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Complete List of Authors:	Mordenti, Attilio; Universita degli Studi di Bologna, DIMEVET Brogna, Nico; Universita degli Studi di Bologna, DIMEVET Merendi, Flavia; Universita degli Studi di Bologna, DIMEVET Formigoni, Andrea; Universita degli Studi di Bologna, DIMEVET Sardi, Luca; Universita degli Studi di Bologna Dipartimento di Scienze Mediche Veterinarie Cardenia, Vladimiro; Universita degli Studi di Bologna, DISTAL Rodriguez Estrada, Maria; Universita degli Studi di Bologna, DISTAL
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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.

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

A.L. Mordenti[1]*, N. Brogna[1], F. Merendi[1], A. Formigoni[1], L. Sardi[1], V. Cardenia[2], M.T. Rodriguez-Estrada[2]

[1] *Dipartimento di Scienze Mediche Veterinarie, Università di Bologna (DIMEVET; Via Tolara di sopra 50; 40064, Ozzano Emilia, Bologna), Italy.*

[2] *Dipartimento di Scienze e Tecnologie Agro-Alimentari, Università di Bologna (DISTAL; Viale G. Fanin 40; 40127, Bologna), Italy.*

*Corresponding author: Attilio Luigi Mordenti; http://orcid.org/0000-0002-4162-6726; Phone: +390512097381; Fax: +390512097373; email: attilio.mordenti@unibo.it

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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 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.

Introduction

In the common consumer perception of beef meat is considered a high-fat food and its consumption may increase the risk of cardiovascular disease and colon cancer in humans (McAfee et al., 2010; McNeill, 2014). Recent nutritional guidelines (EFSA, 2005; U.S. Department of Health and Human Services, 2010) recommend that people enhance their intake with foods rich in polyunsaturated fatty acids (PUFA). Particularly the intake of PUFA n-3 should be increased, so that the western diet n-6/n-3 FA ratio can decrease from 16/1 to 5/1, thus promoting health conditions and preventing the risk of diseases (Moloney et al, 2008). Human health also seems to significantly benefit from the intake of several CLAs, two of which are commonly found in beef and dairy products and are known to possess biological activity: the anti-carcinogenic and anti-atherogenic effects of cis-9, trans-11 CLA and the anti-obesity effects of trans-10, cis-12 CLA have been documented in animal experiments (McAfee et al., 2010; Salter, 2013). With the aim to positively influence the FA composition of beef intramuscular fat, pasture-based farming is the better strategy (Priolo et al., 2001). The possibility to influence the FA content can be achieved also in intensive farming conditions, with cereals and corn silage based diets, by modifying the composition of the lipid fraction of these diets (Pouzo et al., 2016; Scollan et al., 2014; Juarez et al., 2012). However, a higher content of PUFA makes meat more prone to oxidation, which can impact its overall quality and sensory traits (such as colour, flavour, aroma) (Priolo et al., 2001). This may also compromise animal health and could result in the development of unhealthy oxidation products in beef, together with decreased colour stability with a consequent reduction in shelf life (Moloney et al. 2008).

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, Dl-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

Minolta Chromameter CR-200 (Illuminant D65, aperture size 8 mm; Minolta Camera, Osaka, Japan). The LT muscle was then divided into a 50 x 50 x 10 mm thick sample to measure cooking loss; thereafter, in the same cooked sample, Warner-Bratzler Shear Force (WBSF) was evaluated. The WBSF method was assessed also on raw meat. Cooking loss (%) was determined using the Honikel gravimetric method (Honikel, 1998). The WBSF test was carried out on the cooked meat samples, after the cooking loss assessment (AMSA, 1995); the same procedure was carried out on raw meat samples. The second subsample (weight 200 g) was used for proximate analysis; moisture and ash content were determined in ground freeze-dried samples. Crude protein was determined using a Kjeldahl Nitrogen/Protein Analyzer (Gerhardt Vapodest 50, Gerhardt. GMBH; Germany). The ash content was determined by incinerating samples in a muffle furnace at 500 °C. The LT fat content was determined with the Soxhlet method (2055 Soxtec Avanti, Foss Tecator AB, Höganäs, Sweden), according to the AOAC (1992). Intramuscular fat was extracted from LT muscle according to Folch et al. (1957). Fatty acid methyl esters were prepared according to the method proposed by Chistopherson et al. (1969). The upper organic phase was collected and filtered with anhydrous sodium sulphate and 1 µL was injected into a Fisons HRGC 8560 series MEGA2 gas chromatograph (Thermo Electron Corporation, Milan, Italy) equipped with a flame ionization detector and an AS 2000 automatic injection system (Thermo Electron Corporation, Milan, Italy). The column was a Varian (Varian Inc. Ass 1985, USA) CP-SIL 88 WCOT Fused Silica capillary column (100 m x 0.25 mm i.d. x 0.20 µm film thickness). FAME identification was performed by comparing their retention times with those of known standard FAME mixtures (SUPELCO, Sigma Aldrich Inc., St. Louis, MO). Each FAME was expressed as a percentage of the total areas of all FAME peaks. Peroxide value (PV) was determined in the lipid extract (50 mg) from the third subsample, as suggested by Shantha and Decker (1994). This method is based on the ability of peroxides to oxidize ferrous ions to ferric ones. Ammonium thiocyanate then reacts with ferric ions, resulting in a coloured complex that is measured at 500 nm with a spectrophotometer (double-beam UV-visible Jasco model V-550, Jasco International, Tokyo, Japan). PV was calculated from the

absorbance at 500 nm. For the quantitative determination of PV, an 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. TBA-reactive substances (TBARs) were determined in a 2 g sample (ground meat), according to the Witte (1970) method. This method is based on the reaction between thiobarbituric acid and the aldehydes that derive from secondary oxidation of the lipids present in the sample, resulting in a coloured complex that can be measured at 530 nm. TBARs were evaluated at such wavelength 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 ($y = 0.0087x - 0.0051$; $r^2 = 0.999$). The TBARs values were expressed as mg of malonylaldehyde (MDA) per kg of sample. Three replicates were run per sample. All data were analysed by one-way factorial ANOVA with repeated measures, using Tukey's HSD as *post-hoc* test. The dietary treatment was used as the source of variation to determine whether there were any significant differences ($P \leq 0.05$). Pearson's correlation coefficient ($\alpha = 0.05$) was used to separate the means of statistically different parameters and interactions and examine the possible relationships between the degree of unsaturation of the meat and the main oxidative indices. The Statistica version 10 software (StatSoft Inc., Padova, Italy) was used.

Results and discussion

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

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

was significantly higher compared to the B. The n-6/n-3 ratio was significantly higher in group A than in groups B, C, F and G, by 27%, 37%, 25% and 30%, respectively. Regarding the oxidative parameters (Table 3), PV varied from 0.57 to 4.92 meq O_2/kg lipids, being significantly lower in the A diet with respect to the B diet. TBARs values ranged from 0.19 to 0.74 mg MDA/kg meat, remaining below the acceptance threshold of 2 mg MDA/kg beef meat (Campo et al., 2006). No significant effects of dietary supplementation on TBARs levels of fresh meat were observed. No correlations (Pearson test, α=0.05) were found between the results obtained for lipid composition and the oxidative parameters resulting from the seven types of feeding conditions. In general, a low oxidation level was found in all samples, similar to that reported by Boselli et al. (2009), but both PV and TBARs were greater than those reported by other authors (Insani et al., 2008). This could be due to Italian carcass processing practices, which provide for a storage period, aimed at improving meat tenderness and promoting the formation of aroma compounds, which develop during cooking (Rodriguez-Estrada et al., 1997; Boselli et al., 2009). The long-term supplementation of EF seems to favour primary oxidation, even in the presence of vitamin E. Although the dietary supplementation did not have any significant impact on TBARs levels of fresh meat, the protective action of vitamin addition might become evident during meat storage under commercial retail conditions (Cardenia et al., 2011). On the other hand, it might be pointed out that TBARs describe an overall oxidative behaviour, which is useful if compared with PV; however, other specific antioxidant parameters (such as antioxidant enzyme activities and antioxidant capacity) could provide additional information (Mahecha et al., 2011).

Conclusions

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

were relatively low for humans, from a quantitative point of view. Although oxidation parameters did not reflect the antioxidant effect of the latter, this effect might become evident during meat storage (Cardenia et al., 2011).

Disclosure statement

The authors report no conflicts of interest.

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Tables

Table 1: Description of the seven dietary treatments administered to heifers (g as feed)

Groups	Supplementation	Lenght	Description
A (control)		180	Basal diet supplemented with Megalac® and soybean meal (protein 14% and energy 1762 kcal/kg)
B	EF/VE	180	Basal diet plus 250 g EF head^{-1} day^{-1} and 1 g VE head^{-1} day^{-1}
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}
D	CLA/VE	180	Basal diet supplemented with 25 g CLA head^{-1} day^{-1} and 1 g VE head^{-1} day^{-1}
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}
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}
G	EF/CLA	180	Basal diet supplemented with 250 g EF head^{-1} day^{-1}and 25 g CLA head^{-1} day^{-1}

EF, extruded flaxseed; VE, Dl-α tocopheril acetate; CLA, conjugated linoleic acid isomers.

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≤0.05; A and B P≤0.01).

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

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

Fatty acids	Experimental groups							SEM	*P*
(% of total FA)	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

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

SEM= standard error of the mean; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; TBARs = TBA-reactive substances (mg MDA/kg meat); PV = peroxide value (meq O_2/kg lipids)