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Fatty acid profile, meat quality and flavour acceptability of beef from double-muscled Piemontese young bulls fed ground flaxseed

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ABSTRACT

This study was designed to assess the effects of dietary flaxseed on the proximate composition, fatty acid profile, lipid oxidation, colour and flavour of the *longissimus thoracis* muscle of double-muscled Piemontese young bulls. Compared to a control diet, the flaxseed diet had no significant effect on the moisture, protein or ether extract content. Flaxseed modified the fatty acid composition of beef. A more than doubled n-3 polyunsaturated fatty acids (PUFA) content (from 21.1 to 46.7 g/kg of total fatty acids – TFA – in control and flaxseed beef, respectively; $p < .001$) and a lowered n-6/n-3 PUFA ratio (13.13 versus 5.60; $p < .001$) were observed. The total *trans*-octadecadienoic acids were also increased by flaxseed (7.8 *vs* 12.1 g/kg TFA; $p = .001$). The total saturated fatty acids (SFA), monounsaturated fatty acids and PUFA contents, as well as the PUFA/SFA ratio, remained unaffected by the treatment. Lipid oxidation stability tended to be negatively affected by flaxseed ($p = .096$). Such a negative effect was more pronounced as the ageing period advanced. The malondialdehyde content was $\leq$0.72 mg MDA/kg meat, and was therefore below the threshold values for rancidity. Meat colour was not influenced by dietary treatment. The inclusion of flaxseed in the diet increased beef flavour intensity perceived by consumers and did not negatively affect flavour acceptability. Properly combining the choice of animal breed and diet allows a slight improvement of the nutritional value of meat for human consumption.

HIGHLIGHTS

- Proximate composition, colour and flavour of Piemontese beef are not affected by dietary flaxseed
- Dietary flaxseed increases ALA and long-chain n-3 PUFA and lowers the n-6/n-3 PUFA ratio of Piemontese beef
- Flaxseed unprotected from ruminal biohydrogenation does not allow labelling Piemontese beef as source of n-3 FA in the European Union

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Linum usitatissimum L; *mh/mh* genotype; meat quality; lipids; TBARS

Introduction

The food scandals involving beef, the new eating habits of younger generations, the environmental and ethical issues related to animal production, the high prices of beef and the contemporaneous economic recession, are the main reasons why consumers have turned away from beef (Henchion et al. 2014). However, health concerns, mainly those related to the total fat content and to the supposed negative effects due to saturated fatty acids (SFA) and *trans* fatty acids, have the strongest incidence on reducing beef consumption (Mapiye et al. 2015; Ruiz-Núñez et al. 2016).

In late-maturing beef cattle breeds, such as Continental European breeds, the amount of intramuscular fat in young bulls is about 2.5% and is even lower (about 1%) in double-muscled animals (*mh/mh* genotype). Owing to the low fat content, the beef from double-muscled animals has a lower SFA content and a more favourable polyunsaturated/saturated fatty

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*This paper is dedicated to the memory of Professor Gianluigi Destefanis who passed away on April 8, 2018.

acids (PUFA/SFA) ratio than meat from other genotypes (Fiems 2012).

The double-muscled Piemontese breed is the most numerous Italian beef breed. Almost 270,000 cattle (of which about 128,000 are cows) are registered in the Herd Book, the latter being the largest one among the European meat breeds (ANABORAPI 2016). Piemontese meat is greatly appreciated by Italian consumers, especially because of its very low intramuscular fat content.

The results of a study conducted by Brugiapaglia et al. (2014) confirmed the positive charateristics of Piemontese meat (low ether extract content, low concentration of SFA and high PUFA/SFA ratio). However, the traditional feeding system of the Piemontese breed, which is based on high concentrate diets (e.g. a large use of corn and corn by-products), has led to an unfavourable increase in the n-6/n-3 PUFA ratio in the meat.

An excess of n-6 PUFA can interfere with the metabolism of n-3 PUFA, reducing their incorporation into tissue lipids (Daley et al. 2010), thus representing a risk factor for a number of widespread human pathologies (Simopoulos 2011). In recent years, several trials on beef cattle have highlighted that different feeding strategies can be adopted to favour the deposition of n-3 PUFA in muscle tissues in order to obtain a healthier meat (Baba et al. 2016). Flaxseed, which provides large amounts of α-linolenic acid (C18:3 n-3, ALA), has already been successfully used to improve the nutritional value of the PUFA fraction of beef (Scollan et al. 2014).

However, an enrichment in PUFA could induce a greater susceptibility to lipid oxidation (Guyon et al. 2016), a process that could reduce the organoleptic quality of meat, with particular reference to colour and flavour.

To the best of our knowledge, no studies have been carried out to determine the effects of flaxseed on the meat characteristics of Piemontese young bulls. Therefore, the aim of this trial was to evaluate the effect of dietary inclusion of flaxseed on the proximate composition, fatty acid (FA) profile, lipid oxidation, colour and flavour of meat from Piemontese young bulls.

Materials and methods

Animals and dietary treatments

Animal care, handling and experimental procedures were in compliance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

The trial was carried out under practical conditions on a private farm specialised for beef production (Fontanellato, Parma, Italy; latitude: 44°51′ 08″N; longitude: 10°07′34″E; altitude: 65 m a.s.l.). Eighteen male Piemontese calves (4.5 ± 0.59 months) were purchased and transported to the farm, divided into two groups ($n = 9$) and housed in adjacent pens indoors. The two groups were not balanced on a live weight basis, considering the high risks associated with a dominance hierarchy at the feeding rack.

In the pre-experimental period (172 days), all the animals were given the same mixed diet, consisting of a commercial concentrate for fattening cattle (concentrate A: corn, wheat middlings, sunflower meal, roasted dehulled soybean meal, wheat bran, roasted soybean meal, calcium carbonate, cane molasses, canola meal, dicalcium phosphate and sodium chloride), ryegrass hay, corn meal, distillers dried grains with solubles, dried beet pulp and soybean meal (Table 1).

The animals were weighed individually at the beginning of the experimental period. The experimental period lasted 135 days and was divided into two fattening periods (FP1, 66 days; FP2, 69 days) to better fulfil the nutritional requirements of the growing animals. The groups were randomly assigned to two isoenergetic and isonitrogenous diets (Table 1): a control diet (C; initial live weight: 381 ± 18.5 kg) or an experimental diet containing ground flaxseed (*Linum usitatissimum* L.) (FS; initial live weight: 423 ± 23.5 kg) unprotected from ruminal biohydrogenation. The amount of ground flaxseed (dry matter – DM: 917 g/kg; ether extract – EE: 360 g/kg DM; α-linolenic acid: 200 g/kg DM) was set at 10.0% of the diet DM so as not to exceed the maximum content of cyanogenic substances tolerated by ruminants (Petit 2010). The daily feed intake was monitored by group.

Feed analyses

The AOAC International (2000) procedures were used to determine the dry matter (DM: method no. 930.15), ash (method no. 942.05), crude protein (CP: method no. 984.13) and acid detergent fibre (ADF: method no. 973.18). The ether extract (EE) was determined according to method no. 920.39 of AOAC International (2003). The neutral detergent fibre (NDF) was analysed according to Van Soest et al. (1991); α-amylase (Sigma, Sigma–Aldrich Milano, Milano, Italy) was added, but no sodium sulphite, and the results were corrected for the residual ash content. The chemical composition of the feed (g/kg DM) and the FA proportion of the feed

Table 1. Ingredients, proximate composition and fatty acid profile of the experimental diets.

	FP1		FP2	
	C	FS	C	FS
Ingredients (g/kg DM)				
Concentrate A[a]	353	271	313	244
Corn meal	298	316	209	272
Dried sugar beet pulp	181	134	139	70
Ryegrass hay	125	159	116	143
Barley meal	0	0	155	121
Ground flaxseed	0	99	0	100
Soybean meal	44	22	25	5
Concentrate B[b]	0	0	43	46
Proximate composition (g/kg DM)				
DM (g/kg)	861	865	864	868
CP	174	170	172	169
EE	40	76	36	74
Ash	68	62	64	57
NDF	357	356	355	340
ADF	192	192	179	172
NEF (MJ/kg DM)	7.94	8.19	7.89	8.18
Fatty acid composition (g/kg of TFA)				
C14:0	1.60	0.90	0.19	0.10
C16:0	168.50	108.70	17.78	10.96
C18:0	23.10	25.00	2.21	2.48
C18:1 *c*9	268.40	242.30	25.29	23.96
C18:2 n-6	490.00	318.60	49.50	31.97
C18:3 n-3	29.60	292.00	3.27	29.36
Others	18.80	12.40	1.75	1.17

FP: fattening period; C: control diet; FS: flaxseed diet; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; NEF: net energy for fattening; TFA: total fatty acids; *c*: *cis*; Others: C12:0 + C16:1 *t*3 + C16:1 *c*9 + C18:1 *c*11 + C20:0 + C18:3 n-6.

[a]Containing: corn 26%, wheat bran shorts middlings 20%, sunflower meal 15%, rice bran 12%, roasted dehulled soybean meal 9%, wheat bran 7%, roasted soybean meal 4.2%, wheat flour middlings 2%, calcium carbonate 1.5%, cane molasses 1%, canola meal 1%, dicalcium phosphate 0.5%, sodium chloride 0.5%, vitamins and minerals 0.3%. Chemical composition (as fed basis): 18% CP; 5.5% CF (crude fibre); 8.0% cellulose; 7.1% ash; 0.2% Na. Vitamin-mineral composition (given values supplied per kg of concentrate): 88,938 U of vitamin A; 1,338 U of vitamin D_3; 68 mg of vitamin E; 2.5 mg of vitamin B_1; 1.3 mg of vitamin B_2; 1.3 mg of vitamin B_6; 0.011 mg of vitamin B_{12}; 22 mg of vitamin PP; 0.011 mg of biotin; 2.5 mg of pantothenic acid; 750 mg of choline chloride; 54 mg of Mn; 57 mg of Fe; 194 mg of Zn; 12.6 mg of Cu; 0.54 mg of Co; 2.2 mg of I; 0.18 mg of Se.

[b]Containing: corn germ meal 45%, wheat middlings 25%, wheat bran 15%, corn 14.7%, vitamins and minerals 0.3%. Chemical composition (as fed basis): 17.5% CP; 3.4% CF (crude fibre); 7.8% cellulose; 5.6% ash; 0.1% Na. Vitamin–mineral composition (given values supplied per kg of concentrate): 4,000 mg of vitamin E; 5.0 mg of Se.

(g/kg of the total detected FA, analysed as detailed in Renna et al. 2014), are reported in Table 1.

Slaughtering and meat sampling

At an age of 15 months, the animals were transported to the slaughterhouse, individually weighed and then slaughtered, in accordance with the European guidelines (EC No. 1099/2009). The carcasses were split into two sides and chilled at 4 °C. Twenty-four hours *post mortem*, the portion between the 9th and 13th thoracic vertebra of *longissimus thoracis* (LT) muscle was taken from the right side. Each sample was divided into three subsamples of equal length.

Two subsamples were randomly assigned an ageing period of 7 or 10 days. The subsamples were placed in a plastic tray, over-wrapped with a polyvinyl chloride oxygen permeable film and stored in the dark at 4 °C for the designated ageing period.

The remaining subsample was cut into three 2-cm thick steaks which were randomly assigned ageing periods of 2, 7 or 10 days. The steaks were placed in a polystyrene tray, over-wrapped as described before and stored in the dark at 4 °C until lipid oxidation analysis was carried out. The presence of the myostatin gene mutation that is responsible for the double-muscled phenotype (*mh/mh* genotype) in the Piemontese breed was detected in the meat samples using the method described by Di Stasio and Rolando (2005).

Meat analyses

Proximate composition and fatty acid profile

A 2.5 cm thick steak was cut from each 7-day aged subsample, homogenised and divided into two parts. A portion was used to determine the moisture content, according to AOAC method 950.46 (AOAC International 2000). The remaining part was freeze-dried (Edwards MF 1000, Milano, Italy) and then analysed to establish the protein and ether extract contents, and the fatty acid composition.

The nitrogen content was determined by means of the Kjeldahl method (method 928.08; AOAC International 2000) using a Büchi Distillation Unit K-355 (Flawil, Switzerland), and a nitrogen-to-protein conversion factor of 6.25 was used to calculate the protein content.

The ether extract content was determined by means of Soxhlet extraction, without prior acid hydrolysis, using a Büchi Extraction System B-811 (Flawil, Switzerland) with petroleum ether, according to AOAC method 991.36 (AOAC International 2000). The proximate composition results were expressed as g/kg.

Meat fatty acid composition was assessed as reported by Schmid et al. (2009). The fatty acid methyl esters (FAME) were separated and quantified by a high resolution gas chromatograph (Shimadzu GC 2010 Plus, Shimadzu Corporation Analytical Instruments Division, Kyoto, Japan) equipped with a flame-ionisation detector and a CP-Sil 88 capillary column (100 m × 0.25 mm ID, 0.20 μm film thickness; Varian Inc., Lake Forest, CA, USA). The column temperature was held at 45 °C for 5 min, then raised 20 °C min^{-1} up to 195 °C and maintained for 65 min. The temperature of the injector and the flame-ionization detector was maintained at 250 and 280 °C, respectively; the injection volume was 0.1 μl; nitrogen constant linear flow rate was set at 40 ml min^{-1}. Peaks were identified by comparing the retention times with pure FAME standards (Fluka, Sigma and Supelco, Sigma–Aldrich Milano, Milano, Italy; Matreya Inc., Pleasant Gap, PA, USA), as detailed in Renna et al. (2012), and through comparisons with published chromatograms (Juárez et al. 2011; Alves and Bessa 2014; Vahmani et al. 2016). Quantification was assessed using tridecanoic acid (C13:0; Fluka, Sigma–Aldrich Milano, Milano, Italy) as internal standard. The fatty acid content was determined as mg/100 g of LT muscle, and was then expressed as g/100 g of total detected fatty acids.

Lipid oxidation

After each ageing period (2, 7 or 10 days), a sample of 10 g was taken from each steak. Lipid oxidation was assessed by means of measurement of the thiobarbituric acid-reactive substances (TBARS) using the method of Tarladgis et al. (1960), modified by Novelli et al. (1998). The TBARS values were expressed as mg malondialdehyde/kg of muscle tissue (mg MDA/kg).

Colour

Meat colour was measured using a Minolta CR-331C colourimeter with a 25 mm diameter measured area, D65 illuminant and 2° standard observer in the CIELAB space (CIE 1976). The colour measurements were obtained on a freshly cut surface of subsamples aged 7 or 10 days, after 1h of blooming at 4 °C. CIELAB coordinates, Lightness (L*), redness (a*) and yellowness (b*) were recorded and chroma (C*) and hue angle (H*) were calculated as $C^* = (a^{*2} + b^{*2})^{*0.5}$ and $H^* = \tan^{-1}$ (b*/a*).

Consumer sensory evaluation

Two 2.0-cm-thick steaks, cut from each subsample aged 10 days, were vacuum-packaged and stored at −25 °C. Twenty-four hours prior to sensory analysis, the steaks were thawed at 4 °C.

Consumer tests were carried out in individual booths at the Sensory Analysis Laboratory of the Department of Agricultural, Forest and Food Sciences, University of Torino, according to AMSA procedures (AMSA 2015). A total of 63 untrained and regular beef consumers, 39 males and 24 females, ranging in age from 21 to 60 years, were recruited. Informed consents were obtained from all the consumers.

The steaks were cooked on a preheated double plate grill to an internal temperature of 70 °C. After cooking, each steak was cut into 1.3 × 1.3 × 1.9 cm samples which were placed in a foil pouch labelled with a three-digit random number.

The consumers were requested to smell and taste the meat and, then, to rate the perceived flavour intensity (aroma and taste) of each sample using a 9-point, end anchored, intensity scale, where 1 = 'none or extremely bland' and 9 = 'extremely intense' (Miller 1998). The midpoint of the scale (5) was considered a neutral response (i.e. 'neither bland nor intense').

In addition, consumers were asked to rate flavour of each sample as either acceptable or unacceptable. Each consumer evaluated 4 samples (two samples per dietary treatment).

Statistical analyses

The statistical data analyses were carried out using IBM SPSS (IBM Corp. Released 2011; IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY, USA).

The animal was considered as the experimental unit. It was assumed that a general pen effect was negligible due to (i) identical structural characteristics of the pens, (ii) exposition of the animals within the

Table 2. Proximate composition (g/kg meat) of *longissimus thoracis* muscle of Piemontese bulls fed the control (C) and flaxseed (FS) diets ($n = 9$).

	Dietary treatment			
	C	FS	SEM	*p*-value
Moisture	752.9	754.7	1.43	.422
Protein	229.5	231.5	1.35	.343
Ether Extract	5.2	4.6	0.58	.532

SEM: standard error of the mean; *p*: probability.

two pens to identical management practices, (iii) high uniformity of animals, in terms of genotype, physiology and health status, between the pens, (iv) no changes in animal numbers during the trial and no moving of the animals in and out the pens (St-Pierre 2007). We also assumed that, for the considered dependent variables, the individual responses of bulls within the same pen were not correlated as: (i) the same rearing density was used in both pens, no differential feed access due to social behaviour and no competition for space among the animals within the pens occurred; (ii) the correlation of individual responses of animals within pens has been demonstrated to be of low entity for dependent variables measuring meat quality (Jauhiainen et al. 2008).

The proximate and FA compositions were analysed using the GLM procedure, considering the dietary treatment as fixed effect and the cold carcase weight as covariate. The TBARS values were analysed using the GLM procedure for repeated measures over period, with the dietary treatment as fixed factor, the ageing period (days) as repeated factor, and the dietary treatment × ageing period interaction. Colour data were analysed by a split-plot design, considering the dietary treatment effect in the main plot and the day effect in the sub-plot, as well as their interaction. Flavour intensity was analysed using the GLM procedure considering the dietary treatment as fixed effect and the consumer as random effect. Acceptability from flavour data was analysed by chi-square test.

The results were reported as least square means and standard error of the mean. Significance was declared at $p < .05$.

Results and discussion

Proximate composition

The final weight and the feed conversion ratio of the bulls averaged 549 kg and 7.0, respectively. The dietary treatment had no significant effect on the LT proximate composition (Table 2). The very low ether extract values found for Piemontese meat in this trial are due to the lack of acid hydrolysis preceding Soxhlet extraction, and therefore to a lack of phospholipids extraction. The ether extract content of beef from both C-and FS-fed groups was in agreement with previous results of our research group using identical analytical methods (Brugiapaglia and Destefanis 2012; Brugiapaglia et al. 2014) and comparable with the values found on LT muscle of double-muscled Belgian Blue bulls (De Smet et al. 2000).

Some authors have found that the ether extract (Corazzin et al. 2012) and protein (Corazzin et al. 2012; Barahona et al. 2016) increase with the inclusion of flaxseed in the diet, even for the inclusion levels considered in the current trial. However, most of the published literature has shown, in agreement with our findings, that flaxeed does not exert any significant effect on the meat proximate consituents (Juárez et al. 2012; Mapiye, Aalhus et al. 2013; Albertí et al. 2014).

Fatty acid profile

The dietary inclusion of flaxseed significantly modified the FA profile of meat (Table 3), without altering the total intramuscular FA proportion, in agreement with other studies including ALA-rich oilseeds in rations for cattle (Juárez et al. 2011; Mapiye, Aalhus et al. 2013; Mapiye, Turner et al. 2013).

No significant changes were observed for the total SFA, MUFA (monounsaturated fatty acids) and PUFA contents, as previously observed in young bulls of other double-muscled breeds when fed flaxseed (Raes, De Smet et al. 2004; Albertí et al. 2014). The PUFA/SFA ratio was not affected by diet and, for both groups, fell within the recommended values for human nutrition (0.4–1.0) (Jiménez-Colmenero et al. 2012), as generally observed for double-muscled breeds (Scollan et al. 2014; Sevane et al. 2014).

The percentage of PUFA was about double in comparison with that observed in flaxseed-fed young bulls belonging to early-maturing breeds (Mach et al. 2006), but similar to the values reported for Belgian Blue young bulls fed diets rich in n-3 PUFA (Raes et al. 2003). Such a result has to be ascribed to muscle hyperplasia, which determines an increase in the number of cells and, consequently, a high proportion of phospholipids over total lipids (Fiems 2012).

The flaxseed-fed Piemontese bulls showed a higher ALA percentage than Pirenaica bulls (Albertí et al. 2014), a result that is in agreement with the typical high n-3 PUFA proportion over total FA in the meat of this breed, and of leaner animals in general (Aldai et al. 2006; Sevane et al. 2014). Among n-3 PUFA, the proportions of C18:3 n-3 ($p < .001$), C20:5 n-3 ($p < .01$) and C22:6 n-3

Table 3. Fatty acid profile of total lipids in *longissimus thoracis* muscle of Piemontese bulls fed the control (C) and flaxseed (FS) diets ($n = 9$).

	Dietary treatment			
	C	FS	SEM	*p*-value
Individual SFA (g/100 g of TFA)				
C10:0	0.06	0.06	0.009	.855
C12:0	0.05	0.05	0.005	.766
C14:0	1.53	1.45	0.086	.569
C15:0	2.36	2.19	0.298	.701
C15 *anteiso*	0.10	0.11	0.009	.414
C16:0	20.45	18.92	0.531	.078
C17:0	1.23	1.09	0.104	.382
C17 *iso*	0.35	0.35	0.020	.925
C18:0	13.23	15.31	0.560	.028
C18 *iso*	0.02	0.04	0.005	.072
C20:0	0.07	0.10	0.007	.014
C22:0	0.10	0.11	0.012	.635
Individual MUFA (g/100 g of TFA)				
C14:1 *c*9	0.56	0.55	0.050	.818
C16:1 *t*9	0.11	0.11	0.018	.758
C16:1 *c*7	0.45	0.37	0.026	.072
C16:1 *c*9	1.30	0.99	0.080	.124
C17:1 *c*9	0.34	0.38	0.049	.687
C18:1 *t*6-9	0.25	0.29	0.021	.245
C18:1 *t*10-11	2.74	2.47	0.283	.544
C18:1 *t*12	0.57	1.21	0.057	.000
C18:1 *c*9+*t*13-14	22.48	19.21	1.622	.206
C18:1 *c*11+*t*15	1.78	1.59	0.077	.130
C18:1 *c*12	0.36	0.50	0.032	.011
C18:1 *c*15+*t*16	0.36	0.40	0.042	.511
C20:1 *c*11	0.11	0.09	0.006	.024
Individual PUFA (g/100 g of TFA)				
C18:2 *c*9,*t*13+*c*9,*t*14	0.13	0.24	0.028	.016
C18:2 *c*9,*t*12+*t*8,*c*13	0.15	0.23	0.016	.004
C18:2 *t*9,*c*12	0.12	0.20	0.009	.000
C18:2 *t*10,*c*15+*t*11,*c*15	0.24	0.40	0.032	.005
C18:2 n-6 (LA)	21.52	21.86	1.431	.877
C18:2 *c*9,*c*15	0.11	0.09	0.012	.293
C18:2 *c*9,*t*11+*t*7,*c*9+*t*8,*c*10 (CLA)	0.15	0.14	0.008	.514
C18:3 n-6 (GLA)	0.08	0.09	0.008	.822
C18:3 n-3 (ALA)	0.88	2.98	0.140	.000
C20:2 n-6	0.12	0.08	0.008	.005
C20:3 n-9	0.04	0.06	0.004	.003
C20:3 n-6	0.65	0.62	0.051	.729
C20:3 n-3	0.02	0.03	0.006	.362
C20:4 n-6	3.60	3.62	0.255	.974
C20:5 n-3 (EPA)	0.22	0.41	0.039	.007
C22:4 n-6	0.39	0.28	0.028	.020
C22:5 n-3 (DPA)	0.62	0.72	0.086	.424
C22:6 n-3 (DHA)	0.02	0.04	0.005	.010
Groups of FA (g/100 g of TFA)				
Σ SFA	39.56	39.78	0.698	.839
Σ MUFA	31.40	28.14	1.664	.218
Σ C18:1	28.52	25.67	1.647	.274
Σ C18:1 *t*	3.55	3.97	0.310	.379
Σ PUFA	29.04	32.08	1.910	.313
Σ C18:2	22.40	23.16	1.426	.732
Σ C18:2 *t*	0.78	1.21	0.065	.001
Σ n-6 PUFA	26.62	26.97	1.710	.895
Σ n-3 PUFA	2.11	4.67	0.237	.000
Selected FA (mg/100 g meat)				
C16:0	120.75	91.27	10.389	.083
C18:0	76.94	73.87	6.739	.770
C18:1 *c*9+*t*13-14	136.47	94.28	17.186	.128
C18:2 n-6 (LA)	122.53	102.16	5.370	.025
C18:3 n-3 (ALA)	4.90	14.24	0.657	.000
Σ SFA	231.26	191.26	17.535	.156
Σ MUFA	189.00	137.36	21.200	.131
Σ C18:1	172.14	125.38	19.880	.144
Σ C18:1 *t*	20.78	19.11	2.494	.667
Σ PUFA	165.30	150.36	7.031	.184
Σ C18:2	127.78	108.39	5.673	.041
Σ C18:2 *t*	4.61	5.81	0.507	.142
Σ n-6 PUFA	151.62	126.13	6.277	.018
Σ n-3 PUFA	11.85	22.13	0.890	.000
FA ratios and DI				
PUFA/SFA	0.74	0.81	0.057	.408
n-6/n-3 PUFA	13.13	5.60	0.511	.000
DI_{14}*	0.38	0.38	0.047	.920
DI_{16}**	0.06	0.05	0.006	.193
DI_{18}***	1.72	1.28	0.134	.048

TFA: total fatty acids; SEM: standard error of the mean; *p*: probability; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; *c*: *cis*; *t*: *trans*; PUFA: polyunsaturated fatty acids; LA: linoleic acid; CLA: conjugated linoleic acid; GLA: γ-linolenic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; FA: fatty acids; DI: desaturase index.
*DI_{14} = C14:1 *c*9/C14:0.
**DI_{16} = C16:1 *c*9/C16:0.
***DI_{18} = C18:1 *c*9/C18:0.

($p = .01$) were higher in the FS group than in the C group. The overall increase in the concentrations of ALA and its long-chain metabolites was statistically significant, but quantitatively limited, as a result of the typical well-known high biohydrogenation rate of dietary ALA, which lowers the duodenal availability of n-3 PUFA for incorporation in the tissues (Doreau and Ferlay 1994). The docosahexaenoic acid (DHA) content was enhanced by the dietary flaxseed, but its level remained very low, thus confirming that consistent enhancements of DHA are almost exclusively achieved when this fatty acid is supplied directly with the diet. The concentrations of ALA (4.90 and 14.24 mg/100 g of meat in C- and FS-fed bulls, respectively) and EPA + DHA (1.35 and 2.14 mg/100 g of meat in C- and FS-fed bulls, respectively) in Piemontese meat were significantly higher in the FS group than in the C group, but remained in both cases very low. Also in case of FS-fed bulls, such values represented only <1% of the intake recommended by EFSA (2009; 2012) for adult people.

The n-6/n-3 PUFA ratio in the FS group was considerably lower than that of the C group ($p < .001$), and was closer to the recommended value (≤4) for human nutrition (Simopoulos 2011). As previously observed in flaxseed-supplemented Belgian Blue cattle (Raes, Haak et al. 2004), the n-6/n-3 PUFA ratio was found to be above 5. Lower values can be achieved in cattle fed flaxseed (Herdmann et al. 2010), but are difficult to obtain in lean animals, due to the large amount of LA generally found in the phospholipid fraction of their muscles (Raes, Haak et al. 2004).

The sum of conjugated linoleic acid (CLA) isomers C18:2 *c*9,*t*11+*t*7,*c*9+*t*8,*c*10 was unaffected by the

treatment. Its concentration was low, most probably as a consequence of both dietary and genetic reasons. The type of basal diet used, rich in concentrates as commonly occurs in the fattening phase of ruminants, probably led to a *t*10-shifted rumen biohydrogenation pathway, that is usually associated to low rumenic acid (C18:2 *c*9,*t*11) levels (Bessa et al. 2015). Low rumenic acid contents are also usually observed in Piemontese meat (Brugiapaglia et al. 2014) due both to a higher incorporation of CLA in the neutral lipids than in the phospholipids and to a low Stearoyl-CoA desaturase (SCD) activity, which is typical of leaner animals (Sevane et al. 2014).

Many of the detected non-conjugated octadecadienoic isomers are known or thought to be intermediate products of the ruminal biohydrogenation of dietary ALA, which explains their significant increase in the meat of flaxseed-fed cattle in the current and other trials (Juárez et al. 2011; Mapiye, Turner et al. 2013). The sum of *trans* octadecadienoic isomers was higher in the FS meat than in the C meat ($p = .001$), while no differences in the sum content of *trans*-octadecenoic acids were found between groups. It is well-known that *trans* fatty acids increase the risk of cardiovascular disease, but the effect of animal *trans* fatty acids (1–8% of total fats) is still unclear. However, their potential impact on human health is weaker than that of industrial *trans* fats, due to a lower *trans* fatty acids intake from the diet (Brouwer et al. 2013). During GC analysis, the major C18:1 *trans* peak contained a co-elution of C18:1 *t*10 and C18:1 *t*11. As C18:1 *t*10-11 are typically major products of PUFA biohydrogenation, it is apparent that ALA in ground flaxseed was extensively biohydrogenated, which is consistent with the increased C18:0 content when feeding flaxseed. It has been found that C18:1 *t*13–14 can be the major C18:1 *trans* isomer in beef when feeding flaxseed in a barley grain based diet (Juarez et al. 2011). Unfortunately during GC analysis for the current study, C18:1 *t*13–14 co-eluted with C18:1 *c*9, and any changes could not be detected. Interestingly, however, there was an increase in C18:1 *t*12 when feeding flaxseed, and this is consistent with Juarez et al. (2011) where feeding flaxseed in a barley grain based diet increased the content of C18:1 *trans* isomers with double bonds from carbons 12 to 16. Considering the findings of Vahmani et al. (2016) and Juárez et al. (2011), the increased contents of C18:2 *c*9,*t*12+*t*8,*c*13 and C18:2 *c*9,*t*13+*c*9,*t*14 in the meat from FS-fed bulls can be also respectively imputed to the higher contents of C18:1 *t*12 and C18:1 *t*13/*t*14 as precursors for Δ9-desaturase activity. The obtained results further confirm feeding ALA enriched oilseeds in concentrate based diets may shift biohydrogenation away from pathways with intermediates containing *t*10 and *t*11 double bonds.

Among the SFA, only C18:0 and C20:0 were affected by the treatment ($p < .05$), and they were found to be higher in the FS group than in the C one. The contents of *de novo* synthesised SFA (C10:0, C12:0, C14:0 and C16:0) did not differ significantly between the groups in our study. Some authors have reported a significant reduction in SFA (Raes et al. 2003; Mach et al. 2006; Corazzin et al. 2012), which has been ascribed to the inhibitory effects of the lipid supplementation on *de novo* FA synthesis, while others have found no influence on *de novo* synthesised SFA (Raes, De Smet et al. 2004; Albertí et al. 2014). The dietary treatment did not influence the amount of odd- or branched-chain fatty acids to any great extent, in accordance with the general low inhibitory effect of ALA on rumen microbial synthesis (Mapiye, Aalhus et al. 2013).

Lipid oxidation

The average MDA content tended (0.28 *vs* 0.42 mg MDA/kg; $p = .096$) to be lower in the meat of the C group than that of the FS group (Table 4). As expected, the MDA levels increased as the ageing period increased ($p < .001$). A significant interaction between dietary treatment and ageing period was also observed ($p < .05$). In fact, the TBARS values in the C group only increased significantly from d2 to d7, while they increased over the whole ageing period in the FS group.

Some authors have reported that the susceptibility of meat to oxidation mainly depends on the n-3 PUFA content, and in particular on ALA, in the cell membranes (Realini et al. 2004; Daly et al. 2007; Kouba & Mourot 2011). In the current trial, the higher n-3 PUFA content in the meat from the FS-fed animals

Table 4. Thiobarbituric acid reactive substances (TBARS; mg MDA/kg of muscle tissue) values of *longissimus thoracis* muscle of Piemontese bulls fed the control (C) and flaxseed (FS) diets ($n = 9$), during ageing (2, 7 and 10 days at 4 ± 1 °C).

	Dietary treatment			*p*-value		
Ageing	C	FS	SEM	D	AG	D × AG
d2	0.11[a]	0.15[a]	0.008			
d7	0.33[b]	0.39[b]	0.096	.096	.000	.038
d10	0.41[b]	0.72[c]	0.101			

SEM: standard error of the mean; *p*: probability; D: dietary treatment; AG: ageing period; D × AG: interaction between dietary treatment and ageing period. Means with different superscripts (a–c) within each column indicate significant differences among days.

contributed to a greater susceptibility of the muscle to lipid oxidation.

The toxicity of MDA in human nutrition is well known (Watts 1962); however, its level was below the threshold value for rancidity (1–2 mg MDA/kg) in the meat of both groups.

Colour

After 7 and 10 days of ageing, when meat is generally sold at retail, the dietary treatment had no significant effect on CIELAB colour parameters (Table 5).

This result is in agreement with that of the study of Mach et al. (2006), where different lipid sources, including whole flaxseed, at different concentration in the diet, did not influence L*, a* and b* values in meat from Holstein bulls. Similarly, Albertì et al. (2014) reported that adding whole linseed to a concentrate fed to young bulls did not significantly modify meat colour.

Table 5. Colour parameters of *longissimus thoracis* muscle of Piemontese bulls fed the control (C) and flaxseed (FS) diets ($n = 9$) during ageing (7 and 10 days at 4 ± 1 °C).

	Dietary treatment							
	C		FS			*p*- value		
	d7	d10	d7	d10	SEM	D	AG	D × AG
L*	41.24	41.69	41.63	41.55	0.195	.924	.346	.195
a*	27.65	27.64	27.24	27.41	0.377	.633	.841	.817
b*	9.64	9.77	9.29	9.41	0.263	.471	.628	.990
C*	29.29	29.32	28.80	29.00	0.433	.597	.798	.851
H*	19.20	19.48	18.70	18.84	0.304	.375	.499	.815

SEM: standard error of the mean; *p*: probability; D: dietary treatment; AG: ageing period; D × AG: interaction between dietary treatment and ageing period; L*: lightness; a*: redness; b*: yellowness; C*: chroma; H*: hue angle.

Consumer sensory evaluation

The frequency distribution of 252 ratings for the two dietary treatments is reported in Figure 1. Both mode and median values of flavour intensity of FS group were 5, which corresponds to 'neither bland nor intense' on the 9-point intensity scale. For C group the mode and the median values were 3 and 4 respectively, and no ratings were found in the 9th category.

The perceived flavour intensity of FS group was significantly higher than that of C group (Table 6). On the contrary, flavour acceptability was not affected by treatment (Table 7).

Table 6. Flavour intensity of *longissimus thoracis* muscle of Piemontese bulls fed the control (C) and flaxseed (FS) diets ($n = 9$).

	C	FS	SEM	*p*-value
Flavour intensity	4.09	4.69	0.098	.039

SEM: standard error of the mean; *p*: probability. Flavour intensity was evaluated on a 9-point, end-anchored, intensity scale, where 1='none or extremely bland'; 9 = 'extremely intense'.

Table 7. Flavour acceptability of *longissimus thoracis* muscle of Piemontese bulls fed the control (C) and flaxseed (FS) diets.

	Number of ratings (%)			
	C	FS	χ^2	*p*-value
Acceptable	104 (83)	96 (76)	1.19	.275
Unacceptable	22 (17)	30 (24)		
Total	126 (100)	126 (100)		

χ^2: Chi square; *p*: probability.

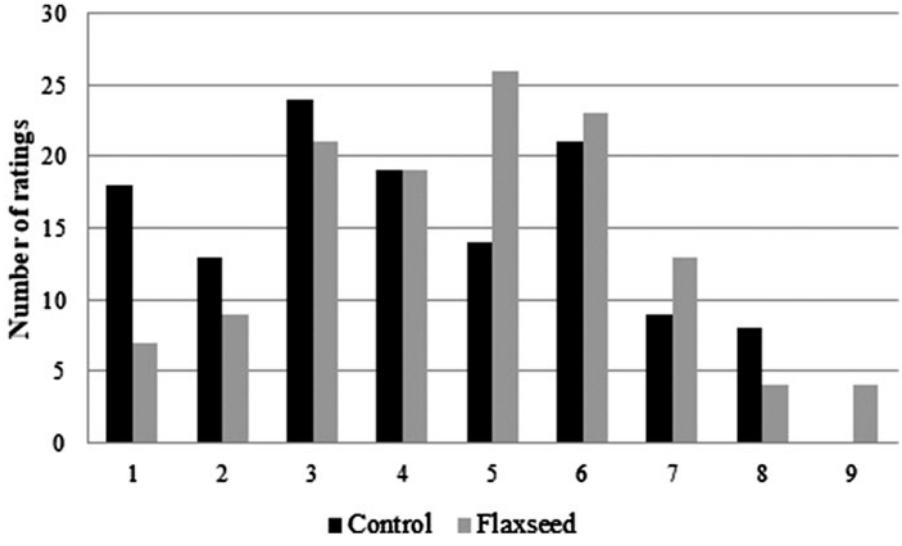

Figure 1. Sensory ratings distribution of flavour intensity. Flavour was evaluated on a 9-point, end-anchored, intensity scale, where 1 = 'none or extremely bland'; 9 = 'extremely intense'.

Beef was designated as acceptable if two-thirds or more of consumers sampling each steak indicated that they were satisfied (Platter et al. 2003). In our study both C group (83%) and FS group (76%) beef resulted in an acceptable rating.

Flavour is a complex attribute of meat palatability and in part depends on meat fat content and composition (Calkins & Hodgen 2007). According to Van Ba et al. (2012), meat with high PUFA content produces unpleasant odours and flavours associated with oxidation.

The higher flavour intensity of FS group may be linked to the differences between treatments in the n-3 PUFA content and most probably to the higher amounts of lower molecular weight unsaturated aldehydes derived from fat oxidation, especially from long chain PUFA (Van Ba et al. 2012).

Literature on how feeding flax to cattle affects palatability is conflicting. Barahona et al. (2016) found that a linseed enriched diet produced meat that had a greater intensity of beef flavour. In contrast, Drouillard et al. (2004) reported no differences in sensory panel observations for juiciness, tenderness or flavour. According to Scollan and Huws (2005) feeding flaxseed, which resulted in meat ALA content of 1.2% of total lipids, had no negative effect on meat organoleptic characteristics. On the contrary, with an increase of the ALA content to 2.8% of the total lipids, an 'abnormal' and 'rancid' beef was recorded (Scollan et al. 2004). These results support the conclusion of Wood et al. (2004) that only when the concentrations of ALA approach 3% of total lipids there are adverse effects on lipid stability, colour or flavour.

After 10 days of ageing, meat of FS group underwent low oxidative deterioration (0.72 mg MDA/kg). Greene and Cumuze (1982) found that detection of MDA by untrained panellists varied from 0.2 to 2 mg MDA/kg and did not necessarily relate to flavour acceptability.

Examining eating quality in 73 beef loins produced using different feeding treatments and containing different concentrations of n-6 and n-3 PUFA, Campo et al. (2006) found that when TBARS values increased, the scores for beef flavour intensity and abnormal flavour increased. This study identifies a TBARS value of 2.3 mg MDA/kg as the point where rancid and other abnormal flavours overpower beef flavour to produce an unacceptable flavour profile in beef.

Conclusions

Our results demonstrate that the inclusion of 10% DM of ground flaxseed can be used in the diets of Piemontese young bulls, without any modifications of the beef proximate composition, colour and flavour acceptability. The dietary flaxseed significantly modified the FA profile of the meat, increasing the *trans*-octadecadienoic acids and the n-3 PUFA contents, and lowering the n-6/n-3 PUFA ratio. The supplies of ALA and of EPA + DHA on a per-serving basis remained very low (i.e. <1% of the recommended intake for adult humans) even in the meat obtained from the FS-fed bulls. Increasing the n-3 PUFA content in the diet of Piemontese young bulls could be a strategic way of enhancing the nutritional value of the derived beef, without forcing consumers to change their eating habits. Alpha-linolenic acid from flaxseed is extensively biohydrogenated; finding ways to increase its ruminal bypass would be a priority when trying to increase the n-3 PUFA content of Piemontese beef, especially if such a strategy would like be adopted by the cattle industry as a way of promoting healthy Piemontese beef.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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