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Fatty acid composition of young Holstein bulls fed whole linseed and rumen-protected conjugated linoleic acid enriched diets

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ABSTRACT

Forty-eight young Holstein bulls were used to evaluate the effect of whole linseed and conjugated linoleic acid (CLA) supplementation on beef fatty acid profile in intramuscular and subcutaneous fat. Animals were fed one of four isoenergetic and isoproteic diets: control (0% linseed, 0% CLA), linseed (10% linseed, 0% CLA), CLA (0% linseed, 2% CLA), and linseed plus CLA (10% linseed, 2% CLA). The fatty acid profile had similar trends in intramuscular and subcutaneous fat when diets were enriched with linseed and/or CLA, increasing the level of CLA*c*9,*t*11 and α-linolenic acid (ALA), and decreasing the *n*-6/*n*-3 fatty acid ratio. Supplementation with linseed improved the fatty acid profile by increasing the proportions of *n*-3 and CLA fatty acids and decreasing the *n*-6/*n*-3 ratio compared with the control. CLA addition achieved similar CLA tissue levels to linseed supplementation, but similar *n*-3 proportions to the control. Linseed plus CLA supplementation was more effective in increasing total polyunsaturated fatty acids and CLA compared with their individual addition. It is concluded that supplementation of whole linseed and/or CLA in bulls resulted in an increase in beef fat of some fatty acids considered to be of potential benefit to human health.

1. Introduction

The manipulation of the fatty acid in ruminant fat to improve its profile is of major importance in meat research, because the fatty acid composition is related to differences in the nutritional value for human consumption. Several studies have shown that animal diet influences the fatty acid composition of meat. Some of the *n*-3 polyunsaturated fatty acid (PUFA) and conjugated linoleic acid (CLA) provide advantages to human health (Dilzer and Park, 2012; Ganesan et al., 2014; Mir et al., 2003). Thus, the supplementation of ruminant diets with PUFA rich lipids would be an effective approach to promote the enrichment of meat with CLA and *n*-3 PUFA.

Linseed is a natural source rich in *n*-3 FA (54.2% α-linolenic acid, ALA) and its seed coat may provide some protection to PUFA against rumen biohydrogenation and thus increase the passage of PUFA into the duodenum (Scollan et al., 2001). The effect of feeding with linseed to beef has been studied by several authors (Mapiye et al., 2013a, 2013b; Razminowicz et al., 2008), and increases of the proportions of ALA, eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) were observed.

CLA *cis-9*, *trans-11* is the major CLA isomer in meat products. The level of CLA *cis-9*, *trans-11* in beef is related to the amount of this isomer produced in the rumen and the synthesis in the tissue, by Δ9-desaturase, from ruminally produced *trans* vaccenic acid (Scollan et al., 2006). The CLA content can be increased by different dietary strategies, such as adding the CLA in rumen-protected forms to prevent rumen biohydrogenation of the CLA (Perfield et al., 2004). In this sense, some studies have evaluated the effect of the inclusion of rumen-ptrotected CLA on the FA profile of meat (Gillis et al., 2004, 2007; Schlegel et al., 2012).

Nevertheless, there is limited information on the effect of the addition of whole linseed plus rumen-protected CLA on the FA profile of beef tissues and on the FA with nutritional interest to human health. Thus, the objective of the current study was to examine the effects of feeding a concentrate diet supplemented with whole linseed, rich in *n*-3 fatty acids, and/or protected CLA on the fatty acid composition of intramuscular fat and subcutaneous

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adipose tissue of beef from young Holstein bulls.

2. Materials and methods

2.1. Animals and feeding

Forty-eight Holstein entire males (initial live weight 240 ± 0.7 kg and 199 ± 4.1 days old) were divided into four groups of twelve calves each, fed at the CITA experimental farm in Aragón (Spain). All four dietary treatments were formulated to be isoenergetic and isoproteic and had similar ether extract (7%) and starch (35%) contents, but differing in the percentage of added linseed and/or CLA (Lutrell® pure, BASF, Germany): control (C, 0% linseed, 0% CLA), whole linseed (L, 10% linseed, 0% CLA), CLA (CLA, 0% linseed, 2% CLA), and whole linseed plus CLA (L+CLA, 10% linseed, 2% CLA). The ingredients and chemical composition of the experimental diets are reported by Albertí et al. (2013). Bulls were fed to appetite and had free access to clean water. Furthermore, bulls were offered barley straw, which is an inextensive source of fiber intended to mitigate the metabolic disorders, such as acidosis, associated with high consumption of concentrates. Animals were cared for in accordance with EU (2010) Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes.

After a finishing period of 123 ± 11.2 days, the bulls (mean live weight 458.4 ± 16.6 kg) were slaughtered at an EU-licensed commercial abattoir following standard procedures. Animal productive performance and carcass characteristics of these animals were reported by Albertí et al. (2013).

2.2. Slaughter and sample collection

Slaughter weight was set at 450 kg live weight. The animals were transported 10 km for slaughter and they were dressed according to commercial practice.

At 24 h after slaughter, *Longissimus thoracis* (LT) steaks (100 g) and a sample of subcutaneous adipose tissue (10 g) were cut at the 6th rib level from the left half-carcass for the intramuscular and subcutaneous FA composition analysis. Fat and meat samples were vacuum-packaged in pouches of polyamide/polyethylene (120 mm and 1 cc/m^2 per 24 h O_2 permeability, 3 cc/m^2 per 24 h CO_2 permeability and 0.5 cc/m^2 per 24 h N_2 permeability, measured at 5 °C and 75% relative humidity) (Vaeseen Schoemarket Ind. Spain) and frozen and stored at −20 °C until lipid analysis. Before analyses, the steaks and SC adipose tissue samples were thawed at 4 °C overnight.

2.3. Fatty acid analysis

Total lipids from feeding samples and beef were extracted and hydrolyzed as described by Whittington et al. (1986) with some modifications by Aldai et al. (2005). All of the samples were analyzed in duplicate. The extracted FA were methylated using 200 μL of trimethylsilyl-diazomethane at 40 °C for 10 min, dried under N_2, dissolved, centrifuged and the supernatant transferred for analysis. The FA methyl esters were analyzed by gas chromatography (Agilent Technologies, Model 7890A, Wilmington, DE, US) using a BPX-70 (SGE U.K. Ltd.) fused-silica capillary column (120 m × 0.22 mm i.d. × 0.2 μm film thickness) and a flame ionization detector (FID) using hydrogen as 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. In these chromatographic conditions, C18:1*t*10 and C18:1*t*11 were unresolved and thus grouped. ChemStation software (Rev. B.03.01) recorded the chromatograms.

Fatty acid methyl esters were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco™ 37 Fame Mix 47885-U, US). For the identification of branched-chain FAME several standards from Matreya Inc. (Pleasant Gap, PA) were used. The isomers of conjugated linoleic acid (CLA) were identified using GC reference standard UC-60M and UC-61M from Nu-Check Prep Inc. (Elysian, MN), and no. 1256 and no. 1257 from Matreya Inc. (Pleasant Gap, PA). Fatty acid proportions were reported as a percentage of total FA identified.

Fatty acids in the intramuscular depot were quantified in muscle using calibration curves of the FAs. Calibration curves were plotted using diluted solutions with a range of concentration such that the concentration of FAs in the sample laid within the range. The curves consisted of a plot of peak area versus concentration. Linear regression analysis of absolute areas versus injected quantities of the FAs was used. The detection (LOD) and quantification (LOQ) limits of the analytical method were calculated from the calibration curves of the selected pure FAME and the mean noise value of ten repetitions of the blank (*n*-hexane) analysis. Fatty acid quantities were expressed as mg FA/100g muscle.

2.4. Statistical analysis

Data were analyzed as a completely randomized design using a general linear model (GLM) procedure (IBM-SPSS version 19 for Windows, 2011). The statistical model included the fixed effects of diet (D) and tissue (T), as well as diet × tissue interaction and residual error. When diet × tissue significant interactions were detected, differences between means were further analyzed by Tukey's test. Differences between means were considered to be significant at $P < 0.05$.

3. Results

3.1. Fatty acid profile of the experimental diets

The profile of the main FA of the different diets is shown in Table 1. Including linseed in the diet (diet L and diet L+CLA) markedly increased the C18:3*n*-3 (ALA) content at the expense of C16:0. Therefore, in the diets L and L+CLA the PUFA total content was significantly higher, whereas their SFA total content was significantly lower, compared with the diets C and CLA. The C18:2*n*-6 (LA) percentage was similar in the C, CLA and L+CLA concentrates (25–27 g/100 g of total FA), whereas in the L concentrate was significantly higher (35 g/100 g of total FA). Moreover, including CLA in the diet markedly increased the CLA*c*9,*t*11 (rumenic acid, RA,), CLA*t*10,*c*12 and CLA*t*9,*t*11 content compared with the C and L concentrates. The addition of linseed plus CLA (diet L+CLA) showed the lowest C18:1*c*9 content, and consequently total monounsaturated fatty acid (MUFA) content, compared with the others diets (C, L and CLA).

3.2. Tissue fatty acid profile

3.2.1. Polyunsaturated fatty acids

Table 2 shows the PUFA proportions (%) in intramuscular and subcutaneous fat of bulls on each dietary treatment. There was no interaction $D \times T$ ($P > 0.05$) for total PUFA. Differences in the total PUFA between the IM and the SC tissue were significant ($P < 0.001$), with higher concentrations of total PUFA in the IM (15.53%) than in the SC (3.75%) tissue.

The effect of diet was significant ($P < 0.05$) for the total PUFA. In the IM tissue, young bulls fed diets containing L plus CLA had higher proportions of total PUFA than bulls fed diets containing