitated by increasing dietary oil level (Yang, Lanari, et al., 2002). On the other hand, the greater dietary PUFA intake with FLAX-2 may have increased degradation of α-tocopherol in the gastrointestinal tract as observed by Villaverde, Cortinas, Barroeta, Martín-Orúe, and Baucells (2004) in poultry.

Table 2
Consumption and content of antioxidant vitamins and ferric reducing-antioxidant power (FRAP) in plasma from pasture-fed cattle no supplemented (CNTRL), supplemented with 0.7% BW corn grain (FLAX-0), supplemented with 0.7% BW corn grain plus 0.125% BW flaxseed (FLAX-1) and supplemented with 0.7% BW corn grain plus 0.250% BW flaxseed (FLAX-2).

Item	CNTRL	FLAX-0	FLAX-1	FLAX-2	SE	*P*-value
Individual intake of antioxidant vitamins						
α-Tocopherol (mg/day)	102.4	122.5	107.2	106.2	7.69	0.29
γ-Tocopherol (mg/day)	97.5^{c}	158.9^{b}	166^{b}	193^{a}	7.92	<0.01
β-Carotene (mg/day)	757	888	758	736	62.4	0.32
Luteín (mg/day)	8407	10,334	8875	8657	695	0.23
Antioxidant status in plasma						
α-Tocopherol (μg/mL)	2.11^{ab}	1.96^{b}	2.37^{a}	1.79^{b}	0.13	0.02
γ-Tocopherol (μg/mL)	0.03	0.03	0.04	0.05	0.01	0.16
β-Carotene (μg/mL)	7.77	6.32	8.99	6.94	0.72	0.07
Lutein (μg/mL)	0.31	0.19	0.27	0.19	0.05	0.31
Retinol (μg/mL)	0.31	0.33	0.32	0.32	0.02	0.93
Total antioxidant activity FRAP (μM Fe^{II})	661	625	620	614	21.5	0.41

Rows with different letters are statistically different ($P < 0.05$).

As reported by Chauveau-Duriot, Doreau, Nozière, and Graulet (2010), beta carotene and lutein were the major carotenoids found in plasma samples and these compounds were incorporated in all treatments, probably coming from forage. Traces of zeaxanthin and 13-cis beta carotene were also observed but not quantified in the present study.

3.2. Antioxidant vitamins and ferric reducing-antioxidant power (FRAP) activity in muscle

As it is shown in Table 3 there was a significant interaction between aerobic exposure and diet ($P = 0.004$) only for α-tocopherol and a significant effect for aerobic exposure × aging time for both tocopherol isomers (α; $P = 0.017$ and γ; $P < 0.0001$), while the interactive effect for the remaining antioxidant vitamins studied were no significant ($P > 0.05$).

In the absence of oxygen (AE = 0), CNTRL and FLAX-1 treatments showed greater concentration of α-tocopherol in beef, similar to the treatment effects observed in plasma. However, after 5 days of aerobic exposure there was a drastic reduction of α-tocopherol in beef, showing a similar decline between diets. Similarly, at 0 days of aerobic exposure the contents of α- and γ-tocopherols were reduced following aging, but at 5 days of aerobic exposure these differences between aging times disappear and the level of α- and γ-tocopherols was lower than its initial values (AE = 0).

The drastic reduction in the concentration of tocopherols with aerobic exposure could be related to the consumption of antioxidant vitamins in meat that interacted to counteract lipid oxidation. Insani et al. (2007) also observed a strong reduction of α-tocopherol content in meat with different initial contents of tocopherols (pasture and grains) when the beef samples were stored for 9 days in retail display conditions. In addition, the levels of α-tocopherol in beef were reduced with aging and almost half of initial content of γ-tocopherol was consumed after 14 days of aging on vacuum ($P < 0.05$), these findings are similar to those reported by Descalzo, Rossetti, Sancho, et al. (2007).

As in plasma, α-tocopherol was, as expected, the most abundant lipid soluble antioxidant vitamin incorporated into the muscle. The initial concentration found in this assay (samples under vacuum by 3 days; PM-3) was lower than those reported previously for *longissimus* muscle (Descalzo, Rossetti, Grigioni, et al., 2007; Yang, Lanari, et al., 2002), but just marginally higher than those reported by Fredrikson-Eriksson and Pickova (2007) for pasture samples. In general, cattle grazed in good quality pasture achieved high concentrations of α-tocopherol in the muscle (Descalzo & Sancho, 2008; Yang, Lanari, et al., 2002). Therefore, as was observed previously, the relative lower initial concentrations of this antioxidant observed in the present study could be attributed to the lower quality of pasture consumed by cattle during the period of supplementation (70 days).

In cattle, β-carotene is the main carotenoid absorbed from the intestine (Yang et al., 1993). Muscle β-carotene content was not different among dietary treatments in the present study (Table 3), in agreement with Mahecha et al. (2009) and as expected from observed plasma β-carotene concentration. In general, muscle levels of β-carotene show a high variability among experiments where cattle were on grazing systems (Descalzo, Rossetti, Grigioni, et al., 2007; Insani et al., 2007; Yang, Brewster, et al., 2002). In this experiment, regardless of dietary treatment or aging period, the overall average of β-carotene was 0.18 ± 0.02 μg/g. As in the present study, Insani et al. (2007) observed no change in the level of β-carotene in beef under retail display conditions when its initial concentration was 0.17 μg/g of meat; however these authors observed a clear trend of declining β-carotene levels when it was 0.74 μg/g of meat, suggesting that the degree of consumption of β-carotene in the muscle may depend on their initial concentrations. As γ-tocopherol and β-carotene, lutein and retinol vitamins also were found in levels one order below that of α-tocopherol. CNTRL and

Table 3
Effect of dietary treatment, postmortem aging times and aerobic exposition on antioxidant vitamins of *longissimus thoracis* muscle.

Antioxidant vitamin (μg/g of fresh meat)	Aerobic exposition (days)	Diet[1]					Postmortem aging (days)				Significance[2]				
		CNTRL	FLAX-0	FLAX-1	FLAX-2	SE	3 days	14 days	56 days	SE	D	P	A	D * A	P * A
α-Tocopherol	0	1.76^{aA}	1.15^{bA}	1.65^{aA}	1.34^{bA}	0.09	1.69^{aA}	1.49^{aA}	1.24^{bA}	0.08	**	*	***	**	*
	5	0.09^{aB}	0.11^{aB}	0.12^{aB}	0.11^{aB}		0.10^{aB}	0.11^{aB}	0.12^{aB}						
γ-Tocopherol[3]	0	0.12	0.13	0.14	0.14	0.02	0.27^{aA}	0.12^{bB}	0.17^{bA}	0.02	ns	*	***	ns	***
	5						0.07^{aB}	0.11^{aB}	0.07^{aB}						
β-Carotene	–	0.19	0.21	0.15	0.17	0.02	0.18	0.19	0.17	0.01	ns	ns	ns	ns	ns
Lutein	–	0.029^{ab}	0.026^{b}	0.033^{a}	0.026^{b}	0.01	0.03	0.03	0.03	0.01	**	ns	ns	ns	ns
Retinol	–	0.07	0.09	0.08	0.07	0.01	0.08	0.08	0.07	0.01	ns	ns	ns	ns	ns

Interactive effect of treatment D × P; D × P × A not significant; P > 0.05.
[1]CNTRL, no supplemented; FLAX-0, supplemented with 0.7% BW of corn grain; FLAX-1, supplemented with 0.7% BW of corn grain plus 0.125% BW of flaxseed; FLAX-2, supplemented with 0.7% of corn grain plus 0.250% BW of flaxseed. D: diet· P: postmortem aging, A: aerobic exposition, D * A: interaction diet * aerobic exposition, P * A: interaction postmortem aging * aerobic exposition, D * P: interaction diet * postmortem aging, D * P * A: interaction diet * postmortem aging * aerobic exposition. [2]ns, no significant; *, ** and *** refer to $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively. Means with different capital letters within the same column (aerobic exposition effect) and different non-capital letters within the same row (dietary treatment or postmortem aging effect) indicate significant differences ($P < 0.05$). [3]Concentrations for CNTRL, FLAX-0, FLAX-1, and FLAX-2 represent the average for 0 and 5 days of Aerobic Exposition.

FLAX-1 treatments showed greater lutein and α-tocopherol concentration in beef, whereas no differences were observed for retinol.

In general, as expected, dietary effects on muscle antioxidant content reflected changes observed on plasma antioxidant content.

FRAP values determine the total reducing capacity or antioxidant capacity of the samples. Mahecha et al. (2011) showed that hydrophilic extracts of *longissimus* muscle had higher antioxidant values than lipophilic extracts, either using FRAP or TEAC methods. Thus, the antioxidant capacity measured by this technique is probably complementary to the action of the lipid-soluble antioxidants. In this work, FRAP was measured in aqueous fractions of muscle extracts. A positive correlation between FRAP and the level of α-tocopherol in the muscle has been reported previously by Descalzo, Rossetti, Grigioni, et al. (2007). Therefore, it was measured here as an indicator of total antioxidant capacity of meat homogenates.

As is shown in Table 4, FRAP level was affected by aging under anaerobic (vacuum packaged) or aerobic conditions (interactive effect PM × AE; P = 0.013). A significant reduction of FRAP activity was thereby observed at 5 days of aerobic exposure in beef from all PM periods with respect to its initial values (AE = 0). In absence of O_2, total FRAP activity of beef was reduced ($P < 0.05$) across PM times in present study (in decreasing order, by days 3, 14 and 56 of aging); this reduction was in agreement with that found by Mahecha et al. (2011), who described a decrease of 18% in aqueous FRAP values of *longissimus* muscle extracts at day 14 of refrigerated storage under similar conditions (vacuum, darkness and 2 °C). Aerobic exposure of meat during 5 days caused a FRAP reduction of 19% in meat aged from 14 days that remained after 56 days of aging. Evidently, the reduction of FRAP could be associated with the reduction of antioxidant vitamin concentration in beef after storage. Increasing periods of aging on vacuum and/or the combination of intermediate periods of storage in vacuum (aging) and subsequent exposure to aerobic conditions thus seem to potentiate the loss of beef total reducing capacity. In addition, antioxidant FRAP activity in beef was affected by the diet (P = 0.005) independently of aging or aerobic exposition periods. Therefore, meat from FLAX-1 and CNTRL showed higher ($P < 0.05$) antioxidant capacity than meat from FLAX-0 and FLAX-2, in coincidence with the lower levels of alpha-tocopherol found in LT muscle from FLAX-0 and FLAX-2 diets (AE = 0).

3.3. Lipid stability (TBARS) of muscle

Lipid oxidation in muscle systems is believed to be initiated at the membrane level in the highly unsaturated phospholipid fraction (Gray & Pearson, 1987). Increasing the muscle concentration of PUFA (which are associated with the cell membrane and are susceptible to lipid oxidation) can therefore result in a significant increase in TBARS levels. As indicated in Table 5, the addition of increasing levels of flaxseed to the diet of steers produced an increase in the peroxidation index in beef, due to an increase of PUFA with more than two double bonds. Fatty acid data of animals analyzed in the present study showed that the addition of increasing levels of flaxseed was also able to increase the proportion of total n − 3 PUFA in beef of corn grain supplemented steers (Table 5). Thus, the total n − 3 PUFA proportion on *longissimus thoracis* was 1.52 in CNTRL, 1.54 in FLAX-0, 1.88 in FLAX-1 and 2.30 in FLAX-2, and the total PUFA proportion was 4.65, 4.96, 5.65 and 6.60, respectively.

Regarding the n − 3 PUFA and nutritional considerations Bjerve, Fischer, Wammer, and Egeland (1989) have proposed an optimal intake of 860–990 mg C18:3n − 3/day and 350–400 mg (C20:5n − 3 + C22:6n − 3)·day^{-1} for adults. Although beef can only provide a small amount recommended intake of n − 3 fatty acids it has been reported that red meat serves as a significant source of n − 3 fatty acids for some populations (Sinclair, Johnson, O'Dea, & Holman, 1994). Taking into account the low level of intramuscular fat reached in present study it is expected that the n − 3 PUFA contribution to human diet would be significantly increased if high levels of intramuscular fat are achieved. Further increasing the content of the n − 3 fatty acids in bovine meat is advisable since many people in Western societies have a dislike to eat fatty fish. McAfee et al. (2011) reported that subjects

Table 4
Effect of dietary treatment, postmortem aging times and aerobic exposition on ferric reducing-antioxidant power and lipid oxidation of *longissimus thoracis* muscle.

Variable	Aerobic Exposition (days)	Diet[1]					Postmortem aging (days)				Significance[2]				
		CNTRL	FLAX-0	FLAX-1	FLAX-2	SE	3d	14d	56d	SE	D	P	A	D*A	P*A
TBARS (mg MDA/kg)	0	0.10^{aB}	0.11^{aB}	0.09^{aB}	0.11^{aB}	0.02	0.10^{aB}	0.10^{aB}	0.10^{aB}	0.03	***	***	***	***	***
	5	0.35^{bA}	0.38^{bA}	0.26^{cA}	0.46^{aA}		0.23^{cA}	0.30^{bA}	0.56^{aA}						
FRAP[3] (μM)	0	510^{a}	471^{b}	515^{a}	478^{b}	10.4	577^{aA}	540^{bA}	467^{cA}	12.68	**	***	***	ns	*
	5						525^{aB}	427^{bB}	425^{bB}						

Interactive effect of treatment D × P; D × P × A not significant; $P > 0.05$.
FRAP = ferric reducing-antioxidant power; tbars = thiobarbituric acid reactive substances.
[1, 2, 3]For references see Table 3. Means with different capital letters within the same column (aerobic exposition effect) and different non-capital letters within the same row (dietary treatment or postmortem aging effect) indicate significant differences ($P < 0.05$).

Table 5
Total fatty acid (FA) content, major classes of FA, individual n − 3 polyunsaturated FA (weight % of total methyl esters) and calculated peroxidation index (%) of *longissimus thoracis* fresh muscle from pasture-fed cattle not supplemented (CNTRL), supplemented with 0.7% BW corn grain (FLAX-0), supplemented with 0.7% BW corn grain plus 0.125% BW flaxseed (FLAX-1) and supplemented with 0.7% BW corn grain plus 0.250% BW flaxseed (FLAX-2).

	Diet					*P*-value
	CNTRL	FLAX-0	FLAX-1	FLAX-2	SE	
Peroxidation index (%)	9.08^{b}	9.73^{b}	11.28^{ab}	13.67^{a}	1.01	0.03
Total FA (g/100 g tissue)	4.74	5.13	3.77	3.53	0.70	0.32
Fatty acid composition (g/100 g total FA)						
Sum of SFA	42.2	40.7	41.4	39.0	0.82	0.09
Sum of MUFA	40.5	42.3	41.9	41.8	0.72	0.53
Sum of PUFA	4.65^{b}	4.96^{b}	5.65^{ab}	6.60^{a}	0.40	0.01
Sum of n − 6 PUFA	3.13^{b}	3.42^{b}	3.77^{ab}	4.30^{a}	0.24	0.02
Sum of n − 3 PUFA	1.52^{b}	1.54^{b}	1.88^{ab}	2.30^{a}	0.17	0.02
n − 6 PUFA/n − 3 PUFA	1.49^{b}	1.71^{a}	1.59^{ab}	1.52^{b}	0.05	0.02
Individual n − 3 PUFA						
C18:3n − 3	0.67^{b}	0.62^{b}	0.77^{b}	0.93^{a}	0.05	<0.01
C20:4n − 3	0.08^{b}	0.09^{b}	0.10^{b}	0.13^{a}	<0.01	<0.01
C20:5n − 3	0.28	0.31	0.37	0.52	<0.01	0.06
C22:5n − 3	0.42	0.47	0.56	0.64	0.06	0.08
C22:6n − 3	0.06	0.06	0.07	0.09	<0.01	0.10

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.
Sum of SFA = C8:0 + C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0; Sum of MUFA = C14:1n − 5 + C16:1n − 7 + C18: 1 cis-9 + C18: 1 cis-11 + C20:1n − 9; Sum of n − 6 PUFA = C18:2n − 6*trans*, *trans* + C18:2n − 6*trans*, *cis* + C18:2n − 6*cis*, *trans* + C18:2n − 6*cis*, *cis* + C20:4n − 6; Sum of n − 3 PUFA = C18:3n − 3 + C20:4n − 3 + C20:5n − 3 + C22:5n − 3 + C22:6n − 3; Sum of PUFA = Sum of n − 3 PUFA + Sum of n − 6 PUFA; peroxidation index = (% dienoic) + (% trienoic × 2) + (% tetraenoic × 3) + (% pentaenoic × 4) + (% hexaenoic × 5).
Rows with different letters are statistically different ($P < 0.05$).

that consumed red meat from grass-fed cattle (containing similar or even lower levels of n − 3 PUFA than in the present study) had not only greater n − 3 PUFA dietary intake, but also plasma and platelet long-chain n − 3 PUFA concentration than subject that consumed red meat from concentrate-fed cattle. Therefore, the highest level of n − 3 PUFA reached in beef from the present study, likely may result in an improvement of consumer health; however it must be further investigated.

The use of different sources of fatty acids can have different effects on the oxidative stability of the meat (Daly, Moloney, & Monahan, 2007; Juárez et al., 2012; Nute et al., 2007). In the present study, three factors (i.e., diet, PM and AE) had significant effects on TBARS values, with *P*-values lower than 0.0001. Two-way interactions (Diet × AE and PM × AE) were highly significant ($P < 0.0001$), but the two-way (Diet × PM) and the three-way interactions (Diet × PM × AE) were not significant, with *P*-values > 0.05.

No significant differences in the TBARS values were observed between dietary treatments on the first day of refrigerated storage. However, TBARS values were increased in all groups with advancing time of refrigerated storage, as a consequence of lipid peroxidation. After 5 days of refrigerated storage, TBARS values were significantly greater than at day 0, and even higher ($P < 0.05$) for the meat of the steers being fed the higher concentration of flaxseed (FLAX-2), mainly due to the higher content of PUFA and the higher peroxidation index (Table 5). This finding is in agreement with Moloney, Kennedy, Noci, Monahan, and Kerry (2012), who reported a similar behavior in the oxidation of meat from lambs fed linseed diets.

Thus, meat from supplemented FLAX-1 diet had the highest lipid stability after storage and FLAX-2 the lowest. This result is interesting because FLAX-1 diet (together with CNTRL) had higher sum of antioxidant vitamins and FRAP activity compared to other dietary treatments. Therefore, concentration of antioxidants incorporated into the muscle in FLAX-1 diet was enough to offset the increased level of peroxidation that could be generated by elevated levels of n3 − PUFA in the muscle, while in FLAX-2 diet, this effect would not have been enough, as it provides an excess of pro-oxidants with higher demand for vitamin E.

Despite the lack of differences ($P > 0.05$) in the level of TBARS between PM aging periods in vacuum packaging (average 0.10 ± 0.03 mg MDA/kg muscle), TBARS levels increased when the aging period was followed by 5 days exposure to oxygen (interaction PM × AE $P < 0.0001$; Table 4). Similar effects have been reported by Yang, Lanari, et al. (2002) in pasture-fed beef aged for 47 days in vacuum followed by 7 days of aerobic storage. These authors attributed their result to the high contents of peroxidable lipids, coupled with aging changes in other muscle components. It is also plausible that denaturation of proteins during aging of beef releases prooxidant metal ions that make lipid and myoglobin more prone to oxidation upon exposure to oxygen. Finally, the loss of FRAP activity in aged beef, as was observed in this study, evidently indicates a loss of reducing power, as well as progressive lipid oxidation during refrigerated retail display.

3.4. Implications of the antioxidant balance on meat color

The oxidation of myoglobin to (brown) metmyoglobin is associated with deterioration of red color or reduction of parameter a* (redness) in meat (Faustman, Chan, Schaefer, & Havens, 1998; McDowell et al., 1996; O'Sullivan et al., 2002). The effect of two-way interactions i.e., DIET × AE (P = 0.047) and PM × AE ($P < 0.0001$) on the a* value were analyzed in this work. As can be seen in Fig. 1a, the initial a* values were similar between diets, except for CNTRL that was slightly ($P = 0.044$) lower than FLAX-2. However, at day 1 of retail display the a* values from all diets were higher than the initial values, but similar between diets. We hypothesize that this increment could be due to the complete transformation from deoxymyoglobin (samples in vacuum) to oxymyoglobin

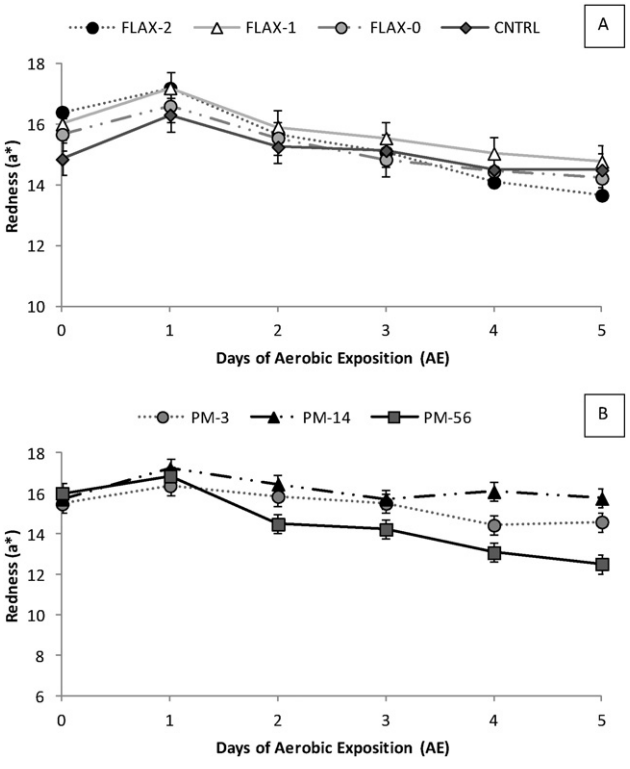

Fig. 1. A) Interactive effect of dietary treatment and days of aerobic exposition (AE) on redness value (means ± SE). CNTRL, not supplemented; FLAX-0, supplemented with 0.7% BW of corn grain; FLAX-1, supplemented with 0.7% BW of corn grain plus 0.125% BW of flaxseed; FLAX-2, supplemented with 0.7% of corn grain plus 0.250% BW of flaxseed. B) Interactive effect of postmortem aging in vacuum (PM) and days of aerobic exposition (AE) on redness value (means ± SE). PM-3, 3 days of postmortem aging on vacuum; PM-14, 14 days of postmortem aging on vacuum and PM-56, 56 days of postmortem aging on vacuum.

(samples in aerobic conditions) during the first day of aerobic exposure, or due to the activation of antioxidant proteins that are normally present in the muscle and react to diminish the action of peroxides. Thereafter, the concomitant molecular deterioration of meat continues under air exposure (Poulson, Suman, Rentfrow, Li, & Beach, 2012). Redness decreased ($P < 0.05$) as retail display continued from 2 to 5 days for all dietary treatments. Similar behavior was reported by Ponnampalam, Trout, Sinclair, Egan, and Leury (2001) in fresh and 28 day-aged lamb muscle exposed for 6 days to retail display conditions. As indicated above, in the current study there was an interaction between diet and day of aerobic exposition. This interaction occurred because redness in FLAX-2 meat decreased more rapidly compared to other treatments from days 2 to 5. This could be associated with the decrease in the antioxidant content that was described previously. As expected, we observed no significant differences in redness between dietary treatments within each day of the display. This means that it is possible to alter the fatty acid composition of the meat without affecting significantly the observable red color at a given display time.

Discoloration in retail meats during display conditions may be a combined function of muscle pigment oxidation and lipid oxidation in the membrane phospholipids (Sherbeck et al., 1995). Chan, Faustman, and Decker (1997) reported that the process of oxymyoglobin oxidation was involved in catalyzing lipid oxidation. In the present study, after 5 days of aerobic exposure, beef from animals on the FLAX-2 diet reached the lowest (13.66) and FLAX-1 the highest (14.78) mean a* values, coincidentally with the lowest and highest antioxidant status respectively. However, these differences were not significant ($P > 0.05$). By contrast, in the absence of O_2 (vacuum-packaged samples), redness values did not differ over time between treatments. These data support the hypothesis that pigment and lipid oxidation may be linked.

The effect of aging (under vacuum) on redness was dependent on the time of aerobic exposure, regardless of dietary treatment. As is shown in Fig. 1b, redness decreased from 2 days to 5 days of aerobic exposure for all aging times. The more aged the meat, the more rapid is the decrease of a* value, with the lowest value recorded (12.52) at day 56 ($P < 0.05$).

Arnold, Arp, Scheller, Williams, and Schaefer (1993) concluded that the optimal concentration of α-tocopherol level in fresh muscle required to delay pigment deterioration was approximately 3.5 μg/g tissue to achieve the maximal antioxidant effect in meat, depending on the muscle in question. Liu, Scheller, Arp, Schaefer, and Williams (1996) found that α-tocopherol concentrations of 0.3-0.35 mg/100 g were required to achieve color stability in beef during refrigerated storage.

In the present study, a deterioration of meat color occurred in parallel with a decrease of the antioxidant status (FRAP activity and antioxidant content) during the aging process and subsequent aerobic exposure. The levels of vitamin E (α plus γ tocopherol) found in LT muscles were lower than those mentioned by previous authors, and hence the antioxidants incorporated through pasture and supplements were not sufficient to maintain initial beef a* values during 5 days of retail display under these experimental conditions. Nevertheless, the lowest a* value observed in this study was higher than the minimum threshold (a*, 11) of acceptability proposed O'Sullivan et al. (2002).

4. Conclusion

The antioxidant vitamin status, lipid oxidation and color stability of beef from grazing cattle were negatively affected during retail display conditions, especially when cattle were supplemented with corn grain (0.7% BW) alone or with corn grain plus a high level of flaxseed (0.250% BW). In contrast, no negative effects were observed on beef antioxidant status or oxidative stability during retail display when a low level of flaxseed (0.125%) was added to the corn grain supplemented to grazing cattle. Negative effects on oxidative stability observed during retail display conditions were accentuated when extending vacuum-package beef aging period.

Therefore, only when a low level of flaxseed was added to the supplement of grazing cattle, the incorporation of natural antioxidants from pasture or supplements with flaxseed generated an adequate antioxidant capacity in the muscle that protected the oxidation given by the highest dietary PUFA content in beef. Further studies are required to better understand the reasons why when adding higher levels of flaxseed to the supplement the incorporation of natural antioxidants from pasture or supplements was not increased and resulted in a lower muscle oxidative stability.

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