Meat Science 89 (2011) 469–477

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

The effects of feeding flaxseed to beef cows given forage based diets on fatty acids of *longissimus thoracis* muscle and backfat

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ARTICLE INFO

Article history:
Received 8 January 2011
Received in revised form 11 May 2011
Accepted 16 May 2011

Keywords:
Cull cow
Flaxseed
Omega-3 fatty acids
Conjugated linoleic acids

ABSTRACT

This study was conducted to investigate changes in fatty acid profiles of beef cows fed grass hay or barley silage based diets, with or without flaxseed supplementation. Both flaxseed and hay feeding increased levels of α-linolenic acid (LNA; 18:3n-3) in *longissimus thoracis* and backfat ($P<0.001$). A forage type by flaxseed level interaction was observed for most LNA biohydrogenation intermediates ($P<0.05$) that indicated feeding hay combined with flaxseed led to the greatest levels of total conjugated linolenic acid, total conjugated linoleic acid, total non-conjugated dienes and total *trans*-18:1. Predominant biohydrogenation intermediates included *t*11,*c*15-18:2, rumenic acid (*c*9,*t*11-18:2) and vaccenic acid (*t*11-18:1).

1. Introduction

Over the past 100 years the n-6/n-3 fatty acid ratio in the human diet in North America has increased from 1–2 to 1 up to 20–30 to 1, a change which has coincided with an increase in the incidence of related chronic diseases including cardiovascular disease (Simopoulos, 1999). As a result of their linkage to human health, regulatory authorities have recently approved food labeling claims for total n-3 fatty acids, for example in Canada this level is ≥300 mg per serving (CFIA, 2003). Although this claim applies to all n-3 fatty acids, these compounds differ in their biological activity with long-chain (LC; >18 carbon) n-3 polyunsaturated fatty acids (PUFA) typically having greater potency (Bailey, 2009; Lunn & Theobald, 2006). The LC n-3 PUFA are most commonly associated with marine sources, but due to their limited intakes in several countries, alternative sources contribute substantially to their intake. For example, in Australia, beef accounts for 28% of LC n-3 PUFA intake (Howe, Meyer, Record, & Baghurst, 2006). Development of beef with enhanced levels of total n-3 fatty acids could, therefore, result in substantial increases in LC n-3 PUFA intake for humans, and provide an opportunity to add value to beef.

Flaxseed contains ~40% oil, and of this 50–60% is linolenic acid (LNA), making it one of the richest plant sources of n-3 fatty acids. Feeding flaxseed is known to increase levels of n-3 fatty acids in pork, poultry and dairy products, and consumption of these products has been demonstrated to help maintain red blood cell n-3 fatty acid levels in humans (Legrand et al., 2010). Feeding cattle flaxseed or flaxseed products also increases n-3 fatty acids in beef (Kronberg, Barcelo-Coblijn, Shin, Lee, & Murphy, 2006; Scollan et al., 2001), but enrichment in adipose tissue and meat is limited by bacterial biohydrogenation in the rumen (Raes, De Smet, & Demeyer, 2004). Biohydrogenation leads to extensive loss of unsaturated fatty acids and the accumulation of partial hydrogenation products such as vaccenic acid (VA, *trans* (*t*)11-18:1) and rumenic acid (RA, *cis* (*c*)9,*t*11-18:2) which have many purported health benefits (Field, Blewett, Proctor, & Vine, 2009; Park, 2009). Thus feeding flaxseed may also present opportunities for producing beef with enhanced levels of partial biohydrogenation intermediates of LNA.

In Canada, finishing youthful cattle (i.e. under 30 months of age) on forage is not common, but mature breeding animals are typically fed greater levels of forage. Among Canadian beef grades, cull cows have been shown to have higher levels of VA, RA and n-3 fatty acids compared to beef finished on high concentrate diets, which may present an economically viable opportunity to produce beef with enhanced levels of beneficial fatty acids (Dugan, Rolland, Aalhus, Aldai, & Kramer, 2008). The objective of the present experiment was to feed flaxseed to cull cows in a relatively high forage diet (i.e. 50:50 forage: concentrate, DM basis) and measure the accumulation of n-3

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0309-1740/$ – see front matter.
doi:10.1016/j.meatsci.2011.05.016

fatty acids and partial biohydrogenation products of PUFA in beef. Moreover, we wished to establish whether feeding barley silage or grass hay would influence beef fatty acid composition, as the influence of forage type on the fatty acid composition of ruminant products has received little attention (Chilliard, Ferlay, & Doreau, 2001), although there is some indication that forage type can interact with flaxseed to modulate milk fatty acid composition in dairy goats and cows (Chilliard & Ferlay, 2004; Shingfield et al., 2005) as well as affect beef fatty acid composition (Aharoni, Orlov, & Brosh, 2004; Mir et al., 2003).

2. Materials and methods

2.1. Animals and diets

Sixty-four British by continental crossbred (>30 months of age) non-lactating, non-pregnant beef cows with body weight (BW) averaging 620 ± 62 kg were used, and the feeding trial was conducted at the Lethbridge Research Centre. Animals were cared for according to the Canadian Council on Animal Care guidelines (CCAC, 1993). Cows were randomly assigned to four diets, with four pens of four cows per diet. Cows had ad libitum access to feed and water.

Diets were formulated to meet or exceed nutrient requirements for mature cows (Table 1; (NRC, 2000)). The diets had a 50:50 forage to concentrate ratio (DM basis) and were fed as total mixed rations. Diets included grass hay control (HC), barley silage control (SC), grass hay plus flaxseed (HF) and barley silage plus flaxseed (SF). Flaxseed was ground with barley in a 7:3 ratio through a 4 mm screen in a hammer mill. The ground material was screened, and whole flaxseeds recovered were estimated to account for approximately 2% of the flaxseed added to the diet. The flaxseed diets contained 15% flaxseed substituted for dry rolled barley (DM basis), resulting in a total dietary lipid content of ~6%. Diets were fed ad libitum for 20 weeks on average.

At the end of the feeding period, cows were shipped the day before slaughter to the Lacombe Research Centre (~6 h) and held overnight with access to water. Cows were slaughtered in groups of 16 with one pen of animals per diet per slaughter date and all animals were slaughtered within 30 d. Carcasses were chilled overnight at 2 °C. At 24 h post mortem, carcasses were knife ribbed between the 12 and 13th rib, and a 2.54 cm loin steak with overlying backfat was removed for fatty acid analyses.

Table 1
Experimental diets, nutrient concentration and fatty acid profiles in lipids extracted from experimental diets.

	Hay control (HC)	Hay + flaxseed (HF)	Silage control (SC)	Silage + flaxseed (SF)
Diet ingredients (g/kg)				
Barley	473.7	327.4	292.8	201.7
Barley silage	0.0	0.0	678.0	682.0
Grass hay	479.1	483.6	0.0	0.0
Flaxseed	0.0	141.3	0.0	87.1
Supplement[a]	47.2	47.6	29.2	29.3
Diet composition				
Dry matter, %	87.76	88.58	54.24	54.56
Crude protein, g/kg	136.5	153.9	126.4	143.7
NEm, Mcal/kg	1.69	1.73	1.77	1.81
NEg, Mcal/kg	1.06	1.08	1.15	1.17
NDF, g/kg	330.9	303.9	333.2	306.3
ADF, g/kg	196.6	199.6	183.9	186.9
Crude fat, g/kg	17.6	63.2	18.1	62.5
Fatty acid, % total FAME				
14:0	0.39	0.09	0.97	0.27
16:0	18.83	7.71	19.72	8.66
*c*9-16:1	0.14	0.07	0.17	0.08
18:0	2.37	2.57	1.93	2.54
*c*9-18:1	16.50	15.10	17.12	15.32
18:2n-6 (LA)	40.55	20.99	43.99	23.06
18:3n-3 (LNA)	19.24	52.46	14.03	48.96
SFA	22.17	10.61	23.18	11.71
MUFA	17.92	15.91	18.74	16.23
PUFA	59.91	73.48	58.09	72.06

NDF: Neutral detergent fiber; ADF: Acid detergent fiber; LA—linoleic acid; LNA; linolenic acid; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

[a] The supplement was composed of: 56.5% barley, 10% canola meal, 2% urea, 25% limestone, 3% salt, 0.066% VIT E 500, 1% premix, 0.05% flavor and 2.5% molasses, which provided to diets in 5% (in DM) and supply to 1 kg diet (in DM) with additional: 14.67 mg copper, 58.32 mg zinc, 26.73 mg manganese, 0.66 mg iodine, 0.23 mg cobalt, 0.29 mg selenium, 4825 IU vitamin A, 478 IU vitamin D and 32 IU vitamin E.

2.2. Fatty acid analysis

From the loin steak collected, 5 g of subcutaneous fat was sampled and the *longissimus thoracis* (LT) muscle was comminuted using a Robot Coupe Blixir BX3 (Robot Coupe USA Inc., Ridgeland, MS). Subcutaneous fat and a 15–20 g subsample of LT were stored at −80 °C for subsequent fatty acid analyses. Prior to analyses, lipid extractions from LT, fatty acid methylations from subcutaneous fat and LT, and GC and Ag+−HPLC analyses were conducted as described by Juárez et al. (2011). Specifically, subcutaneous fat (50 mg) was freeze-dried and directly methylated with 0.5 N sodium methoxide (Cruz-Hernandez et al., 2004). Intramuscular lipids were extracted from the meat samples with 2:1 chloroform: methanol using a 20:1 solvent to sample ratio (Folch, Lees, & Stanley, 1957). To derivatize all meat lipid classes, extracts were methylated using 5% methanolic HCl, and to correct for CLA isomerization, separate methylations with 0.5 N sodium methoxide were conducted. Fatty acid methyl esters (FAME) were analyzed using GC (acid and basic methylations) according to Kramer, Hernandez, Cruz-Hernandez, Kraft, and Dugan (2008) and CLA isomer analysis by Ag+−HPLC (basic methylation) according to Cruz-Hernandez et al. (2004). Additional biohydrogenation products of LNA, specifically *t*11,*t*15-18:2 and *c*9,*t*11, *t*15-18:3, were identified based on their published GC/MS characterization (Gómez-Cortés, Bach, Luna, Juárez, & de la Fuente, 2009) and their relative retention compared to known fatty acids.

2.3. Statistical analysis

Data were analyzed using the MIXED procedure of SAS version 9.2 (SAS, 2009) and the model included flaxseed supplementation, forage type and their interaction with slaughter date and pen nested within the interaction as random factors. Pen was used as the experimental unit. Significances were reported at $P<0.05$ and trends were reported at $P<0.10$. Fatty acids with concentrations less than 0.05% of total FAME were not reported in tables.

3. Results and discussion

3.1. Diets

The diet compositions are summarized in Table 1. Among the diets the amount of net energy, neutral detergent fiber and acid detergent fiber were similar. Crude protein contents met or exceeded cow requirements (NRC, 2000). Crude fat contents of flaxseed containing diets were approximately 45 g/kg greater than control diets. In the HC and SC diets, linoleic acid (LA, 18:2n-6) was the most abundant fatty acid at 40.6 and 44.0% of total fatty acids, respectively, followed by LNA, 16:0 and *c*9-18:1. In flaxseed diets (HF and SF), LNA was the most abundant fatty acid accounting for 52.5 and 49.0% of total fatty acids, respectively. Levels of PUFA biohydrogenation intermediates were negligible for all diets.