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Effect of dietary inclusion of different lipid supplements on quality and oxidative susceptibility of beef meat

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Keywords:	beef quality, flaxseed, CLA, vitamin E, lipid fatty acid composition and oxidation
Abstract:	The aim of this study was to evaluate the impact of dietary extruded flaxseed (EF) and/or rumen-protected (rp) lipids on beef meat quality and oxidation. Sixty-three crossbred heifers (Charolais X Limousin) were evenly distributed into seven experimental groups, balanced in terms of age and BW. The feeding groups differed in both, the dietary lipid source (EF and/or rp conjugated linoleic acid (CLA), supplemented with vitamin E (VE)) and the supplementation length (90 or 180 days before slaughter); the same total amount of lipids was administered to the animals. With the respect of the control group, the α -linolenic acid content significantly increased and the n-6/n-3 ratio decreased in cattle groups fed with EF. The results also

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	<p>show that providing CLA and/or EF did not lead to an increase in CLA isomers (CLAs) in the lipid fraction of the meat. Our finding show that administering EF for a shorter period with respect to longer, moderately enhance the nutritional value of the meat with regard to fat composition. No significant effects of dietary supplementation on TBA-reactive substances (TBARs) levels of fresh meat were observed; however, the protection provided by vitamin supplementation might become evident during meat storage under commercial retail conditions.</p>

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3 1 **Effect of dietary inclusion of different lipid supplements on quality and oxidative**
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5 2 **susceptibility of beef meat**
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35 17 **Abstract**
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39 19 The aim of this study was to evaluate the impact of dietary extruded flaxseed (EF) and/or rumen-
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41 20 protected (rp) lipids on beef meat quality and oxidation. Sixty-three crossbred heifers (Charolais X
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43 21 Limousin) were evenly distributed into seven experimental groups, balanced in terms of age and
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51 25 of the control group, the α -linolenic acid content significantly increased and the n-6/n-3 ratio
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53 26 decreased in cattle groups fed with EF. The results also show that providing CLA and/or EF did not
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55 27 lead to an increase in CLA isomers (CLAs) in the lipid fraction of the meat. Our finding show that
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3 27 administering EF for a shorter period with respect to longer, moderately enhance the nutritional
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5 28 value of the meat with regard to fat composition. No significant effects of dietary supplementation
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7 29 on TBA-reactive substances (TBARs) levels of fresh meat were observed; however, the protection
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9 30 provided by vitamin supplementation might become evident during meat storage under commercial
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11 31 retail conditions.
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13 32 14 15 33 **Introduction**

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18 34 In the common consumer perception of beef meat is considered a high-fat food and its consumption
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20 35 may increase the risk of cardiovascular disease and colon cancer in humans (McAfee et al., 2010;
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22 36 McNeill, 2014). Recent nutritional guidelines (EFSA, 2005; U.S. Department of Health and Human
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24 37 Services, 2010) recommend that people enhance their intake with foods rich in polyunsaturated
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26 38 fatty acids (PUFA). Particularly the intake of PUFA n-3 should be increased, so that the western
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28 39 diet n-6/n-3 FA ratio can decrease from 16/1 to 5/1, thus promoting health conditions and
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30 40 preventing the risk of diseases (Moloney et al, 2008). Human health also seems to significantly
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32 41 benefit from the intake of several CLAs, two of which are commonly found in beef and dairy
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34 42 products and are known to possess biological activity: the anti-carcinogenic and anti-atherogenic
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36 43 effects of cis-9, trans-11 CLA and the anti-obesity effects of trans-10, cis-12 CLA have been
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38 44 documented in animal experiments (McAfee et al., 2010; Salter, 2013). With the aim to positively
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40 45 influence the FA composition of beef intramuscular fat, pasture-based farming is the better strategy
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42 46 (Priolo et al., 2001). The possibility to influence the FA content can be achieved also in intensive
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44 47 farming conditions, with cereals and corn silage based diets, by modifying the composition of the
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46 48 lipid fraction of these diets (Pouzo et al., 2016; Scollan et al., 2014; Juarez et al., 2012). However, a
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48 49 higher content of PUFA makes meat more prone to oxidation, which can impact its overall quality
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50 50 and sensory traits (such as colour, flavour, aroma) (Priolo et al., 2001). This may also compromise
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52 51 animal health and could result in the development of unhealthy oxidation products in beef, together
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54 52 with decreased colour stability with a consequent reduction in shelf life (Moloney et al. 2008).
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Therefore, the addition of suitable dietary protections, such as vitamin E (VE), may be important to control oxidation, especially during storage (Juarez et al., 2012). In fact, VE supplementation can lengthen the retail shelf-life of beef by 1.6 to 5 days (Nassu et al., 2011b). The aim of this study was to improve the meat nutritional properties by evaluating the impact of these dietary lipid treatments on beef meat quality (total fat content, FA composition and oxidation susceptibility).

Materials and methods

The Scientific Ethics Committee on Animal Experimentation of the University of Bologna approved the experimental protocol of this study (n.: 71674-X/6 - All.: 63). Sixty-three crossbred heifers (Charolais X Limousin), approximately 11 months old and 380 kg weight, were randomly distributed into seven groups. Each group received a basal diet included (kg/head/d): corn silage (8), beet pulp silage (5), corn meal (2.5), straw (0.8) and a concentrate (2). Feeding was enriched with various experimental supplements (EF, D α -tocopheril acetate (VE), CLAs (LUTA-CLA 60[®] (BASF) guarantees a minimum of 56% of conjugated linoleic acid methyl ester (28% cis-9, trans-11 CLA and 28% trans-10, cis-12 CLA)) at different doses, leading to 7 independent diet groups (Table 1). The diets of the experimental groups differed with respect to the lipid source and to the length of administration but, considering their entire experimental period (180 days), the same total amount of lipids was administered to the animals. When animals reached an average BW of 675 kg, they were transported to a slaughtering facility (Unipeg, Reggio Emilia). Twenty-four hours after slaughtering, a three-rib sample (20 cm of thickness from the 7th-9th thoracic vertebrae) of *Longissimus thoracis et lumborum* muscle (LT), was taken from the half right carcass of each of the 63 heifers. The muscle samples were vacuum-packaged and wet-aged for 14 days in a refrigerator at 2-4 °C. At the end of the aging period, each sample was cut into three subsamples, then vacuum-packaged, rapidly frozen and stored at -20 °C. At the time of analysis, the 63 beef samples were thawed in 3 different work sessions. Each first subsample was deboned and the first and last slices were cut and discarded. After 30 min of exposure to air, colour (L*, a*, b*) was measured using a

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3 79 Minolta Chromameter CR-200 (Illuminant D65, aperture size 8 mm; Minolta Camera, Osaka,
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5 80 Japan). The LT muscle was then divided into a 50 x 50 x 10 mm thick sample to measure cooking
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7 81 loss; thereafter, in the same cooked sample, Warner-Bratzler Shear Force (WBSF) was evaluated.
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9 82 The WBSF method was assessed also on raw meat. Cooking loss (%) was determined using the
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11 83 Honikel gravimetric method (Honikel, 1998). The WBSF test was carried out on the cooked meat
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13 84 samples, after the cooking loss assessment (AMSA, 1995); the same procedure was carried out on
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15 85 raw meat samples. The second subsample (weight 200 g) was used for proximate analysis; moisture
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17 86 and ash content were determined in ground freeze-dried samples. Crude protein was determined
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19 87 using a Kjeldahl Nitrogen/Protein Analyzer (Gerhardt Vapodest 50, Gerhardt. GMBH; Germany).
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21 88 The ash content was determined by incinerating samples in a muffle furnace at 500 °C. The LT fat
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23 89 content was determined with the Soxhlet method (2055 Soxtec Avanti, Foss Tecator AB, Höganäs,
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25 90 Sweden), according to the AOAC (1992). Intramuscular fat was extracted from LT muscle
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27 91 according to Folch et al. (1957). Fatty acid methyl esters were prepared according to the method
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29 92 proposed by Christopherson et al. (1969). The upper organic phase was collected and filtered with
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31 93 anhydrous sodium sulphate and 1 µL was injected into a Fisons HRGC 8560 series MEGA2 gas
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33 94 chromatograph (Thermo Electron Corporation, Milan, Italy) equipped with a flame ionization
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35 95 detector and an AS 2000 automatic injection system (Thermo Electron Corporation, Milan, Italy).
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37 96 The column was a Varian (Varian Inc. Ass 1985, USA) CP-SIL 88 WCOT Fused Silica capillary
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39 97 column (100 m x 0.25 mm i.d. x 0.20 µm film thickness). FAME identification was performed by
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41 98 comparing their retention times with those of known standard FAME mixtures (SUPELCO, Sigma
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43 99 Aldrich Inc., St. Louis, MO). Each FAME was expressed as a percentage of the total areas of all
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45 100 FAME peaks. Peroxide value (PV) was determined in the lipid extract (50 mg) from the third
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47 101 subsample, as suggested by Shantha and Decker (1994). This method is based on the ability of
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49 102 peroxides to oxidize ferrous ions to ferric ones. Ammonium thiocyanate then reacts with ferric ions,
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51 103 resulting in a coloured complex that is measured at 500 nm with a spectrophotometer (double-beam
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53 104 UV-visible Jasco model V-550, Jasco International, Tokyo, Japan). PV was calculated from the
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3 105 absorbance at 500 nm. For the quantitative determination of PV, an Fe(III) standard calibration
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5 106 curve was used with a concentration range of 0.1-5 µg/mL ($y = 0.0282x - 0.0003$; $r^2 = 0.999$). PV
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7 107 was expressed as meq of O₂ per kg of fat. Three replicates were run per sample. TBA-reactive
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9 108 substances (TBARS) were determined in a 2 g sample (ground meat), according to the Witte (1970)
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11 109 method. This method is based on the reaction between thiobarbituric acid and the aldehydes that
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13 110 derive from secondary oxidation of the lipids present in the sample, resulting in a coloured complex
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15 111 that can be measured at 530 nm. TBARS were evaluated at such wavelength with a double-beam
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17 112 UV-visible spectrophotometer (Jasco model V-550, Jasco International, Tokyo, Japan), and they
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19 113 were calculated from the absorbance. For the quantitative determination of TBARS, a 1,1,3,3-
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21 114 tetramethoxypropane standard calibration curve was used with a concentration range of 0.045-0.113
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23 115 µg/mL ($y = 0.0087x - 0.0051$; $r^2 = 0.999$). The TBARS values were expressed as mg of
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25 116 malonylaldehyde (MDA) per kg of sample. Three replicates were run per sample. All data were
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27 117 analysed by one-way factorial ANOVA with repeated measures, using Tukey's HSD as *post-hoc*
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29 118 test. The dietary treatment was used as the source of variation to determine whether there were any
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31 119 significant differences ($P \leq 0.05$). Pearson's correlation coefficient ($\alpha = 0.05$) was used to separate
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33 120 the means of statistically different parameters and interactions and examine the possible
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35 121 relationships between the degree of unsaturation of the meat and the main oxidative indices. The
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37 122 Statistica version 10 software (StatSoft Inc., Padova, Italy) was used.
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124 **Results and discussion**

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46 125 Table 2 shows the chemical composition of the LT muscle: no significant increase in the lipid
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48 126 content of meat from heifers that had received flaxseed, or CLA, was noted. This result is in
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50 127 agreement with Juarez et al. (2012), who administered 10% of ground flaxseed to feedlot steers for
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52 128 129 days. WBSF data here obtained, can be classified as "very tender", according to the Destefanis
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54 129 et al. (2008) WBSF scale (category n. 5; <3.36 kg). As observed by Juarez et al. (2012) and Nassu
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56 130 et al. (2011b), meat tenderness was not affected by lipid supplementation. Regarding the colour

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3 131 measurements performed in the aged meat, L value (lightness) of group C was significantly less
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5 132 bright than those of B, F and G groups. This finding might be related to those presented by Priolo et
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7 133 al. (2001) who have noted by the linear regression between objective L value and time on pasture
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9 134 that was significant ($P < 0.001$; $r^2 = 0.74$). The cause of this effect is extremely difficult to evaluate
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11 135 because several factors play an important role, such as: carcass fatness, meat ultimate pH, animal
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13 136 age, carcass weight and intramuscular fat content. Table 3 shows LT muscle fatty acid profile, in
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15 137 particular for α -linolenic acid content, we observed a dose effect in groups supplemented with EF
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17 138 compared with the A diet (C, D and E, average 0.31%). Moreover the α -linolenic acid content in a
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19 139 short term EF supplementation (90 days, C, average 0.71%) is to prefer than a long term
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21 140 supplementation (180 days, B, F and G, average 0.54%). Furthermore, Pearson test ($\alpha=0.05$),
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23 141 between EF increasing doses (0, 250 and 500 g/head/day) and α -linolenic acid level in lipid meat,
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25 142 was statistically significant ($r = 0.74825$). Priolo et al. (2001) reported the variation of α -linolenic
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27 143 acid in intramuscular fat of cattle: grazing for six months on pasture has an effect of increasing
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29 144 intramuscular α -linolenic acid by 50%. However, considering the lipid meat content shown in Table
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31 145 3, these differences were relatively low from a quantitative point of view. Considering these results,
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33 146 administering the same amount of flaxseed in a shorter time may be the best strategy (Nassu et al.,
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35 147 2011a) to reduce partially the n-6/n-3 ratio (calculated on all FA detected) as noticed in previous
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37 148 studies (Mordenti et al., 2005; Mordenti et al., 2013). Regarding the two main CLAs contents, our
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39 149 results indicate that administering rp CLA, with or without EF, do not have had effectiveness on
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41 150 both CLAs contents as compared to A group; obviously the CLAs contents were not influenced by
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43 151 the treatment length. This finding is in agreement with what has been reported by other authors
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45 152 (Schiavon et al., 2011) and might have been due to an CLA utilization directly by animals, reducing
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47 153 body mass (trans-10, cis-12 CLA) and improving feed efficiency (cis-9, trans-11 CLA). Among
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49 154 PUFA, arachidonic acid content was significantly higher ($P \leq 0.05$) in group A than in groups B and
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51 155 D. The content of eicosatrienoic acid (C20:3 n-3 and n-6) was significantly higher ($P \leq 0.05$) in
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53 156 group A than in groups B, D and G, and the A group amount of n-3 docosapentaenoic acid (DPA)

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3 157 was significantly higher compared to the B. The n-6/n-3 ratio was significantly higher in group A
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5 158 than in groups B, C, F and G, by 27%, 37%, 25% and 30%, respectively. Regarding the oxidative
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7 159 parameters (Table 3), PV varied from 0.57 to 4.92 meq O₂/kg lipids, being significantly lower in the
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9 160 A diet with respect to the B diet. TBARs values ranged from 0.19 to 0.74 mg MDA/kg meat,
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11 161 remaining below the acceptance threshold of 2 mg MDA/kg beef meat (Campo et al., 2006). No
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13 162 significant effects of dietary supplementation on TBARs levels of fresh meat were observed. No
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15 163 correlations (Pearson test, $\alpha=0.05$) were found between the results obtained for lipid composition
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17 164 and the oxidative parameters resulting from the seven types of feeding conditions. In general, a low
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19 165 oxidation level was found in all samples, similar to that reported by Boselli et al. (2009), but both
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21 166 PV and TBARs were greater than those reported by other authors (Insani et al., 2008). This could be
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23 167 due to Italian carcass processing practices, which provide for a storage period, aimed at improving
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25 168 meat tenderness and promoting the formation of aroma compounds, which develop during cooking
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27 169 (Rodriguez-Estrada et al., 1997; Boselli et al., 2009). The long-term supplementation of EF seems
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29 170 to favour primary oxidation, even in the presence of vitamin E. Although the dietary
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31 171 supplementation did not have any significant impact on TBARs levels of fresh meat, the protective
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33 172 action of vitamin addition might become evident during meat storage under commercial retail
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35 173 conditions (Cardenia et al., 2011). On the other hand, it might be pointed out that TBARs describe
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37 174 an overall oxidative behaviour, which is useful if compared with PV; however, other specific
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39 175 antioxidant parameters (such as antioxidant enzyme activities and antioxidant capacity) could
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41 176 provide additional information (Mahecha et al., 2011).

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43 178 **Conclusions**

44 179 The results obtained in the present study confirm that the diets that included EF supplementation
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46 180 proved to be effective at improving the n-3 FA and lowering the n-6/n-3 ratio in lipid fraction of
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48 181 beef meat. For this aspect, a shorter period of supplementation (90 days) was a more successful
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50 182 strategy than a longer one (180 days). However, considering the lipid meat content, these quantities

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3 183 were relatively low for humans, from a quantitative point of view. Although oxidation parameters
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5 184 did not reflect the antioxidant effect of the latter, this effect might become evident during meat
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7 185 storage (Cardenia et al., 2011).
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11 187 **Disclosure statement**

13 188 The authors report no conflicts of interest.
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4 **1 Tables**5
6 **2 Table 1: Description of the seven dietary treatments administered to heifers (g as feed)**
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8 Groups	Supplementation	Lenght	Description
9 A (control)		180	Basal diet supplemented with Megalac® and soybean meal (protein 14% and energy 1762 kcal/kg)
10 B	EF/VE	180	Basal diet plus 250 g EF head ⁻¹ day ⁻¹ and 1 g VE head ⁻¹ day ⁻¹
11 C	Control + EF/VE	90 + 90	Basal diet (first 90 days) followed by basal diet supplemented with 500 g EF head ⁻¹ day ⁻¹ and 2 g VE head ⁻¹ day ⁻¹
12 D	CLA/VE	180	Basal diet supplemented with 25 g CLA head ⁻¹ day ⁻¹ and 1 g VE head ⁻¹ day ⁻¹
13 E	Control + CLA/VE	90 + 90	Basal diet (first 90 days) followed by basal diet supplemented with 50 g CLA head ⁻¹ day ⁻¹ and 2 g VE head ⁻¹ day ⁻¹
14 F	EF/CLA/VE	180	Basal diet supplemented with 250 g EF head ⁻¹ day ⁻¹ , 25 g CLA head ⁻¹ day ⁻¹ and 1 g VE head ⁻¹ day ⁻¹
15 G	EF/CLA	180	Basal diet supplemented with 250 g EF head ⁻¹ day ⁻¹ and 25 g CLA head ⁻¹ day ⁻¹

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25 **3** EF, extruded flaxseed; VE, DL- α tocopheril acetate; CLA, conjugated linoleic acid isomers.
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Table 2: Composition (% of DM) and physical properties of the *Longissimus* muscle in heifers

Item	Experimental groups							SEM	P
	A	B	C	D	E	F	G		
Dry Matter	26.9	28.1	26.8	27.2	26.6	27.8	27.2	0.15	ns
Protein	79.0	77.6	78.2	79.3	80.2	78.3	80.3	0.57	ns
Ether extract	13.6	15.3	14.6	12.7	11.9	14.1	12.7	0.56	ns
Cooking loss (%)	30.1	28.9	30.3	30.2	30.2	28.3	29.1	0.37	ns
WBSF fresh (kg)	1.84	1.75	2.10	2.08	2.08	1.89	1.76	0.06	ns
WBSF cooked (kg)	3.38	3.52	3.55	3.23	3.77	4.29	3.56	0.10	ns
Color at day 14									
L value	42.7 ^{ab}	44.6 ^a	40.9 ^b	42.9 ^{ab}	43.3 ^{ab}	44.5 ^a	45.4 ^a	0.35	≤ 0.05
a* value	20.9	21.8	21.3	20.5	20.7	20.2	20.0	0.23	ns
b* value	11.0	11.8	10.5	10.4	10.8	10.6	11.2	0.19	ns

Values in the same row with different superscripts are different (a and b, $P \leq 0.05$; A and B $P \leq 0.01$).

SEM=standard error of the mean; WBSF = Warner-Bratzler Shear Force

1 Table 3: Fatty acid content, fatty acid classes and oxidative parameters of *Longissimus* muscle in
 2 heifers

Fatty acids (% of total FA)	Experimental groups							SEM	P
	A	B	C	D	E	F	G		
C14:0	2.95	3.05	3.18	3.33	3.24	2.95	2.80	0.08	ns
C16:0	28.34	28.61	28.22	29.32	28.51	28.54	27.76	0.25	ns
C16:1 n-7	2.67	2.55	2.45	2.57	2.47	2.74	2.45	0.05	ns
C18:0	17.43	19.22	19.67	19.54	19.51	17.44	19.21	0.26	ns
C18:1 n-9	35.92	36.17	33.59	34.83	34.45	35.97	37.48	0.36	ns
C18:1 n-7	1.94 ^{ab}	1.59 ^{ab}	1.99 ^a	1.65 ^{ab}	1.84 ^{ab}	1.68 ^{ab}	1.43 ^b	0.14	≤ 0.05
C18:2 n-6	6.20	4.91	6.04	5.00	5.59	6.29	4.86	0.21	ns
C18:3 n-3	0.29 ^{Bb}	0.54 ^{Ac}	0.71 ^{Aa}	0.33 ^{Bb}	0.30 ^{Bb}	0.56 ^{Ac}	0.52 ^{Ac}	0.02	≤ 0.05
CLA cis9,trans11	0.34	0.41	0.40	0.33	0.35	0.34	0.36	0.03	ns
CLA trans10,cis12	0.16	0.15	0.14	0.13	0.12	0.15	0.16	0.01	ns
C20:4 n-6	1.10 ^a	0.48 ^b	0.93 ^{ab}	0.57 ^b	0.88 ^{ab}	0.83 ^{ab}	0.58 ^b	0.05	≤ 0.05
C20:5 n-3	0.10 ^{ab}	0.06 ^b	0.12 ^a	0.07 ^b	0.08 ^{ab}	0.11 ^a	0.06 ^b	0.01	≤ 0.05
C22:5 n-3	0.14 ^A	0.06 ^B	0.11 ^{AB}	0.09 ^{AB}	0.11 ^{AB}	0.11 ^{AB}	0.09 ^{AB}	0.01	≤ 0.01
C22:6 n-3	0.29	0.16	0.29	0.18	0.23	0.29	0.19	0.02	ns
SFA	50.01 ^B	52.26 ^{AB}	52.51 ^A	53.61 ^A	52.83 ^A	50.21 ^B	51.14 ^B	0.83	≤ 0.01
MUFA	41.08	40.80	38.53	39.53	39.29	40.89	41.88	0.89	ns
PUFA	8.91	6.94	8.96	6.86	7.88	8.89	6.98	0.74	ns
PUFA/SFA	1.00 ^A	0.91 ^{AB}	0.91 ^{AB}	0.87 ^B	0.90 ^{AB}	1.00 ^{AB}	0.96 ^{AB}	0.03	≤ 0.01
n-6/n-3	8.99 ^a	6.54 ^b	5.70 ^b	8.63 ^a	8.98 ^a	6.76 ^b	6.26 ^b	0.46	≤ 0.05
TBARs	0.70	0.26	0.19	0.44	0.21	0.32	0.73	0.23	ns
PV	0.54 ^b	4.11 ^a	0.85 ^b	2.80 ^{ab}	1.07 ^{ab}	4.28 ^a	2.59 ^{ab}	1.45	≤ 0.05

3 Values in the same row with different superscripts are different (a and b, P≤0.05; A and B P≤0.01).

1 SEM= standard error of the mean; SFA = saturated fatty acids; MUFA = monounsaturated fatty
2 acids; PUFA = polyunsaturated fatty acids; TBARs = TBA-reactive substances (mg MDA/kg
3 meat); PV = peroxide value (meq O₂/kg lipids)
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