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Adipose tissue and muscle fatty acid profiles of steers fed red clover silage with and without flaxseed

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ABSTRACT

The current study examined the effects of inclusion of flaxseed in a red clover silage diet on growth, carcass quality, adipose tissue and muscle fatty acid (FA) profiles of beef steers. Twelve British × Continental crossbred steers (363 ± 26.5 kg) were randomly assigned to two pens and fed diets containing 70% red clover silage, 15% steam rolled barley/vitamin–mineral supplement and either additional 15% steam rolled barley or 15% triple rolled flaxseed (all DM basis) for 215 d. Diet had no effect ($P > 0.05$) on average daily gain or carcass quality atributes. Overall, FA profiles in all tissues followed similar trends when flaxseed was added to the diet but intramuscular fat had the highest proportions of *n*-3 and *n*-6 polyunsaturated FA (PUFA) while subcutaneous fat had largest proportions of conjugated linoleic acids (CLA) and perirenal fat had the highest proportions of *trans*-18:1 isomers, particularly vaccenic acid (VA). Feeding flaxseed increased ($P < 0.05$) percentages of α-linolenic acid (ALA; 1.1–1.6% of total FA), total *n*-3 PUFA, conjugated linolenic acids, non-conjugated linoleic acids (mostly *t*11,*c*15-18:2), and led to unprecedented increases in CLA (mainly rumenic acid, RA; 1.1–2.9%), and *trans*-18:1 isomers (chiefly VA; 5.9–9.5%) in muscle and adipose tissues. Flaxseed also reduced individual and total *n*-6 PUFA, *cis*-monounsaturated FA, branched-chain FA and saturated FA (mainly 16:0) in beef lipids. Overall, feeding flaxseed in a red clover silage diet enhanced the levels of FA with a positive health image (ALA, 2-fold; RA, 3 to 4-fold and VA, 4 to 5-fold) and reduced the levels of less desirable FA (*n*-6 PUFA; 14:0, 16:0) in muscle and adipose tissues without adverse effects on growth and carcass quality. Differences in tissue FA composition were also noted, and high levels of VA in perirenal fat in particular have potential for use in testing for health effects and development of value added beef and other food products.

1. Introduction

Owing to increased consumer awareness of the linkage between diet and health, research has focused on manipulating the fatty acid (FA) composition of beef to achieve a FA profile consistent with consumer perceptions and health recommendations. Among the FA, considerable attention has been given to omega-3 (*n*-3) polyunsaturated FA (PUFA)

Abbreviations: ADG, average daily gain; ALA, α-linolenic acid; BCFA, branched-chain fatty acid; *c*, *cis*; CLA, conjugated linoleic acid; CLnA, conjugated linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FAME, fatty acid methyl ester; LA, linoleic acid; LT, longissimus thoracis; MUFA, monounsaturated fatty acid; *n*-3, omega-3; *n*-6, omega-6; NCLA, non-conjugated linoleic acid; OCFA, odd-chain fatty acid; PUFA, polyunsaturated fatty acid; RA, rumenic acid; SFA, saturated fatty acid; *t*, *trans*; VA, vaccenic acid.

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such as α-linolenic acid (18:3*n*-3, ALA), eicosapentaenoic acid (20:5*n*-3, EPA) and docosahexaenoic acid (22:6*n*-3; DHA) and rumen derived PUFA biohydrogenation products, mainly rumenic acid (*c*9,*t*11-18:2, RA) and its precursor vaccenic acid (*t*11-18:1, VA). Omega-3 PUFA (ALA, EPA and DHA) have been reported to reduce risk of cardiovascular disease, Alzheimer disease, obesity, type II diabetes, osteoporosis, dry eye syndrome (McAfee et al., 2010; McNeill and Van Elswyk, 2012), and depression in adolescents with eating disorders (Swenne et al., 2011). Rumenic acid, the main natural isomer of conjugated linoleic acid (CLA) has been shown to possess cardio-protective, anti-carcinogenic, anti-atherogenic, anti-inflammatory and positive immune modulatory properties (Benjamin and Spener, 2009). Vaccenic acid may impart health benefits beyond those associated with its desaturation to RA including reducing pro-inflammatory cytokines and platelet aggregation in humans (Sofi et al., 2010), and plasma triglycerides in animal models (Roy et al., 2007). In this context, a range of nutritional strategies including feeding oilseeds rich in PUFA (*e.g.*, ALA or linoleic acid) have been investigated for their potential to increase *n*-3 PUFA, VA and RA, but the reported net increases have not been substantial (see reviews by Dugan et al., 2011; Mir et al., 2003).

Initial attempts to increase VA and RA in beef included feeding PUFA sources in high grain diets, however, this approach promotes use of a PUFA biohydrogenation pathway with intermediates other than VA and RA (Dugan et al., 2011; Mir et al., 2003). This shift does not only limit the net amount of VA and RA, but also leads to changes in blood lipids in animal models which may negatively impact cardiovascular health (Roy et al., 2007). Recent potential strategies to improve beef fatty acid composition include feeding flaxseed in diets with an increased amount of forage (50% of DM; Nassu et al., 2011) over an extended period (20 weeks, He et al., 2012). Noteably, the type of forage can have overriding effects on levels of PUFA biohydrogenation intermediates with feeding grass hay yielding higher levels of VA and RA compared to barley silage (Nassu et al., 2011; He et al., 2012). Given the differences in tissue *trans* FA composition (Wistuba et al., 2007), examining the effects of forage type, level and duration of feeding in flaxseed supplemented diets on the levels of VA and RA in different beef tissues could also be important.

Feeding red clover silage, a rich source of polyphenol oxidase, has been shown to reduce the extent of lipolysis and biohydrogenation of dietary PUFA in the rumen (Van Ranst et al., 2011) and increase recovery of ALA in meat, as opposed to grass silage where polyphenol oxidase is absent (Lee et al., 2009). Inclusion of flaxseed in a red clover diet might, therefore, increase recovery of ALA and PUFA biohydrogenation intermediates in beef. To our knowledge, there have been no studies examining the effects of feeding flaxseed in a red clover silage diet on *n*-3 PUFA and biohydrogenation intermediates in different beef tissues. The objective of the current study was, therefore, to determine the effects of feeding flaxseed in a diet with a high level of red clover silage (70% of DM) to steers on growth and carcass quality attributes, adipose tissue and muscle FA composition.

2. Materials and methods

2.1. Animals and diets

Twelve British × Continental crossbred steers with an initial mean body weight of 363 ± 26.5 kg (mean ± standard deviation) were randomly assigned to two pens of six animals and fed experimental diets for 215 d from December 2010 to July 2011 at the Lacombe Research Centre, Alberta, Canada. Diets contained 70% red clover silage, 15% steam rolled barley/vitamin–mineral supplement and either additional 15% steam rolled barley or 15% triple rolled flaxseed (all DM basis). Steers in each pen were group fed and were capable of feeding at the feed bunk at the same time (0.8 m of space at the bunk per animal). Steers were fed to appetite and had free access to clean water. Animals were cared for in accordance with guidelines established by the Canadian Council on Animal Care (CCAC, 1993).

2.2. Feed sampling and analysis

Feed samples were collected weekly and stored at −20 °C, then pooled monthly before determination of DM, minerals, crude fat, crude protein (AOAC, 2003), and acid detergent fibre (Van Soest et al., 1991). Fatty acids from the finishing total mixed ration were extracted and methylated as described by Sukhija and Palmquist (1988) and analysed according to Dugan et al. (2007).

2.3. Growth performance, carcass quality measurements

Individual steer weights were measured monthly and average daily gain (ADG) was calculated by dividing each animal's weight gain by days on-test. Steers were slaughtered at target ultrasound subcutaneous fat depth of 6–8 mm between the 12th and 13th rib over the right *longissimus thoracis* (LT) muscle of each animal. Ultrasound backfat thickness was measured monthly by a certified ultrasound technician using an Aloka 500V diagnostic real-time ultrasound with a 17 cm 3.5 Mhz linear array transducer (Overseas Monitor Corporation Ltd., Richmond, B.C., Canada) following procedures described by Brethour (1992) and used to target slaughter date.

At slaughter, animals were stunned, exsanguinated and dressed in a commercial manner at the Lacombe Research Centre abattoir and samples of kidney (perirenal) fat were collected from the cranial–central part of the perirenal fat 20 min *post-mortem* during evisceration and stored at −80 °C until analysed for FA. Carcasses were split and chilled overnight at 4 °C. At 24 h *post-mortem*, carcasses were knife-ribbed at the Canadian grade site (between the 12th and the 13th ribs on the left side) and assessed for grade fat thickness, rib-eye area, estimated lean yield (Canadian Food Inspection Agency, 1992) and marbling (American Meat Science Association, 1990) by two certified beef graders. A 50 mm steak was removed from the grade site (caudal end of the LT), trimmed of epimysium tissue, subcutaneous and intermuscular fat, and comminuted using a Robot Coupe Blixir BX3 (Robot Coupe