



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.

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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 lincatine (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.

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, Gomersoro, 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 \times 200 mm, 3- μ m 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 μ l of trimethylsilyl-diazomethane at 40 °C for 10 min, dried under N₂, 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 \times 0.22 mm i.d. \times 0.2 μ m 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-

Table 1

Ingredients in the three concentrates fed to young Pirenaica bulls.

	C	L	L + E
<i>Ingredient (% feed)</i>			
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
<i>Chemical analysis (%DM basis)</i>			
Crude protein	16.4	16.6	16.6
Ether extract	5.6	5.6	5.6
Crude fiber	6.8	7.9	7.9
<i>Fatty acid composition (% total fatty acids)</i>			
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 Na₂SO₄, 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).

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