

Review: *Trans*-forming beef to provide healthier fatty acid profiles

Michael E. R. Dugan¹, Noelia Aldai², Jennifer L. Aalhus¹, David C. Rolland¹, and John K. G. Kramer³

¹Lacombe Research Centre, Agriculture and Agri-Food Canada, Lacombe, Alberta, Canada T4L 1W1 (e-mail: mike.dugan@agr.gc.ca); ²Instituto de Ganadería de Montaña, CSIC-ULE, Finca Marzanas, 24346, Grulleros, León, Spain; and ³Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada N1G 5C9. Received 28 April 2011, accepted 15 August 2011.

Dugan, M. E. R., Aldai, N., Aalhus, J. L., Rolland, D. C. and Kramer, J. K. G. 2011. **Review: *Trans*-forming beef to provide healthier fatty acid profiles.** Can. J. Anim. Sci. **91**: 545–556. *Trans* fatty acids are found naturally in foods, particularly in those derived from ruminant animals, such as beef and dairy cattle. Over the past few decades, human consumption of *trans* fatty acids has increased, but this has been mainly from products containing partially hydrogenated vegetable oils. The correlation of *trans* fatty acid consumption with diseases such as coronary heart disease has been cause for concern, and led to recommendations to reduce their consumption. *Trans* fatty acids, however, have differing effects on human health. Therefore, in foods produced from ruminant animals, it is important to know their *trans* fatty acid composition, and how to enrich or deplete fatty acids that have positive or negative health effects. This review will cover the analysis of *trans* fatty acids in beef, their origin, how to manipulate their concentrations, and give a brief overview of their health effects.

Key words: *Trans*, vaccenic acid, rumenic acid, biohydrogenation, beef

Dugan, M. E. R., Aldai, N., Aalhus, J. L., Rolland, D. C. et Kramer, J. K. G. 2011. ***Trans*-formation du bœuf pour une meilleure composition en acide gras.** Can. J. Anim. Sci. **91**: 545–556. Les acides gras *trans* existent à l'état naturel dans les aliments, particulièrement ceux issus des ruminants, tels les bovins de boucherie ou les bovins laitiers. Au cours des dernières décennies, on a assisté à une hausse de la consommation d'acides gras *trans* chez l'être humain, mais ceux-ci venaient essentiellement de produits renfermant des huiles végétales partiellement hydrogénées. La corrélation entre la consommation d'acides gras *trans* et certaines maladies comme l'insuffisance coronaire suscite des préoccupations et a entraîné la formulation de recommandations visant à en réduire la consommation. Cependant, les effets des acides gras *trans* sur la santé varient. Par conséquent, il est important de connaître la composition des acides gras *trans* des aliments dérivés des ruminants, ainsi que la façon d'accroître la concentration des acides gras bénéfiques et de diminuer celle des acides gras qui ne le sont pas. Cet article présente l'analyse des acides gras *trans* du bœuf, leur origine, les méthodes pour en modifier la concentration et un aperçu de l'incidence de ces acides sur la santé.

Mots clés: *Trans*, acide vaccénique, acide ruménique, biohydrogénation, bœuf

The occurrence of *trans* (*t*) double bonds in beef fatty acids has been known for over 80 yr, and for decades these were not considered to be of practical significance. Potential bioactivities of these fatty acids have recently driven development of methods for their analyses, and investigations into how their concentrations can be increased to provide health benefits to consumers. The present review will cover current methods for analyzing fatty acids containing *trans* double bonds, the origin of these fatty acids in beef, how their concentrations can be manipulated, and a brief overview of their health effects.

ANALYSIS OF FATTY ACIDS IN BEEF WITH *TRANS* DOUBLE BONDS

Prior to conducting an experiment where fatty acids from beef are to be analyzed, there are a number of important issues that need to be addressed. These include timing of sample collection, which tissue to sample, sampling procedure, weight of sample required, and how samples will be processed and stored prior to

further analyses. There are published methods on sample preparation, but their coverage is beyond the scope of the present review. Readers are directed to authoritative texts and reviews on the subject (Kramer et al. 2001; Christie 2003; Leray 2011). Irrespective of the preparation methods used, however, it is imperative that verifiable and accurate analytical methods are used that yield reliable results on which scientifically sound predictions and conclusions can be made.

The evaluation of beef fat composition is primarily based on determining its fatty acid composition. Most frequently, this involves derivatizing the fatty acids in the various lipid classes to their fatty acid methyl esters (FAME), which can then be analyzed by gas chromatography (GC), high performance liquid chromatography or a combination of both. Acid catalyzed methylation,

Abbreviations: CLA, conjugated linoleic acid; CLnA, conjugated linolenic acid; DDGS, dried distiller's grain plus solubles; FAME, fatty acid methyl esters; GC, gas chromatography; PUFA, polyunsaturated fatty acid; RA, rumenic acid; VA, vaccenic acid

typically with 5% methanolic HCl at 80°C for 1 h (Christie 2003), is a good general purpose method for preparing FAME. This procedure will derivatize most lipid classes, but it can result in *trans,trans* and methoxy artifacts of conjugated linoleic acid (CLA; Kramer et al. 1997; Murrieta et al. 2003). On the other hand, base-catalyzed methylation, typically with 0.5 N sodium methoxide at 50°C for 15 min (Yurawecz et al. 1999), will not form artifacts, but does not derivatize free fatty acids, N-acyl or ether linked lipids (Cruz-Hernandez et al. 2004, 2006b). For comprehensive analysis of beef fatty acids, a combination of acid- and base-catalyzed methylation procedures has therefore been recommended (Cruz-Hernandez et al. 2006b; Kramer et al. 2008). Analysis after mild alkaline hydrolysis followed by methylation of FFA with (trimethylsilyl) diazomethane (Aldai et al. 2006) or methanolic H₂SO₄ at a reduced temperature (O'Fallon et al. 2007) are also potential alternatives. When applying any method, however, there is always some variation between analysts and laboratories. It is, therefore, important to ensure the method chosen is working properly, for example, by checking to see if hydrolysis or methylations are complete or if any of the fatty acids are being unduly affected during derivatization.

When analyzing FAME, the method used is often tailored to the level of detail required while trying to keep things simple, accurate and economical. If the goal is to measure major fatty acids in beef, this can be done using GC with a short column (e.g., 30 m) in less than 20 min. Major fatty acids present at more than 3–4% of total FAME in beef lipids typically differ in carbon chain length and/or number of double bonds. Relatively large differences in boiling point and polarity between the major fatty acids allows for their relatively simple analyses using GC. Although knowing the concentration of major fatty acids in beef is of considerable importance, current emphasis is on being able to analyze geometric and positional isomers of fatty acids that are generally present at low concentrations. To do so, long highly polar GC columns (i.e., 100–120 m) and multiple separations are conducted using complementary temperature programs (Kramer et al. 2008). In addition, silver ion chromatography (thin layer chromatography, high performance liquid chromatography or solid phase extraction) have been employed to resolve FAME containing different numbers of double bonds and *cis/trans* (*c/t*) configurations (Cruz-Hernandez et al. 2004; Kramer et al. 2008). Currently, the use of ionic GC columns is also being investigated to provide complementary GC separations (Delmonte et al. 2011). The major limitation in GC analysis of FAME has been the lack of authentic standards for a number of biohydrogenation intermediates. Using GC/MS can overcome some of the difficulties, but there are challenges when using electron impact MS while trying to identify fatty acids with more than one double bond and when double bonds are separated by more than one methylene group

(Christie 2011). The use of chemical ionization (e.g., acetonitrile) and dimethylloxazoline (DMOX) derivatives is promising, but extensive libraries of mass spectra under these conditions are not presently available. Developing methods for fatty acid analyses is still a dynamic area of research due to the ongoing need for improved techniques, identification of biohydrogenation intermediates, and to support development of authentic standards. Breakthroughs in analyses will be key to furthering our understanding of beef fatty acid composition, and how it is influenced by diet, genetics and environment.

When beginning to analyze beef fatty acids, it is advisable to use a published method where attention has been placed on providing analytical details and identifying FAME for which there are no standards. Using published procedures where fatty acids were positively identified by employing complimentary techniques in the absence of standards allows one to compare retention times, and relative elution order, as a guide to tentatively identify fatty acids during analyses. It is important, however, that the methods used are applicable to the samples being investigated. For example, if one wishes to use a method to analyze FAME from the duodenal digesta of cattle, a complementary (i.e., second) GC run is not necessary to quantify the individual *trans*-18:1 isomers (Loor et al. 2004). However, a complimentary GC run with a different temperature program is typically required for beef fat, as it contains a high content of oleic acid (*c*9-18:1), which can overlap and interfere with *trans*-18:1 isomer analysis.

Another practical consideration when analyzing FAME is that the chromatographic conditions of the column can change slightly over time and lead to shifts in retention times, and in certain cases to changes in elution order. In such cases, it is useful to be able to analyze fractions of a sample that have been separated using a complementary chromatographic procedure, for example, solid phase extraction with silver nitrate impregnated silica (Kramer et al. 2008). This is a very valuable tool, especially when considering the region in the GC chromatogram from the *trans*-18:1 isomers to linoleic acid (18:2n-6; Fig. 1).

THE ORIGIN OF TRANS FATTY ACIDS IN BEEF

Current knowledge of polyunsaturated fatty acid (PUFA) biohydrogenation in the rumen has been summarized by Dawson and Kemp (1970), Harfoot and Hazelwood (1997), Jenkins et al. (2008) and most recently by Lourenço et al. (2010). Rumen bacteria were originally thought to hydrogenate PUFA because, under anoxic conditions in the rumen, they could act as terminal electron acceptors and maintain oxidation/reduction cycling of nucleotides required for substrate level phosphorylation (i.e., adenosine triphosphate synthesis). A more recent and widely accepted explanation is that PUFA are toxic to bacteria, and biohydrogenation neutralizes their effects.

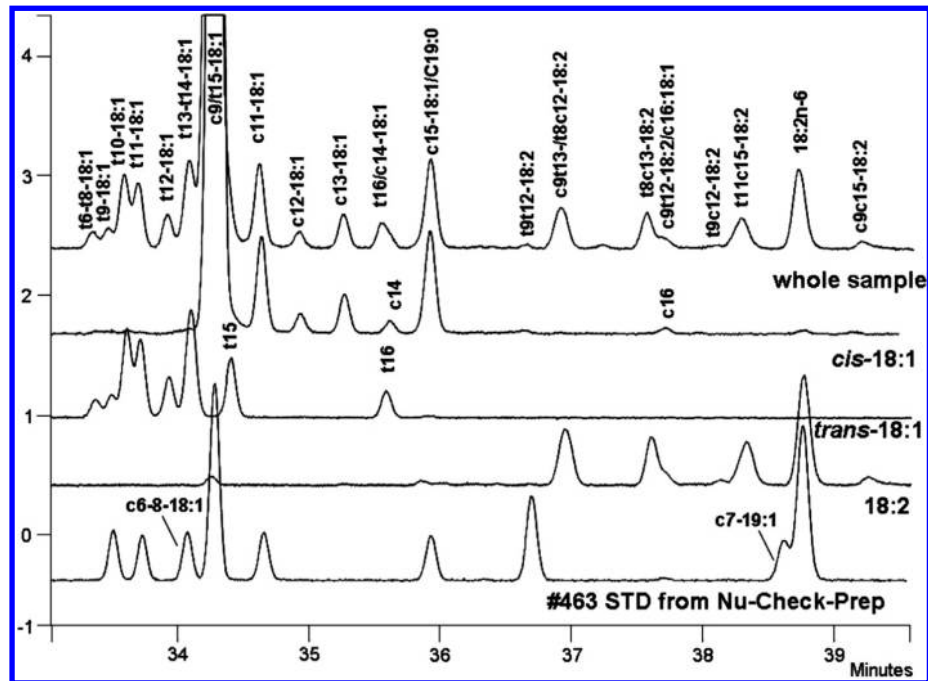


Fig. 1. Partial gas chromatograms (*trans*-18:1 to 18:2n-6) of fatty acid methyl esters (FAME) from different fractions eluted from Ag^+ -solid phase extraction. FAME were from backfat from steers finished on 70% barley, 15% hay, 10% flax for 90 d (Juárez et al. 2011a). Samples were analyzed using a Varian CP-3800 GC with a 1079 split/splitless injector equipped with a flame ionization detector and a Varian CPSil88-100 m column with 0.25 μm internal diameter and 0.2 μm film thickness. Samples (1 μL FAME at 1 mg mL^{-1} hexane) were injected using a Varian CP-8400 Autosampler, a 20:1 split, an injector temperature of 250°C and a detector temperature of 250°C. The initial temperature was 45°C held for 4 min, increased to 175°C at 13°C min^{-1} and held for 27 min, increased to 215°C at 4°C min^{-1} and held for 35 min.

The discovery of *trans* double bonds in ruminant products dates back to Bertram (1928), who found traces of a solid unsaturated fatty acid in fat from beef, mutton and butterfat. This finding indicated the presence of a *trans* fatty acid in ruminant products, and Bertram elegantly characterized this as *t*11-18:1, which he named vaccenic acid (VA). Later, Booth et al. (1935) found a seasonal change in butterfat with an increased absorbance at 230 nm in the summer, a wavelength now known to be indicative of conjugated linoleic acid (CLA). Body fats are in part derived from the diet, and also from endogenous synthesis, but large differences in dietary influences on body fat composition led Shorland (1950) to classify animals as either heterolipoid (lacking resemblance to dietary fat) or homolipoid (animals that readily incorporate dietary fatty acids into their fat depots). Essentially, monogastric animals are homolipoid, while ruminants are heterolipoid. Changes in the composition of dietary fats, when ingested by ruminants, and formation of saturated fatty acids and fatty acids containing *trans* double bonds, were eventually attributed to the biohydrogenation activity of rumen microbes (Reiser 1951; Shorland et al. 1955). The diversity of intermediates produced during biohydrogenation of the major PUFAs in feed was subsequently investigated (Shorland et al. 1957),

and pathways for linolenic acid (Ward et al. 1964; Wilde and Dawson 1966; Kepler and Tove 1967) and linoleic acid (Kepler et al. 1966) biohydrogenation were proposed. Even at this time, however, it was recognized that the diversity of biohydrogenation intermediates could not be accounted for by a single pathway in isolated organisms, and that the process was likely more complex when carried out by mixed rumen flora (Kepler et al. 1966).

Animal metabolism of fatty acids consists mainly of synthesis by elongation and desaturation and degradation by oxidation. However, fatty acid metabolism in the rumen depends primarily on the diet, rumen conditions and the predominant species of lipid-metabolizing microorganisms. The findings of a single experiment might, therefore, not be able to be extrapolated to other experiments unless feeding, management and resulting rumen conditions are similar. Even at this, individual animal variation can be considerable (Kraft et al. 2008; Aldai et al. 2011). For example, consider an experiment where a conventional western Canadian finisher diet was fed to steers containing 85% ground barley, 15% barley silage and a 5% vitamin/mineral/protein supplement (dry matter basis; Dugan et al. 2010). In this study, the two major *trans*-18:1 isomers found in brisket fat were *t*10- and *t*11-18:1, and these averaged 1.17 and 0.85% of

total FAME, respectively. On average most animals had more *t*10- than *t*11-18:1; however, some had more *t*11- than *t*10-18:1 (Fig. 2). These findings are contrary to the generally held belief that ruminant products, including beef, contain *t*11-18:1 as their predominant *trans*-18:1 isomer (Wolff 1995). Wood (1983) actually found more *t*10- than *t*11-18:1 in fresh beef and beef products in the United States, and suggested this may have been due to the inclusion of partially hydrogenated vegetable oils in the diet of cattle. Wolff (1995), however, indicated that because the higher concentration of *t*10-18:1 had been found across a range of processed foods containing beef it implied that most of the beef in the United States were fed hydrogenated fats, a possibility that he felt was unlikely. He further suggested that improved analytical methods for *trans* fatty acid analysis would have avoided confusion between the isomers. However, it appears the findings of Wood (1983) were correct, since, under certain feeding conditions, *t*10-18:1 can be the predominant *trans*-18:1 isomer found in beef.

MANIPULATING TRANS FATTY ACIDS CONCENTRATIONS IN BEEF

Pariza et al. (1979) found an extract from pan-fried ground beef inhibited mutagenesis, and the active compound in the extract was later characterized as CLA (Ha et al. 1987). The finding that fat from beef could have such a positive health effect, after years of recommendations by health authorities to reduce intakes of saturated fatty acids from animal products, was exciting news for beef researchers and the industry. These discoveries led to considerable scientific activity to enrich concentrations of CLA in beef. The enrichment of beef with appreciable quantities of CLA has, however, been difficult to accomplish.

PUFA in Low Forage-to-Concentrate Ratio Diets Can be a Bad Combination

It is widely known that beef is a natural source of CLA, and it is derived from dietary PUFA. However, because CLA is an intermediate and not the end product of PUFA biohydrogenation in the rumen, trying to increase its concentrations presents challenges. Typically, the concentration of CLA in beef is less than 1% of total fatty acids. As reviewed by Moloney et al. (2001), Mir et al. (2003), and Raes et al. (2004a), concentrations of CLA can be increased in beef by increasing the forage to concentrate ratio, and by feeding fresh grass instead of grass silage. Supplementation of the diet with PUFA-rich oils or oilseeds has also led to increases in CLA (Mir et al. 2003; Raes et al. 2004a). Up to 6% PUFA-rich oil has been added to diets leading to relatively large increases in CLA compared with original concentrations. However, since the original concentrations of CLA were low, the absolute increases were not appreciable.

Griinari et al. (2000) provided evidence that the majority of the main natural isomer of CLA [rumenic acid (RA), *c*9,*t*11-18:2] found in ruminant products does not originate directly from the rumen. Instead, only small amounts of CLA escape the rumen and *trans*-18:1 fatty acids are the main biohydrogenation intermediates available to the animal. Absorbed VA is subsequently desaturated in the tissues of ruminants by Δ^9 -desaturase to form RA. Therefore, challenges to increase CLA in beef have been to produce rumen conditions favorable for bacterial species that synthesize VA, while preventing conditions leading to complete biohydrogenation to stearic acid (18:0). Dugan et al. (2007) found a greater accumulation of *t*10-18:1 than VA in Canadian feedlot beef finished with a high barley diet (73% ground barley, 22% barley silage as-fed), consistent with the findings of Wood (1983) for USA beef and

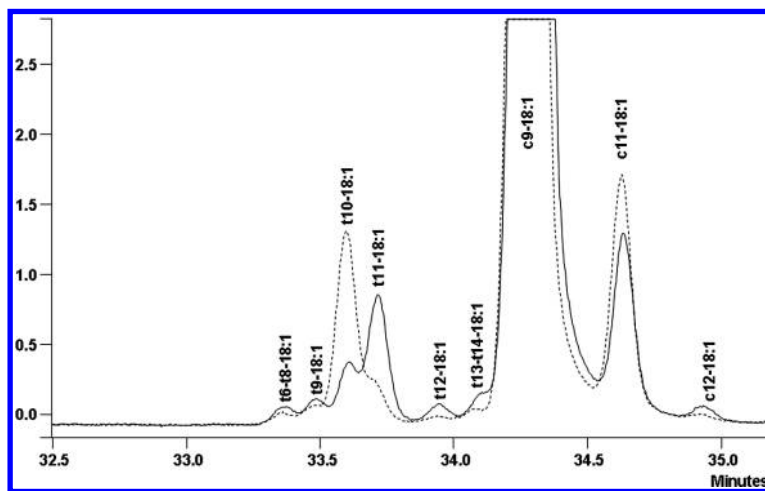


Fig. 2. Partial gas chromatograms of fatty acid methyl esters showing the *trans*-18:1 region of brisket fat from steers finished on a barley diet, with one animal high in *t*10-18:1 and one high in *t*11-18:1 (Dugan et al. 2010). Equipment and chromatographic conditions as outlined in Fig. 1.

Purchas et al. (2005) in USA beef compared with New Zealand grass finished beef. Dugan et al. (2008) also found more $t10-18:1$ than VA, and less RA in backfat from youthful Canadian beef (<30 mo of age) compared with cull cows (>30 mo of age) collected from commercial packing plants. These results again likely related to the higher proportion of forage in cow diets. The finding of more $t10-18:1$ in youthful Canadian beef was further supported by similar findings in a retail survey of Canadian grade A and AA (i.e., youthful) strip loin steaks and associated backfat (Aldai et al. 2009). Ground beef collected during the same survey, however, had more VA than $t10-18:1$, which would indicate the use of either Canadian cull cows or imported grass-finished beef in ground beef production (Aldai et al. 2010d). A survey of beef from the United States conducted by Leheska et al. (2008) comparing the fatty acid composition of grain vs. grass-finished beef also found that grain fed beef had less CLA and more $t10-18:1$ than VA as compared with grass-finished beef.

A shift towards deposition of $t10-18:1$ when feeding grain is consistent with changes seen in beef fat in Germany when cattle are fed concentrate vs. grazed on pasture (Dannenberger et al. 2004). In this report, however, VA remained the predominant *trans*-18:1 isomer, which is likely due to the relatively low intake of concentrate compared with that typically fed to beef cattle in North America. Raes et al. (2004b) fed concentrate with limited barley (13%) and wheat (20%) with straw as a roughage source, and was able to maintain a higher proportion of VA than $t10-18:1$ in both rumen contents and adipose tissue. Limiting the amount of barley in the concentrate portion of the diet to 34.5% in a 60:40 roughage to concentrate diet (DM basis) was also found to maintain a higher concentration of VA than $t10-18:1$ in duodenal digesta (Lee et al. 2005). Essentially, if cattle are fed too high a level of grain with highly fermentable starch, there is a shift in rumen microflora, a reduction in rumen pH and a shift in the biohydrogenation pathways towards producing $t10-18:1$ instead of VA (Bauman and Griinari 2003). Accentuated increases in $t10-18:1$ may also occur when feeding grains, since the final step in the biohydrogenation that leads to stearic acid is influenced by pH (Troegeler-Meynadier et al. 2006). The type of grain and level of processing can, therefore, play a major role in determining the isomeric profile of *trans*-18:1 produced in the rumen (Mohammed et al. 2010). The shift from VA to $t10-18:1$ when cattle are switched from pasture to a high barley diet can also be quite rapid, as Aldai et al. (2011) found that as little as 1 mo of concentrate feeding was enough to reduce VA from 2.4 to 1.8% and increase $t10-18:1$ from 0.5 to 2.8% in loin muscle of genetically lean cattle.

Corn is generally considered to have a slower ruminal fermentation rate than barley or wheat. When Fritsche et al. (2001) fed a diet with 70% corn they observed 0.7% VA and 0.22% $t10-18:1$ in beef subcutaneous fat.

Duckett et al. (2002) fed steers a diet with 80% conventional corn and found a greater duodenal flow of VA than $t10-18:1$. The amount of VA increased when high-oil corn was substituted for conventional corn, but when corn oil was added to conventional corn at the same rate as in the high-oil corn, the flow of $t10-18:1$ was more than 300% higher than the flow of VA. Beaulieu et al. (2002) and Dhiman et al. (2005) added soybean oil to a corn-based diet and found greater concentrations of total *trans*-18:1 in adipose tissue, but no change was observed in RA, leading them to speculate the increase in *trans* fatty acids was related to isomers other than VA. When Madron et al. (2002) supplemented a corn-based diet with up to 25.6% extruded full fat soybeans, however, muscle concentrations of $t10-$ and $t11-18:1$ increased, but VA remained the predominant isomer. In contrast, when Hristov et al. (2005) fed diets with 78.6% dry rolled barley, supplemented with either 5% safflower or high oleic acid safflower, they found only 0.6% VA and more than 4.5% $t10-18:1$ in adipose tissue for both diets. Aldai et al. (2010c) also found including 3% soybean oil in an 85% ground barley diet with barley straw as the forage source led to even higher rates of $t10-18:1$ accumulation in beef, reaching 11.6% in backfat. As previously mentioned, the accumulation of *trans*-18:1 isomers can be related to the effects of low pH on biohydrogenation pathways, but the type and level of oil can also inhibit biohydrogenation (Troegeler-Meynadier et al. 2006), further increasing *trans*-18:1 in beef. The longer chain PUFA in fish oil (i.e., docosahexaenoic acid) are known to be particularly effective at reducing biohydrogenation rates (Lee et al. 2005), but their effectiveness is also influenced by the composition of other oils in the diet (Duckett and Gillis 2010). The accumulation of *trans*-18:1 when feeding PUFA may reflect inhibition at the enzymatic level (Troegeler-Meynadier et al. 2006), or direct inhibition of the bacteria involved in the final step of hydrogenation of *trans*-18:1 to stearic acid (Lourenço et al. 2010).

Reducing the $t10-18:1$ to VA Ratio

The *trans* fatty acid content of foods has recently been under much scrutiny, as industrially produced *trans* fats are known to negatively impact cardiovascular health (Mensink et al. 2003). Foods derived from ruminants are currently exempt from requiring labeling of their *trans* fatty acid contents in Canada, and in many other countries (Health-Canada 2006; Duham 2009). This decision was largely based on the assumption that VA and RA are the main *trans* fatty acids in ruminant fats, and that they have a neutral or possible net health benefit for consumers. The finding that VA is not the main *trans* fatty acid isomer in youthful Canadian beef has resulted in investigations to try to limit the production of less desirable *trans* fatty acids, particularly $t10-18:1$. To this end, feeding buffer (sodium sesquicarbonate) in a barley grain-based diet to neutralize rumen

pH was found to maintain a lower $t10/t11-18:1$ ratio in beef, but the effectiveness was lost over the feeding period (Aldai et al. 2010e). In-feed antibiotics may also inhibit bacteria that produce specific *trans-18:1* isomers, but Aldai et al. (2008) found that several antibiotics, such as tylosin, chlorotetracycline and sulfamethazine, were ineffective at altering the concentration of $t10-18:1$ in barley fed beef cattle. The lack of response, however, may have been related to the relatively low level of PUFA in the diets, as Mir et al. (2008) found including tylosin in a barley diet supplemented with sunflower seeds caused a preferential increase in $t10-18:1$. Consistent with this, the ionophore monensin has also been shown to increase $t10-18:1$ in milk fat when dairy cows were fed concentrate containing supplementary sunflower seed oil (Cruz-Hernandez et al. 2006a).

Modification of beef *trans* fatty acid profiles is possible by increasing the forage to concentrate ratio, but along with this there can be reductions in animal performance and fat deposition (Aldai et al. 2011). The feeding of large amounts of grain, however, has recently become somewhat less economical due to its competitive use for ethanol production in North America. As a result, the feeding of wheat dried distiller's grain plus solubles (DDGS) has increased in western Canada, which has been beneficial for two reasons. First, up to 40% wheat DDGS can be substituted for ground barley in beef finisher diets with little impact on animal performance (Gibb et al. 2008), carcass traits (Walter et al. 2010) or meat quality (Aldai et al. 2010a). Second, substituting increasing levels of wheat DDGS for ground barley in finisher diets results in a linear increase in VA, and a linear reduction in $t10-18:1$ in subcutaneous fat (Dugan et al. 2010). Production of ethanol from wheat removes much of the starch from the grain, leaving relatively high levels of protein, fiber and oil (Walter et al. 2010). These changes in composition result in a feedstuff with a feeding value similar to that of ground barley, but with a less rapid rumen fermentation that results in shifts towards $t10-18:1$ producing bacterial species. Sources of DDGS are not, however, considered equal since the results when feeding wheat DDGS have not always been consistent, and substituting corn DDGS for ground barley has been found to increase the $t10/t11-18:1$ ratio (Aldai et al. 2010b).

Increasing Vaccenic and Rumenic Acids

Ip et al. (1994) estimated humans need to consume 3 g of CLA a day to be effective against developing breast cancer, and this provided an initial target for enriching animal products. There is still, however, a clear need for short- and long-term trials to study the effects of individual isomers of CLA (Benjamin and Spener 2009). Such experiments should also consider the contribution of VA and to RA synthesis. As mentioned previously, finding ways to appreciably increase concentrations of VA and RA in beef has been challenging. More success has been found in enriching levels

of these fatty acids in milk. For example, Lock and Bauman (2004) have produced milk fat with 4.7% CLA and 12.1% VA by feeding a corn-based total mixed ration that included 2% soybean oil and 1% fish oil. Differences in CLA content between beef and dairy products may reflect differences in the diet consumed, intakes and the passage rates of digesta between these cattle types, as well as differences in the rate of CLA synthesis in mammary tissue as compared with adipocytes and muscle (Aharoni et al. 2004). In addition, in beef there may be more of a dilution effect related to endogenous fatty acid synthesis. It is apparent, however, that supplying a PUFA source in combination with a high forage diet over an extended duration will result in the accumulation of biohydrogenation intermediates in beef. Duckett et al. (2009) fed supplemental corn oil or corn grain to steers grazing on endophyte-free tall fescue. Steers finished on pasture had 2.82% VA in their backfat, supplementation with corn on pasture resulted in 2.28% VA, while supplementing corn oil increased VA to 6.19%. Subcutaneous RA concentrations responded similarly with 0.74% on pasture, 0.72% when pasture was supplemented with corn, and 1.14% when corn oil was provided to pastured cattle.

To achieve increased concentrations of biohydrogenation intermediates in beef, inhibition of the final step of biohydrogenation from *trans-18:1* to 18:0 is a necessity. Reducing rumen pH by feeding grain has been demonstrated to inhibit ruminal lipolysis and biohydrogenation in dairy cattle (Chilliard et al. 2007). Since lipolysis is the first step that enables biohydrogenation, reducing the extent of lipolysis also has the potential to promote bypass of dietary lipids. Juárez et al. (2011a) fed 10% rolled flaxseed along with 63% ground barley, and 22% alfalfa brome hay to steers over a 90-d finishing period, and increased total omega-3s in beef loin muscle from 0.95 to 2.05% of total fatty acids. This level was ~ fourfold lower than found in pork when feeding pigs (i.e., a monogastric animal with limited biohydrogenation) the same level of flaxseed for 77 d (Juárez et al. 2011b). Comparing results from these two species indicates that even when feeding an oilseed such as flaxseed to cattle on a high-grain diet, biohydrogenation processes are still quite active. In support of this, Juárez et al. (2011a) found increased concentrations of PUFA biohydrogenation products in beef when flaxseed was included in the diet, but increases in VA were only marginal (0.64 to 0.78%), while RA remained unchanged. Instead, there was actually a decrease in $t10-18:1$ accompanied by relatively large increases in other *trans-18:1* isomers ($t13/t14$, $t15$, $t16-18:1$), and atypical 18:2 isomers ($c9,t13/t8,c12-$, $t8,c13-$, $t11,c15-$ $c9,c15-$ and $c12,c15-18:2$) derived from the biohydrogenation of linolenic acid. The health effects of many of these biohydrogenation intermediates in humans are unknown, and their evaluation will be important if flaxseed is to be fed to cattle to increase omega-3 fatty

acids or their biohydrogenation product concentrations in beef.

Incorporating flaxseed in a diet with a high proportion of ground barley, therefore, does not appear to be a viable approach for increasing the content of VA or RA in beef. However, some increases in CLA in beef have been found when feeding flaxseed together with high levels of forage (Enser et al. 1999; Raes et al. 2003; De La Torre et al. 2006; Barton et al. 2007). The type of forage when feeding flaxseed has also been found to significantly effect deposition of biohydrogenation intermediates in beef (Nassu et al. 2011). Inclusion of 15% flaxseed in a diet with 50% grass hay increased RA in beef loin muscle fatty acids from 0.26 to 0.55% and VA from 0.6 to 2.2%. In contrast, inclusion of the same level of flaxseed with barley silage as the forage source only increased RA from 0.2 to 0.28%, and VA from 0.48 to 0.8%. Again, feeding flaxseed to cattle also resulted in relatively large increases in the concentrations of unique metabolites of linolenic acid biohydrogenation including most *t*-18:1 isomers and several 18:2 fatty acids (*c*9,*t*13/*t*8,*c*12-, *t*8,*c*13-, *t*11,*c*15-, *c*9,*c*15- and *c*12,*c*15-18:2). As a result, when feeding flaxseed together with hay, only 42% VA was found in total *trans*-18:1 and concentrations of non-conjugated non-methylene interrupted 18:2 exceeded concentrations of total CLA by 2.4-fold.

Feeding flaxseed in combination with grass hay appears to be a way to increase concentrations of VA and RA in beef, but these increases were muted by increases in other linolenic acid metabolites of unknown physiological significance (Nassu et al. 2011). Interestingly, He et al. (2011) analyzed backfat biopsies from the same animals over the course of the experiment and found increases in VA from weeks 12 to 20 were substantially higher than from weeks 6 to 12, indicating increased rates of adipose tissue accumulation over time. Positive effects of a longer feeding period have also been demonstrated by analyses conducted in the authors' laboratory for a recent study conducted at the Agriculture and Agri-Food Canada, Brandon Research Centre by Hushton Block (unpublished data). In this study, heifers were fed 15% flaxseed in a diet with 70% grass hay for 250 d, and the concentration of RA reached 1.31 and 2.42% and VA reached 7.1 and 8% in loin muscle and backfat, respectively. Clearly increases in the content of VA and RA in beef is dependent on relatively high levels of oilseed (up to 6% added oil) in the diet, the type of forage included in the diet, and the duration of the feeding period (Fig. 3.). However, when feeding flaxseed in a high hay diet, accumulation of aforementioned unique metabolites of linolenic acid biohydrogenation products occurs including *t*11,*t*15-, *c*9,*t*13/*t*8,*c*12-, *t*8,*c*13-, *t*11,*c*15-, *c*9,*c*15-, *c*12,*c*15-18:2 and *t*11,*c*13-CLA and two conjugated linolenic acid isomers (*c*9,*t*11,*t*15- and *c*9,*t*11,*c*15-CLnA). From a nutritional perspective, it is also interesting to note the concentrations of biohydrogenation intermediates can actually

exceed concentrations of essential fatty acids (linoleic acid, 18:2n-6 and linolenic acid, 18:3n-3).

HEALTH EFFECTS OF FATTY ACIDS WITH TRANS DOUBLE BONDS

The effects of fatty acids with *trans* double bonds on health have been extensively reviewed (Odegaard and Pereira 2006; Combe et al. 2007; Gebauer et al. 2007; Malpuech-Brugere et al. 2009; Smith et al. 2009). Key indicators of *trans* fatty acid effects on human health have been drawn from epidemiological studies and further controlled studies. On the whole, there is little doubt *trans* fatty acids have adverse effects on cardiovascular disease and may have effects on diabetes, cancer and other diseases. Efforts to understand the effects of *trans* fatty acids on human health have, however, been confounded because *trans* fatty acid isomers do not have the same (or equal) physical, biological and health properties.

In the past decade, a great deal of emphasis has been placed on limiting the intake of *trans* fatty acids through regulations and recommendations because of their association with cardiovascular disease. One source of *trans* fats in human diets arises from the consumption of industrially produced partially hydrogenated vegetable oils, which can contain variable concentrations (Stender et al. 2006) and a broad spectrum of *trans* fatty acid isomers (Wolf et al. 1998; Cruz-Hernandez et al. 2004). A second major source of *trans* fats is from ruminant meat and milk, but there has been some belief that these are mainly in the form of VA and RA. Both VA (Field et al. 2009) and RA (Field and Schley 2004; Bhattacharya et al. 2006) have demonstrated health effects related to their anti-cancer activity and potential effects on coronary heart disease and immune function. Commercially produced grain-fed beef in Canada (Aldai et al. 2009) and the United States (Leheska et al. 2008) has, however, been surveyed and shown to contain more *t*10-18:1 than VA, and many other PUFA biohydrogenation intermediates.

Establishing the bioactivity of individual fatty acids with *trans* double bonds is of considerable importance, as this will help direct research to their selective enrichment or depletion in beef. Typically most of the investigations of *trans* fatty acids have dealt with RA and its comparison with the other CLA isomers such as *t*10,*c*12-18:2, and the *trans*-18:1 isomer VA compared with *t*10-18:1. The consumption of *t*10,*c*12-18:2 was originally thought to have beneficial effects because of its ability to decrease body fat while increasing lean muscle mass in animal models (Dugan et al. 1997, 2001), but its consumption has also been linked to insulin resistance in men with metabolic syndrome (Riserus et al. 2002). It has also been linked to insulin resistance and inflammation in human adipocytes, and these have been related to release of intracellular calcium (Kennedy et al. 2010). Results from rabbits fed butter enriched with either *t*10-18:1, or combined with VA and RA,

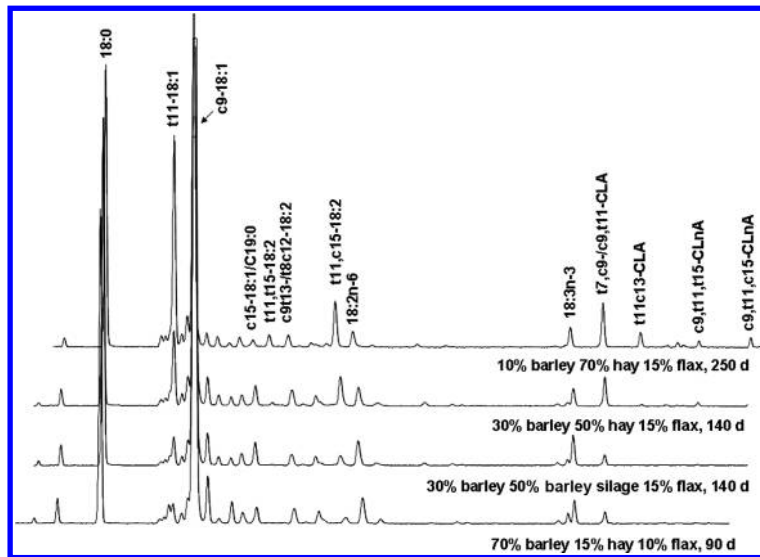


Fig. 3. Partial gas chromatograms of fatty acid methyl esters showing the *trans*-18:1 to the conjugated linolenic acid (CLnA) region from backfat from cattle finished for different durations on flaxseed containing diets with different forages and forage to concentrate ratios. Equipment and chromatographic conditions as outlined in Fig. 1.

showed that *t*10-18:1 negatively effects plasma lipoprotein profiles and enhances aortic fatty streak formation (Bauchart et al. 2007; Roy et al. 2007). In humans, studies focused on feeding dairy products enriched with VA and RA have shown limited effects relative to control (Desroches et al. 2005; Tricon et al. 2006), but levels may have been too low to elicit responses. Compared with industrially produced partially hydrogenated vegetable oil, however, VA and RA may have some beneficial effects in humans (Chardigny et al. 2008), but not when consumed at elevated levels (Motard-Belanger et al. 2008).

The effects of one fatty acid, or group of related fatty acids, and their relationships to health need to be considered in the context of the whole diet consumed, and one must also consider that theories are subject to change based on the weight of available evidence. For example, consumption of *trans*-18:1 fatty acids from partially hydrogenated vegetable oils has been accepted as a factor contributing to the risk of developing cardiovascular disease, but concentrations of *trans*-18:2 isomers (*c*9,*t*12-18:2 and *t*9,*c*12-18:2) that have in general been ignored, have been shown to be more associated with heart attacks (Baylin et al. 2003) and sudden cardiac death (Lemaitre et al. 2006) than *trans*-18:1 isomers. Whole foods derived from ruminants also have PUFA biohydrogenation metabolites other than *t*10-18:1, VA and RA that may impact health, but their effects are largely unknown. The number of potential biohydrogenation products increases as the number of double bonds increase in a fatty acid, and just as for CLA isomers, these biohydrogenation metabolites may have divergent health effects. The biological effects of a number of plant derived conjugated linolenic

acid (CLnA) isomers with adjacent conjugated double bonds have recently been investigated, and similar to CLA, these may have a range of effects on obesity, cardiovascular health and cancer (Hennessy et al. 2011). Only trace amounts of these di-conjugated CLnA isomers have been detected in milk fat (Destaillets et al. 2005). More common in ruminant fats are the mono-conjugated CLnA derivatives of α -linolenic acid, such as *c*9, *t*11, *c*15- and *c*9, *t*11, *t*15-18:3 (Bessa et al. 2007; Gómez-Cortés et al. 2009). The biological properties of these mono-conjugated CLnA derivatives have not been thoroughly investigated. As previously noted, feeding flaxseed to beef cattle can increase the concentrations of these mono-conjugated CLnA metabolites. In addition, feeding flaxseed can substantially increase concentrations of *t*11,*c*15-18:2, which could possibly act as a precursor for CLnA synthesis via Δ 9-desaturase activity.

Overall, fatty acids in beef with *trans* double bonds can have positive, neutral and negative effects on human health. The biological effects of all these PUFA metabolites in ruminant fats and their possible interactions have yet to be investigated. Producing beef with meaningfully enhanced concentrations of biohydrogenation products with potential human health benefits remains a challenge, but recent developments in feeding strategies have shown promise. Despite individual animal variation, progress has been made in recent years to understand the dynamics of the rumen environment and how to manipulate it to increase the population of rumen bacteria that produce desired fatty acids. Being able to consistently produce beef with enhanced concentrations of specific fatty acids, or combinations thereof, and their biohydrogenation

products in a whole food matrix is a goal for future studies. Development of these capabilities might then provide motivation for the beef industry to shift towards producing beef with more VA and RA than *t*10-18:1 and CLA isomers other than RA, or perhaps more CLnA or *t*11,*c*15-18:1 or other biohydrogenation products that have yet to be characterized. Perhaps one day specialized beef products with customized fatty acid profiles might become part of an individual's diet, tailored to suit their genetic predispositions, or specific health concerns.

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