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Effect of including linseed in a concentrate fed to young bulls on intramuscular fatty acids and beef color

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ABSTRACT

The effect of varying concentrate composition to include 5% linseed and 200 IU of vitamin E on the growth performance, fatty acid composition, and muscle color during shelf life was assessed in 46 young Pirenaica bulls finished to two fatness levels. Adding 5% linseed lowered the dressing rate without altering daily gain or carcass classification. It likewise did not alter the total saturated, monounsaturated, or polyunsaturated fatty acids in the intramuscular fat, though the percentage of α-linolenic acid and n − 3 fatty acids increased significantly while the n − 6 fatty acid to n − 3 fatty acid ratio decreased. Higher subcutaneous fat cover depth at slaughter increased the total percentage of oleic acid and monounsaturated fatty acids without affecting the percentage of saturated or polyunsaturated fatty acids. Adding 200 IU of vitamin E in addition to linseed did not alter the color of film-wrapped fresh meat during storage in darkness.

1. Introduction

Consumers have shown heightened interest in lean meats (Ngapo & Dransfield, 2006) with low saturated and high polyunsaturated fatty acid contents (Scollan et al., 2006) because of evidence that the fatty acid composition of the diet is a cardiovascular risk factor (EFSA, 2010). Moreover, compared with long chain n − 6 polyunsaturated fatty acids, long chain n − 3 polyunsaturated fatty acids lower the risk of cardiovascular disease in humans (Palmquist, 2009).

The intramuscular fat content of the *Longissimus dorsi* muscle in Spanish beef varies from 0.9 to 3.2% (Christensen et al., 2011; Insausti, Goñi, Petri, Gorraiz, & Beriain, 2005), thus qualifying as lean meat. However, the fattening systems employed, based on concentrates rich in corn and soybean meal, can result in a highly unbalanced intramuscular fat profile with a high proportion of linoleic acid, which can raise the n − 6 to n − 3 ratio to values between 16 and 25 (Blanco et al., 2010; Insausti, Beriain, Alzueta, Carr, & Purroy, 2004).

Adding linseed to concentrates used to fatten cattle has proved effective in increasing the percentage of n − 3 fatty acids in the intramuscular fat (Scollan, Dhanoa, et al., 2001). Some international animal nutrition guidelines limit the use of linseed owing to the presence of antinutritional factors, phytic acid and phytoestrogens, linamarin (cyanogenic glucoside), and linatine (de Blas, Mateos, & Rebollar, 2003; Ewing, 1998). Thus, EFSA (2007) has made a general recommendation of 5% whole linseed in concentrate for cattle and a maximum of 7.5% linseed cake for calves, and 20% for yearlings.

Increasing the polyunsaturated fatty acid content of meat can reduce its stability to oxygen and heighten its susceptibility to rancidity, resulting in variations in sensory quality and loss of color shelf life (Faustman, Sun, Mancini, & Suman, 2010; Nute et al., 2007). Enriching the diet with antioxidants such as vitamin E is one way to combat these adverse effects. Liu, Lanari, and Schaefer (1995) recommended a concentration of 1.2 μg α-tocopherol/g muscle to achieve a significant increase in color display life. Polyunsaturated fatty acids being more prone to oxidation during the display of meat, lipids destabilize the metmyoglobin MbFe(III) molecule when meat is on display, resulting in lipid oxidation by a mechanism involving direct exposure of the heme group to the lipids (Baron, Skibsted, & Andersen, 2002). Subsequently, the products of these reactions promote oxidation by myoglobin and fatty acids (Faustman et al., 2010), decreasing the shelf life of beef. Color, fat, and cut have been identified as intrinsic cues directly related to the perception of beef quality at the time of purchase (Banovic, Grunert, Barreira, & Fontes, 2009), and decisions by consumers are very likely determined in large measure by meat color.

The object of this study was therefore to evaluate the effect of adding whole linseed and vitamin E to concentrate fed to young bulls slaughtered at two fat cover depths on the fatty acid profile of the intramuscular fat and on beef color behavior during storage.

2. Materials and methods

2.1. Animals and diets

The experiment was carried out using 46 young bulls of the Pirenaica breed. The bulls (278 ± 42.1 kg BW) were allotted to six experimental

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groups by body weight, ensuring that the mean weight of each group was comparable. The bulls in three groups were fattened to a dorsal fat layer 3-mm thick on average, and the other three groups were fattened to a dorsal fat layer thickness of 4 mm. The groups were finished with a diet of control concentrate [C] (n = 7), linseed-containing concentrate [L] (n = 8), or linseed plus vitamin E-containing concentrate [L + E] (n = 8) (Table 1). All animals were allowed ad libitum access to concentrate and barley straw.

Animals were weighed early in the morning every two weeks, and the average daily weight was then calculated. Concentrate intake was monitored by group. Subcutaneous fat thickness was measured by ultrasound at the 4th dorsal vertebra using an Aloka model SSD-900 apparatus with a 7.5-MHz multifrequency electronic linear array probe (5 to 10 MHz) having a 62-mm scan width (model UST 5710-7.5, Aloka Spain, Madrid, Spain).

When the young bulls reached the end point they were transported to a licensed abattoir 20-min distant, without a fasting period. Each group of bulls was held in lairage in a separate pen for under 2 h and afterward slaughtered according to EU Regulations. The carcasses were chilled at 2 °C for 24 h in a cooling room. The *Longissimus dorsi* muscle was removed and sampled for subsequent analysis. One 2 cm-thick steak was taken at the level of the vertebra at T5 and set aside for proximate analysis and vitamin E determination. Another 2 cm-thick steak was taken at the level of the vertebra T6 for fatty acid analysis. The steaks were vacuum packaged and frozen at −20 °C for further analysis. Two 3 cm-thick steaks were sampled between the vertebrae at T7 and T9 for instrumental color determination.

Table 1
Ingredients in the three concentrates fed to young Pirenaica bulls.

	C	L	L + E
Ingredient (% feed)			
Barley grain, ground	22.6	23.4	23.2
Corn grain, ground	35.0	33.0	33.0
Gluten feed	10.0	10.0	10.0
Bran	4.0	4.0	4.0
Soybean meal	13.8	11.9	11.9
Soybean skin	5.0	5.0	5.0
Whole linseed	0.0	5.0	5.0
Beet pulp	4.0	4.0	4.0
Calcium soap[a]	0.4	0.0	0.0
Animal fat[b]	2.0	0.6	0.6
Calcium carbonate	1.5	1.5	1.5
Dicalcium phosphate	0.3	0.2	0.2
Sodium bicarbonate	0.5	0.5	0.5
Sodium chloride	0.5	0.5	0.5
Vitamin mineral premix[c]	0.2	0.2	0.2
Rumalato[d]	0.2	0.2	0.2
Vitamin E premix[e]	0.0	0.0	0.2
ME[f], MJ/kg DM	12.9	12.7	12.7
Chemical analysis (%DM basis)			
Crude protein	16.4	16.6	16.6
Ether extract	5.6	5.6	5.6
Crude fiber	6.8	7.9	7.9
Fatty acid composition (% total fatty acids)			
C16:0	14.30	9.29	9.16
C18:0	4.67	2.97	2.83
C18:1, c9	15.79	12.30	11.51
C18:2, c9, c12	62.24	62.35	64.04
C18:3, c9, c12, c15	0.42	11.86	10.89
n−6	62.24	62.35	64.04
n−3	0.42	11.86	10.89
n−6 to n−3 ratio	148.62	5.26	5.88

C: control concentrate; L: linseed-supplemented concentrate; L + E: linseed + 2% vitamin E-supplemented concentrate.

[a] Fatty acid (FA) composition of the calcium soap: 46.5% palmitic acid, 37.8% oleic acid, 8.6% linoleic acid, 4.4% stearic acid, 1.1% myristic acid, 0.4% arachidic acid, 0.3% α-linolenic acid, 0.2% lauric acid, and 0.2% palmitoleic acid.

[b] Animal fat mix: 50% tallow and 50% lard, containing: 40.9% oleic acid, 23.0% palmitic acid, 12.7% linoleic acid, 12.2% stearic acid, 3.4% palmitoleic acid, 1.0% α-linolenic acid, and 0.4% margaric acid.

[c] Vitamin mineral premix, content per kg: 10 mg vitamin E, 7000 IU vitamin A, 1500 IU vitamin D3, 500 mg Na_2SO_4, 100 mg MgO, 40 mg Zn, 30 mg Mg, 5 mg Fe, 2 mg Cu, 0.5 mg I, 0.5 mg Co, 0.2 mg Se, and 0.3 mg butylated hydroxytoluene.

[d] Salts of organic acids.

[e] Vitamin E premix content: 10% α-tocopherol acid per kg.

[f] Calculated according to MAFF (1975).

2.2. Chemical analysis

Concentrate samples were analyzed for dry matter and ash according to official AOAC methods (AOAC, 2000). Nitrogen was determined using a protein analyzer (model NA2100, CE Instruments, ThermoQuest Italia, Rodano, Italy), and the ether extract using an ANKOM model XT10 extractor. Crude fiber was analyzed using an ANKOM model 200 fiber analyzer (Ankom Technology, Gomensoro, S.A., Madrid, Spain).

Muscle samples too were analyzed for dry matter and ash according to official AOAC methods (AOAC, 2000). The remaining muscle was ground and freeze-dried, and afterwards the nitrogen and ether extract were analyzed, using the same methodology as in the concentrate analysis. To determine the α-tocopherol content of the muscle, 1 g of *Longissimus dorsi* muscle was treated with a saponification solution and the non-saponifiable matter recovered by petroleum ether extraction (Liu, Scheller, & Schaefer, 1996). Samples were analyzed using an Agilent model 1100 HPLC chromatograph (Agilent Technologies España S.L., Las Rozas, Spain) equipped with a quaternary pump, an Atlantis dC18 4.6 mm × 200 mm, 3-μm capillary column [Waters Cromatografía, S.A., Cerdanyola del Vallès, Spain], and a fluorescence detector (excitation λ = 295 nm; emission λ = 340 nm). The mobile phase was an ACN: water mixture (95:5) with 0.1% TFA.

Before fatty acid analysis, steaks were thawed at 2 ± 1 °C for 24 h. The total lipids in the meat were extracted and hydrolysed as described by Whittington, Prescott, Wood, and Enser (1986) with certain modifications or optimizations (Aldai, Murray, Nájera, Troy, & Osoro, 2005). Samples were taken in duplicate, 0.5–1 g for fatty acid analysis, and saponified after flushing with nitrogen. The extracted fatty acids (FAs) were methylated using 200 μL of trimethylsilyl-diazomethane at 40 °C for 10 min, dried under N_2, dissolved, and centrifuged, and the supernatant was transferred for analysis. Fatty acid methyl esters were stored at −80 °C for later chromatographic analysis. Analysis was by gas chromatography using a BPX-70 (SGE U.K. Ltd.) fused-silica capillary column (120 m × 0.22 mm i.d. × 0.2 μm film thickness). The fatty acid methyl esters were separated by gas chromatography (Agilent model 7890) using a flame ionization detector (FID) and hydrogen as the carrier gas. The oven temperature was initially set at 50 °C, and gradually ramped up to 240 °C, where it remained to the end of the cycle. The entire process took about 45 min. Fatty acid methyl esters were identified on the basis of similar peak retention times using standards where available (Sigma Chemical Co. Ltd., Poole, UK). Fatty acids were quantified using tricosanoic acid methyl ester (C23:0), added prior to saponification, as an internal standard. Column response and linearity were checked using a mixture of fatty acids (C16:0, C18:0, C18:1n−9, C18:2n−6, relative to internal standard C23:0, Sigma Chemical Co. Ltd., Poole, UK).

2.3. Instrumental color determination

Meat samples were placed on a polystyrene tray, wrapped in oxygen-permeable film that was not in contact with the surface of the meat, and held at 4 °C in the dark. For the color measurements samples were placed on a standard white tile. Color readings were taken at two randomly selected locations on the cranial surface of each piece to obtain a representative mean value. Muscle color was measured in the CIELAB space (CIE, 1986) with a measured area diameter of 8 mm, specular component included, and 0% UV, D65 standard illuminant, observer angle 10°, and zero and white calibration using a Minolta model CM-

2006d spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan) at storage times of 24 h, 48 h, 6 d, 9 d, and 14 d. Lightness (L*), redness (a*), and yellowness (b*) were recorded, and hue angle (H*) and chroma (C*) indexes were calculated as $H^* = \tan^{-1}(b^* / a^*)$ expressed in degrees, and $C^* = (a^{*2} + b^{*2})^{0.5}$ (MacDougall, 1986; Wyszecki & Stiles, 1982). The relative oxymyoglobin, metmyoglobin and myoglobin contents were calculated from measurements taken at wavelengths of 473, 525, 572, and 730 nm (AMSA, 1991; Krzywicki, 1979).

2.4. Statistical analysis

A two-way (3 × 2) analysis of variance was performed using the SAS GLM procedure (SAS Institute Inc., Cary, NC, USA) to determine the effect of diet and fat cover depth on animal performance and on the fatty acid profile of the intramuscular fat. Statistical differences between treatments were assessed using Tukey's test, significance being determined at $P < 0.05$. The MIXED procedure was applied to calculate the least square means and the standard error for meat color during storage, with diet, fat cover depth, and time as fixed effects and animal as the random effect in the model.

3. Results and discussion

The ingredients and chemical composition of the concentrates, including the key fatty acid composition, are summarized in Table 1. The three concentrates were formulated to be isocaloric and isoproteic. Compared with the two concentrates that contained 5% linseed, the fatty acid (FA) composition of the control concentrate had a higher percentage of saturated and monounsaturated FAs and a lower percentage of polyunsaturated FAs. The result was a polyunsaturated to saturated FA ratio of 3:2 in the control diet and nearly 6 in the linseed-containing concentrates. The percentage of n − 3 fatty acids was less than 1 in the control diet and 11 in the other two diets, yielding a very high n − 6 to n − 3 ratio in the control group and a ratio of between 5 and 6 in groups L and L + E. The percentage of α-linolenic acid (ALA, 18:3 n − 3 c9, c12, c15) was 0.4 in the control diet and about 11 in the other two diets, while the percentage of linoleic acid (C18:2 n − 6 c9, c12) was similar in both types of concentrate (62.2 vs. 62.4 and 64.0).

3.1. Animal performance

The average weight of the calves, aged 203 ± 31.6 days, at the start of the experiment was 278 ± 42.1 kg, with non-significant differences between the various dietary and fat cover depth groups (Table 2). There were no significant differences in slaughter weight between the dietary groups ($P = 0.35$), mean weight being 441 ± 57.1 kg at a mean age of 319 ± 42.9 days, corresponding to the yearling commercial category designation. By contrast, there were significant differences ($P < 0.001$) for weight and age at slaughter with fat cover depth. The young bulls slaughtered at a dorsal fat layer thickness of 3 mm had a lower weight (402 kg) and were younger (289 days) than the young bulls slaughtered at a fat cover thickness of 4 mm (481 kg and 349 days). The average daily gain of the young bulls was not significantly different between the diets or levels of fattening at slaughter ($P > 0.05$). The daily weight gain values were somewhat lower than expected based on the potential exhibited by the Pirenaica breed according to results previously obtained with animals finished using concentrate (Albertí, Sañudo, & Santolaria, 1995; Albertí et al., 1997; Blanco, Villalba, Ripoll, Sauerwein, & Casasús, 2009; Piedrafita et al., 2003), which consistently attained 1.7 kg/d. Still, the gain was similar to results obtained for animals fed a concentrate diet and slaughtered at less than 500 kg live weight (Panea, Olleta, Sañudo, Campo, & Piedrafita, 1999). The lower growth recorded in the current experiment might be attributable to the time of year when the experiment took place, in the summer, when high indoor temperatures could depress feed intake (Koknaroglu, Otles, Mader, & Hoffman, 2008), thus lowering the daily gain (Hoffman & Self, 1973; Mader, Holt, Hahn, Davis, & Spiers, 2002).

Concentrate intake expressed as dry matter by the bulls slaughtered at a fat cover depth of 3 mm was 5.5 kg/d for the control group, 6% lower than the value for the groups that included linseed in the diet (5.8 kg/d group L; 5.9 kg/d group L + E). Concentrate intake levels for the experimental groups slaughtered at a fat thickness of 4 mm were similar (6.2 kg/d group C, 5.9 kg/d group L, and 6.2 kg/d group L + E) but slightly higher than for their counterparts slaughtered at the lower fat thickness.

The dressing percentage for the young bulls in group C was 62.6%, significantly higher ($P < 0.05$) than the value of 60.4% for the bulls in group L, while at 61.1% the dressing percentage for the young bulls in group L + E was intermediate between the two. The young bulls slaughtered at the higher of the two fat cover depths had a dressing percentage one point better than that for the bulls slaughtered with the thinner fat layer, although the difference was not significant ($P > 0.05$). This unexpected lower dressing percentage for the bulls fed linseed-containing concentrate might be a result of the high mucilage content of linseed. Mucilage can absorb large amounts of water and thus lead to increased gut fill, decreasing the yield. Dressing performance in animals of the same breed fed similar diets tends to rise with slaughter weight (Sami, Augustini, & Schwarz, 2004; Short et al., 1999), since the internal organs and skin contribute proportionally less to body weight. This improvement in dressing performance is not linear, however, being more evident at lower slaughter weights. Breed also has a significant effect. Highly specialized beef breeds like Belgian Blue, Limousin, and Piemontese have dressing percentage values above 63%, beef breeds values around 60–62%, rustic breeds around 56–59%, and dairy breeds like Holsteins values below 55% (Albertí et al., 2008; Chambaz, Scheeder, Kreuzer, & Dufey, 2003; Piedrafita et al., 2003).

There were no significant differences in final muscle pH at 24 h between the dietary or fat cover groups (Table 2). The mean pH of 5.5 ± 0.06 reflects the low levels of stress before slaughter, ensuring no detrimental effects on meat quality (De Smet, Raes, & Demeyer, 2004).

Concentrate type did not affect the chemical composition of the meat, with no significant differences in dry matter, protein, fat, or minerals being found (Table 3). However, the bulls in group C had a lower percentage of intramuscular fat (0.9%) in the *Longissimus dorsi* muscle than the bulls in the other groups that were fed linseed (1.3%), though

Table 2
Performance and slaughter traits of young Pirenaica bulls (mean values).

	Concentrate (CN)			Fat cover (F)		Pooled S.E.	P-value		
	C	L	L + E	3 mm	4 mm		CN	F	CN × F
N	14	16	16	23	23				
Initial live weight, kg	288.6	273.5	273.5	278.8	277.4	15.93	0.568	0.921	0.999
Slaughter weight, kg	450.2	429.4	446.5	402.0b	481.3a	15.19	0.350	0.001	0.943
Average daily gain, kg	1.39	1.35	1.52	1.42	1.42	0.073	0.064	0.956	0.943
Dressing %	62.6a	60.4b	61.1ab	60.8	61.8	0.75	0.022	0.089	0.698
pH (24 h)	5.51	5.48	5.48	5.49	5.48	0.023	0.211	0.605	0.731

a–b: values with different letters in the same row statistically different at $P < 0.05$.
C: control concentrate group; L: linseed-supplemented concentrate group; L + E: linseed + 2% vitamin E-supplemented concentrate group.

Table 3
Meat composition (fresh matter) of the *Longissimus dorsi* muscle from young Pirenaica bulls fed three concentrate diets and slaughtered at two dorsal fat cover depths.

	Concentrate (CN)			Fat cover (F)		Pooled S.E.	P-value		
	C	L	L + E	3 mm	4 mm		CN	F	CN × F
Dry matter (%)	24.5	25.0	24.7	24.3b	25.1a	0.31	0.180	0.004	0.327
Crude protein (%)	22.3	22.3	22.0	22.0	22.4	0.37	0.559	0.175	0.355
Ether extract (%)	0.9	1.3	1.3	1.0b	1.4a	0.25	0.118	0.020	0.646
Vitamin E (mg/kg)	0.81b	1.06ab	1.52a	0.97b	1.32a	0.207	0.005	0.050	0.787

a–b: values with different letters in the same row statistically different at $P < 0.05$.
C: control concentrate group; L: linseed-supplemented concentrate group; L + E: linseed + 2% vitamin E-supplemented concentrate group.

the difference was not statistically significant. The lack of effect of linseed or vitamin E inclusion in the concentrates on intramuscular fat content is in agreement with previous results with similar diets on steers of Juárez et al. (2012). The percentage of dry matter in the meat from the young bulls slaughtered at a subcutaneous dorsal fat thickness of 4 mm was higher (25.1%, $P < 0.05$) than in the meat from the bulls slaughtered at a fat layer thickness of 3 mm (24.3%) and the percentage of intramuscular fat was also higher (1.4% vs. 1.0%, respectively). Extending finishing time generally increases fatness, and this is more evident for high-energy diets (Bruns, Pritchard, & Boggs, 2004; Sami et al., 2004). The low intramuscular fat percentage in these bulls confirms that Pirenaica cattle are a late-developing breed, consistent with previous results (Albertí et al., 2008; Indurain, Beriain, Goñi, Arana, & Purroy, 2006).

Vitamin E concentration in the muscle varied significantly with diet ($P < 0.01$) and fat cover depth ($P < 0.05$). Thus, the meat from the animals in the L + E group had a statistically higher level of vitamin E (1.52 mg/kg) than the meat from the animals in group C (0.81 mg/kg), which received no added ingredients. The value was also higher, though not significantly so, than in the meat from the bulls in group L (1.06 mg/kg), which did not receive added vitamin E. Deposition of vitamin E in the muscle represented an 88% increase over that in the animals in group C. The meat from the animals slaughtered at a subcutaneous fat thickness of 4 mm had a higher level of vitamin E deposition (1.32 mg/kg) than the meat from the other bulls (0.97 mg/kg). Since vitamin E is fat-soluble, the higher fattening level allowed more vitamin E to be deposited in those animals. In addition, vitamin E deposition is known to take place slowly (Arnold et al., 1992) so prolonging the finishing time might have helped increase vitamin E levels in the meat. The vitamin E concentration of 1.32 mg/kg would appear to be apt to ensure meat color stability, since other studies have reported that a minimum of 1.2 mg of α-tocopherol/kg is needed to increase shelf life (Liu et al., 1995).

3.2. Effect of concentrate and fat cover on the fatty acid profile of the intramuscular fat

The differences in concentrate composition did not significantly alter the percentage or content of saturated FAs (SFAs), monounsaturated FAs (MUFAs), or polyunsaturated FAs (PUFAs) in the intramuscular fat (Tables 4 and 5). The mean percentage values for the SFAs ranged from 23.2 to 24.9 g/100 g for palmitic acid, 19.2 to 20.7 mg/100 g for stearic acid and 2.1 to 2.4 mg/100 g for myristic acid, and these values were similar to those reported in other animals fed concentrate (de la Fuente et al., 2009). However, the total percentage of SFAs was higher than that recorded in other studies (Insausti et al., 2004; Moreno, Keane, Noci, & Moloney, 2008; Noci, O'Kiely, Monahan, Stanton, & Moloney, 2005; Partida, Olleta, Sañudo, Albertí, & Campo, 2007; Realini, Duckett, Brito, Dalla Rizza, & De Mattos, 2004; Varela et al., 2004). These discrepancies with respect to those other studies are attributable to differences in sex, breed and precocity, diet or production system, or slaughter weight. The percentage of SFAs in the total FAs was similar in the two fat cover groups.

Percentage of MUFAs in the intramuscular fat was not significantly different ($P > 0.05$) in the various groups. However, the bulls in the higher fat cover group had 32.7% MUFAs, higher than the 29.1% in the 3-mm fat cover group. This represented an increase of 69% in the MUFA content in the meat with respect to the low fat cover group (490.6 g/kg vs. 289.9 mg/kg $P > 0.001$). These results are in agreement with the FA composition reported in other studies for finished cattle as the percentage of the intramuscular fat increased (Moreno et al., 2008; Okumura et al., 2012) and in a comparison of young bulls from early and late-maturing breeds (Cuvelier et al., 2006). The significant increase recorded here was mainly ascribable to the increase in oleic acid, which rose from 17.9 mg/100 g to 21.3 mg/100 g ($P < 0.05$), a finding consistent with other studies (Camfield, Brown, Lewis, Rakes, & Johnson, 1997; Indurain et al., 2006; Moreno et al., 2006; Wood et al., 2008) and indicative of reduced levels of biohydrogenation caused by decreased ruminal pH, mainly for concentrate diets (Duckett, Wagner, Yates, Dolezal, & May, 1993). Increasing the percentage of MUFAs, and especially oleic acid, in beef fat is nutritionally beneficial to consumers, since this type of fat does not raise levels of low-density cholesterol or risk factors for coronary heart disease (EFSA, 2005).

Group L + E had a higher PUFA content than groups C and L, though the differences were not significant. An increase in PUFAs in animals fed a vitamin E-enriched diet including linseed was also reported by Juárez et al. (2011), who suggested alterations in hydrogenation.

The proportion of PUFAs in the young Pirenaica bulls in this experiment was much higher than in those of other Spanish breeds slaughtered at the same weight after finishing similar to that of the control group (Insausti et al., 2004). This high proportion of PUFAs could be partly attributable to the fact that Pirenaica are a late-maturing breed and the carcass and meat have a low overall fat content (Indurain et al., 2006), and therefore the proportion of phospholipids in the cell wall is greater than that of neutral lipids. Belgian Blue bulls fed diets containing linseed also had a high percentage (17.3%) of PUFAs in the intramuscular fat (Raes et al., 2004). Percentage of PUFAs tends to be lower in early-maturing breeds (Insausti et al., 2004). The percentage of PUFAs deposited in young Holstein bulls fed concentrate containing linseed ranged from 14.3% to 17.6% (Mach et al., 2006), and Holstein steers fed supplemented grass silage deposited 15.7% PUFAs (Warren, Scollan, Enser, et al., 2008).

The intramuscular fat in the bulls in group L contained 13.9% of n − 6 PUFAs, slightly less than the 16.6% in group L + E and also less than the 18.4% in the intramuscular fat in the in group C. However, these differences were not statistically significant. The n − 3 PUFA content and percentage in the animals fed a linseed-containing diet increased significantly, from 16.9 mg/100 g, or 1.9% of the FAs in group C to 31.7 mg/100 g (2.48%) in group L and 42.0 mg/100 g (3.1%) in group L + E ($P < 0.026$). Thus, linseed-containing diets increased the proportion of n − 3 PUFAs and tended to decrease the proportion of n − 6 PUFAs, significantly lowering the n − 6 to n − 3 ratio. The mean ratio for the two groups with linseed in the diet was 5.4, compared with 9.6 ($P < 0.001$) for the control group. Other studies have ascribed this increase in the proportion of n − 3 PUFAs in the intramuscular fat in animals fed feed containing whole linseed to partial protection of the FAs from biohydrogenation by the seed coat (Scollan, Dhanoa, et al.,

Table 4
Percentage fatty acid content (g/100 g fatty acids) of the intramuscular fat in the *Longissimus dorsi* muscle from young Pirenaica bulls fed three concentrate diets and slaughtered at two dorsal fat cover depths.

Fatty acid	Concentrate (CN)			Fat cover (F)		Pooled S.E.	P-value		
	C	L	L + E	3 mm	4 mm		CN	F	CN × F
SFAs	51.09	50.92	48.29	50.56	49.45	2.359	0.387	0.495	0.441
MUFAs	28.20	32.18	31.52	29.06b	32.74a	1.944	0.110	0.024	0.504
PUFAs	20.66	16.81	20.12	20.31	17.75	3.150	0.409	0.356	0.729
n − 6	18.38	13.87	16.57	17.29	14.84	2.774	0.267	0.316	0.667
n − 3	1.88b	2.48ab	3.09a	2.59	2.45	0.409	0.026	0.740	0.888
n − 6 to n − 3 ratio	9.64a	5.65b	5.23b	6.87a	6.39b	0.279	0.001	0.032	0.001
14:0	2.12	2.44	2.24	2.21	2.36	0.281	0.524	0.549	0.830
16:0	24.87	24.16	23.23	23.92	24.15	1.053	0.315	0.888	0.530
18:0	20.69	20.35	19.25	20.97	19.06	1.328	0.493	0.072	0.488
18:1-t11	6.54	6.71	6.78	6.74	6.63	0.900	0.963	0.970	0.383
18:1-c9	17.36	20.76	20.03	17.89b	21.30a	1.963	0.205	0.041	0.594
18:2-c9,t11	0.12	0.20	0.20	0.16	0.19	0.018	0.618	0.390	0.111
18:2-c9,c12	13.80	10.15	11.97	12.71	10.89	2.013	0.207	0.299	0.673
18:3-c9, c12, c15	0.51b	1.34a	1.65a	1.27	1.15	0.186	0.001	0.532	0.997
20:5-c5,c8,c11,c14,c17	0.20	0.27	0.28	0.27	0.24	0.068	0.478	0.653	0.832
22:5-c7,c10,c13,c16,c19	0.44	0.45	0.49	0.49	0.43	0.106	0.897	0.586	0.733
22:6-c4,c7,c10,c13,c16,c19	0.06	0.04	0.06	0.06	0.05	0.018	0.531	0.809	0.888

SFAs: saturated fatty acids.MUFAs: monounsaturated fatty acids.PUFAs: polyunsaturated fatty acids.a–b: values with different letters in the same row statistically different at $P < 0.05$.C: control concentrate group; L: linseed-supplemented concentrate group; L + E: linseed + 2% vitamin E-supplemented concentrate group.

Mean n−6 to n−3 ratio values explaining interaction between concentrate and fat cover depth, by group.

n − 6 to n − 3 ratio	C	L	L + E
3 mm	9.31	6.41	5.94
4 mm	9.97	4.79	4.92

C: control concentrate group; L: linseed-supplemented concentrate group; L + E: linseed + 2% vitamin E-supplemented concentrate group.

2001). The results of other experiments have shown that adding 10% ground linseed augmented the proportion of n − 3 PUFAs through an increase in the 18:3 n − 3 PUFAs (Juárez et al., 2011). Also, adding 8% whole, crushed, or ground linseed increased the concentration of n − 3 PUFAs in the phospholipid fraction and 18:3 n − 3 PUFAs in the neutral lipid fraction of the intramuscular fat (Maddock et al., 2006). Levels of n − 6 PUFAs in the intramuscular fat of the young bulls in the present study were above 14%, while concentrations of n − 3 PUFAs were between 2 and 3%, because, of the two main PUFAs, C18:2 n − 6 is deposited faster and in higher concentrations in the muscle than C18:3 n − 3 (Wood et al., 2008). The n−6 to n−3 ratios for the linseed-containing diets were lower than the ratio values of 14.7, 9.0, and 6.3 recorded for Holstein bulls fed concentrate containing 3.6%, 11.2%, and 18.0% linseed (Mach et al., 2006). Furthermore, the n − 6 to n − 3 ratio in the linseed-containing dietary groups was lower than the ratio recorded in Holstein bulls fed concentrate supplemented with mixed lard and tallow or with palm oil compounds or standard commercial concentrate (de la Fuente et al., 2009; Insausti et al., 2004; Partida et al., 2007). However, while adding linseed lowered the n − 6 to n − 3 ratio to close to or less than 5 compared with a commercial fattening diet, the ratio was not as low as for animals fed supplemented grass (French et al., 2000), grass or grass silage with different flax or fish oil supplements (Noci, Monahan, Scollan, & Moloney, 2007; Scollan, Choi, et al., 2001; Scollan, Enser, Gulati, Richardson, & Wood, 2003; Warren, Scollan, Enser, et al., 2008), or corn silage supplemented with linseed (Maddock et al., 2006; Raes et al., 2004).

At the higher fat cover depth the percentage of n − 6 PUFAs dropped, though not significantly, from 17.3% to 14.8%, while the percentage of

Table 5
Fatty acid content of the intramuscular fat in the *Longissimus dorsi* muscle (mg/100 g fresh matter) from young Pirenaica bulls fed three concentrate diets and slaughtered at two dorsal fat cover depths.

Fatty acid	Concentrate (CN)			Fat cover (F)		Pooled S.E.	P-value		
	C	L	L + E	3 mm	4 mm		CN	F	CN × F
SFAs	442.76	692.78	646.66	487.95b	733.50a	126.515	0.125	0.024	0.714
MUFAs	248.74	438.91	441.46	289.88b	490.65a	89.655	0.061	0.001	0.508
PUFAs	188.46	213.51	268.28	196.37	258.41	59.609	0.369	0.189	0.521
n − 6	168.17	175.24	219.98	166.36	214.32	50.267	0.493	0.223	0.454
n − 3	16.95b	31.67ab	41.96a	25.75	37.07	8.703	0.024	0.115	0.633
14:0	18.23	33.48	30.49	22.22b	34.47a	6.657	0.068	0.032	0.695
16:0	215.44	327.46	315.78	233.98b	354.32a	61.764	0.152	0.024	0.741
18:0	178.79	278.07	251.93	197.87b	287.12a	50.875	0.145	0.042	0.706
18:1-t11	59.33	91.81	85.81	65.39b	96.88a	17.596	0.168	0.031	0.476
18:1-c9	150.46	284.88	290.44	179.46b	324.36a	64.164	0.060	0.010	0.467
18:2-c9,t11	1.47b	3.57a	2.91ab	1.77b	3.79a	0.753	0.024	0.003	0.271
18:2-c9,c12	125.41	128.21	159.54	122.06	156.79	36.531	0.536	0.226	0.442
18:3-c9, c12, c15	4.38b	17.27a	23.05a	13.24	18.31	4.756	0.002	0.191	0.387
20:5-c5,c8,c11,c14,c17	1.80	3.23	3.58	2.57	3.35	0.911	0.142	0.274	0.787
22:5-c7,c10,c13,c16,c19	4.06	5.48	6.45	4.72	6.19	1.628	0.343	0.242	0.629
22:6-c4,c7,c10,c13,c16,c19	0.57	0.45	0.71	0.49	0.66	0.218	0.451	0.344	0.820

SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids.
a-b: values with different letters in the same row statistically different at $P < 0.05$.
C: control concentrate group; L: linseed-supplemented concentrate group; L + E: linseed + 2% vitamin E-supplemented concentrate group.

n−3 PUFAs did not change, and the n−6 to n−3 ratio decreased from 6.9 to 6.4 ($P < 0.03$). However, there was an interaction between diet and fat cover, because in the control group the n−6 to n−3 ratio increased with fattening, from 9.3 to 9.9, while in groups L and L + E the ratio decreased from 6.4 to 4.8 and from 5.5 to 5.2, respectively.

3.2.1. Individual fatty acids

The bulls slaughtered at the high fat cover depth tended to deposit more PUFAs in the meat (258.4 mg/100 g vs. 196.4 mg/100 g, $P > 0.05$) than the bulls with less fat cover, though the percentage relative to the total FAs tended to be lower (17.7 g/100 g vs. 20.3 g/100 g, $P > 0.05$).

The bulls in the groups fed a linseed-containing diet had a higher percentage of α-linolenic acid levels than the control bulls (1.3 and 1.6 vs. 0.51; $P < 0.001$) and three and four times the content in the meat (17.3 g/100 g and 23.0 g/100 g vs. 4.4 mg/100 g in group C, $P < 0.05$). Fat cover depth did not significantly affect either the α-linolenic acid content in the meat or the percentages of this acid in the total FAs.

Linoleic acid was the main PUFA. Neither percentage levels nor content in the meat changed significantly with concentrate composition or fat cover depth (mean values: 11.84 g/100 g FAs; 138.6 g/100 g muscle). Percentage of linoleic acid values varied substantially between animals in the same group, especially in the animals with the 3-mm dorsal fat layer (CV 54%), but the degree of variation tended to decrease in the animals with 4 mm of dorsal fat (CV 31%).

The content of conjugated linoleic acid isomer c9,t11 in the meat from groups L and L + E was twice that for the control group. Greater fat cover depth heightened both this difference and its level of significance. The relative percentage of long chain FAs and the content in the meat were both low and did not vary with diet or fat cover depth.

The findings for the relative percentage composition of the intramuscular FA content in this study were in agreement with the results for late-maturing breeds. Thus, the proportion of PUFAs tends to be higher at lower degrees of fat cover, with the ratio of MUFAs increasing and the proportion of SFAs either increasing or holding more or less steady as fat deposition proceeds (Duckett et al., 1993). The results of this experiment show that including linseed in concentrate fed to young bulls increased the deposition of α-linolenic acid and n−3 fatty acids and thus significantly lowered the n−6 to n−3 ratio. Therefore, the FA content in the meat of the bulls fed concentrate with linseed was within the ranges recommended for consumers. Moreover, at the higher fat cover depth percentage deposition of oleic acid in the meat rose more than that of the SFAs, further lowering the n−6 to n−3 ratio, and hence levels in the meat in line with recommendations for a healthy diet.

3.3. Color of beef packaged in oxygen-permeable film wrap during storage

Beef color measurements taken over the 14-day storage period indicated that color was not influenced by the type of diet. Subcutaneous fat cover depth exerted a partial influence, but meat color was mainly affected by storage time (Table 6). The meat was bright red in color, characterized by a high lightness value (42.5), a low redness value (11.7), and a relatively high yellowness value (14.0) [data not shown], with a suitable chroma value of 18.1 and a high hue value of 50.0. The lightness and chroma values of the meat at 48 h were similar to those recorded for the meat of yearling bulls and females of the same breed fed commercial concentrate slaughtered at 478 kg and stored in comparable conditions (Albertí, Ripoll, Casasús, Panea, & Blanco, 2011). Meat color attained its optimum appearance at 24 h; afterwards lightness and hue remained stable, while redness, yellowness, and chroma gradually decreased. The acceptable color values over the storage period and the lack of influence of linseed were consistent with the results obtained in other studies on finishing with concentrate diets including 10% or 11% linseed (Grobbel, Hunt, Seyfert, & Drouillard, 2006; Mach et al., 2006).

The meat from the young bulls slaughtered at a dorsal fat thickness of 4 mm had lower lightness ($P < 0.05$) and higher chroma ($P < 0.01$) values than the meat from the animals in the 3-mm dorsal fat cover thickness group, though the meat from both groups of carcasses had chroma values close to 18, indicating that meat appearance should earn good consumer acceptability (MacDougall, 1982). Mandell, Maclaurin, and Buttenhan (2001) found that the a* and b* values of the lean color of Holstein bulls tended to increase as carcass weight and back fat increased. Similarly, Dunne, Keane, O'Mara, Monahan, and Moloney (2004) and Warren, Scollan, Nute, et al. (2008) also found that beef brightness values tended to decrease and the a* and b* and chroma values tended to increase, though not always consistently, with live weight and age.

Changes in myoglobin during the storage period followed the expected trend. Metmyoglobin increased gradually from 2.8% at 24 h to 14.7% at 336 h at the expense of oxymyoglobin, which decreased from 67.5% to 57.2%, while deoxymyoglobin held steady at around 29%, similar to the results obtained in other studies (Lagerstedt, Lundström, & Lindahl, 2011). The percentage of metmyoglobin increases during storage as a result of oxidation of the pigments and is manifested by the appearance of dark or discolored areas; at the same time, the L*, a*, b*, and chroma values decrease (Camo, Lorés, Djenane, Beltrán, & Roncalés, 2011; Insausti et al., 1999; Lagerstedt et al., 2011), while hue increases sharply (Liu, Scheller, Arp, Schaefer, & Frigg, 1996). Consumers reject meat at metmyoglobin levels of 20% (MacDougall, 1982), and hence, with metmyoglobin levels at 15% after 14 days in this experiment, meat appearance was still acceptable. This finding was also consistent with the chroma values, because various authors have reported that consumers prefer chroma values higher than 17–18, similar to the present results (MacDougall, 1982; Ripoll, Panea, & Albertí, 2012).

The absence of any effect of diet on the proportions of the SFAs, MUFAs, and PUFAs in the intramuscular fat may account for the color stability of the meat. Thus, there was no intrinsic factor like high PUFA rancidity levels contributing to oxidation of the heme pigments. Additionally, light is known to be a potent oxidant, with photons helping to trigger oxidation, but since the meat was stored in darkness in this experiment, light did not act to heighten oxidation (Djenane, Sánchez-Escalante, Beltrán, & Roncalés, 2001). Therefore, as an indicator of shelf life, the meat color exhibited the expected pattern for satisfactory appearance and demonstrated that including 5% linseed in concentrate

Table 6
Beef color of the *Longissimus dorsi* muscle from young Pirenaica bulls fed three concentrate diets and slaughtered at two fat cover depths during storage.

	Concentrate (CN)			Fat cover (F)		Time in d (T)					Pooled S.E.	P-value						
	C	L	L + E	3 mm	4 mm	1	2	6	9	14		CN	F	T	CN × F	CN × T	F × T	CN × F × T
Lightness (L*)	42.2	42.4	42.6	43.1a	41.7b	42.2	42.1	42.7	42.8	42.2	0.25	0.890	0.044	0.066	0.617	0.614	0.001	0.764
Chroma (C*)	18.1	18.1	18.3	17.8b	18.6a	18.8a	18.4a	18.1ab	18.1ab	17.6b	0.29	0.748	0.008	0.001	0.536	0.246	0.101	0.469
Hue (h*)	49.4	50.6	50.6	50.8	49.6	50.5	50.1	50.0	50.0	50.4	0.29	0.491	0.167	0.648	0.964	0.278	0.148	0.468
Metmyoglobin (%)	7.9	8.5	8.4	7.3b	9.2a	2.8e	4.5d	9.1c	10.1b	14.7a	0.32	0.835	0.043	0.001	0.196	0.498	0.759	0.175
Oxymyoglobin (%)	62.1	62.2	63.1	62.7	62.3	67.3a	65.1b	61.8c	61.0c	57.2d	0.42	0.464	0.589	0.001	0.157	0.456	0.396	0.762
Myoglobin (%)	30.0	29.3	28.5	30.0a	28.5b	29.9ab	30.4a	29.0b	28.9bc	28.1c	0.57	0.141	0.021	0.007	0.952	0.406	0.898	0.954

a–d: values with different letters in the same row statistically different at $P < 0.05$.
C: control concentrate group; L: linseed-supplemented concentrate group; L + E: linseed + 2% vitamin E-supplemented concentrate group.

did not significantly alter meat color. Further, adding a higher than usual dose of vitamin E to the concentrate did not improve meat color stability. These results are in agreement with those of O'Sullivan et al. (2003), who found that dietary regimen had a lower effect on the shelf life of overwrapped meat than on that of meat packaged in a modified atmosphere because of the different changes in color taking place. While chroma values were initially higher in MAP (Vatansever et al., 2000), MAP subsequently induced lipid and myoglobin oxidation (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010). The results reported here also indicate that varying the concentrate composition to include linseed or vitamin E had a lower effect than storage time on the color of meat from finished young bulls, bearing out the findings reported by Juárez et al. (2012).

4. Conclusions

Adding linseed to the concentrate increased the percentage of n − 3 fatty acids, mainly α-linolenic acid, in the intramuscular fat and lowered the n − 6 to n − 3 ratio, thus yielding healthier beef for consumption. Vitamin E seemed to act synergistically with linseed to improve the fatty acid profile. Accordingly, augmenting the fat cover depth, and hence fat deposition, at slaughter raised the percentage of monounsaturated fatty acid content thanks to an increase in oleic acid. The alterations in the polyunsaturated fatty acid content did not have any detrimental effects on beef color stability during storage. In addition to these results, it needs to be considered that meat acceptance by consumers is conditioned by sensory quality and lipid oxidation, which will be the subject of a subsequent study.

Therefore, including linseed in concentrate for beef fattening would appear to be both advisable from a nutritional and health standpoint and commercially attractive to consumers.

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