Effect of dietary supplementation on lipid photoxidation in beef meat, during storage under commercial retail conditions

EFFECT OF DIETARY SUPPLEMENTATION ON LIPID PHOTOXIDATION IN BEEF MEAT, DURING STORAGE UNDER COMMERCIAL RETAIL CONDITIONS

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Abstract

The effects of feeding composition on the photosensitized oxidation of lipids from beef meat, were evaluated during storage under commercial retail conditions. Feeding was enriched with linseed oil (LO), Dl-α tocopheryl acetate (vE) and conjugated linoleic acid (CLA) at different doses and provided for diverse periods, resulting in 7 diet groups (A-G). After slaughtering and 2 weeks of holding period, meat slices were packed in vessels with transparent shrink film and exposed to white fluorescent light for 8 h at 8 °C. Total cholesterol oxidation products (COPs) level varied from 4.0 to 13.0 μg/g of lipids, which corresponded to 0.1-0.6% oxidized cholesterol. The lowest peroxide value (PV) in the diet added with vE and LO for 90 days was found. Light exposure only had a significant impact on thiobarbituric acid reactive substances (TBARs). In general, Dl-α tocopheryl acetate supplemented for 90 days improved the oxidative stability of beef meat stored under commercial retail conditions.

Keywords: Beef meat; Diet; Storage; Lipid oxidation; Cholesterol oxidation; CLA; Dl-α tocopheryl acetate

1. Introduction

Over the past few years, the consumer needs have driven the beef meat market towards the production of leaner and healthier meat. Several strategies have been undertaken to modulate the lipid fraction of beef meat, as it influences its nutritional and sensory quality. To enable the differentiation of beef meat based on quality, the definition of the latter has become increasingly complex as it encompasses the physical intrinsic (color, shape, appearance, tenderness, juiciness, flavor) and extrinsic quality traits (brand, quality mark, origin, healthiness, production environment, etc.) (Scollan, Hocquette, Nuerberg, Dannenberger, Richardson, & Moloney, 2006).

Diet formulation with vegetable oils that contain an elevated percentage of unsaturated fatty acids (UFA), should result in healthier meat products (Mitchaothai et al., 2007). In fact, it is recommended that total fat, saturated fatty acids (SFA), n-6 polyunsaturated fatty acids (n-6 PUFA), n-3 PUFA and *trans* fatty acids (TFA) should contribute <15–30%, <10%, <5–8%, <1–2% and <1% of total energy intake, respectively (EFSA, 2010). Reduction of the SFA intake (which are known to raise total and low-density lipoprotein (LDL) cholesterol) and increase of the n-3 PUFA consumption is particularly encouraged (Simopoulos, 2006). On the other hand, beef meat is also a dietary source of conjugated linoleic acid (CLA) (Ritzenthaler, McGuire, Falen, Schultz, Dasgupta, & McGuire, 2001). The dominant CLA in beef is the *cis*-9,*trans*-11 isomer, which has been found to display several health promoting biological properties, including antitumoral and anticarcinogenic activities (De la Torre et al., 2006). CLA *cis*-9,*trans*-11 isomer is mainly associated with the triacylglycerol lipid fraction and, therefore, is positively correlated with the level of fatness. The content of CLA *cis*-9,*trans*-11 isomer in beef is related to the amount of this isomer produced in the rumen and the one synthesized in the tissue, by delta-9 desaturase, from ruminally produced vaccenic acid (18:1 *trans*-11). The latter is the major *trans* 18:1 isomer in beef and, as the precursor of tissue CLA in both animals and man, it

should be considered as a neutral or beneficial *trans*-isomer (Wood et al., 2004).

However, a higher degree of FA unsaturation is known to favor meat oxidation (Boselli, Caboni, Rodriguez-Estrada, Gallina Toschi, Daniel, & Lercker, 2005; Boselli, Cardenia, Rodriguez-Estrada, 2012). Lipid oxidation has a great impact on the overall quality of muscle foods, since meat color, texture, nutritional value and safety are negatively affected (Williams, Frye, Frigg, Schaefer, Scheller, & Liu, 1992). In addition, aldehydes, ketones and carboxylic acids are generated by this degradation process, thus leading to undesirable odors and flavors (Mottram, 1987).

Oxidation in muscle originates at the cell membrane, where a large amount of PUFA is present (Wood et al., 2004). However, the cell membrane contains other unsaturated lipophilic molecules, such as cholesterol, that can also oxidize. A wide range of cholesterol oxidation products (COPs) can be generated by chemical, photosensitized and enzymatic oxidation (Lercker and Rodriguez-Estrada, 2002; Smith, 1996). Photosensitized oxidation could be critical in beef meat due to its high content of heme pigments (mainly myoglobin and hemoglobin) (Boselli et al., 2012), which act as photosensitizers thus promoting lipid oxidation. When assessing lipid oxidation in beef meat, however, it is very important to consider that it is usually subjected to a holding period for few days at 3–6 °C to improve its tenderness and promote the formation of aroma compounds; this may lead to a higher initial cholesterol oxidation degree in the untreated meat slices, even before being subjected to photoxidation (Boselli, Rodriguez-Estrada, Fedrizzi, & Caboni, 2009). Large attention has been focused on COPs as they are likely to be involved in lipid metabolism, various chronic and degenerative diseases, and disturbance of cell functionality (Garcia-Cruset, Carpenter, Codony, & Guardiola, 2002; Osada, 2002; Schroepfer, 2000; Otaegui-Arrazola, Menendez-Carreño, Ansorena, & Astiasaran, 2010). Although COPs are usually present in low amounts in raw muscle food, their concentrations tend to dramatically increase after exposure to prooxidant agents or after being highly processed (Hur,

88 Park, & Joo, 2007; Kerry, Gilroy, & O'Brien, 2002; Otaegui-Arrazola et al., 2010). Under this
89 situation, dietary supplementation or addition of antioxidants (such as vitamin E) could be an
90 important strategy to extend their shelf–life, by reducing or preventing lipid peroxidation
91 (Williams et al., 1992). To the best of our knowledge, no study has been performed on the
92 photosensitized oxidation of beef meat, as related to the dietary supplementation.
93 The aim of this work was to evaluate the effect of dietary supplementation on lipid
94 oxidation in beef meat, during storage under commercial retail conditions. Particular attention
95 was addressed to oxidation of fatty acids and cholesterol.
96
97 **2 Materials and methods**
98 *2.1 Reagents and solvents*
99 Ammonium thiocyanate (NH_4SCN, ≥ 97.5%), barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$, ≥
100 99%), ethylenediamine-tetraacetic acid (EDTA) disodium salt (100%±1%), iron (II) sulfate
101 heptahydrate ($FeSO_4 \cdot 7H_2O$, ≥ 99.0%), trichloroacetic acid (≥ 99%) and diethyl ether, were
102 supplied by Carlo Erba Reagenti (Rodano, Italy). Chloroform, *n*-hexane, methanol and ethanol
103 were purchased from Merck (Darmstadt, Germany). Silylating agents (pyridine,
104 hexamethyldisilazane and trimethylchlorosilane) and double distilled water were supplied by
105 Carlo Erba (Milan, Italy). Anhydrous sodium sulfate and potassium hydroxide were purchased
106 from BDH (Poole, England) and Prolabo (Fontenay, France), respectively. The standard mixture
107 of fatty acid methyl esters (GLC 463) was supplied by Nu-Chek (Elysian, MN, USA).
108 Tridecanoic acid methyl ester (purity: 99%), cholest-5-en-3β,19-diol (19-hydroxycholesterol, 19-
109 HC) (purity: 99%) and cholest-5-en-3β,7α-diol (7α-hydroxycholesterol, 7α-HC) (purity: 99%)
110 were purchased from Steraloids (Newport, Rhode Island, USA). (24*S*)-ethylcholest-5,22-dien-
111 3β-ol (stigmasterol) (purity: 95%), β-sitosterol (purity: 60%), campesterol (purity: 37.5%),
112 cholest-5-en-3β,7β-diol (7β-hydroxycholesterol, 7β-HC) (purity: 90%), 5α,6α-epoxy-cholestan-

113 3β-ol (α-epoxycholesterol, α-EC) (purity: 87%), 5β,6β-epoxy-cholestan-3β-ol (β-
114 epoxycholesterol, β-EC) (purity: 80%), cholestan-3β,5α,6β-triol (cholestanetriol, triol) (purity:
115 99%), cholest-5-en-3β-ol-7-one (7-ketocholesterol, 7-KC) (purity: 99%) and cholest-5-en-3β-ol
116 (cholesterol) (purity: 99%), were purchased from Sigma (St. Louis, MO, USA). N°1 filters (70
117 mm diameter) were used (Whatmann, Maidstone, England). Aminopropyl solid-phase extraction
118 (SPE) cartridges (Strata NH_2-55mm, 70A, 500 mg/3 mL) from Phenomenex (Torrence, CA,
119 USA) were utilized for sterol oxides purification.
120 The phosphate buffer used for the TBARs determination was prepared by adding 65.8 mL
121 of 0.5 M NaH_2PO_4 and 111 mL of 0.5 M $Na_2HPO_4 \cdot H_2O$ (water solutions) in a 500 mL
122 volumetric flask. pH was controlled, taken to neutrality (either with the acid or the basic
123 solution), and then taken to volume with water. To delay oxidation and prevent the prooxidative
124 effect of metals, proper amounts of EDTA and ascorbic acid were added to the buffer to reach a
125 final concentration of 0.1% (w/v) for both of them.
126 The silylation mixture was prepared with dried pyridine, hexamethyldisilazane and
127 trimethylchlorosilane (all from Sigma) at a ratio of 5:2:1 by volume.
128
129 *2.2 Sampling, packaging and set-up of the photosensitized oxidation experiment*
130 Fifty-six male heifers of race Charolaise, approximately 10 month old and 400 Kg weight,
131 were randomly separated in seven groups. Each group received corn silage (8 Kg/head/day), beet
132 pulp silage (5 Kg/head/day), corn meal (2.5 Kg/head/day) and straw (0.8 Kg/head/day). Feeding
133 was enriched with various ingredients (linseed oil, Dl-α tocopheryl acetate, conjugated linoleic
134 acid ((CLA) *cis*-9, *trans* 11 and *trans*-10, *cis*-12 isomers) at various doses, leading to 7
135 independent diet groups: control (A), linseed oil/ Dl-α tocopheryl acetate for 180 days (B),
136 linseed oil/ Dl-α tocopheryl acetate for 90 days (C), CLA/ Dl-α tocopheryl acetate for 180 days

137 (D), CLA/ Dl-α tocopheryl acetate for 90 days (E), linseed oil/CLA/ Dl-α tocopheryl acetate for
138 180 days (F) and linseed oil/CLA for 180 days (G), as shown in Table 1.

139 Heifers were slaughtered six month later, after reaching approximately 650 Kg weight. The
140 carcasses were refrigerated for 24 h before muscle samples were taken. The *longissimus*
141 *lumborum* (LL) muscle was excised from the carcass and freed from the superficial lipids; the
142 meat sample was divided longitudinally and the terminal parts were excluded, since the diameter
143 was not homogeneous. The muscle samples were vacuum-packed and wet-aged for 2 weeks at 4
144 °C. At the end of the aging period, the LL muscle was then cut into three pieces, trimmed of
145 surface adipose tissue and 168 subsamples were obtained; the meat slice was 1 cm thick and had
146 a weight ranging 100-150 g (thin slices).

147 Each thin slice was packed in a polyethylene vessel, which was wrapped with a transparent
148 shrink film (14 μm thickness) with 10445 mL/m^2/24 h of oxygen permeability. The packed slices
149 were subjected to the following storage conditions:

150 (a) Fifty-six vessels were immediately frozen (-20 °C), which represented T0;

151 (b) Fifty-six vessels were stored in the dark at 8 °C for 8 h (T8D) in a bench refrigerator;

152 (c) Fifty-six vessels were stored at 8 °C under a daylight lamp for 8 h (T8L), in a bench
153 refrigerator. The daylight lamp had a temperature and power of 3800 °K, 1200 Lux and 36 W
154 (Osram, Milan, Italy), respectively. The lamps were located 1.5 m above the samples.

155

156 *2.3 Lipid extraction*

157 Lipids were extracted according to a modified version (Boselli et al., 2005) of the method
158 described by Folch, Lees, and Sloane-Stanley (1957). The frozen samples were minced and 15 g
159 were homogenized with 200 mL of a chloroform:methanol solution (1:1, v/v) in a glass bottle
160 with screw-cap. The bottle was kept in an oven thermostated at 60 °C for 20 min before adding
161 100 mL chloroform. After 3 min of homogenization, the content of the bottle was filtered

through filter paper to eliminate the solid residue, which consisted mostly of proteins. The filtrate was mixed thoroughly with 100 mL of a 1 M KCl solution and left overnight at 4 °C in order to obtain phase separation. The lower phase containing the lipids was collected and dried with a rotary evaporator. The fat content was determined gravimetrically. Two lipid extractions were performed for each sample.

2.4 Gas-chromatographic determination of fatty acid composition

About 20 mg of lipid extract were methylated with 200 μL of diazomethane (Fieser, & Fieser, 1967); 1.01 mg of tridecanoic acid methyl ester was added (as internal standard), and the mixture was transmethylated with 40 μL of 2 N KOH in methanol (European Commission, 2002), vortexed for 1 min, left standing for 5 min, and centrifuged at 1620 *x g* for 5 min. Supernatant was transferred to a vial before being injected into a gas chromatograph coupled to a flame ionization detector (GC-FID). The GC-FID instrument was a GC8000 series (Fisons Instruments, Milan, Italy) interfaced with a computerized system for data acquisition (Chromcard Data System, ver. 2.3.1, Fisons Instruments). A J&W HP88 fused-silica column (100 m x 0.25 mm x 0.2 μm film thickness) (Agilent Technologies, Santa Clara, CA, USA) coated with 88% cyanopropyl aryl siloxane was used. Oven temperature was programmed from 100 °C to 180 °C at a rate of 3 °C/min, kept at 180 °C for 10 min, and then taken to 240 °C at a rate of 3 °C/min; the final temperature was kept for 30 min. The injector and detector temperatures were both set at 250 °C. Helium was used as carrier gas at a constant pressure of 260 KPa. The split ratio was 1:50. Tridecanoic acid methyl ester was used as internal standard for FA quantification, and peak identification was carried out by comparing the peak retention times with those of the GLC 463 FAME standard mixture. The GC response factor of each fatty acid was calculated by using the GLC 463 FAME standard mixture and the internal standard (13:0). The limit of detection (LOD) of FAMEs was 0.0038 mg, whereas the limit of

quantification (LOQ) was 0.01 mg. LOD and LOQ were calculated as a signal-to-noise ratios equal to 3:1 and 10:1, respectively.

The quantification of FAME was carried out according to the following formula:

$C_i = (A_i \times C_{is}) / (A_{is} \times P \times K_{ris})$

where, A_i is the fatty acid peak area; A_{is} is the internal standard area, C_i is the fatty acid concentration, C_{is} is the concentration of internal standard, P is the dry weight of the lipid sample and K_{ris} is the response factor.

2.5 Peroxide value (PV)

PV was determined in 50 mg of lipid extract, as suggested by Shantha and Decker (1994). This method is based on the ability of peroxides to oxidize ferrous ions to ferric ions. Ammonium thiocyanate reacts with ferric ions, resulting in a colored complex that can be measured spectrophotometrically. PV was evaluated at 500 nm with a double-beam UV-visible spectrophotometer (Jasco model V-550, Jasco International, Tokyo, Japan), and it was calculated from the absorbance. For the quantitative determination of PV, a Fe(III) standard calibration curve was used with a concentration range of 0.1-5 μg/mL ($y = 0.0282x - 0.0003$; $r^2 = 0.999$). PV was expressed as meq of O_2 per kg of fat. Three replicates were run per sample.

2.6 TBA-reactive substances (TBARs)

TBARs value was determined in 2 g of sample (ground meat) according to a modified method of Witte, Krause, & Bailet (1970). This method is based on the reaction between the thiobarbituric acid with aldehydes that derive from secondary oxidation of lipids present in the sample, resulting in a colored complex that can be measured spectrophotometrically. TBARs were evaluated at 530 nm with a double-beam UV-visible spectrophotometer (Jasco model V-550, Jasco International, Tokyo, Japan), and they were calculated from the absorbance. For the

quantitative determination of TBARs, a 1,1,3,3-tetramethoxypropane standard calibration curve was used with a concentration range of 0.045-0.113 μg/mL ($y = 0.0087x - 0.0051$; $r^2 = 0.999$). TBARs value was expressed as mg of malonylaldehyde (MDA) per kg of sample. Three replicates were run per sample.

2.7 Determination of sterols

About 200 mg of extracted lipids were added with known amounts of the internal standard solution (0.140 mg of betulinol and 0.0125 mg of 19-hydroxycholesterol for the determination of total sterols and COPs, respectively). Subsequently, the sample was dried under nitrogen and treated with 10 mL of 1 N KOH solution in methanol to perform a saponification at room temperature for 18 h (Sander, Addis, Park, & Smith, 1989). For the extraction of the unsaponifiable matter, 10 mL of water and 10 mL of diethyl ether were added to the samples, which were shaken, and the diethyl ether fraction was separated; the extraction with 10 mL of diethyl ether was repeated twice. The three portions of diethyl ether were pooled, treated with 5 mL of a 0.5 N KOH solution, and extracted. The resulting ethereal extract was washed twice with 5 mL of water. The ether solution was finally evaporated in a rotary evaporator, after elimination of excess water by addition of anhydrous sodium sulfate. The unsaponifiable matter was then diluted in 1 mL of *n*-hexane:isopropanol (4:1, v/v). One-tenth of the unsaponifiable matter was used for the determination of total sterols, whereas the remaining part was utilized for COP analysis.

The determination of total sterols (sum of free and esterified) was performed by gas chromatography coupled to a flame ionization detector (GC-FID), after sample silylation (Sweeley, Beutley, Mokita, & Wells, 1963). The GC-FID instrument was an HRGC 5300 model (Carlo Erba), which was equipped with a split-splitless injector. A CP-SIL 5CB Low Bleeds/MS (30 m x 0.32 mm i.d. x 0.25 μm film thickness) (Varian Chrompack, Middelburg, The

Netherlands) was used. The oven temperature was programmed from 265 °C to 280 °C at 0.5 °C/min and then from 280 °C to 325 °C at 4 °C/min; the injector and detector temperatures were both set at 325 °C. Helium was used as carrier gas at a flow of 2.9 mL/min; the split ratio was 1:15. Two replicates were run per sample.

Sterols were identified by comparing their retention times and mass spectra with those of the corresponding standards, using GC-FID and GC-MS (same analytical conditions as COPs; see paragraph 2.8). Sterols were then quantified according to the following expression:

$$C_i = (A_i \times C_{is})/(A_{is} \times P \times K_{ris})$$

where, A_i is the sterol peak area; A_{is} is the internal standard (betulinol) area, C_i is the sterol concentration, C_{is} is the concentration of the internal standard, P is the dry weight of the lipid fraction and K_{ris} is the response factor.

2.8 Determination of cholesterol oxidation products (COPs)

Regarding the determination of COPs, the remaining 9/10 of the unsaponifiable matter were purified by NH_2 SPE according to Rose-Sallin, Hugget, Bosset, Tabacchi, and Fay (1995). COPs were eluted with acetone. The purified fraction was then silylated (Sweeley et al., 1963), dried under nitrogen stream and dissolved in 50 µL of *n*-hexane. One µL of the silylated sterol oxides was injected into a GC coupled to a mass spectrometer (GC-MS) Shimadzu QP-2010 Plus (Kyoto, Japan). The system was fitted with a capillary Zebron ZB-5 column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Phenomenex, Torrance, CA, USA), coated with 5% phenyl-95% dimethylpolysiloxane, and interfaced with a computerized system for data acquisition (GC solution ver.2, Shimadzu, Kyoto, Japan). The oven temperature was kept at 250 °C for 3 min, then taken from 250 °C to 280 °C at 2 °C/min, kept at 280 °C for 12 min, and then taken from 280 °C to 320 °C at 1.7 °C/min; the final temperature was kept for 20 min. The injector and transfer line temperatures were set at 325 °C and 230 °C, respectively. Helium was used as

carrier gas at a flow rate of 0.41 mL/min; the split ratio was 1:15. The filament emission current was 70 eV. A mass range from 50 to 550 *m*/*z* was scanned at a rate of 1500 amu/s. Acquisition was performed with total ion current (TIC) and single ion monitoring (SIM) modes.

The identification of COPs was confirmed by comparing their retention times and mass spectra with those of the corresponding standards. COPs were quantified using 19-hydroxycholesterol as internal standard, by using the SIM acquisition mode. The *m/z* ratios used for identification and quantification of each COP are reported in Table 2. Their response factors were evaluated with respect to the corresponding internal standards. GC-MS LOD and LOQ of COPs were 0.08 μg and 0.28 μg, respectively. LOD and LOQ were calculated as a signal-to-noise ratio equal to 3:1 and 10:1, respectively. Two replicates were run per sample.

2.9 Statistical analysis

The data are reported as mean values of independent replicates of each analytical determination (Tables 3-4). Factorial analysis of variance (ANOVA) was performed for data from the crossed treatments, in order to study the influence of the different diets and storage conditions, as well as their interactions, on sterol composition and the oxidative parameters. Tukey's honest significance test was performed at a 95% confidence level ($p \leq 0.05$), in order to separate means of statistically-different parameters and interactions. Pearson correlation coefficients (α=0.05) were used to examine possible relationships between oxidation parameters of beef meat slices over the whole data set. A principal component analysis (PCA) was also carried out. Statistical analysis of the data was performed by SPSS 16.0.1 (2007, IBM-SPSS Inc., Chicago, Illinois, USA).

3. Results and discussion

3.1. Total fatty acid composition

Lipid content of muscle (4.6-6.1%) was similar (P>0.05) among treatments (data not shown). Table 3 shows the FA composition of total lipids (mean values expressed as mg/100 mg of lipids). In general, the most abundant FA was oleic acid (~ 37-41% of total FA; 13.4-23.8 mg/100 mg of lipids, which corresponded to 0.4-1.8 g/100 g of meat), followed by palmitic (~ 25-27%; 9.9-19.9 mg/100 mg of lipids, which corresponded to 0.4-1.6 g/100 g of meat), stearic (~ 18-21%; 8.0-14.8 mg/100 mg of lipids, which corresponded to 0.3-1.2 g/100 g of meat), myristic (~ 2-3%; 1.4-3.5 mg/100 mg of lipids, which corresponded to 0.04-0.18 g/100 g of meat) and palmitoleic acids (~ 2%; 1.5-2.7 mg/100 mg of lipids, which corresponded to 0.005-0.002 g/100 g of meat). Among long-chain PUFA (48.5-57.4 mg/100 mg of lipids, which corresponded to 0.04-0.30 g/100 g of meat), linoleic (1.30-4.35 mg/100 mg of lipids, which corresponded to 0.06-0.21 g/100 g of meat) and arachidonic acid (0.12-0.81 mg/100 mg of lipids, which corresponded to 0.008-0.030 g/100 g of meat) were the most abundant, whereas docosahexaenoic (DHA) were detected at trace levels (< 0.01 mg/100 mg of lipids).

The diets did not significantly impact the amount of ∑CLA in meat (Table 3); however, diet C led to a significant (P<0.05) increase α-linolenic acid, whereas diet E reported the highest level of linoleic acid. Moreover, no significant effect of diets on the amount of *trans*-vaccenic acid was observed, except for diet B. These FA changes might be related to the biohydrogenation effect of the rumen enzymes on the dietary LO. During storage, lipid oxidation mainly affected PUFAs, since linoleic, arachidonic, eicosapentaenoic and docosapentaenoic acids significantly decreased, leading to an increase of CLA; however, no photoxidation effect was noted.

The factorial analysis of the single FA content evidences some significant interaction between diet G and storage; in fact, after storage, ∑CLA content was significantly higher than those found in other treatments. When diet F was used, the level of arachidonic and eicosapentaenoic acids dropped to 0.28 and 0.04 mg/100 mg of lipids, respectively, after

312 photoxidation. Saturated (SFA; 0.89-3.13 g/100 g meat), monounsaturated (MUFA; 0.76-2.6
313 g/100 g meat), and polyunsaturated (PUFA; 0.04-0.30 g/100 g of meat) fatty acids accounted for
314 49-55, 40-46, and 3-9% of total FA, respectively (Table 4). The n-6/n-3 FA ratio varied from 4
315 to 16, which reflects the current Western diet trend, being much higher than the corresponding
316 nutritional recommendations (1 < n-6/n-3 < 4) (Simopoulos, 2006).

317 The Δ-desaturase index [(20:2 n-6 + 20:4 n-6 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3/18:2 n-6 +
318 18:3 n-3 + 20:2 n-6 + 20:4 n-6 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3) × 100] is useful to evaluate the
319 activities of both Δ5- and Δ6-desaturases, which are enzymes that catalyze the formation of n-6
320 and n-3 PUFA. No significant effect of diet and storage was found on the activity of both Δ5-
321 and Δ6-desaturases, since its index (9.8-24.4) did not significantly change. However, some trend
322 was detected when diets A, E and G were used, since the Δ-desaturase index was higher as
323 compared with other dietary treatments.

324 In general, beef meat FA composition was partially (P<0.05) affected by feedings, since
325 LO and vE supplementation for shorter period led to a higher α-linolenic acid content without
326 influencing the content of palmitic, stearic and oleic acids. However, no significant differences
327 in CLA levels were found when LO and/or CLA with and without vE were supplied.
328 Nevertheless, a decrease of PUFAs during the storage was observed.

329

330 *3.2 Sterols content*

331 Total sterol content (Table 5) ranged from 399 to 1343 mg/100 g lipids, which
332 corresponded to 283 and 514 mg/kg of meat. Total cholesterol was about 98% of total sterols,
333 followed by campesterol (38% of phytosterols), β-sitosterol (27%), stigmasterol (19%) and 5-
334 avenasterol (16%). The highest amount of sterols was found in untreated meat samples obtained
335 with diet A, being cholesterol (942.8 mg/100 g lipids, which corresponded to 0.05 g/100 g of
336 meat), campesterol (1.04-7.86 mg/100 g lipids, which corresponded to < 0.0002 g/100 g of meat)

and β-sitosterol (1.07-20.09 mg/100 g lipids, which corresponded to < 0.0003 g/100 g of meat) the most representative sterols. Total cholesterol content of the samples ranged from 0.3 to 1.8 mg/100 mg of lipids, which corresponded to 0.03-0.05 g/100 g of meat; such a large variation might be due to the heterogeneous structure of the muscle.

However, both diet and storage conditions did not significantly impact (P>0.05) sterol level, except for cholesterol, probably due to enzymatic activity (such as cholesterol oxidase) that converts cholesterol into other compounds different from COPs, thus leading to a decrease of cholesterol amount (MacLachlan, Wotherspoon, Ansel, & Brooks, 2000); no main effect of diet was evident, though. Moreover, photosensitized oxidation did not significantly (P>0.05) impact the cholesterol content, even though a decreasing trend during the storage was observed.

Finally, it must be noted that the relative presence of the single phytosterols in the raw beef meat did not correspond to the sterol composition of the dietary oil sources (linseed and soybean oil), where β-sitosterol is usually about 2-4 times higher than campesterol. It might be possible that phytosterols have been selectively absorbed in different amount and/or have been partially metabolized/degraded by beef cattle, converting them into other compounds (Rozner & Garti, 2006). In fact, in humans, campesterol is more absorbed than β-sitosterol (Lutjohann, Bjorkhem, Beil, & von Bergmann, 1995), which seems to be related to the structure and length of the sterol side-chain.

3.3 Lipid oxidation

The effect of photosensitized oxidation on lipids from beef meat obtained with different diets was evaluated by PV and TBARs (Table 6). PV ranged from 0.51 to 5.91 meq O_2/kg lipid and was markedly lower in A, C and E diets than in the other treatments. After 8 h of storage under dark conditions, PV significantly increased from 1.75 (T0) to 4.08 (T8D) meq O_2/kg lipid; while after light exposure it significantly (P<0.05) dropped to 3.29 (T8L) meq O_2/kg due to

362 hydroperoxide breakdown induced by light. On the other hand, no interaction effect between diet
363 and storage was found (Table 6). The lack of significant differences could be partly ascribed to
364 the high data dispersion observed. However, when 2 g of vE were added to feed for 90 days, PV
365 were lower than in the other treatments, while LO and CLA led to a PV increase.

366 In general, it might be pointed out that PV values found do not represent a problem from
367 the lipid oxidation standpoint. In addition, these data are similar to reported in literature. Boselli
368 et al. (2009) found comparable PV levels in beef meat when exposed to fluorescent light for 8 h
369 at 4 °C.

370 TBARs varied from 0.19 to 2.53 mg malonaldehyde (MDA)/kg meat, which corresponded
371 to diets C-T0 and A-T8L, respectively (Table 6). Diets B (LO/vE for 180 days) and E (CLA/vE
372 for 90 days) lead to the lowest formation of TBARs (Table 6). The main effect of storage time
373 was investigated, but some interactions between diet and storage time were detected; in fact,
374 control diet significantly (p<0.05) increased the TBARs level under the different storage
375 conditions. TBARs formation was affected by light exposure as it led to a 10% increase of
376 TBARs level. Samples kept at dark showed a four times higher TBARs content with respect to
377 those found in fresh meat (T0), which could be to the simultaneous action of both autoxidation
378 and enzymatic oxidation mechanisms that lead to the formation and demolition of peroxides
379 during storage (Boselli et al., 2012). In the present study, the level of lipid oxidation in beef
380 remained below the acceptable threshold of 2 mg MDA/kg beef (Campo et al., 2006), except for
381 A-T8D, C X T8D, G X T8D, A-T8L and G-T8L diets. However, data found were higher than
382 those reported by Insani et al. (2008). This could be attributed to Italian slaughtering and
383 processing practices, which imply a holding period of a few days at 3–6 °C, aimed at improving
384 meat tenderness and promoting the formation of aroma compounds or their precursors that
385 develop during cooking (Rodriguez-Estrada, Penazzi, Caboni, Bertacco, and Lercker, 1997;
386 Boselli et al., 2009).

387

388 *3.4 Cholesterol oxidation products (COPs)*

389 Table 6 shows the average value of total COPs as related to feeding, storage conditions and
390 their interaction. In general, the total COPs levels found in the beef slices ranged from 11.72 to
391 39.12 mg/kg of lipids, which corresponded to 0.5-2.1 mg/kg of meat and were lower than those
392 reported by other authors (Boselli et al., 2009).

393 COPs did not significantly (P>0.05) changed during storage, but an increasing trend on
394 COPs formation was noted after light exposure. A similar trend was reported by Boselli et al.
395 (2009), which observed that prolonged light exposure (8 h) led to an increase of PV even if
396 COPs remained constant; this could be ascribed to the higher rates of degradation of these
397 products with respect to their formation. As already mentioned, the extent of initial cholesterol
398 oxidation found here could be due to the long holding period to which the meat was subjected
399 (15 days). A similar effect of the holding period on the oxidative quality of beef meat has
400 previously been reported (Rodriguez-Estrada et al., 1997; Boselli et al., 2009).

401 The main COPs detected were 7α-HC (1.5-7.5 mg/kg lipids; which corresponded to 0.03-
402 0.52 mg/kg of meat), 7β-HC (2.1-9.2 mg/kg lipids, which corresponded to 0.05-1.02 mg/kg of
403 meat), β-CE (1.7-9.1 mg/kg lipids, which corresponded to 0.01-0.67 mg/kg of meat), α-CE (1.2-
404 5.2 mg/kg lipids, which corresponded to 0.03-0.38 mg/kg of meat), and 7-KC (3.2-10.5 mg/kg
405 lipids, which corresponded to 0.06-0.87 mg/kg of meat). Dominant COPs in both irradiated and
406 unirradiated raw beef meat were the same as those reported by Boselli et al. (2009), confirming
407 the role of 7-KC as marker of cholesterol oxidation in raw muscle food (Boselli et al., 2012). In
408 general, CLA and vE supplied for 90 days (diet D) led to lower amounts of single COPs as
409 compared with other diets. In addition, α-CE and β-CE significantly decreased during storage
410 under both dark and light exposure conditions, which could be ascribed to epoxy-ring opening in
411 presence of water and acidic conditions, with the consequent generation of triol; however, the

412 latter was not detected under the analytical conditions used. No significant effect of diet x
413 storage interaction was detected, even though some trend was observed; the latter tendency was
414 confirmed by the other oxidation parameters (PV and TBARs), though.

415 The cholesterol oxidation ratio (%OR, calculated as % COPs/cholesterol) varied from 0.1
416 to 0.4% in fresh meat (T0) samples, whereas it ranged from 0.2 to 0.5% and from 0.2 to 0.6% in
417 T8D and T8L samples, respectively. CLA and vE supplied for 90 d led to the lowest %OR
418 among all dietary treatments. Although no significant effect of light exposure on %OR was
419 observed, it must be noticed that meat obtained with diet F (LO, CLA and vE for 180 d) showed
420 the highest cholesterol oxidation (0.6% OR). The lack of the pro-oxidant effect of light on %OR
421 is in contrast with the PV and TBARs data, as they evidenced a significant impact (P>0.05) of
422 light exposure on general lipid oxidation. Therefore, it seems that, under the experimental dietary
423 conditions tested, light exposure affected more the fatty acid fraction than cholesterol.

424 According to the threshold of toxicological concern (TTC) for unclassified compounds,
425 which corresponds to 0.15 μg per person per day (Kroes et al., 2004), COPs levels found in fresh
426 and photoxidized meats (45-213 μg/100 g of meat) might represent a risk for human health and
427 thus further research is needed required to better ascertain their toxicity levels.

428 To limit light absorption and its overall impact on lipid oxidation, it would be necessary to
429 use appropriate packaging material (with a wavelength transmission range between 490 and 589
430 nm and/or with aluminum layers as light and gas barrier) (Bekbölet, 1990; Boselli et al., 2012)
431 and conditions (modified atmosphere and vacuum) (Boselli et al., 2012).

432

433 *3.5 Correlations and Principal Component Analysis (PCA)*

434 A correlation study (Pearson test, α=0.05) was performed on the results obtained for lipid
435 composition, oxidative parameters and the crossed treatments deriving from the different types

436 of feedings and three storage conditions. For better data comprehension, only significant
437 correlations are here discussed.
438 Oleic acid was indirectly correlated to stearic acid (r=-0.604, p=0.004). Cholesterol was
439 positive correlated (r=0.783, p=0.000) to all sterols, due to their similar pathway of origin but it
440 was negatively correlated to TBARs (r=-0.601, p=0.004). The latter resulted positively
441 correlated to CLA (r=0.662, p=0.001), which could be ascribed to its higher susceptibility to
442 oxidation. A positive, linear correlation was found between PV and TBARs (r=0.480, p=0.001),
443 which supports the well-known strict interdependence between hydroperoxides and their
444 demolition/evolution compounds. All COPs were correlated to PV (r=0.554, p=0.009),
445 especially 7α-HC (r=0.652, p=0.001), 7β-HC (r=0.568, p=0.001) and 7-KC (r=0.517, p=0.016).
446 As expected, positive correlations were observed between 7α-/7β-HC and 7-KC (r=0.884,
447 p=0.001), as they derive from the same oxidation pathways. In fact, the 7-oxysterols (7α-HC,
448 7β-HC and 7-KC) originate from 7-hydroperoxide demolition (Lercker et al., 2002).
449 Principal component analysis performed with total fatty acid and sterols composition,
450 oxidative parameters was able to distinguish different clusters (Figure 1), explaining in two
451 principal components 59.21% of total variance. The first principal component clearly separated
452 PUFAs from myristic acid, while the second principal component grouped COPs with MUFA
453 separating them from the cluster TBARs-SFA. The principal component analysis (Figure 2)
454 performed with crossed treatment was able to recognize cluster trends by the distribution on
455 principal component scores. The diets C, E and A X T0 with PUFAs (linoleic, arachidonic,
456 eicosapentaenoic, docosapentaenoic and n-6 fatty acids) were completely separated from other
457 treatments by the first principal component, while diet B X T0 was well separated by the second
458 principal component, confirming that COPs behavior was mainly correlated to PV instead of
459 TBARs.
460

4. Conclusions

Photoxidation of raw beef meat obtained with seven different diets, was studied under different storage conditions. In general, both PV and TBARs increased after storage at 8 °C under darkness conditions, while exposure to light led to hydroperoxide breakdown with a consequent rise of TBARs. In general, vE increased the oxidative stability of the photoxidized beef meat, regardless of the other feed ingredients. Beef meat obtained with diets C and E was more stable from the oxidative standpoint, probably due to the meat enrichment with vE and the shortest supplementation period (90 days). Although cholesterol oxidation rate was 0.1-0.6% of total cholesterol, COPs contents of fresh and photoxidized meats (45-213 μg/100 g of meat) were above the TTC for unclassified compounds (0.15 μg per person per day; Kroes *et al.*, 2004), so it is of outmost importance to adopt suitable storage and packaging strategies to limit light absorption and its overall impact on meat lipid oxidation.

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596 **Table 1.** Description of the seven dietary groups.

Diet Groups	Supplementation	Days	Description
A (control)	-	180	Base diet with corn silage (protein 14% and energy 1762 Kcal/kg)
B	LO/vE	180	Based diet plus 1 g vE $head^{-1}$ day^{-1} and 250 g LO $head^{-1}$ day^{-1}
C	Control + LO/vE	90+90	Base diet (90 days) followed by based diet integrated with 2 g vE $head^{-1}$ day^{-1} and 500 g LO $head^{-1}$ day^{-1}
D	CLA/vE	180	Based diet integrated with 1 g vE $head^{-1}$ day^{-1} and 5 g CLA $head^{-1}$ day^{-1}
E	Control + CLA/vE	90+90	Base diet (90 days) followed by based diet integrated with 2 g vE $head^{-1}$ day^{-1} and 10 g CLA $head^{-1}$ day^{-1}
F	LO/CLA/vE	180	Based diet integrated with 1 g vE $head^{-1}$ day^{-1}, 5 g CLA $head^{-1}$ day^{-1} and 250 g LO $head^{-1}$ day^{-1}
G	LO/CLA	180	Based diet integrated with 5 g CLA $head^{-1}$ day^{-1} and 250 g LO $head^{-1}$ day^{-1}

597

598 Abbreviations: CLA, conjugated linoleic acid (*cis*-9, *trans* 11 and *trans*-10, *cis*-12 isomers); LO,
599 linseed oil; vE, Dl-α tocopheryl acetate.

600

601

602 **Table 2.** Retention time and characteristic mass fragmentation (*m/z*) of TMS-ether derivatives of

603 cholesterol oxides, obtained by GC/MS coupled to a ZB-5 column.

Oxysterols	Characteristic ions (*m/z*)	Retention time (min)
7α-hydroxycholesterol-TMS	**456** 457 458 546	20.07
7β-hydroxycholesterol-TMS	**456** 457 458 546	23.85
5,6β-epoxycholesterol-TMS	356 **384** 445 474	24.39
5,6α-epoxycholesterol-TMS	**366** 384 459 474	24.99
7-ketocholesterol-TMS	131 367 **472** 514	30.80
19-hydroxycholesterol-TMS (IS)	145 **353** 366 456	22.62

604 Note: ions in bold were used for quantification purposes

605

606

607

608 **Table 3.** Fatty acid composition of raw meat (expressed as mg/100 mg of lipids) as related to diets and storage conditions.

Factor	14:0		16:0		16:1		18:0		18:1 *t*11		18:1		18:2 n-6		18:3 n-3		∑CLA		20:4 n-6		20:5 n-3		22:5 n-3	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	2.60	0.06	26.64	0.35ab	2.22	0.05ab	19.17	0.34	0.97	0.07b	38.46	0.23ab	4.82	0.37a	0.30	0.02d	0.18	0.04	0.66	0.12ab	0.07	0.01	0.18	0.03
B	2.90	0.06	26.44	0.23ab	2.25	0.10ab	19.20	0.50	1.87	0.20a	40.00	0.57ab	3.85	0.23abc	0.48	0.04abc	0.22	0.05	0.37	0.03abc	0.04	0.00	0.13	0.01
C	2.77	0.02	26.27	0.21ab	1.86	0.02b	20.76	0.18	1.40	0.13ab	37.36	0.32b	4.55	0.27ab	0.61	0.04a	0.23	0.06	0.52	0.05abc	0.06	0.01	0.14	0.03
D	2.90	0.08	27.37	0.17a	2.03	0.13ab	20.52	0.50	1.20	0.04b	37.54	0.45ab	3.29	0.15bc	0.41	0.03bcd	0.20	0.05	0.30	0.03bc	0.04	0.01	0.11	0.01
E	2.68	0.08	26.61	0.10ab	2.03	0.04ab	20.35	0.61	1.22	0.17b	37.22	1.19b	5.01	0.49a	0.36	0.03cd	0.19	0.05	0.72	0.13a	0.07	0.02	0.21	0.04
F	2.55	0.13	25.74	0.31b	1.98	0.11ab	18.82	0.98	1.06	0.07b	40.42	1.06a	4.42	0.50ab	0.48	0.04abc	0.20	0.05	0.55	0.11abc	0.07	0.01	0.19	0.03
G	2.94	0.15	26.85	0.52ab	2.27	0.11a	19.59	0.69	1.47	0.13ab	39.83	0.21ab	2.59	0.35c	0.54	0.03ab	0.25	0.06	0.28	0.02c	0.03	0.00	0.11	0.01
Stat. signif.	ns		<0.05		<0.05		ns		<0.05		<0.05		<0.05		<0.01		ns		<0.05		ns		ns	
Storage																								
T0	2.71	0.07	26.81	0.2	2.14	0.1	19.12	0.3	1.15	0.1b	38.55	0.46	4.89	0.34a	0.50	0.03	0.01	0.00c	0.67	0.09a	0.08	0.01a	0.21	0.02a
T8D	2.76	0.06	26.27	0.2	2.07	0.1	19.97	0.4	1.13	0.1b	38.93	0.63	3.80	0.16b	0.43	0.03	0.28	0.01b	0.39	0.03b	0.04	0.00b	0.12	0.01b
T8L	2.82	0.06	26.60	0.2	2.07	0.1	20.22	0.4	1.66	0.1a	38.59	0.44	3.54	0.25b	0.44	0.03	0.34	0.01a	0.40	0.03b	0.04	0.00b	0.12	0.01b
Stat. signif.	ns		ns		ns		ns		<0.05		ns		<0.01		ns		<0.05		ns		<0.05		<0.01	
D x S																								
A X T0	2.47	0.07	26.87	0.43	2.25	0.16	17.98	0.49	0.81	0.04f	38.12	0.04	6.25	0.21a	0.35	0.03b	0.02	0.00d	1.12	0.05a	0.12	0.02a	0.31	0.02a
B X T0	2.94	0.13	26.78	0.45	2.38	0.19	18.78	0.81	1.55	0.07bcde	40.22	0.95	4.03	0.54bcd	0.52	0.06ab	0.01	0.00d	0.41	0.07bcd	0.05	0.01ab	0.15	0.02abcd
C X T0	2.71	0.06	26.67	0.32	1.84	0.02	20.42	0.45	1.32	0.15bcdef	36.93	0.51	5.14	0.05abc	0.68	0.05a	0.01	0.00d	0.68	0.11abcd	0.08	0.03ab	0.21	0.04abcd
D X T0	2.83	0.20	27.26	0.38	2.05	0.23	20.14	0.55	1.18	0.03cdef	38.30	0.23	3.68	0.17bcd	0.45	0.09ab	0.01	0.00d	0.37	0.10bcd	0.05	0.02ab	0.13	0.04abcd
E X T0	2.60	0.06	26.34	0.04	1.99	0.10	19.98	0.79	1.06	0.13def	37.13	2.67	5.92	1.39ab	0.37	0.04ab	0.01	0.00d	0.94	0.36ab	0.10	0.04ab	0.28	0.11abc
F X T0	2.53	0.33	26.54	0.61	2.16	0.25	17.30	1.03	0.88	0.03ef	39.40	1.27	5.88	1.12ab	0.58	0.09ab	0.02	0.00d	0.90	0.23abc	0.12	0.02a	0.29	0.05ab
G X T0	2.88	0.33	27.19	1.11	2.29	0.24	19.27	1.13	1.21	0.02cdef	39.74	0.26	3.35	0.23bcd	0.54	0.07ab	0.01	0.00d	0.29	0.04d	0.03	0.01b	0.11	0.01bcd
A X T8D	2.60	0.10	26.11	0.86	2.18	0.01	20.08	0.01	1.06	0.09def	38.63	0.57	4.02	0.10abcd	0.28	0.01b	0.24	0.03c	0.41	0.02bcd	0.04	0.00ab	0.11	0.00cd
B X T8D	2.89	0.10	26.25	0.46	2.18	0.24	19.56	1.33	1.44	0.11bcdef	39.90	1.22	3.71	0.36abcd	0.51	0.06ab	0.31	0.00abc	0.32	0.02cd	0.03	0.00b	0.11	0.00cd
C X T8D	2.79	0.00	26.00	0.43	1.92	0.03	20.77	0.17	1.27	0.12bcdef	37.86	0.75	4.20	0.64abcd	0.57	0.08ab	0.32	0.01abc	0.42	0.07bcd	0.05	0.00ab	0.12	0.01bcd
D X T8D	2.91	0.16	27.42	0.37	2.05	0.29	20.36	0.88	1.10	0.04def	36.91	0.98	3.20	0.25bcd	0.38	0.05ab	0.27	0.01bc	0.29	0.01d	0.04	0.00b	0.10	0.01cd
E X T8D	2.75	0.18	26.57	0.05	2.07	0.06	20.58	1.48	0.78	0.21f	37.35	2.24	4.60	0.37abc	0.30	0.01b	0.26	0.03bc	0.60	0.12abcd	0.06	0.02ab	0.18	0.03abcd
F X T8D	2.42	0.10	24.98	0.38	1.91	0.20	18.39	2.41	1.04	0.17cdef	42.57	2.85	3.98	0.23abcd	0.44	0.05ab	0.24	0.00c	0.46	0.05bcd	0.06	0.01ab	0.17	0.03abcd
G X T8D	2.94	0.29	26.58	1.11	2.20	0.21	20.07	1.28	1.25	0.03cdef	39.27	0.14	2.86	0.05cd	0.50	0.05ab	0.34	0.00abc	0.23	0.01d	0.03	0.00b	0.10	0.00cd
A X T8L	2.72	0.10	26.94	0.54	2.23	0.04	19.45	0.03	1.04	0.15def	38.63	0.47	4.18	0.28abcd	0.29	0.02b	0.28	0.03bc	0.45	0.03bcd	0.05	0.01ab	0.13	0.02abcd

B X T8L	2.88	0.12	26.28	0.34	2.19	0.12	19.26	0.65	2.61	0.19a	39.87	1.22	3.80	0.46abcd	0.42	0.08ab	0.34	0.03abc	0.38	0.06bcd	0.05	0.00ab	0.12	0.01bcd
C X T8L	2.81	0.00	26.13	0.35	1.82	0.05	21.10	0.18	1.60	0.36bcd	37.28	0.47	4.32	0.46abcd	0.59	0.07ab	0.36	0.00ab	0.47	0.01bcd	0.05	0.00ab	0.08	0.04d
D X T8L	2.97	0.08	27.44	0.20	1.98	0.28	21.07	1.31	1.32	0.03bcdef	37.41	0.95	2.98	0.19cd	0.39	0.05ab	0.33	0.01abc	0.24	0.01d	0.03	0.01b	0.10	0.01cd
E X T8L	2.69	0.21	26.94	0.15	2.04	0.08	20.50	1.27	1.81	0.11bc	37.19	2.22	4.52	0.41abc	0.42	0.06ab	0.30	0.05abc	0.63	0.13abcd	0.06	0.02ab	0.19	0.03abcd
F X T8L	2.69	0.27	25.70	0.21	1.88	0.18	20.76	1.21	1.26	0.03bcdef	39.29	0.63	3.41	0.24bcd	0.43	0.04ab	0.34	0.03abc	0.28	0.01d	0.04	0.00b	0.10	0.01cd
G X T8L	3.01	0.28	26.79	0.80	2.32	0.22	19.42	1.62	1.96	0.07ab	40.49	0.23	1.55	0.77d	0.57	0.07ab	0.40	0.00a	0.32	0.03cd	0.04	0.00ab	0.13	0.00bcd
Stat. signif.	ns		ns		ns		ns		<0.05		ns		<0.01		<0.01		<0.05		<0.01		<0.001		<0.01	

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610 Results are expressed as mean and SE, standard error of the fifty-six independent samples (n=56). Abbreviations: A, control diet; B, diet
611 supplemented with LO/vE 180 days; C, diet supplemented with LO/vE for 90 days; D, diet supplemented with CLA/vE for 180 days; E, diet
612 supplemented with CLA/vE for 90 days; F, diet supplemented with LO/CLA/vE for 180 days; G, diet supplemented with LO/CLA for 180 days;
613 ∑CLA, *c*9,*t*11 + *t*10,*c*12; T0, fresh raw meat; T8D, samples stored at dark; T8L, samples exposed at light; Stat. signif., statistical significance; a,
614 b, c, d, e, f, statistically different means (Tukey's test; $p \leq 0.05$).

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Table 4. Total saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) (expressed as % of total FA), and n-6/n-3 in raw beef meat, as related to diets and storage conditions

Factor	Σ SFA		Σ MUFA		Σ PUFA		n-6/n-3	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	51.30	0.52bc	42.06	0.41	6.52	0.51ab	14.61	0.61a
B	51.14	0.42bc	43.39	0.68	5.33	0.30abc	8.29	0.71b
C	53.17	0.21ab	40.35	0.37	6.40	0.32ab	7.56	0.10bc
D	54.13	0.48a	41.19	0.43	4.58	0.17bc	8.79	0.76b
E	52.60	0.81abc	40.37	1.27	6.90	0.68a	13.41	0.84a
F	50.02	1.08c	43.60	1.17	6.23	0.67ab	8.78	0.23b
G	52.52	0.24abc	43.33	0.25	4.01	0.29c	5.44	0.66c
Stat. signif.	<0.05		ns		<0.05		<0.05	
Storage								
T0	51.52	0.37	41.49	0.51	6.64	0.48a	10.1	0.8
T8D	52.03	0.58	42.60	0.64	5.35	0.20b	9.5	0.7
T8L	52.82	0.41	42.04	0.50	5.14	0.27b	9.0	0.8
Stat. signif.	<0.05		ns		<0.01		ns	
Diet x storage								
A X T0	49.96	0.03ab	41.19	0.20	8.51	0.20a	16.06	1.61a
B X T0	50.88	0.46ab	43.35	1.14	5.36	0.74abcd	7.87	0.13ef
C X T0	53.03	0.69ab	39.64	0.55	7.09	0.13abc	7.70	0.29ef
D X T0	53.65	0.03ab	41.17	0.45	4.90	0.44abcd	8.95	1.30bcdef
E X T0	51.85	0.82ab	39.87	2.76	7.97	2.01abc	14.09	1.17abc
F X T0	49.02	0.08b	42.38	1.51	8.15	1.55ab	9.48	0.42bcdef
G X T0	52.25	0.30ab	42.81	0.01	4.51	0.34bcd	6.54	0.36ef
A X T8D	51.77	1.11ab	42.82	1.05	5.39	0.06abcd	13.88	0.38abcd
B X T8D	51.33	1.07ab	43.40	1.53	5.26	0.47abcd	7.40	0.12ef
C X T8D	53.01	0.01ab	41.02	0.80	5.96	0.79abcd	7.38	0.05ef
D X T8D	53.79	0.61ab	41.67	0.44	4.52	0.18bcd	8.93	1.71bcdef
E X T8D	52.91	1.80ab	40.67	2.39	6.36	0.56abcd	14.46	0.34ab
F X T8D	48.54	2.95b	45.77	3.12	5.67	0.18abcd	8.73	0.10bcdef
G X T8D	52.44	0.03ab	42.85	0.11	4.28	0.08cd	5.96	0.48ef
A X T8L	52.16	0.81ab	42.18	0.46	5.66	0.34abcd	13.90	0.50abcd
B X T8L	51.20	0.85ab	43.42	1.37	5.38	0.55abcd	9.60	2.15bcdef
C X T8L	53.48	0.02ab	40.38	0.48	6.14	0.50abcd	7.61	0.14ef
D X T8L	54.95	1.38a	40.73	1.26	4.32	0.12cd	8.50	1.50cdef
E X T8L	53.04	1.89ab	40.58	2.42	6.38	0.53abcd	11.67	2.17abcde
F X T8L	52.49	0.71ab	42.64	0.96	4.87	0.25abcd	8.13	0.10def
G X T8L	52.44	0.70ab	44.33	0.05	3.23	0.65d	3.84	1.70f
Stat. signif.	<0.01		ns		<0.05		<0.05	

Results are expressed as mean and SE, standard error of the fifty-six independent samples (n=56). Abbreviations: A, control diet; B, diet supplemented with LO/vE 180 days; C, diet supplemented with LO/vE for 90 days; D, diet supplemented with CLA/vE for 180 days; E, diet supplemented with CLA/vE for 90 days; F, diet supplemented with LO/CLA/vE for 180 days; G, diet supplemented with LO/CLA for 180 days; T0, fresh raw meat; T8D, samples stored at dark;

T8L, samples exposed at light; Stat. signif., statistical significance; a, b, c, d, e, f, statistically different means (Tukey's test; $p \leq 0.05$).

Table 5. Sterols contents of raw beef meat (mg/100 g of lipids), as related to diets and storage conditions.

Factor	Cholesterol			Campesterol			Stigmasterol			β-sitosterol			Δ^5avenasterol			TOT Sterols		
Diet	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
A	942.77	92.97		4.06	0.26		1.11	0.28		4.07	0.45		0.86	0.35		952.87	93.60	
B	664.73	63.83		3.57	0.42		1.81	0.54		3.06	0.51		0.94	0.26		674.11	64.88	
C	855.32	109.16		3.28	0.35		1.67	0.28		2.65	0.34		0.77	0.25		863.69	109.86	
D	807.13	134.40		3.50	0.64		1.38	0.53		2.55	0.34		0.92	0.30		815.48	135.11	
E	910.86	159.92		3.88	0.52		2.36	1.05		4.31	1.54		1.04	0.30		922.46	160.17	
F	705.34	114.46		3.24	0.58		1.84	0.44		3.80	1.49		1.31	0.56		715.53	116.62	
G	752.22	93.60		3.42	0.41		2.08	0.24		2.68	0.40		0.93	0.22		761.33	94.22	
Stat. Signific.	ns			ns			ns			ns			ns			ns		
Storage																		
T0	1097.25	82.11	a	4.58	0.31	a	2.04	0.21		3.92	0.66	ab	1.41	0.29		1109.20	82.64	a
T8D	673.34	42.69	b	3.04	0.22	b	2.23	0.56		3.03	0.71	a	0.78	0.11		682.43	42.68	b
T8L	636.54	55.17	b	3.05	1.47	b	1.00	0.18		2.98	0.33	b	0.70	0.18		644.28	55.70	b
Stat. Signific.	<0.001			ns			ns			<0.001			ns			<0.001		
Diet x storage																		
A X T0	1321.47	112.35	a	4.92	0.17		1.56	0.54		5.37	0.34		1.58	0.94		1334.90	111.53	a
B X T0	880.93	114.33	ab	4.96	0.71		2.73	0.73		4.19	1.13		1.60	0.57		894.38	116.42	ab
C X T0	1188.40	163.89	ab	4.03	0.32		2.06	0.44		3.16	0.54		1.35	0.55		1198.99	164.74	ab
D X T0	1001.69	233.83	ab	4.07	1.03		1.19	0.36		1.49	0.33		0.00	0.00		1008.45	235.14	ab
E X T0	1331.96	413.00	a	4.74	1.35		1.86	0.59		3.04	0.74		1.33	0.60		1342.94	415.26	a
F X T0	1109.44	191.50	ab	5.06	1.31		2.44	0.67		7.45	4.08		2.80	1.46		1127.19	196.30	ab
G X T0	846.86	181.08	ab	4.27	0.73		2.45	0.38		2.76	0.71		1.23	0.10		857.56	181.74	ab
A X T8D	785.85	58.95	ab	3.50	0.45		0.79	0.36		2.83	0.69		0.58	0.22		793.55	59.49	ab
B X T8D	573.92	79.99	ab	2.74	0.61		1.96	1.32		2.30	0.63		1.06	0.24		581.96	80.43	ab
C X T8D	823.24	132.79	ab	3.61	0.75		1.88	0.34		2.69	0.13		0.41	0.41		831.83	133.54	ab
D X T8D	558.32	96.80	ab	2.49	0.56		2.31	1.43		2.30	0.23		0.94	0.08		566.36	96.07	ab
E X T8D	699.62	79.07	ab	3.37	0.81		4.18	3.14		6.44	4.55		0.94	0.40		714.54	74.67	ab
F X T8D	530.28	125.31	ab	2.62	0.55		2.38	1.31		1.56	0.44		1.00	0.26		537.84	126.16	ab
G X T8D	743.89	178.88	ab	3.03	0.46		2.06	0.41		2.65	0.75		0.52	0.34		752.14	179.60	ab
A X T8L	721.00	81.13	ab	3.76	0.37		0.97	0.57		4.00	0.72		0.43	0.43		730.16	82.24	ab
B X T8L	539.35	42.12	ab	3.02	0.28		0.75	0.51		2.71	0.75		0.16	0.16		545.98	42.43	ab
C X T8L	546.32	67.02	ab	2.30	0.41		1.11	0.56		2.11	0.78		0.46	0.18		552.30	67.34	ab
D X T8L	861.39	316.87	ab	3.95	1.63		0.63	0.63		3.86	0.40		1.81	0.67		871.65	319.58	ab
E X T8L	701.01	125.89	ab	3.54	0.46		1.05	0.41		3.45	1.62		0.86	0.63		709.91	125.69	ab
F X T8L	394.08	56.83	b	1.94	0.23		0.74	0.49		1.61	0.59		0.39	0.24		398.75	57.72	b
G X T8L	692.67	110.83	ab	2.86	0.63		1.76	0.40		3.11	0.79		0.82	0.53		701.22	112.89	ab
Stat. Signific.	<0.05			ns			ns			ns			ns			<0.05		

Results are expressed as mean and SE, standard error of the fifty-six independent samples (n=56). Abbreviations: A, control diet; B, diet supplemented with LO/vE 180 days; C, diet supplemented with LO/vE for 90 days; D, diet supplemented with CLA/vE for 180 days; E, diet supplemented with CLA/vE for 90 days; F, diet supplemented with LO/CLA/vE for 180 days; G, diet supplemented with LO/CLA for 180 days; T0, fresh raw meat; T8D, samples stored at dark;

637 T8L, samples exposed at light; Stat. signif., statistical significance; a, b, statistically different
638 means (Tukey's test; $p \leq 0.05$).

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640 **Table 6.** Effects of diets and storage conditions on the average of peroxide value (PV, meq O_2/kg fat), TBARs (mg MDA/kg meat), single and

641 total COP contents (mg/kg of lipids), and cholesterol oxidation ratio (OR, %) of raw beef meat.

Factor	PV			TBARs			7α-HC		7β-HC		β-CE			α-CE			7-KC		Total COPs		Ratio COPs %		
Diet	Mean	SE		Mean	SE		Mean	SE	Mean	SE	Mean	SE		Mean	SE		Mean	SE	Mean	SE	Mean	SE	
A	2.39	0.36	cd	1.96	0.13	a	4.72	0.86	6.53	1.14	4.07	0.57		2.88	0.53		6.95	1.15	25.16	3.33	0.32	0.05	abc
B	4.70	0.37	a	0.70	0.13	d	5.04	0.65	5.57	0.71	5.00	1.12		2.95	0.58		7.16	1.27	25.72	4.21	0.36	0.03	ab
C	1.83	0.21	d	1.40	0.12	bc	2.75	0.46	2.93	0.57	3.56	0.60		1.72	0.51		4.20	0.73	15.15	2.30	0.21	0.05	bc
D	2.75	0.35	bcd	1.24	0.13	bc	5.80	1.62	6.89	2.15	4.84	0.90		3.45	1.08		9.41	3.40	30.42	8.68	0.36	0.06	ab
E	2.18	0.26	cd	0.66	0.12	d	2.53	0.43	2.39	0.48	3.23	0.80		1.49	0.67		3.88	0.40	13.53	2.06	0.15	0.02	c
F	4.12	0.40	ab	0.92	0.16	cd	4.01	0.72	5.10	0.71	5.40	0.99		2.73	0.61		6.39	1.07	23.64	3.71	0.43	0.07	a
G	3.22	0.30	bc	1.87	0.12	ab	4.47	0.38	5.74	0.88	4.49	0.87		2.15	0.61		7.16	0.91	24.00	2.83	0.35	0.06	ab
Stat. Signific.	<0.001			<0.05			ns		ns		ns			ns			ns		ns		<0.001		
Storage																							
T0	1.75	0.18	c	0.43	0.09	c	3.75	0.55	4.54	0.59	6.55	0.62	a	3.51	0.43	a	6.65	0.81	24.99	2.76	0.25	0.03	
T8D	4.08	0.26	a	1.57	0.09	b	4.34	0.38	5.04	0.64	3.78	0.39	b	1.85	0.37	b	5.48	0.52	20.49	1.93	0.33	0.04	
T8L	3.25	0.19	b	1.76	0.08	a	4.48	0.73	5.48	0.98	2.78	0.37	b	2.09	0.51	b	7.22	1.53	22.06	3.92	0.35	0.04	
Stat. Signific.	<0.001			<0.001			ns		ns		<0.01			<0.01			ns		ns		ns		
Diet x Storage																							
A X T0	0.51	0.06	f	0.70	0.32	bcd	1.69	0.25	2.66	1.16	4.51	0.59	abc	3.16	1.16		3.36	0.45	15.38	3.39	0.13	0.03	b
B X T0	3.56	0.22	abcd	0.26	0.07	cd	7.02	1.31	7.26	3.50	9.12	1.81	a	5.22	0.91		10.49	3.16	39.12	8.50	0.43	0.05	ab
C X T0	0.52	0.04	f	0.19	0.05	d	1.48	0.22	2.22	0.80	4.47	0.72	abc	2.35	1.02		3.80	1.27	14.33	3.11	0.13	0.03	b
D X T0	1.57	0.36	def	0.44	0.14	cd	5.05	1.22	5.72	1.32	6.44	1.91	abc	3.50	0.54		7.69	1.79	28.40	6.42	0.30	0.00	ab
E X T0	1.08	0.14	ef	0.21	0.05	d	1.50	0.52	2.05	0.84	5.20	1.87	abc	2.79	1.89		4.06	1.04	15.60	5.62	0.10	0.00	b
F X T0	2.28	0.52	bcdef	0.44	0.20	cd	4.51	2.22	6.29	1.93	8.91	1.71	ab	4.04	1.40		9.01	2.44	32.76	9.04	0.30	0.07	ab
G X T0	2.76	0.59	bcdef	0.74	0.20	abcd	4.96	0.83	5.56	1.24	7.20	1.60	abc	3.54	0.78		8.10	1.82	29.37	5.97	0.38	0.08	ab
A X T8D	4.14	0.67	abc	2.33	0.44	ab	6.20	1.37	8.32	1.78	5.05	1.12	abc	2.84	1.29		7.56	1.79	29.97	6.54	0.40	0.07	ab
B X T8D	5.91	0.67	a	0.83	0.20	abcd	4.44	0.86	4.85	0.85	3.69	1.22	abc	2.00	1.16		5.93	1.37	20.91	5.34	0.33	0.06	ab
C X T8D	2.15	0.25	cdef	2.04	0.29	abc	3.06	0.81	2.65	1.42	3.34	1.49	abc	1.18	0.78		3.17	0.78	13.39	4.31	0.17	0.07	ab
D X T8D	4.12	0.82	abc	1.48	0.22	abcd	4.87	1.46	5.79	1.87	4.99	0.51	abc	2.69	1.02		5.79	1.03	24.13	5.50	0.45	0.10	ab
E X T8D	2.20	0.42	cdef	0.77	0.15	abcd	3.65	0.98	2.29	1.25	1.74	1.02	c	1.47	0.50		4.04	0.68	11.72	3.06	0.15	0.03	ab
F X T8D	5.75	0.38	a	1.05	0.30	abcd	3.45	0.28	4.14	0.27	3.08	0.24	abc	1.38	0.80		3.99	0.53	16.04	1.46	0.37	0.09	ab
G X T8D	4.26	0.55	abc	2.47	0.64	ab	4.70	0.44	7.25	2.23	4.59	0.67	abc	2.90	1.02		7.86	1.75	27.29	2.30	0.48	0.13	ab
A X T8L	2.53	0.25	bcdef	2.53	0.52	a	6.28	1.23	8.60	1.51	2.65	0.99	bc	2.66	0.24		9.93	1.98	30.12	4.44	0.43	0.06	ab
B X T8L	4.63	0.73	ab	0.89	0.20	abcd	3.66	0.43	4.58	0.53	2.20	0.61	c	1.62	0.59		5.05	0.51	17.12	1.43	0.33	0.03	ab
C X T8L	2.83	0.23	bcdef	1.74	0.35	abcd	3.70	0.90	3.91	0.94	2.88	0.92	abc	1.63	0.97		5.62	1.62	17.74	5.13	0.33	0.10	ab

D X T8L	2.57	0.19	bcdef	1.57	0.37	abcd	7.47	4.87	9.16	6.57	3.11	1.75	abc	4.24	3.31	4.45	1.55	38.73	26.81	0.33	0.14	ab
E X T8L	3.25	0.42	bcde	0.89	0.14	abcd	2.44	0.22	2.82	0.35	2.76	0.56	abc	1.70	0.30	3.55	0.42	13.28	1.72	0.20	0.04	ab
F X T8L	4.33	0.66	abc	1.12	0.26	abcd	4.08	0.71	4.88	0.99	4.22	1.20	abc	2.78	0.56	6.18	1.41	22.13	4.48	0.60	0.12	a
G X T8L	2.64	0.22	bcdef	2.40	0.63	ab	3.74	0.65	4.41	0.84	1.70	0.46	c	1.18	0.79	5.51	1.19	15.36	3.04	0.23	0.05	ab
Stat. Signific.	<0.001			<0.001			ns		ns		<0.01			ns		ns		ns		<0.01		

Results are expressed as mean and SE, standard error of the fifty-six independent samples (n=56). Abbreviations: A, control diet; B, diet supplemented with LO/vE 180 days; C, diet supplemented with LO/vE for 90 days; D, diet supplemented with CLA/vE for 180 days; E, diet supplemented with CLA/vE for 90 days; F, diet supplemented with LO/CLA/vE for 180 days; G, diet supplemented with LO/CLA for 180 days; T0, fresh raw meat; T8D, samples stored at dark; T8L, samples exposed at light; Stat. signif., statistical significance; a, b, c, d, e, f, statistically different means (Tukey's test; $p \leq 0.05$).

Figure captions

Figure 1. PCA loadings' plot from oxidative parameters, sterol and fatty acid composition

Figure 2. PCA score plot of oxidative parameters, sterol and fatty acid composition and crossed treatments