

Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef

A.M. Descalzo^{*}, E.M. Insani, A. Biolatto, A.M. Sancho, P.T. García,
N.A. Pensel, J.A. Josifovich

Instituto Tecnología de Alimentos, Centro de Investigación en Agroindustria, Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, CC77 (B1708WAB) Morón, Pcia de Buenos Aires, Argentina

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Abstract

Argentine meat has been traditionally produced on pasture. However, to comply with some market requirements, grain finishing is becoming more common among producers. The main goal of the present work was to study lipid oxidation in fresh meat from animals fed different diets in relationship with their antioxidant vitamin status. Attributes were evaluated in beef from pasture or grain-fed animals with (PE and GE) or without supplementation (P and G) with vitamin E (500 UI/head/day). Fresh meat produced on grain (G and GE) had higher fat (4.0 ± 1.6 and 4.7 ± 1.4 g/100 g) and cholesterol content (51.0 ± 3.0 and 52.0 ± 4.0 mg/100 g) than meat from pasture (P and PE) fed animals (2.7 ± 1.2 to 2.9 ± 1.1 g/100 g and 48.0 ± 5.0 to 49.0 ± 4.0 mg/100 g of intramuscular fat and cholesterol respectively). Fatty acid composition was clearly affected by diet. Beef from pasture-fed cattle had higher percentage of linolenic acid, less linoleic acid and, overall, higher percentage of polyunsaturated fatty acids than beef from grain-fed animals ($P < 0.05$).

Thiobarbituric acid reactive substances number and volatile levels of hexanal, pentanal, heptanal, octanal and 3-methylbutanal were higher in grain than in pasture samples ($P < 0.05$). P + PE meat had higher content of antioxidant vitamins than G + GE samples ($P < 0.001$). Values ranged from: 15.92 ± 3.48 (G) to 17.39 ± 4.29 (GE) and 25.3 ± 10.0 (P) to 21.98 ± 5.11 (PE) $\mu\text{g/g}$ of ascorbic acid; from 1.05 ± 0.73 (G) to 1.76 ± 0.97 (GE) and 3.08 ± 0.45 to 3.91 ± 0.74 $\mu\text{g/g}$ of α -tocopherol; and from 0.06 ± 0.03 (G) to 0.05 ± 0.01 (GE) and 0.45 ± 0.21 (P) to 0.63 ± 0.27 (PE) $\mu\text{g/g}$ of β -carotene. In addition, principal component analysis clearly separated grain from pasture samples regardless of their supplementation with vitamin E. This level of supplementation did not improve the antioxidant status of fresh meat ($P > 0.05$). We conclude that pasture diet contributes natural antioxidants in sufficient amounts and is an efficient way to prevent lipid oxidation in fresh beef.

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1. Introduction

Oxidative damage is the major non-microbial factor responsible for quality deterioration of muscle foods.

Particularly, the oxidative status of meat is indicated by its colour stability and susceptibility to rancidity.

Lipid oxidation in muscle foods is initiated by stressors arising from both internal and external sources. The most important stressors are the reactive oxygen species (ROS) including free radicals and peroxides (Morrisey, Sheehy, Galvin, Kerry, & Buckeley, 1998).

^{*} Corresponding author. Tel.: +54 11 46210446/57; fax: +54 11 46212012.

E-mail address: adescalzo@cnia.inta.gov.ar (A.M. Descalzo).

During handling, processing and storage of fresh meat, released endogenous iron is partially responsible for the catalysis of lipid oxidation (Gutteridge, Paterson, Segal, & Halliwell, 1981; Halliwell & Gutteridge, 1986).

Propagation of lipid peroxidation in membranes promotes myoglobin oxidation resulting in colour deterioration, formation of rancid odours and other off-flavours in fresh meat (Gorelik & Kanner, 2001). Red meat is specially susceptible to the prooxidant effect of haem proteins in combination with endogenous iron (Monahan, Crackel, Gray, Buckeley, & Morrissey, 1993).

The action of ROS is opposed by enzymatic and non-enzymatic antioxidant defence systems (Halliwell, 1997). Among the non-enzymatic antioxidants, vitamin E, and more specifically α -tocopherol, is widely recognised as enhancer of the antioxidant activity in meat. Many authors described its usefulness in diminishing discoloration of meat induced by oxidation (Arnold, Arp, Scheller, Williams, & Schaefer, 1993; Faustman, Chan, Schaefer, & Havens, 1998). Moreover, several authors reported a negative correlation between oxidation and α -tocopherol levels in fresh bovine meat (Gatellier, Hamelin, Durand, & Renerre, 2001; Jakobsen & Bertelsen, 2000; Lawlor, Sheehy, Buckley, & Morrissey, 2000; Renerre, Poncet, Mercier, Gatellier, & Métro, 1999).

Supranutritional supplementation with vitamin E to finishing steers improved beef colour as well as suppressed lipid oxidation and stabilised cell integrity. Therefore, supplementation of animal diets with vitamin E has been strongly proposed as a production strategy (Arnold et al., 1993; Faustman et al., 1989, 1998; Liu, Lanari, & Schaefer, 1995; Mitsumoto, Arnold, Schaefer, & Cassens, 1993, 1995).

Nevertheless, in addition to α -tocopherol content, some other factors that influence oxidative stability in muscle should be considered. For example, the contribution of polyunsaturated fatty acids content (PUFA > 18:2), upon the balance between pro-oxidant/antioxidant compounds (Jensen, Lauridsen, & Bertelsen, 1998).

β -carotene is another important fat soluble antioxidant that quenches sites localised within the hydrophobic region of biological membranes, contrasting with the scavenging activity of α -tocopherol close to the membrane surface (Fukuzawa, Inokami, Tokumura, Terao, & Suzuki, 1998). Therefore, although β -carotene is less reactive than α -tocopherol, both antioxidants can exert a co-operative antioxidant activity at different positions within the membrane (Tsuchihashi, Kigoshi, Iwatsuki, & Niki, 1995).

Pasture consumed by cattle is known to supply vitamin E requirements in addition to other natural antioxidants (Gatellier, Mercier, & Renerre, 2004). On the other hand, studies have shown that pasture feeding led to increased values of highly unsaturated fatty acids

(García, Pensel, Margaria, Olga Rosso, & Machado, 1999; Larick & Turner, 1989; Melton, Black, Davis, & Backus, 1982; Yang, Lanari, Brewster, & Tume, 2002b). Argentine beef has been traditionally produced on pasture. However, in order to comply with some market requirements, regarding consumer's preference for meat with more marbling, grain supplementation is becoming more common among producers.

During post-slaughter, cellular antioxidant defences may no longer be tightly activated. Therefore, a considerable antioxidant status must be achieved before slaughter in order to maximise the protection of muscle lipids against peroxidation. The aim of this work was to determine naturally occurring antioxidant activity in fresh Argentine beef achieved by different production systems, and their relationship with lipid oxidation parameters.

2. Materials and methods

2.1. Animals and diets

This study was part of a project conducted to determine the influence of finishing diets on meat quality and oxidative stability of fresh and aged meat of cross-breed steers.

Pasture: Twenty crossbreed steers were grown on green natural pastures composed mainly by tall fescue (*Festuca arundinacea* Schreb.), rescue grass (*Bromus catharticus*), alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.) under continuous grazing at a forage rate of 1000/2000 kg dried pasture/ha. Ten pasture-fed animals were used as control and another 10 animals of this group received 500 units/head/day of vitamin E (all-rac- α -tocopheryl acetate, Roche, Argentina) using wheat bran (up to 1.5 kg/head) as vehicle.

Grain diet: Twenty crossbreed steers were separated in groups of 10 animals and individually fed. Ten steers were fed with 5 kg corn/day/animal + 6 kg hay/day/animal (control group) and the other 10 animals received the same diet plus a supplement 500 units/day of vitamin E as indicated for pasture-fed cattle.

All animals were grown at INTA experimental station located at Pergamino in Buenos Aires Province, Argentina. As growth rate differed for grain or pasture-fed animals (Table 1), pasture-fed cattle continued on trial until slaughter weight was reached.

Animals were slaughtered after reaching approximately 480 kg weight. Serum was collected immediately before the slaughter. *Psoas major* muscle (PM) was selected as the sample muscle due to its elevated susceptibility to oxidative deterioration during storage, which might cause colour alterations in both fresh and aged meat (Eikelenboom, Hoving-Bolinka, Kluitmana, Houben, & Klont, 2000; Rhee, Ziprin, Ordonez, & Bohac,

Table 1
Production parameters and plasma content of α -tocopherol and β -carotene

	Pasture		Grain	
	Control (P)	Supplemented (PE)	Control (G)	Supplemented (GE)
Daily weight gain ^b (kg)	0.456 ± 0.075a	0.522 ± 0.082a	0.708 ± 0.096b	0.789 ± 0.102b
Yield (%)	61.1a	60.5a	59.2a	59.1a
Carcass weight ^b (kg)	123.2 ± 2.78a	124.4 ± 3.02a	122.8 ± 5.23a	124.4 ± 4.28a
Plasma levels of α -tocopherol (μ g/ml) ^{a,b}	4.08 ± 0.71a	3.68 ± 0.82a	0.84 ± 0.19b	3.59 ± 0.65a
Plasma levels of β -carotene (μ g/ml) ^{a,b}	8.00 ± 0.63a	10.81 ± 1.59a	1.42 ± 0.14b	2.89 ± 0.83b

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

^a Plasma levels at slaughter time.

^b Values are mean ± standard deviation.

1988), and due to its high commercial value. Right and left PM muscles from each animal were cut into four longitudinal pieces. Each quarter was randomly distributed among storage times, vacuum packaged and immediately refrigerated at 1–2 °C for use in ageing experiments. Fresh samples (24 h post-slaughter) were kept at –80 °C until analysis.

2.2. Lipid oxidation measurement

To assess the amount of lipid oxidation, the content of thiobarbituric acid reactive substances (TBARS) number was determined using the acid precipitation technique described by Pensel (1990). Briefly, triplicate aliquot samples of PM (10 g), were chopped and processed in an stomacher type homogeniser during 180 s in bags containing 50 ml trichloroacetic acid (Merck, Darmstadt, Germany) solution (10% w/v). Slurries were filtered, an equal volume of 2-thiobarbituric acid (Sigma–Aldrich, St. Louis, MO, USA) 0.02 M was added, and samples were incubated at 25 °C overnight until pink colour development. TBARS were determined at maximum absorption (530 nm) and concentrations were calculated using 1,1,3,3-tetraethoxypropane (Sigma–Aldrich, St. Louis, MO, USA) as standard within the range from 0 to 0.5 μ M. Results were expressed as mg of malonaldehyde (MDA) equivalents/kg of fresh meat.

2.3. α -Tocopherol and β -Carotene content of muscles and plasma samples

α -Tocopherol and β -Carotene were extracted as described by Buttriss and Diplock (1984). PM samples were chopped and homogenised for 2 min at 3000 rpm with an UltraTurrax type homogeniser in potassium phosphate buffer pH 7.2, with addition of 1% pyrogallol (Sigma–Aldrich St. Louis, USA) in ethanol. Saponification was performed for 30 min at 70 °C with 10 N KOH (Merck Química, Argentina). Then, samples were extracted twice with *n*-hexane (J.T. Baker, USA, HPLC grade), evaporated under nitrogen flow, resuspended in absolute ethanol (J.T. Baker, Mexico, HPLC grade) and filtered through a 0.45 micropore nylon membrane

before injection. Plasma samples were extracted with *n*-hexane as described for meat, but without the saponification step.

All samples were analysed by reverse phase high-performance liquid chromatography (HPLC).

2.3.1. HPLC conditions

The HPLC pump (model P4000) with a membrane vacuum degasser and a 20 μ l loop injector were purchased from TSP (Thermo Separation Products Inc., USA), and connected to an Alltima C18 column (250 mm × 4.6 mm), 5 μ m particle size. The electrochemical detector (Decade, Antec Leyden, The Netherlands) was equipped with a flowcell with Ag/AgCl and glassy carbon reference and working electrodes respectively.

The mobile phase used for electrochemical detection was modified from the technique described by de Rijke et al. (1997). The flow-rate was 1 ml/min and the reference cell was set at +700 mV. Recovery of α -tocopherol and β -carotene was 98%. Calibration curves were performed with DL- α -tocopherol (Merck, Darmstadt, Germany), and β -carotene standards (Sigma–Aldrich, St. Louis, USA) diluted in absolute ethanol.

2.4. Volatile determination: static head space-gas chromatography

Aliquot samples of grounded PM (5 g) were placed into a 27-ml headspace vial, adding 100 μ l of internal standard, 4-heptanone (Merck-Schuchardt, Germany), of 500 μ g/ml and sealed with a crimp-top caps with TFE/silicone septa seals. Volatile compounds levels were determined by headspace analysis on a Shimadzu series 14B gas-liquid chromatograph (GLC), equipped with a flame ionisation detector (FID) and a model HSS-2B headspace sampler. Volatile compounds were separated by using a poly (20%-diphenyl-8 0%-dimethylsiloxane) glass capillary column (60 m × 0.53 mm i.d. × 0.50 μ m) film thickness (Supelco Park, Bellefonte, PA). The carrier and make-up gas was high-purity nitrogen at a pressure of 13 and 50 kPa, respectively. High-purity hydrogen (65 kPa) and compressed air (45 kPa) were supplied to the flame ionisation detector. The injector and detector

temperatures were 280 °C. The column oven temperature was programmed at increasing temperature from 4 to 80 °C at a rate of 2 °C/min, then from 80 to 150 °C at a rate of 5 °C/min and finally from 150 to 250 °C at a rate of 40 °C/min and held for 10 min. The different components were identified by comparison of retention times with those of standards (Sigma–Aldrich, St. Louis, USA). Meat samples were equilibrated in the headspace sampler for 60 min at 90 °C prior to injection. Injection volume was 0.8 ml, and the split ratio was 1:1. The output signal from detector was integrated automatically with a Class-VP Software. Quantitative determinations were based on the known amount of added standard.

2.5. Fat analyses

For fat content aliquot samples of 10 g each, trimmed of external fat, were minced carefully, dried and extracted in a Tekator apparatus using hexane as the extraction solvent according to official methods (AOAC Official methods of analysis, 1990). Lipids from aliquot samples of 5 g each were extracted according to Folch, Lees, and Sloane-Stanley (1957). Fatty acid methyl esters (FAME) were prepared according to Pariza, Park, and Cook (2001) and measured using Chrompack CP 900 equipment fitted with a flame ionisation detector. Separation of FAME was performed on a WCOT fused silice capillary column (CP-Sil 88 coating, 100 m × 0.25 mm i.d.), using N₂ as carrier gas. The oven temperature was programmed at 70 °C for 4 min, increased from 70 to 170 °C at a rate of 13 °C/min and then increased from 170 to 200 °C at 1 °C/min. Individual fatty acids were identified by comparing relative retention times with individual fatty acids standards (PUFA-2 Animal Source, Supelco, USA). Analytic results were expressed as percentages of total fatty acids.

2.6. Ascorbic acid determination

Ascorbic acid was determined in muscle homogenates by the ferric reduction reaction with addition of ascorbate oxidase as indicated by Benzie and Strain (1999). Homogenates were centrifuged at 10,000g for 30 min. Aliquots from supernatant were analysed with or without incubation in the presence of ascorbate oxidase (Sigma–Aldrich, St. Louis, USA) (0.88 U/ml at 37 °C, 15 min). Differences in ferric reduction activity due to ascorbic acid were determined, and concentrations were calculated from an ascorbic acid (Sigma–Aldrich, St. Louis, USA) calibration curve in the range from 100 to 1000 µM.

2.7. Statistical analysis

All values are reported as the mean ± standard deviation for 10 measurements on each animal group. Vol-

tile determinations were performed over five samples. Treatments were compared by analysis of variance using the GLM procedure (SAS 8.0, SAS Institute, Inc., Cary, NC) for a fixed effect model with two basal diets and two supplement levels: $Y_{ijk} = \text{mean} + \text{diet}_i + \text{supplement}_j + (\text{diet} \times \text{supplement})_{ij} + \varepsilon_{ijk}$.

Mean values were compared using the Tukey test at a significance level of 0.05.

Principal component analysis (PCA) was performed to describe the relationship between variables and their influence over grain and pasture-fed samples using the statistical software SPSS v. 11.5 (Chicago, IL).

3. Results and discussion

3.1. α -Tocopherol and β -Carotene contents in plasma and muscle homogenates

Vitamin E supplementation did not affect carcass quality or yield characteristics. As expected, the grain production system resulted in a major daily weight gain, but carcasses yielded the same at slaughter time with 480 kg live weight (Table 1).

Vitamin E supplementation increased fourfold the α -tocopherol content in plasma of grain-fed animals, which reached levels equivalent to those found for the pasture-fed group. However, no increment was detected in supplemented pasture-fed group when compared to its respective control. Only unsupplemented grain-fed animals presented lower levels of plasma α -tocopherol. Similar results were described by Yang, Brewster, Lanari, and Tume (2002a), but these authors found that supplemented grain-fed animals showed levels of plasma α -tocopherol even higher than the pasture-fed group.

On the contrary, results obtained for PM muscle did not resemble the tendencies found for plasma (Table 2). However, this observation is in agreement with previous reports indicating that α -tocopherol content in plasma may not be used as an indicator of muscle vitamin E concentration (Liu et al., 1995).

In muscle samples, pasture-fed steers contained higher amounts of α -tocopherol than the grain-fed group. Even after supplementation with vitamin E, meat from grain-fed animals did not reach the α -tocopherol levels found in meat from pasture-fed cattle. Table 2 shows a twofold difference between pasture and grain diets ($P < 0.0001$).

Only a small but not significant increase was observed in meat from supplemented pasture-fed cattle respect to the control group ($P > 0.05$). This result clearly indicates that the diet and not the supplement was the main factor for vitamin E incorporation in muscle under the experimental conditions employed (500 IU/animal/day).

As pointed out by Liu et al. (1995), the strategy for supplementing beef cattle is to achieve sufficient concen-

Table 2
Levels of antioxidant vitamins, TBARS and volatiles in fresh beef^a

Treatment	TBARS (mgMDA/kg)	Ascorbic acid ($\mu\text{g/g}$)	α -Tocopherol ($\mu\text{g/g}$)	β -Carotene ($\mu\text{g/g}$)	SFA (g/100 g)	MUFA (g/100 g)	PUFA (g/100 g)
Grain	0.28 \pm 0.10a	15.92 \pm 3.48a	1.50 \pm 0.73a	0.06 \pm 0.03a	45.49 \pm 3.86a	37.83 \pm 4.35a	7.29 \pm 2.59a
Grain + E	0.26 \pm 0.10a	17.39 \pm 4.29a	1.76 \pm 0.97a	0.05 \pm 0.01a	44.10 \pm 2.08a	37.83 \pm 2.57a	8.19 \pm 2.03a
Pasture	0.09 \pm 0.03b	25.30 \pm 10.23b	3.08 \pm 0.45b	0.45 \pm 0.21b	42.85 \pm 2.9a	34.17 \pm 1.51b	10.31 \pm 2.25b
Pasture + E	0.10 \pm 0.03b	21.98 \pm 5.11b	3.91 \pm 0.74b	0.63 \pm 0.27b	44.33 \pm 3.8a	32.85 \pm 3.37b	8.68 \pm 1.70b
<i>Significance level of ANOVA^b</i>							
Diet	****	***	****	****	NS	****	**
Supplement	NS	NS	*	NS	NS	NS	NS
Diet \times Suppl.	NS	NS	NS	NS	NS	NS	NS

Treatment	PUFA > 18:2 (g/100 g)	3-Methylbutanal ($\mu\text{g/g}$)	Pentanal ($\mu\text{g/g}$)	Hexanal ($\mu\text{g/g}$)	Heptanal ($\mu\text{g/g}$)	Octanal ($\mu\text{g/g}$)
Grain	2.54 \pm 0.01a	0.13 \pm 0.03	0.21 \pm 0.07b	1.62 \pm 0.49a	0.78 \pm 0.32a	0.66 \pm 0.20a
Grain + E	2.86 \pm 0.64a	0.14 \pm 0.05	0.13 \pm 0.03a	1.30 \pm 0.37a	0.61 \pm 0.2a	0.58 \pm 0.14a
Pasture	4.44 \pm 0.99b	ND	0.12 \pm 0.05a	0.96 \pm 0.52b	0.37 \pm 0.29b	0.31 \pm 0.19b
Pasture + E	3.64 \pm 0.72b	ND	0.15 \pm 0.06a	1.16 \pm 0.24b	0.50 \pm 0.24b	0.39 \pm 0.16b
<i>Significance level of ANOVA^b</i>						
Diet	****	–	*	*	*	**
Supplement	NS	–	NS	NS	NS	NS
Diet \times Suppl.	NS	–	NS	NS	NS	NS

Means with different letters in the same row differ ($P < 0.05$).

TBARS: 2-thiobarbituric acid reactive substances; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids (PUFA > 18:2:PUFA with more than two double bonds); ND: not detected.

^a Values are means \pm standard deviation.

^b Probability for diet, supplement and interaction: ****: $P < 0.0001$, ***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$.

tration of α -tocopherol in muscle as to maximise its antioxidant efficacy. The ideal concentration will be the minimum quantity within the tissue that provides for the maximal suppression of lipid and protein oxidation in fresh beef.

Supplementation of basal diets with 500 UI vitamin E/animal/day was suggested by Liu et al. (1995) in order to achieve an ideal α -tocopherol concentration of 3.5 $\mu\text{g/g}$ fresh beef. However, this was not the case in our experiment using PM muscle (Table 2). Grain-fed animals supplemented with vitamin E reached 1.76 $\mu\text{g/g}$ of α -tocopherol in muscle and had no significant differences ($P > 0.05$) compared to the control grain diet (1.50 $\mu\text{g/g}$). Regarding pasture-fed animals, α -tocopherol mean levels were 3.91 and 3.08 $\mu\text{g/g}$ for supplemented and control diets respectively. The values achieved with pasture diets are in agreement with Faustman et al. (1989), who postulated 3 μg α -tocopherol/g tissue as the threshold level which prevents colour deterioration of fresh beef. Likewise, Arnold et al. (1993) proposed critical concentrations for *longissimus lumborum* and *gluteus medius* muscles of 3.3 and 3.8 $\mu\text{g/g}$ respectively, in order to produce beef with extended colour and lipid stability.

In other experiences, Yang et al. (2002a) used a dietary intake of about 2500 UI/head/day of α -tocopherol acetate for grain-fed animals to achieve equivalent levels of vitamin E as those supplied by a quality pasture diet (4.3–6.1 $\mu\text{g/g}$ tissue).

β -carotene levels in plasma were significantly higher for pasture than for grain-fed animals. Furthermore, plasma β -carotene was not affected by dietary supplementation with vitamin E at the level used (500 UI/head/day) (Table 1).

This result was also observed in muscle (Table 2). Pasture-fed steers incorporated significantly higher amounts of β -carotene into muscle tissues than grain-fed animals. Concentrations found were 0.63–0.45 and 0.06–0.05 $\mu\text{g/g}$ for meat from pasture and grain-fed cattle respectively. Apparently, pasture consumed by animals provided enough β -carotene to allow its incorporation into the tissue. As shown in Table 2, there was almost a 10-fold difference in tissue levels among pasture and grain basal diets ($P < 0.0001$).

However, other authors found that supplementation of diets with vitamin E affects the deposition of β -carotene in different tissues. Yang et al. (2002a), showed that β -carotene contents in plasma, liver, fat and muscles decreased with supplementation of pasture diet with 2500 UI/head/day of α -tocopherol acetate.

In the present study, the level of supplementation used (500 UI vitamin E/animal/day) did not interfere with the incorporation of β -carotene into the PM muscle. Apparently, vitamin contents in the present study were not high enough to promote competition between α -tocopherol and β -carotene for their receptors in membranes, as postulated by Yang et al. (2002a).

3.2. Lipid oxidation

Measurement of TBARS levels (Table 2), showed significant differences in lipid oxidation levels between meat from pasture and grain-fed animals. Samples from pasture-fed cattle, were three times less oxidised than grain ones ($P < 0.0001$). This result is in agreement with other authors (Lynch, Kerry, Buckley, Faustman, & Morrissey, 1999; Mercier, Gatellier, & Renerre, 2004). As expected, natural antioxidants protected beef against lipoperoxidation.

Fig. 1 shows the graphical correlation between vitamin content (α -tocopherol and β -carotene) vs. TBARS in meat. Samples from pasture and grain-fed animals were clearly separated when both variables were considered. Both antioxidants correlated inversely with TBARS and moreover, they showed the same cut-off value of 0.16 mg malonaldehyde equivalents/kg tissue, that discriminated pasture from grain-fed groups.

Samples with values higher than 2.5 μg of vitamin E/g tissue fitted into the pasture group, and showed TBARS levels under the cut-off value (Fig. 1(a)).

A similar relationship was found for β -carotene content in muscle tissues and TBARS. Samples with β -carotene concentrations higher than 0.15 $\mu\text{g}/\text{g}$ belonged to pasture group and showed lower TBARS levels (Fig. 1(b)). In a preliminary report, we described an inverse correlation between lipid oxidation and vitamin contents in PM samples (Descalzo et al., 2000).

The obtained results might indicate that both antioxidants could exert a co-operative effect to diminish lipid oxidation at membrane level, thus obtaining lower TBARS levels in pasture-fed beef.

3.3. Ascorbic acid contents in *Psoas major* muscle

Pasture diet also favoured the incorporation of ascorbic acid into muscles (Table 2). All pasture (supplemented and control) group presented significantly higher values of ascorbic acid in comparison with all grain (supplemented and control) group. Again, supplementation of diets with vitamin E did not influence the presence of ascorbic acid in PM muscle. Differences should be attributed to the nature of basal diets. Commonly, ascorbic acid is added post-mortem to ground beef in order to improve red colour and to extend the shelf life of meat through its interaction with α -tocopherol and other antioxidants (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2002; Mitsumoto et al., 1991). However, results are controversial. Realini, Duckett, Brito, Dalla Rizza, and De Matos (2004) found that addition of ascorbic acid to ground beef did not alter lipid oxidation but delayed metmyoglobin formation therefore improving redness retention during display.

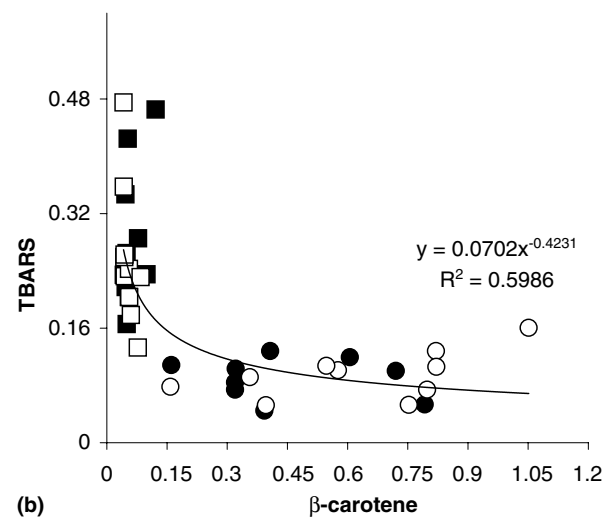
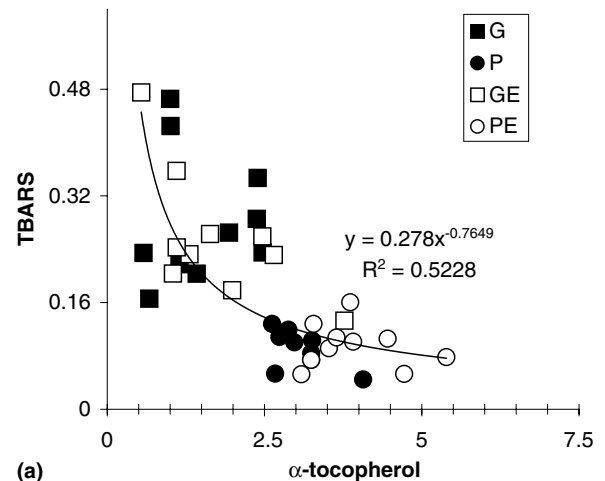


Fig. 1. Relationship between TBARS (mg MDA equivalents/kg muscle) and antioxidant vitamins: α -tocopherol ($\mu\text{g}/\text{g}$ muscle), and β -carotene ($\mu\text{g}/\text{g}$ muscle) in fresh PM samples from pasture and grain-fed cattle with (PE and GE) or without (P and G) vitamin E supplement ($n = 10$).

Endogenous ascorbic acid content in muscle (21.98–25.30 $\mu\text{g}/\text{g}$ for pasture and 15.92–17.39 $\mu\text{g}/\text{g}$ for grain meat) were lower than concentrations added to improve meat stability (500–1000 $\mu\text{g}/\text{g}$, Realini et al., 2004). Nevertheless, contribution of endogenous ascorbic acid to the overall antioxidant status in PM beef cannot be disregarded.

3.4. Fatty acid composition and volatile production

In accord with previous results, grain-fed samples had higher fat and cholesterol contents than pasture-fed ones (Marmer, Maxwell, & Williams, 1984). No statistical differences were found due to supplementation with vitamin E (Table 3). The differences in the fat content should be considered for the interpretation of fatty acids analysis in total lipid fractions of meat and their

relationship with volatile production to assess the oxidative stability of muscle.

For total lipids of PM (Table 2), grain-fed beef contained the highest percentage of monounsaturated fatty acids while pasture-fed beef had the highest percentage of polyunsaturated fatty acids. There were no differences among diets for saturated fatty acids. Percentages of PUFA ranged from 8.68–10.31% for pasture-fed cattle and from 7.29% to 8.19% for grain-fed animals ($P < 0.05$). PUFA percentages in PM were below the values found in other muscles (Yang et al., 2002b). Nevertheless, the percentage of PUFA was significantly more prevalent in muscles from pasture-fed steers respect to grain-fed ones, as described by other authors (García et al., 1999; Yang et al., 2002b).

Statistical differences in individual fatty acids were found between diets for, oleic 18:1c ($n - 9$), trans 18:1, linolenic (18:3 $n - 3$), and 22:4 $n - 6$ acid (Table 3).

When only basal diet was considered (without taking into account the supplementation effect), grain-fed meat presented a significantly higher ($P < 0.05$) content of palmitic, oleic, and 22:4 acids. On the other hand, pasture-fed meat showed predominantly higher percentages of palmitoleic, trans 18:1, linolenic, and 22:5 fatty acids.

As expected the $n - 6/n - 3$ fatty acid ratio was lower for pasture than for grain-fed animals (Table 3). Pasture samples showed values between 3.72 and 4.13, whereas

grain samples showed values between 5.73 and 6.56. Differences in $n - 6/n - 3$ fatty acid ratio was principally due to the higher content of linolenic $n - 3$ acid in meat from pasture-fed animals and conversely, the higher 22:4 $n - 6$ found in grain samples. A decrease of the ratio $n - 6/n - 3$ in beef from animals produced in pasture was also reported by other authors (García et al., 1999; Yang et al., 2002b).

When the amounts of PUFA $> 18:2$ in muscle were considered, concentrations were equivalent in meat produced on pasture or grain. However, TBARS values in pasture samples were lower than in grain samples. This could indicate that natural antioxidants found in pasture could protect meat from oxidation.

Volatile compounds were determined in order to complement the results of oxidative status. Overall, they were more prevalent in meat from grain-fed animals.

As expected, hexanal was the most abundant volatile followed by heptanal, octanal, and to a lesser extent pentanal and 3-methyl butanal (Table 2).

All the aldehydes associated with oxidation processes correlated positively with TBARS levels.

Octanal and 3-methyl butanal presented correlation coefficients of 0.91 and 0.78 with TBARS respectively. Octanal derived mainly from oleic ($n - 9$) acid (Piyeteau et al., 2000). Meat from grain-fed animals contained higher amounts of oleic acid than pasture

Table 3

Fatty acid profile of total lipids, cholesterol and intramuscular fat content of fresh PM muscles of pasture or grain-fed steers with or without vitamin E supplement

	Pasture		Grain	
	Control ($n = 10$)	Supplemented ($n = 10$)	Control ($n = 10$)	Supplemented ($n = 10$)
IMF %	2.7 ± 1.24a	2.9 ± 1.10a	4.7 ± 1.4b	4.1 ± 1.6b
Chol (mg/100 g)	49 ± 4.0a	48 ± 5.0a	52 ± 4.0b	51 ± 3.0b
<i>Fatty acid</i>				
14:0	2.2 ± 0.3a	2.0 ± 0.2a	2.0 ± 0.3a	2.1 ± 0.43a
15:0 + 14:1	1.6 ± 0.3a	1.6 ± 0.3a	1.2 ± 0.4a	1.3 ± 0.5a
16:0	22.0 ± 1.9a	22.4 ± 1.3a	25.0 ± 1.8a	24.0 ± 1.7a
16:1	3.8 ± 0.3a	3.9 ± 0.6a	3.6 ± 0.2a	3.3 ± 0.2a
17:0	0.4 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.1a
17:1	0.6 ± 0.2a	0.6 ± 0.2a	0.5 ± 0.2a	0.4 ± 0.2a
18:0	19.1 ± 2.3a	19.8 ± 2.4a	18.2 ± 3.1a	19.1 ± 1.8a
18:1 t	4.2 ± 0.6a	4.2 ± 0.8a	2.8 ± 0.5b	2.9 ± 0.4b
18:1 c	29.5 ± 2.3a	29.7 ± 2.8a	34.3 ± 4.2b	34.5 ± 2.6b
18:2 $n - 6$	5.4 ± 1.1a	5.4 ± 1.4a	4.7 ± 1.7a	5.3 ± 1.4a
18:3 $n - 3$	1.4 ± 0.1a	1.3 ± 0.3a	0.7 ± 0.2b	0.7 ± 0.1b
20:3 $n - 6$	0.4 ± 0.1a	0.4 ± 0.1a	0.3 ± 0.1a	0.4 ± 0.1a
20:4 $n - 6$	1.6 ± 0.6a	1.6 ± 0.6a	1.2 ± 0.2a	1.4 ± 0.4a
20:5 $n - 3$	tr	tr	tr	tr
22:4 $n - 6$	0.03 ± 0.01a	0.04 ± 0.01a	0.10 ± 0.04b	0.12 ± 0.06b
22:5 $n - 3$	0.6 ± 0.1a	0.5 ± 0.2a	0.4 ± 0.2a	0.4 ± 0.1a
22:6 $n - 3$	tr	tr	tr	tr
$n - 6/n - 3$	7.43/2	7.44/1.8	6.3/1.1	7.22/1.1
Ratio ^a	3.72a	4.13a	5.73b	6.56b

Means with different letters in the same row differ ($P < 0.05$).

Chol (Cholesterol content); IMF: Intramuscular Fat.

^a Ratio of all $n - 6$ /all $n - 3$ fatty acids.

samples (1.41–1.64 vs. 0.79–0.86 g/100 g beef). Consequently, the presence of octanal should be reflecting the susceptibility of beef from grain-fed cattle to lipid oxidation.

Hexanal and pentanal are typical volatiles resulting from highly unsaturated fatty acids in raw meat (Elmore, Mottram, Enser, & Wood, 1999; Estévez, Morcuende, Ventanas, & Cava, 2003). Both volatile compounds presented a positive correlation with TBARS measurement (0.53 and 0.54 respectively). 3-methylbutanal was found only in grain samples whereas values for pasture meat were under the detection limit. A possible explanation for 3-methylbutanal production could be that there is a relationship between lipid oxidation and the Strecker degradation pathway. Aldehydes produced by lipid oxidation may undergo Maillard reaction with amino compounds in meat, producing dicarbonyl compounds that may then react with amino acids forming Strecker degradation products (Anderson & Lingnert, 1998). An oxidized meat sample would therefore contain quantifiable amounts of 3-methylbutanal as determined by the head-space volatile detection method.

This result could be an indirect indicator of protein damage due to interaction with lipid oxidation products. This agrees with previous results where we reported that grain-fed argentine beef presented higher values of protein oxidation than pasture meat (Insani, Eyherabide, Descalzo, Sancho, & Pensel, 2000).

3.5. Descriptive principal component analysis

PCA was applied to the set of antioxidant compounds: α -tocopherol, β -carotene and ascorbic acid, together with oxidation parameters: TBARS and volatiles. PCA makes it possible to describe the main variations observed among data sets without any hypothesis or any external information concerning the studied sample. Therefore, this kind of analysis allowed determining the relationship among dependent experimental variables and their influence over the meat sample population.

The first two principal components accounted for 79.65% of the total variation. As shown in Fig. 2, PCA scores for pasture samples were described by higher contents of antioxidant compounds. Conversely, scores for grain samples were associated with higher volatile production and TBARS number. At a first glance, the principal component 1 (62.44% of the total variation) separated meat samples according to their differences in antioxidant/oxidation parameters. Basal diets were evidently grouped along the principal component 1 and a good discrimination between beef from pasture and grain-fed animals was again observed. In this PCA assay, supplementation was not differentiated within the basal diets.

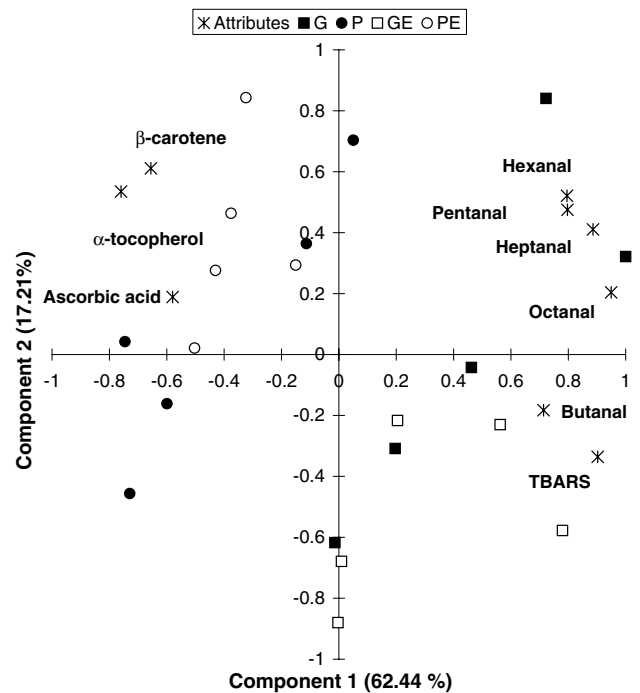


Fig. 2. Similarity map in a principal component analysis (PCA) assay, defined by the principal components 1 and 2 for TBARS, volatiles: hexanal, pentanal, octanal, heptanal and 3-methyl butanal and vitamins: α -tocopherol, β -carotene and ascorbic acid. PM samples from pasture and grain-fed cattle with (PE and GE) or without (P and G) vitamin E supplement ($n = 5$).

4. Conclusions

Pasture diet improved the oxidative stability in PM muscle. As well, it contributed to reduce the $n - 6/n - 3$ fatty acids ratio in intramuscular fat and consequently may improve the nutritional quality of beef.

Principal component analysis differentiated meat from pasture or grain-fed animals when oxidation and antioxidant parameters were considered together. This effect was not influenced by supplementation of diets with 500 UI vitamin E/head/day. In addition, this work supports research indicating that dietary finishing mode has a very important impact upon the balance between natural antioxidants and oxidation development in fresh meat.

Further implication of antioxidant enzymes and tissue redox state on oxidative stability of meat from pasture or grain-fed animals are in progress in our laboratory.

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