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Dietary influence on the m. longissimus dorsi fatty acid composition of lambs in relation to protein source

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ABSTRACT

Dietary lipid effect, as a consequence of protein supplement, on lamb m. *longissimus dorsi* fatty acid composition was investigated, with emphasis on biohydrogenation intermediates. Crossbred lambs (White Swedish Landrace × Texel) were fed a barley-based diet without (CON) or with protein supplements including peas (PEA), rapeseed cake (RC) or hempseed cake (HC). The HC diet resulted in the highest muscle 22:6n-3 proportion, with the RC diet being similar ($P<0.05$). Protein supplement did not affect the *c*9,*t*11 conjugated linoleic acid (CLA) proportion, however the HC diet increased some minor CLA isomers, including *t*10,*c*12 CLA ($P<0.05$). The *t*10-18:1 and total *trans*-18:1 were lowest for the RC diet ($P<0.05$), likely relating to rumen conditions and precursor availability. The saturated, monounsaturated and branched-chain fatty acids were largely unaffected by protein supplement. In conclusion, feeding the RC diet lowered the *t*10-18:1 and total *trans*-18:1 in meat, and modestly increased 22:6n-3 content. The direction of these changes would be beneficial, making the RC diet the preferred protein supplement; however the magnitude of the changes in the present experiment may not be sufficient to have an impact on human health.

1. Introduction

Shorter production seasons in northern Europe encourage year-round indoor finishing of market lambs. Intensive feeding with concentrate-based diets has the added advantage of increasing animal production performance, in terms of average daily gain and feed efficiency, compared to forage-only diets (Jacques, Berthiaume, & Cinq-Mars, 2011). Cereal-based diets, however, generally require additional protein supplementation to meet the animals' nutritional requirements. Soybeans are largely imported into Europe, yet are the predominant protein supplement used in commercial livestock rations. Interest has increased in using locally produced legumes and oilseed cakes in animal rations to reduce reliance on imported feeds and associated environmental concerns. Feeding oilseed cakes and legumes also have the potential to supply polyunsaturated fatty acid (PUFA), which can either bypass or be subject to biohydrogenation in the rumen.

Dietary PUFA bypassing the rumen can reduce the proportion of hypercholesterolemic saturated fatty acid (SFA), namely 16:0, while increasing proportions of hypocholesterolemic monounsaturated (MUFA) and PUFA in ruminant meat (Demeyer & Doreau, 1999; Williams, 2000). Moreover, the proportion of 18:2n-6 and 18:3n-3 PUFA in the supplements can also influence the n-6/n-3 ratio in meat (Palmquist, 2009). The majority of dietary PUFA undergoes biohydrogenation, whereby dietary concentrations can affect the rate, extent and pathway used for biohydrogenation (Demeyer & Doreau, 1999). Lamb can be one of the richest sources of PUFA biohydrogenation intermediates in the human diet (Schmid, Collomb, Sieber, & Bee, 2006), and of these, there has been interest in increasing the content of vaccenic (*t*11-18:1) and rumenic acids (*c*9,*t*11-CLA) due to their purported health effects (Field, Blewett, Proctor, & Vine, 2009; Park & Pariza, 2007). Production strategies to improve animal performance can, however, be counter-productive in terms of fatty acid composition. Feeding concentrate high in rapidly fermentable starch can shift biohydrogenation pathways towards *t*10-18:1 instead of vaccenic and rumenic acid (Bauman, Baumgard, Corl, & Griinari, 1999; Radunz et al., 2009), which is undesirable as *t*10-18:1 has been found to negatively impact blood cholesterol profiles in laboratory animals (Bauchart et al., 2007; Roy et al., 2007).

Previously, the growth performance of lambs supplemented with peas was similar to those supplemented with either faba beans or soybean meal, with the added benefit of higher PUFA content and lower n-6/n-3 ratio of the meat, owing to the higher 18:3n-3 content of peas (Scerra et al., 2011). Limited reporting of biohydrogenation products indicated that the protein supplements did not affect the *t*11-18:1 concentration, however the pea diet increased the *c*9,*t*11-CLA by more than three-fold. This finding is unusual in that muscle concentrations of *c*9,*t*11-18:2 and *t*11-18:1 have been shown to be

Abbreviations: BCFA, branch chained fatty acids; CLA, conjugated linoleic acid; DM, dry matter; FAME, fatty acid methyl esters; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid.

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highly correlated in lamb (Nuernberg et al., 2005) and in beef (Aldai, Dugan, Rolland, & Kramer, 2009). Differences in diet fermentation kinetics may have contributed to these unusual findings (Moate, Boston, Jenkins, & Lean, 2008). Overall, the effects of protein supplements on levels of PUFA biohydrogenation products in lamb have not been intensively investigated. In Sweden, locally produced peas, rapeseed cake and hempseed cake are readily available protein supplements and their effects on animal performance were recently compared (Karlsson & Martinsson, 2011). The present study is an extension of Karlsson and Martinsson (2011), and reports protein supplement effects on fatty acid composition of lamb m. *longissimus dorsi*. In addition to examining the consistency of pea effects on biohydrogenation profiles in lamb, the effects of the unsaturated fatty acid (UFA) content of hempseed cake and rapeseed cake supplements were of interest. Hempseed is unique compared to typical oilseeds, having significant proportions of 18:3n-6 and 18:4n-3 (Callaway, 2004), which, if integrated, may circumvent the initial rate limiting step towards the formation of long-chain PUFA in tissues (Guillou, Zadravec, Martin, & Jacobsson, 2010). Rapeseed, on the other hand, is rich in *c*9-18:1, with a favourably low n-6/n-3 ratio. Consequently, the overall objective was to determine which protein source would promote the healthiest lamb fatty acid profile while providing optimal animal performance.

2. Materials and methods

2.1. Animal diets and sample collection

Experimental diet compositions and animal production performance were reported by Karlsson and Martinsson (2011). Briefly, forty-eight crossbred (White Swedish Landrace × Texel) ewe lambs, 87 ± 9 days old, were divided into four treatment groups and fed experimental diets for 8 weeks. The basal diet was barley-based and included 100 g hay/kg diet on a dry matter (DM) basis. Supplements replaced a proportion of the barley to balance the protein content of the supplemented diets. The non-supplemented control (CON) diet contained 13.0 MJ metabolisable energy (ME), 112 g/kg DM crude protein (CP) and 26 g/kg DM crude fat (CF). Supplemented diets included pea (PEA, 13.4 MJ/kg DM, 161 g/kg DM CP, 22 g/kg DM CF), rapeseed cake (RC, 13.4 MJ/kg DM, 162 g/kg DM CP, 63 g/kg DM CF) or hempseed cake (HC, 12.2 MJ/kg DM, 160 g/kg DM CP, 47 g/kg DM CF). The CF content of oilseed supplemented diets was near the recommended 6% limit for healthy rumen function (Bauman, Perfield, de Veth, & Lock, 2003). Barley and peas were crushed, rapeseed cake was expeller-pressed and heat treated, whereas hempseed cake was cold-pressed. All diets included 6 g/kg DM mineral–vitamin premix. Animals were fed twice daily and had free access to water (Karlsson & Martinsson, 2011) Feedstuffs were sampled weekly and composited for fatty acid analysis. Lambs were slaughtered commercially and m. *longissismus dorsi* samples were collected randomly from 22 lambs (PEA and RC diets, n = 6; CON and HC diets, n = 5). Samples were frozen and transported on dry ice and stored at −80 °C until further analysis.

2.2. Lipid extraction and fatty acid analysis

Feedstuffs were dried at 60 °C for 20 h and ground through a 1 mm screen using a hammer mill (Slagy 200, Kamas Kvarnmaskiner AB, Malmö, Sweden). Feedstuff samples (0.5 g) were directly methylated according to the procedure of Sukhija and Palmquist (1988). Lipids from meat samples (5 g) were extracted by homogenising in hexane:isopopanol (3:2 v/v) (Hara & Radin, 1978). Total lipid extract was determined gravimetrically. Fatty acid methyl esters (FAME) were prepared from 10 mg of extracted lipid using both base (sodium methoxide, at 50 °C) and acid (methanolic HCl, at 80 °C) catalysed methylation as described by Cruz-Hernandez et al. (2004).

The FAME, including CLA isomers, were then analysed using methods described by Cruz-Hernandez et al. (2004) and Kramer, Hernandez, Cruz-Hernandez, Kraft, and Dugan (2008). For GC analysis, 1 μL of FAME (1 mg/ml) was injected using a 20:1 split onto a CP-Sil88 column (100 m, 25 μm ID, 0.2 μm film thickness; Varian Inc., Walnut Creek, CA, US) in a CP-3800 gas chromatograph equipped with a 1079 split/splitless injector (Varian Inc., Walnut Creek, CA, US). Hydrogen was used as the carrier gas under constant pressure (25 psi, initial flow rate of 1 ml/min) and injector and flame ionisation detector temperatures were held at 250 °C. Each sample was analysed using two GC temperature programmes as described by Kramer et al. (2008) to permit analysis of non-CLA FAME including individual *trans*-18:1 isomers. The CLA isomers were analysed by HPLC (Prostar 230 HPLC, equipped with a Prostar 410 autosampler and a Prostar 335 photo-diode-array detector; Varian Inc., Walnut Creek, CA, US) using three 250 mm × 4.6 mm Chromspher 5 lipids columns (Varian Inc., Walnut Creek, CA, US) hooked in series in a TS-43 column oven (Phenomenex ThermaSphere, Torrance, CA, US) maintained at 25 °C. The flow rate of mobile phase (hexane with 0.1% acetonitrile, 0.5% diethyl ether in hexane) was 1 ml/min. Samples (10 μl) were injected at a concentration of 25 mg/ml and CLA isomers were detected at 233 nm as described by Cruz-Hernandez et al. (2004).

Separation of *t*7,*c*9- and *c*9,*t*11-CLA by conventional GC/Ag^+-HPLC was compared to a rapid method using a 30 m SLB IL 111 GC column (0.25 mm, 0.2 μm film thickness, Supelco Inc., Bellefonte, PA, US) as described by Turner, Rolland, Aldai, and Dugan (2011). For FAME identification, the Nu-Chek Prep Inc. (Elysian, MN, US) GLC-463 and UC-59M CLA and Supelco bacterial acid mix (47080-U, Sigma-Aldrich, Oakville, ON, CAN) standards were used. The CLA isomers and other FAME not included in the standards were identified by their retention times and elution orders reported in literature (Cruz-Hernandez et al., 2004; Delmonte et al., 2011).

2.3. Statistical analysis

Muscle fatty acid data were analysed by one-way ANOVA as a completely randomised design using the Proc Mixed procedure of SAS v9.2 (SAS, 2001). Diet was used as the treatment effect, with individual animal as the experimental unit. The Kenward–Rogers option of SAS was used to calculate the degrees of freedom with statistical significance was set at $P<0.05$. Muscle fatty acid least square means and standard error of the means are presented in the tables. Comparison of *t*7,*c*9-CLA as a proportion of combined *t*7,*c*9- and *c*9,*t*11-CLA using conventional GC/Ag^+-HPLC and a 30 m SLB IL-111 ionic column were made using the Proc Reg and Proc Corr procedures of SAS v9.2 (2001).

3. Results and discussion

3.1. Dietary effects on muscle fatty acids

A summary of diet composition as reported by Karlsson and Martinsson (2011) and the fatty acid profiles of the feedstuffs are presented in Table 1. Supplemented diets were isonitrogenous, but varied in metabolisable energy and fat content. Nutrient differences between the diets complicate evaluations of the meat fatty acid profile, but were considered inherent factors of the trial when comparing high oil and high starch protein supplements. As a consequence, the RC and HC diets had about a 2-fold higher crude fat content than the CON and PEA diets, having foreseeable influence on the meat fatty acid profile. The RC diet provided the most *c*9-18:1 and had appreciable amounts of 18:2n-6 and 18:3n-3. The HC diet provided the most 18:2n-6, with an 18:3n-3 amount similar to the RC diet, and was unique in providing 18:3n-6 and 18:4n-3. The n-6/n-3 ratio of RC and HC diets were similar, being lower than that of PEA and CON diets.