Animal (2012), **6:11**, pp 1888–1896 Contributions by N. Aldai © The Animal Consortium, contribution by J.A.Basarab
© Government of Alberta, and contributions by C. Mapiye, M.E.R. Dugan, M. Juárez, V.S. Baron, T. Turner, X. Yang and J.L. Aalhus
© Her Majesty the Queen in Right of Canada, as represented by the Minister of Agriculture and Agri-Food Canada 2012
doi:10.1017/S1751731112000730

animal

Influence of α-tocopherol supplementation on *trans*-18:1 and conjugated linoleic acid profiles in beef from steers fed a barley-based diet

C. Mapiye[1], M. E. R. Dugan[1†], M. Juárez[1], J. A. Basarab[2], V. S. Baron[1], T. Turner[1], X. Yang[1], N. Aldai[3] and J. L. Aalhus[1]

[1]Lacombe Research Centre, Agriculture and Agri-Food Canada, 6000 C&E Trail, Lacombe, Alberta T4L 1W1, Canada; [2]Alberta Agriculture and Rural Development, Lacombe Research Centre, 6000 C & E Trail, Lacombe, Alberta T4L 1W1, Canada; [3]Food Science and Technology, Faculty of Pharmacy, University of Basque Country, 01006 Vitoria-Gasteiz, Spain

(Received 29 August 2011; Accepted 15 February 2012; First published online 3 April 2012)

The current study was conducted to determine the effect of different α-tocopherol (vitamin E) inclusion levels on trans*(*t*)-18:1 and conjugated linoleic acid (CLA) profiles in subcutaneous and intramuscular fat of steers fed a barley-based diet. Fifty-six feedlot steers were offered a barley-based finisher diet (73% steam rolled barley, 22% barley silage and 5% supplement as-fed basis) with four levels of supplementary* DL*-α-tocopheryl acetate (340, 690, 1040 or 1740 IU/steer per day) for 120 days. Adding vitamin E to the diet had little effect on the overall fatty acid composition of intramuscular fat. The proportion of individual and total* t,t- *and* cis*(*c*),*t*-CLA,* n*-3 fatty acids, total polyunsaturated fatty acids (PUFA), mono-unsaturated fatty acids and saturated fatty acids to PUFA ratio in subcutaneous fat were not influenced (*$P > 0.05$*) by dietary vitamin E supplementation. Increasing levels of vitamin E led to linear reductions in* t6-/t7-/t*8-18:1 and* t*10-18:1 (*$P < 0.05$*), and linear increase in* t*11-/*t*10-18:1 ratio (*$P < 0.05$*) in subcutaneous fat. The content of 20:3*n*-6 and total* n*-6 in subcutaneous fat decreased (*$P < 0.05$*) linearly with increasing amounts of vitamin E. The subcutaneous fat* n*-6:*n*-3 ratio showed a quadratic (*$P < 0.05$*) response to vitamin E. In conclusion, although vitamin E supplementation has some potential to reduce* t*10-18:1 formation and increase* t*11-/*t*10-18:1 ratio in subcutaneous fat of cattle fed barley-based diets, the changes in the present study were limited and may not have been sufficient to impact on human health.*

Keywords: CLA, vitamin E, *t*10-18:1 shift, *t*11-18:1, *trans* fatty acids

Implications

Feeding high levels of concentrates results in increased *trans*(*t*)10-18:1 and decreased *t*11-18:1 (vaccenic acid) deposition in beef. High intake of *trans* fatty acids other than *t*11-18:1 is, according to observational studies, associated with an increased risk of coronary heart disease in humans, whereas *t*11-18:1 may have independent beneficial human health effects and these are in part mediated through its conversion to *cis*(*c*)9, *t*11-18:2 (rumenic acid). Vitamin E has been postulated to modify rumen microbial populations and consequently fatty acid biohydrogenation. The current trial, therefore, aimed to determine the level of vitamin E supplementation that would optimally prevent the shift from *t*11-18:1 to *t*10-18:1 and consequently improve the healthfulness of beef fed high-grain diets. Current findings could have implications for the beef industry if regulations are imposed requiring *trans* fat labelling of ruminant products with increased levels of *trans* fatty acids other than *t*11-18:1. The beef industry using a new trademark ('E-Beef') may find the information useful for product differentiation.

Introduction

Beef from animals fed high-concentrate diets contains more *trans*(*t*)10-18:1 than *t*11-18:1 (vaccenic acid; Dugan *et al.*, 2007; Aldai *et al.*, 2009). Inclusion of increased amounts of grain in beef finisher diets creates a rumen environment conducive for biohydrogenation of polyunsaturated fatty acids (PUFA) through the *t*10-18:1 instead of *t*11-18:1 pathway resulting in an increased rate of *t*10-18:1 deposition (Hristov *et al.*, 2005; Aldai *et al.*, 2010). For humans, total *trans* fatty acid intake is associated with an increase in coronary

† E-mail: duganm@agr.gc.ca

heart disease (Oh *et al.*, 2005); however, studies on effects of individual *t*18:1 isomers are limited (Field *et al.*, 2009; Wang *et al.*, 2012). In rabbits, feeding butter enriched with *t*10-18:1 had unhealthy effects on blood lipoprotein profiles, whereas effects of feeding butter enriched with *t*11-18:1 were limited (Bauchart *et al.*, 2007; Roy *et al.*, 2007). In humans, intake of dairy products enriched with *t*11-18:1 and *cis*(*c*)9, *t*11-18:2 (rumenic acid) also had limited effects on blood lipid profiles (Tricon *et al.*, 2006; Sofi *et al.*, 2010), however, some unhealthy changes were noted when relatively high levels were consumed (Chardigny *et al.*, 2008). Vaccenic acid, however, may have other positive health influences either independently, or by desaturation to rumenic acid (Field *et al.*, 2009; Wang *et al.*, 2012), which has many potential health benefits mostly relating to its anticarcinogenic, immune modulatory and anti-inflammatory properties (Benjamin and Spener, 2009). Currently, nutritional strategies that substantially prevent the *t*10-18:1 shift (i.e. increases in *t*10-18:1 and reductions in *t*11-18:1) in grain-fed beef are few, if any. Development of such strategies would, therefore, be beneficial to both producers and consumers.

Vitamin E (DL-α-tocopherol) which is primarily active as an antioxidant has been reported to protect PUFA from free-radical attack *in vivo* and *post mortem* in animal tissues (Morrissey *et al.*, 1994; Gabryszuk *et al.*, 2007). It has also been shown to prevent the *t*11- to *t*10-18:1 shift in plasma (Kay *et al.*, 2005) and milk (Pottier *et al.*, 2006) in cattle. Recently, Juárez *et al.* (2010) showed that feeding 1051 IU vitamin E/animal per day during the finishing period can reduce *t*10-18:1 and increase *t*11-18:1 in cattle subcutaneous fat. Vitamin E might, therefore, be an efficient way to lower the concentration of fatty acids, which pose human health risk when feeding high-concentrate diets. In addition, dietary supplementation of vitamin E improves colour and lipid stability of beef (Lee *et al.*, 2008; Nassu *et al.*, 2011a), and has potential to reduce carcinogenic heterocyclic amines in cooked meat (Liao *et al.*, 2009). These benefits give vitamin E comparative advantage over other dietary strategies for manipulating fatty acid profiles such as use of buffers (Aldai *et al.*, 2010), dried distillers grains with solubles (Dugan *et al.*, 2010), condensed tannins (Vasta *et al.*, 2009) and saponins (Brogna *et al.*, 2011). That could also economically justify its inclusion in beef finisher diets. However, the dietary concentration of vitamin E required to optimally prevent the *t*10-18:1 shift in beef fed high-concentrate diets has not been established. The objective of the current study was to determine the effect of graded levels of vitamin E supplementation on *t*18:1 and conjugated linoleic acid (CLA) in subcutaneous and intramuscular fat of steers fed a barley-based diet.

Material and methods

Animals and diets

Fifty-six British × Continental crossbred steers estimated to be between 8 and 10 months of age were purchased commercially. Animals were fed barley silage and over a 1-month 7-step transition period were placed on experimental finishing diets. After the transition period, animals weighed 430±14.5 kg and were stratified by weight into eight pens (four levels of dietary vitamin E; two pens/vitamin E level; seven steers/pen and $n = 14$ steers/treatment) at the Lacombe Research Centre of Agriculture and Agri-Food Canada. All finisher diets contained 73% steam rolled barley, 22% barley silage and 5% supplement (Table 1; as-fed basis). The supplement provided 340 IU DL-α-tocopheryl acetate/animal per day. Four experimental finishing diets were created by top-dressing and hand mixing additional vitamin E (0, 350, 700 or 1400 IU DL-α-tocopheryl acetate/steer per day). To ensure that all the feed provided each day was consumed, it was provided at ~2.5% of BW once daily, which resulted in 10% to 15% orts after 18 h and complete consumption by 24 h. Diets were randomly assigned to pens. 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 experimental diets for an average of 120 days. Dietary treatments and experimental procedures were approved by the Lacombe Research Centre Animal Care Committee. Animal care was in compliance with the principles and guidelines established by the Canadian Council on Animal Care (1993).

Feed analysis

Feed samples were collected weekly and stored at −20°C, then pooled monthly before analyzed for dry matter and CP according to Association of Official Analytical Chemists (AOAC, 2003), and acid detergent fibre and neutral detergent fibre as described by Van Soest *et al.* (1991). Fatty acid methyl esters (FAMEs) from the finishing total mixed ration were prepared as described by Sukhija and Palmquist (1988) and analyzed using the chromatographic conditions reported by Dugan *et al.* (2007).

Sample collection

Steers were slaughtered at the Lacombe Research Centre abattoir over three slaughter dates within a 1-month period (two steers/pen per treatment per slaughter day for the first and third kill, with three steers/pen per treatment for the second kill) at target ultrasound backfat measurements of 6 to 8 mm. Ultrasound backfat thickness (mm) was measured by a certified ultrasound technician between the 12th and 13th rib over the *longissimus* muscle on the right side of each animal using an Aloka 500V diagnostic real-time ultrasound with a 17 cm 3.5 MHz linear array transducer (Overseas Monitor Corporation Ltd, Richmond, BC, Canada) following procedures described by Brethour (1992). At slaughter, final live weights were recorded and animals were stunned, exsanguinated and dressed in a commercial manner. Carcass quality traits were also measured at slaughter and results were reported by Nassu *et al.* (2011a). At ~40 min *post mortem*, during evisceration, a cube of subcutaneous fat (5 cm × 5 cm × the thickness of the subcutaneous fat) was collected from the grade site (12th rib) and stored at −80°C for subsequent subcutaneous fatty acid determinations. At 24 h *post mortem*, the left *longissimus thoracis* muscle was removed from the carcass. A steak 2.5 cm thick was dissected from the posterior end of the

12th rib, comminuted using a Robot Coupe Blixir BX3 (Robot Coupe USA Inc., Ridgeland, MS, USA) and frozen (−80°C) pending intramuscular fatty acid analysis.

Fatty acid analysis

Subcutaneous fat samples (50 mg) were freeze-dried and directly methylated with sodium methoxide according to Cruz-Hernandez *et al.* (2004). Intramuscular fatty acids were extracted from 1 g of freeze-dried meat samples using a mixture of chloroform–methanol (2 : 1 v/v) according to Folch *et al.* (1957). Lipid aliquots (10 mg) from each muscle sample were methylated separately using acidic (5% methanolic HCl) and basic (0.5 N sodium methoxide) reagents (Kramer *et al.*, 2008). Internal standard (1 ml of 1 mg 23:0 methyl ester/ml toluene) was added before the addition of the methylating reagent.

The majority of FAMEs were analyzed using the gas chromatography (GC) and Ag^+-HPLC methods outlined by Cruz-Hernandez *et al.* (2004) except *t*18:1 isomers, which were analyzed using two complementary GC temperature programmes instead of analyses after preparatory Ag^+-TLC separation at 120°C (Dugan *et al.*, 2007; Kramer *et al.*, 2008). The first temperature programme was as previously described (temperature programme A; 45°C held for 4 min, increased at 13°C/min to 175°C and held for 27 min, then increased at 4°C/min to 215°C and held for 35 min) by Cruz-Hernandez *et al.* (2004). The second programme (temperature programme B; 45°C held for 4 min, increased at 13°C/min to 150°C and held for 47 min, then increased at 4°C/min to 215°C and held for 35 min) improved the resolution of some *t*18:1 isomers (*t*6-8 to *t*11-18:1) and allowed for the analysis of the remaining *t*18:1 isomers by difference (Kramer *et al.*, 2008).

For the identification of FAME by GC, the reference standard no. 461 from Nu-Check Prep Inc. (Elysian, MN, USA) was used. Branched-chain FAMEs were identified using a GC reference standard BC-Mix1 purchased previously from Applied Science (State College, PA, USA). For CLA isomers, the UC-59M standard from Nu-Chek Prep Inc. was used as it contained all four positional CLA isomers. Individual CLA isomers were obtained from Matreya Inc. (Pleasant Gap, PA, USA). The *t*18:1 and CLA isomers not included in the standard mixtures were identified by their retention times and elution orders, as reported previously (Cruz-Hernandez *et al.*, 2004; Kramer *et al.*, 2008). All chemicals and solvents were of analytical grade. The FAMEs were quantified using chromatographic peak area and internal standard (23:0 ME)-based calculations. Fatty acid concentrations were reported as percentage of total fatty acids identified.

Statistical analyses

Statistical analyses for subcutaneous and intramuscular fatty acids were performed using the PROC MIXED procedure of Statistical Analysis Systems Institute (SAS, 2009). The model incorporated dietary vitamin E dosage (340, 690, 1040 or 1740 IU/steer per day) as the main effect and the random effects of kill and pen. As pen was not significant, it was removed from the model. Individual animal was used as an experimental unit. The polynomial CONTRAST statement was included in the model to test for linear and quadratic effects of increasing dietary levels of vitamin E on subcutaneous and intramuscular fatty acid composition. The level of statistical significance for all analyses was set at $P \leq 0.05$.

Results

Diet composition

Ingredients and chemical composition of the diets are shown in Table 1. Diets contained 1.9% total fatty acids. Linoleic acid (18:2*n*-6, LA; 10%) was the most abundant fatty acid followed by 16:0 (3.8%) and *c*9-18:1 (3%).

Subcutaneous fat fatty acid profiles

Table 2 shows effects of vitamin E on fatty acid profiles in subcutaneous fat of steers fed high levels of barley. Dietary vitamin E concentration had no effect ($P > 0.05$) on total fatty acids (mg/g fat), percentages of total branched chain fatty acids (BCFA), mono-unsaturated fatty acids (MUFA) and

Table 1 *Composition of the basal total mixed ration*

Variable	Mean	s.d.
Diet ingredients (% as-fed basis)		
Steam rolled barley	72.8	
Barley silage	22.0	
Supplement[a]	4.36	
Canola oil	0.39	
Molasses	0.39	
Mold inhibitor	0.08	
Vitamin E (DL-α-tocopheryl acetate (IU/head per day)) from the supplement	340	
Nutrient composition (DM basis, $n = 4$)		
DM (%)	73.5	1.5
CP (%)	13.7	0.3
ADF (%)	15.7	1.1
NDF (%)	26.6	0.2
TDN (%)[b]	75.0	0.1
ME (MJ/kg)[c]	11.3	0.1
NEg (MJ/kg)[c]	4.81	0.07
NEm (MJ/kg)[c]	7.41	0.03
FA composition (mg/g, DM basis, $n = 4$)		
16:0	3.76	0.17
18:0	0.29	0.15
*c*9-18:1	3.06	0.26
*c*11-18:1	0.20	0.18
18:2*n*-6	10.0	0.5
18:3*n*-3	1.16	0.03
Total FAs	19.0	0.9

s.d. = standard deviation; DM = dry matter; ADF = acid detergent fibre; NDF = neutral detergent fibre; TDN = total digestible nutrients; ME = metabolisable energy; NEg = net energy for gain; NEm = net energy for maintenance; FA = fatty acid.

[a]Supplement contained 93% DM, 23% CP, 2.7% crude fat, 4.0% crude fibre, 8.7% calcium, 2.2% phosphorus, 1.3% sodium, 0.7% potassium, 0.3% sulphur, 0.2% magnesium, 2800 mg/kg zinc, 2200 mg/kg manganese, 963 mg/kg iron, 907 mg/kg copper, 48.5 mg/kg iodine, 17 mg/kg cobalt, 14.5 mg/kg selenium, 241 000 IU/kg vitamin A and 45 000 IU/kg vitamin D.

[b]For finishing diets, TDN (%) = 92.2 to 1.12 × ADF.

[c]ME and NEm and NEg values were calculated according to NRC (1996).

Table 2 *Effect of graded levels of vitamin E on FA profiles (% of total FAs) in subcutaneous fat of steers fed a barley-based diet*

Variable	Vitamin E level (IU DL-α-tocopheryl acetate/animal per day)				s.e.m.	*P*-value	
	340	690	1040	1740		Linear	Quadratic
Σ FA (mg/g fat)	90.5	88.2	89.2	88.6	1.12	0.38	0.48
Σ BCFA	1.37	1.40	1.38	1.38	0.04	0.92	0.60
Σ SFA	40.7	42.1	40.4	39.5	0.8	0.08	0.33
Σ MUFA	53.4	52.2	53.9	53.5	0.9	0.57	0.86
Σ *cis*-MUFA	50.4	49.1	50.8	50.3	0.8	0.72	0.88
Σ *t*18:1	3.35	3.53	3.03	3.19	0.16	0.22	0.64
*t*6/*t*7/*t*8-	0.206	0.222	0.188	0.179	0.012	0.035	0.73
*t*9-	0.261	0.273	0.244	0.239	0.012	0.068	0.91
*t*10-	1.97	1.92	1.79	1.621	0.137	0.047	0.90
*t*11-	0.401	0.469	0.481	0.497	0.037	0.077	0.32
*t*12-	0.097	0.113	0.100	0.095	0.005	0.28	0.12
*t*13/*t*14-	0.225	0.223	0.213	0.212	0.011	0.26	0.72
*t*15-	0.111	0.111	0.100	0.117	0.009	0.66	0.31
*t*16-	0.068	0.080	0.070	0.069	0.005	0.70	0.35
*t*11-/*t*10-	0.205	0.238	0.298	0.313	0.034	0.003	0.28
Σ CLA	0.520	0.490	0.496	0.490	0.031	0.99	0.19
Σ *t,t*-CLA	0.045	0.043	0.047	0.045	0.002	0.54	0.68
Σ *c-/t* CLA	0.452	0.443	0.448	0.443	0.028	0.86	0.95
*t*7,*c*9-18:2	0.084	0.093	0.089	0.081	0.006	0.54	0.25
*t*9,*c*11-18:2	0.071	0.065	0.071	0.066	0.005	0.61	0.94
*t*10,*c*12-18:2	0.012	0.013	0.011	0.012	0.001	0.19	0.57
*t*11,*c*13-18:2	0.010	0.010	0.009	0.009	0.001	0.79	0.98
*c*9,*t*11-18:2	0.246	0.250	0.252	0.268	0.017	0.36	0.83
Σ *n*-3	0.228	0.235	0.227	0.225	0.011	0.68	0.75
18:3*n*-3	0.196	0.216	0.207	0.200	0.011	0.94	0.19
20:3*n*-3	0.021	0.018	0.022	0.022	0.003	0.52	0.75
20:5*n*-3	nd	nd	nd	nd			
22:5*n*-3	nd	nd	nd	nd			
22:6*n*-3	nd	nd	nd	nd			
Σ *n*-6	1.95	2.05	1.85	1.79	0.08	0.054	0.73
18:2*n*-6	1.74	1.93	1.73	1.70	0.06	0.22	0.29
20:2*n*-6	0.033	0.037	0.035	0.032	0.004	0.74	0.44
20:3*n*-6	0.072	0.065	0.067	0.059	0.004	0.018	0.86
20:4*n*-6	0.045	0.036	0.040	0.040	0.004	0.51	0.16
Σ PUFA	2.14	2.26	2.07	2.03	0.08	0.16	0.65
n-6/*n*-3	7.71	8.39	8.06	7.47	0.24	0.19	0.035
SFA/PUFA	0.054	0.055	0.053	0.051	0.003	0.37	0.77

Σ FA = total fatty acids; s.e.m. = standard error of mean; BCFA = branched chain fatty acids; SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; CLA = conjugated linoleic acid; nd = not detected; PUFA = polyunsaturated fatty acids; *n*-3 = omega-3 FAs; *n*-6 = omega-6 FAs.
Σ BCFA = sum of iso-15:0 + anteiso-15:0 + iso-17:0 + anteiso-17:0 + iso-18:0;
ΣSFA = sum of 12:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0;
Σ *c*-MUFA = sum of *c*9-14:1 + *c*9-15:1 + *c*7-16:1 + *c*9-16:1 + *c*11-16:1 + *c*13-16:1 + *c*9-17:1 + *c*9-18:1 + *c*11-18:1 + *c*12-18:1 + *c*14-18:1 + 1 *c*5-18:1 + *c*10-19:1 + *c*9-20:1 + *c*11-20:1;
Σ *t*18:1 = Σ*t*-MUFA = sum of *t*6/*t*7/*t*8- + *t*9- + *t*10- + *t*11- + *t*12- + *t*13/*t*4- + *t*15- + *t*16;
Σ MUFA = *c*-MUFA + *t*-MUFA;
Σ *n*-3 = sum of 18:3*n*-3 + 20:3*n*-3;
Σ *n*-6 = sum of 18:2*n*-6 + 20:2*n*-6 + 20:3*n*-6 + 20:4*n*-6;
n-6/*n*-3 = (sum of 18:2*n*-6 + 20:3*n*-6 + 20:4*n*-6)/(sum of 18:3*n*-3 + 22:5*n*-3);
Σ PUFA = sum of 18:3*n*-3 + 18:2*n*-6 + 20:2*n*-6 + 20:3*n*-3 + 20:3*n*-6 + 20:4*n*-6;
Σ *t,t*-CLA = sum of *t*6,*t*8-18:2 + *t*10,*c*12-18:2;
Σ *c,t*-CLA = sum of *t* 12,1 *c* 4-18:2 + *c* 9, *t*11-18:2;
Σ CLA = sum of *t*6/*t*8-18:2 + *t*9,*c*11-18:2 + *t*10,*c*12-18:2 + *t*12,*c*14-18:2 + *c*9,*t*11-18:2;
*t*11-/*t*10- = *t*11-18:1/ *t*10-18:1 ratio;
SFA/PUFA = (sum of 12:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0)/(sum of 18:3*n*-3 + 18:2*n*-6 + 20:2*n*-6 + 20:3*n*-3 + 20:3*n*-6 + 20:4*n*-6).

cis-MUFA in subcutaneous fat. The total amount of saturated fatty acids (SFA) in subcutaneous fat tended to decrease linearly with increasing vitamin E supplementation ($P < 0.10$).

The dominant *t*18:1 isomers in subcutaneous fat were *t*10-18:1 and *t*11-18:1. Increasing levels of dietary vitamin E led to a linear decrease in the content of *t*6-/*t*7-/*t*8-18:1 and

*t*10-18:1 ($P < 0.05$). The subcutaneous fat proportion of *t*11-18:1 tended to increase linearly with vitamin E supplementation ($P < 0.10$). The *t*11-/*t*10-18:1 ratio also increased linearly ($P < 0.05$) with vitamin E concentration.

The proportion of total *t,t*- and *c,t*-CLA, and the sum of all CLA isomers in subcutaneous fat were unaffected ($P > 0.05$) by dietary vitamin E supplementation. The dominant CLA isomer was *c*9,*t*11-18:2 followed by *t*7,*c*9-18:2 and together they accounted for 68% of CLA. Consistent with total CLA, no changes in individual CLA isomers were found with increasing vitamin E supplementation ($P > 0.05$).

Vitamin E level in the diet also had no influence ($P > 0.05$) on total and individual *n*-3 fatty acids in subcutaneous fat. There was a linear decrease in total *n*-6 fatty acids with increasing vitamin E supplementation ($P < 0.05$, Table 2). This was related to a linear decline in 20:3*n*-6 ($P < 0.05$) in subcutaneous fat and the lowest value was recorded for the 1740 IU vitamin E/day treatment. The subcutaneous fat *n*-6:*n*-3 ratio, however, increased and then decreased in a quadratic ($P < 0.05$) fashion with steers supplemented with 690 and 1740 IU/day having the largest and smallest values, respectively. There were no differences ($P > 0.05$) in proportions of total PUFA content and SFA/PUFA ratio in subcutaneous fat across vitamin E treatments.

Intramuscular fatty acid profiles

As shown in Table 3, adding vitamin E to the diet had little effect on the overall fatty acid composition of intramuscular fat. Total fatty acids (mg/g fat) and proportions of total BCFA, SFA, *cis*-MUFA and MUFA in intramuscular were not influenced ($P > 0.05$) by vitamin E concentration. Vitamin E supplementation in the diet also had no effect ($P > 0.05$) on total *t*18:1 isomers. The major *t*18:1 isomers remained *t*10- and *t*11-18:1, however, neither their levels nor the *t*11-/*t*10-18:1 ratio in intramuscular fat were affected by vitamin E supplementation ($P > 0.05$). The content of *t*6-/*t*7-/*t*8-18:1 and *t*12-18:1, however, exhibited linear and quadratic patterns, respectively ($P < 0.1$; Table 3).

There were no differences ($P > 0.05$) in concentrations of individual, total *t,t*- and *c,t*-CLA in intramuscular fat across vitamin E treatments. The total CLA content in intramuscular fat, however, responded quadratically ($P < 0.05$) to vitamin E concentration with steers that received 340 and 1040 IU/day having the highest and lowest values, respectively (Table 3). Intramuscular fat proportions of total and individual *n*-3 and *n*-6 fatty acids, total PUFA, *n*-6/*n*-3 and SFA/PUFA ratios were unaffected ($P > 0.05$) by addition of vitamin E to the diet.

Discussion

For humans, high total *trans* fatty acid intake is associated with an increase in coronary heart disease (Oh *et al.*, 2005), and as such health authorities from several countries, including Canada, have recommended reductions in their intake, and have made labelling of total *trans* fatty acids in foods mandatory. Individual *trans* fatty acid isomers can, however, have differing effects and notably both *t*11-18:1 and rumenic acid have many potential health benefits mostly relating to their anticarcinogenic, immune modulatory and anti-inflammatory properties (Benjamin and Spener, 2009; Field *et al.*, 2009; Wang *et al.*, 2012). Owing to increasing demand for health-promoting foods, developing strategies to produce beef products with low *t*10-18:1 but enriched with *t*11-18:1 is important for the beef industry. Overall, there are very few studies that have attempted to prevent the *t*10-18:1 shift in beef by incorporating vitamin E into finisher diets containing high levels of grain. Dietary fatty acid values in the present study are in accordance with trials where barley-based finisher diets have been fed (Dugan *et al.*, 2008; Nassu *et al.*, 2011b) and are indicative of the relatively high 18:2*n*-6 content in barley grain. Although the concentration of vitamin E in the basal diet except for the protein supplement was not measured, Nassu *et al.* (2011a) via regression analysis estimated that it provided 770 IU of vitamin E/animal per day. It is important to take total basal level of vitamin E in the diet into account when considering vitamin E supplementation.

The result that *t*10-18:1 and *t*11-18:1 were the most abundant *t*18:1 isomers in subcutaneous and intramuscular fat is consistent with most available literature on conventionally fed beef (Kraft *et al.*, 2008; Alfaia *et al.*, 2009; Aldai *et al.*, 2010). The observed linear reductions in *t*6/*t*7/*t*8- and *t*10-18:1 and linear increase in *t*11-18:1 in subcutaneous fat with increasing levels of vitamin E agrees with earlier reports by Juárez *et al.* (2010 and 2011). Similar observations were made in cattle plasma (Kay *et al.*, 2005) and milk (Kay *et al.*, 2005; Pottier *et al.*, 2006). The mode of action of vitamin E has not been clarified yet. Martin and Jenkins (2002) and Pottier *et al.* (2006), however, suggested that vitamin E might have inhibitory and stimulatory effects on the growth and function of bacteria that produce *t*10- and *t*11-18:1, respectively. Given that ruminal environmental stress is an important factor for the synthesis of *trans* fatty acids in the rumen (Härtig *et al.*, 2005), vitamin E may in some way alleviate this stress, although it may not be through its typical role as an anti-oxidant, given the highly reduced environment of the rumen.

Another mechanism of action of α-tocopherol could be linked to its structural resemblance to compounds such as α-tocopherolquinol and deoxy-α-tocopherolquinol, which act as endogenous electron donors in the biohydrogenation of rumenic acid to *t*11-18:1 (Hughes and Tove, 1980a and 1980b), thus increasing the synthesis of fatty acids by this pathway. In this regard, it has been suggested that vitamin E can act as an electron donor to restore α-tocopherolquinol and deoxy-α-tocopherolquinol or act in place of the electron donors to provide the electrons for the reduction of the *cis* bond of the conjugated diene or get metabolised to these donors by microorganisms in the rumen (Pottier *et al.*, 2006). These explanations suggest that the modes of action of vitamin E could be multiple and variable. More research is, therefore, suggested to ascertain the mechanism(s) by which dietary vitamin E influences biohydrogenation pathways from PUFA to *t*18:1 isomers, and also from *t*18:1 isomers to 18:0 (stearic acid).

Table 3 *Effect of graded levels of vitamin E on FA profiles (% of total FAs) in intramuscular fat of steers fed a barley-based diet*

	Vitamin E level (IU DL-α-tocopheryl acetate/animal per day)					*P*-value	
Variable	340	690	1040	1740	s.e.m.	Linear	Quadratic
Σ FA (mg/g fat)	42.7	45.7	45.82	45.5	4.1	0.57	0.52
Σ BCFA	1.27	1.24	1.21	1.23	0.06	0.49	0.47
Σ SFA	41.2	41.6	40.9	41.3	0.78	0.94	0.83
Σ MUFA	48.8	48.3	48.9	48.3	0.5	0.61	0.89
Σ *cis*-MUFA	46.4	45.6	46.4	46.6	0.6	0.54	0.57
Σ *t*18:1	2.68	2.81	2.69	2.49	0.13	0.18	0.37
*t*6-/*t*7-/*t*8-	0.136	0.147	0.139	0.116	0.010	0.085	0.18
*t*9-1	0.215	0.215	0.204	0.200	0.009	0.18	0.88
*t*10-	1.418	1.44	1.30	1.32	0.09	0.30	0.77
*t*11-	0.386	0.420	0.368	0.369	0.038	0.38	0.82
*t*12-	0.103	0.094	0.091	0.100	0.006	0.79	0.082
*t*13-/*t*14-	0.208	0.205	0.198	0.202	0.008	0.52	0.51
*t*15-	0.068	0.060	0.068	0.055	0.006	0.18	0.72
*t*16-	0.068	0.065	0.070	0.070	0.005	0.68	0.90
*t*11-/*t*10-	0.253	0.300	0.301	0.300	0.036	0.31	0.34
Σ CLA	0.771	0.698	0.687	0.730	0.036	0.51	0.047
Σ *tt*-CLA	0.066	0.058	0.058	0.059	0.005	0.33	0.25
Σ *c*/*t*-CLA	0.703	0.652	0.631	0.653	0.036	0.36	0.23
*t*7,*c*9-18:2	0.132	0.125	0.112	0.112	0.009	0.10	0.48
*t*9,*c*11-18:2	0.075	0.069	0.068	0.068	0.005	0.41	0.52
*t*10,*c*12-18:2	0.017	0.018	0.015	0.016	0.001	0.26	0.70
*t*11,*c*13-18:2	0.011	0.013	0.012	0.014	0.002	0.41	0.85
*c*9,*t*11-18:2	0.392	0.349	0.375	0.394	0.025	0.57	0.20
Σ *n*-3	0.685	0.768	0.790	0.732	0.056	0.63	0.13
18:3*n*-3	0.269	0.297	0.280	0.273	0.013	0.76	0.17
20:3*n*-3	nd	nd	nd	nd			
20:5*n*-3	0.104	0.113	0.132	0.118	0.013	0.41	0.25
22:5*n*-3	0.253	0.282	0.287	0.266	0.023	0.82	0.26
22:6*n*-3	0.050	0.040	0.045	0.047	0.004	0.94	0.31
Σ *n*-6	4.45	4.61	4.28	4.33	0.36	0.46	0.97
18:2*n*-6	3.35	3.47	3.26	3.20	0.29	0.27	0.78
20:2*n*-6	0.065	0.070	0.060	0.060	0.005	0.25	0.95
20:3*n*-6	0.284	0.251	0.246	0.259	0.021	0.42	0.15
20:4*n*-6	0.647	0.682	0.721	0.650	0.073	1.0	0.32
22:4*n*-6	0.120	0.111	0.121	0.110	0.011	0.57	0.89
Σ PUFA	5.33	5.37	5.04	5.07	0.44	0.33	0.78
n-6/*n*-3	6.23	6.08	5.69	5.89	0.28	0.31	0.34
SFA/PUFA	0.131	0.130	0.129	0.126	0.014	0.66	0.99

Σ FA = total fatty acids; s.e.m. = standard error of mean; BCFA = branched chain fatty acids; SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; CLA = conjugated linoleic acid; nd = not detected; PUFA = polyunsaturated fatty acids; *n*-3 = omega-3 FAs; *n*-6 = omega-6 FAs.
Σ BCFA = sum of iso-15:0 + anteiso-15:0 + iso-17:0 + anteiso-17:0 + iso-18:0;
Σ SFA = sum of 12:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0;
Σ *c*-MUFA = sum of *c*9-14:1 + *c*9-15:1 + *c*7-16:1 + *c*9-16:1 + *c*11-16:1 + *c*13-16:1 + *c*9-17:1 + *c*9-18:1 + *c*11-18:1 + *c*12-18:1 + *c*14-18:1 + 1 *c*5-18:1 + *c*10-19:1 + *c*9-20:1 + *c*11-20:1;
Σ *t*-18:1 = Σ*t*-MUFA = sum of *t*6/*t*7/*t*8- + *t*9- + *t*10- + *t*11- + *t*12- + *t*13/ *t* 4- + *t*15- + *t*16;
Σ MUFA = *c*-MUFA + *t*-MUFA;
Σ *n*-3 = sum of 18:3*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3;
Σ *n*-6 = sum of 18:2*n*-6 + 20:2*n*-6 + 20:3*n*-6 + 20:4*n*-6 + 22:4*n*-6;
n-6/*n*-3 = (sum of 18:2*n*-6 + 20:2*n*-6 + 20:3*n*-6 + 20:4*n*-6 + 22:4*n*-6)/(sum of 18:3*n*-3 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-3);
Σ PUFA = sum of 18:3*n*-3 + 18:2*n*-6 + 20:2*n*-6 + 20:5*n*-3 + 20:3*n*-6 + 20:4*n*-6 + 20:5*n*-3 + 22:4*n*-6 + 22:6*n*-3;
Σ *t,t*-CLA = sum of *t*6,*t*8-18:2 + *t*10, *c*12-18:2;
Σ *c*-/*t*-CLA = sum of *t*12, *c*14-18:2 + *c* 9,*t*11-18:2;
Σ CLA = sum of *t*6/*t*8-18:2 + *t*9,*c*11-18:2 + *t*10,*c*12-18:2 + *t*12,*c*14-18:2 + *c*9,*t*11-18:2;
*t*11-/*t*10- = *t*11-18:1/*t*10-18:1 ratio;
SFA/PUFA ratio = (sum of 12:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0)/(sum of 18:3*n*-3 + 18:2*n*-6 + 20:2*n*-6 + 20:5*n*-3 + 20:3*n*-6 + 20:4*n*-6 + 20:5*n*-3 + 22:4*n*-6 + 22:6*n*-3).

The degree of response of *t*10- and *t*11-18:1 to vitamin E in subcutaneous fat was low in magnitude compared with results obtained by Juárez *et al.* (2010), and at no time did *t*11-18:1 exceed 32% of *t*10-18:1. As the highest rate of vitamin E supplementation in the current study was about 700 IU more than that used by Juárez *et al.* (2010) and fed for a longer period (120 *v.* 90 days), marked responses in *t*18:1 isomers were anticipated. The inconsistency in these results could, however, be attributed to differences in the type of forage used in the basal diet (barley silage *v.* alfalfa/brome hay) for these trials. Feeding flaxseed with hay led to greater accumulations of *t*18:1 isomers in plasma (He *et al.*, 2011), backfat and muscle (Nassu *et al.*, 2011b) of cattle than feeding flaxseed with barley silage. Kong *et al.* (2010) also reported dissimilarities in rumen microbial communities between cull cows fed a hay-based diet and those fed a silage-based diet. Such differences in endogenous microbial communities, which may be in part related to forage borne microbes, as well as alterations in endogenous ruminal microbes could be responsible for changing ruminal biohydrogenation of *trans* fatty acids. In addition, as compared with hay, ensiling may enhance lipolysis (Daley *et al.*, 2010), degrade antioxidants and alter plant secondary compounds in the forage, factors that could also modify the biohydrogenation activity of rumen bacteria (Bagheripour *et al.*, 2008). Substituting barley silage with grass hay in the basal diet might, therefore, be a valuable tool in preventing *t*10-18:1 shift and investigation into different forage types merit further investigation.

The observation that *t*11-/*t*10-18:1 ratio in subcutaneous fat increased linearly with vitamin E supplementation could imply that inclusion of vitamin E in high-concentrate rations is a potential strategy to improve beef healthfulness from a human nutrition perspective. The *t*11-/*t*10-18:1 ratio reflects the relative flow of PUFA through the major biohydrogenation pathways (i.e. *t*10- or *t*11-18:1) with a higher ratio being regarded as healthier for consumers than a lower ratio (Aldai *et al.*, 2010).

The finding that vitamin E did not influence total *t*18:1 in subcutaneous and intramuscular fat disagrees with earlier work (Juárez *et al.*, 2010 and 2011) where addition of vitamin E to the control diet decreased total *t*18:1. The contrast may also be related to differences in the forage component of the basal diet used in these trials. Potentially the positive and negative changes in individual *t*18:1 isomers observed in the current study were either equal or not high enough to elicit changes in total *t*18:1.

In Canada, beef is currently exempted from *trans* labelling based on the assumption that it contains relatively high amounts of *t*11-18:1 and rumenic acid (Health Canada, 2006). In the current study, average values for total *t*18:1, *t*10-18:1 and *t*11-18:1 per serving in regular ground beef (70% lean, 30% subcutaneous fat) would be 0.96, 0.53 and 0.14 g per 100 g of serving compared with 0.12, 0.062 and 0.017 g per 100 g of serving, respectively for lean steak. Thus, the total *t*18:1 content for regular ground beef would be greater than the current labelling requirements of 0.2 g per 100 g of serving) in Canada (Health Canada, 2006). It is also important to note that, on average, *t*10-18:1 and *t*11-18:1 would constitute about 52% and 15% of total *t*18:1, respectively, in both regular ground beef and lean steak. Current results also indicate that feeding a high barley-grain diet like most concentrate-based diets (Dugan *et al.*, 2007; Kraft *et al.*, 2008; Leheska *et al.*, 2008) increased levels of *trans* fatty acids other than *t*11-18:1 in beef compared with high-forage diets. In this context, it could, therefore, be important to re-examine the exclusion of *t*18:1 isomers from compulsory labelling of *trans* fatty acids in ruminant-derived foods. It also highlights the need for studying effects of individual *t*18:1 isomers, as their health effects in humans may differ significantly.

The lack of effect of vitamin E observed for *cis*-MUFA and total MUFA in the present experiment is in line with earlier studies in cattle (Juárez *et al.*, 2011) and sheep (Chen *et al.*, 2008). The negative linear trend observed between SFA and vitamin E supplementation in subcutaneous fat concurs with previous findings where feeding vitamin E and soybean oil (Chen *et al.*, 2008) or corn oil (Chen *et al.*, 2010) decreased total SFA in *longissimus* muscle of sheep. These results may be explained by the antioxidant effects of vitamin E on desaturase enzyme activity involved during *de novo* biosynthesis of fatty acids (Chen *et al.*, 2008 and 2010). Inclusion of vitamin E has been reported to increase Δ^9- and Δ^6-desaturase activity and consequently alter fatty acid profiles in rodents liver tissues (Özkan *et al.*, 2005). Assessment of the effects of vitamin E supplementation on activity of lipogenic enzymes in ruminants could, therefore, be worth exploring.

In accordance with current results, Juárez *et al.* (2011) found that the inclusion of vitamin E supplementation had no influence on key individual CLAs in subcutaneous and intramuscular fats. It is important to note that *c*9,*t*11-18:2 and *t*11-18:1 in subcutaneous fat followed similar numerical linear trends, verifying the prominent precursor–product relationship reported between these two fatty acids (Griinari *et al.*, 2000; Daley *et al.*, 2010). The finding that the percentage of total CLA in intramuscular fat was quadratically related to vitamin E concentration is difficult to explain, as no linear or quadratic effects were found for individual CLA isomers. According to Chen *et al.* (2010), adding vitamin E and corn oil to the diet increased concentrations of *t*11-18:1, *c*9,*t*11-18:2 and total CLA in the intramuscular fat of sheep.

The observation that inclusion of vitamin E did not affect the level of individual and total *n*-3 fatty acids in subcutaneous fat agrees with Juárez *et al.* (2011). Juárez *et al.* (2011), however, observed that feeding vitamin E plus flaxseed increased levels of 18:3*n*-3. Overall, literature suggests that vitamin E modifies the biohydrogenation of CLA and *n*-3 fatty acids when combined with PUFA-rich dietary sources (Chen *et al.*, 2008 and 2010; Juárez *et al.*, 2011). In this view, it might also be important to evaluate the effect of combining high levels of vitamin E with other rumen biohydrogenation modifiers such as buffers (Aldai *et al.*, 2010), dried distillers grains plus solubles (Dugan *et al.*, 2010) and plant secondary compounds (e.g. tannins (Vasta *et al.*, 2009)

and saponins (Brogna *et al*., 2011)), which have been shown to avert the *t*10-18:1 shift and improve CLA and *n*-3 fatty acid profiles in meat.

The observed decline in total *n*-6 with increasing vitamin E supplementation may be due to the observed decrease in 20:3*n*-6 in subcutaneous fat as vitamin E levels increased. Speculatively, these results might mean that vitamin E actually promotes the first step in *n*-6 fatty acid biohydrogenation or utilisation of 20:3*n*-6 increases with increasing amounts of dietary vitamin E. However, changes in *n*-6 fatty acids were less than 0.3% of total fatty acids. A quadratic response in the *n*-6:*n*-3 ratio was also found with increasing vitamin E supplementation and 18:2*n*-6 showed a similar numeric trend, however, an explanation for this is not immediately apparent. Overall, the responses of *n*-6 fatty acids in subcutaneous fat to increasing vitamin E levels did not provoke changes in total PUFA across vitamin E treatments. Similarly, Juárez *et al.* (2011) found that vitamin E had no effect on total PUFA. In contrast to these results, Chen *et al.* (2008 and 2010) reported that sheep supplemented with vitamin E and an oil source had higher total PUFA above that observed with oil alone. This suggests that combining vitamin E and an oil might elevate PUFA levels in beef fed high-concentrate diets.

The observation that addition of vitamin E to the diet had little effect on the fatty acid composition of intramuscular fat compared with subcutaneous fat could be a consequence of differences in sensitivity to dietary changes between the two tissues. Subcutaneous adipose tissue is relatively more sensitive to changes in diet and rumen function compared with muscle (Dugan *et al*., 2008). This is because subcutaneous fat has a high proportion of neutral lipids that accumulate greater levels of PUFA biohydrogenation products relative to polar lipids (Fritsche *et al*., 2001; Nuernberg *et al*., 2005).

Conclusions

Supplementation of vitamin E in finisher diets containing barley grain and silage as a basal diet has some potential to limit *t*10-18:1 deposition and promote a healthier balance between *t*11- and *t*10-18:1 in subcutaneous fat. Practical recommendations will, however, need to take into account dietary concentration of vitamin E, forage type, forage-to-concentrate ratio, presence of other rumen biohydrogenation modifiers, differences in tissues, cost of supplementation and the economic benefit under each production circumstance.

Acknowledgements

Authors are indebted to the Alberta Meat and Livestock Agency (ALMA) for financing the current research. Special thanks go to the AAFC–Lacombe Research Centre Beef Unit staff, meat processing staff and the meat quality technical team for their skilled assistance with this project. Drs C. Mapiye and T. Turner gratefully acknowledge the receipt of NSERC fellowships funded through ALMA. Dr N. Aldai thanks the Spanish Ministry of Science and Innovation for the contract through the 'Ramón y Cajal (RYC-2011-08593)' program.

References

Aldai N, Dugan MER, Rolland DC and Kramer JKG 2009. Survey of the fatty acid composition of Canadian beef: backfat and *longissimus lumborum* muscle. Canadian Journal of Animal Science 89, 315–329.

Aldai N, Dugan MER, Kramer JKG, Robertson WM, Juárez M and Aalhus JL 2010. *Trans*-18:1 and conjugated linoleic acid profiles after the inclusion of buffer, sodium sesquicarbonate, in the concentrate of finishing steers. Meat Science 84, 735–741.

Alfaia CPM, Alves SP, Martins SIV, Costa ASH, Fontes CMGA, Lemos JPC, Bessa RJB and Prates JAM 2009. Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. Food Chemistry 114, 939–946.

Association of Official Analytical Chemists (AOAC) 2003. Official methods of analysis, 14th edition. AOAC, Washington, DC, USA.

Bagheripour E, Rouzbehan Y and Alipour D 2008. Effects of ensiling, air-drying and addition of polyethylene glycol on *in vitro* gas production of pistachio by-products. Animal Feed Science and Technology 146, 327–336.

Bauchart D, Roy A, Lorenz S, Chardigny JM, Ferlay A, Gruffat D, Sébédio JL, Chilliard Y and Durand D 2007. Butters varying in *trans* 18:1 and *cis*-9, *trans*-11 conjugated linoleic acid modify plasma lipoproteins in the hypercholesterolemic rabbit. Lipids 42, 123–133.

Benjamin S and Spener F 2009. Conjugated linoleic acids as functional food: an insight into their health benefits. Nutrition and Metabolism 6, 36.

Brethour JR 1992. The repeatability and accuracy of ultrasound in measuring backfat of cattle. Journal of Animal Science 70, 1039–1044.

Brogna DMR, Nasri S, Salem HB, Mele M, Serra A, Bella M, Priolo A, Makkar HPS and Vasta V 2011. Effect of dietary saponins from *Quillaja saponaria* L. on fatty acid composition and cholesterol content in muscle *Longissimus dorsi* of lambs. Animal 5, 1124–1130.

Canadian Council on Animal Care 1993. Guide to the care and use of experimental animals, vol. 1, 2nd edition. Canadian Council on Animal Care, Ottawa, Ontario, Canada.

Chardigny J-M, Destaillats F, Malpuech-Brugere C, Moulin J, Bauman DE, Lock AL, Barbano DM, Mensink RP, Bezelgues J-B, Chaumont P, Combe N, Cristiani I, Joffre F, German JB, Dionisi F, Boirie Y and Sebedio J-L 2008. Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the *trans* Fatty Acids Collaboration (TRANSFACT) study. American Journal of Clinical Nutrition 87, 558–566.

Chen XJ, Mao HL, Lin J and Liu JX 2008. Effects of supplemental soybean oil and vitamin E on carcass quality and fatty acid profiles of meat in Huzhou lamb. Acta Agriculturae Scandinavica Section A – Animal Sciences 58, 129–135.

Chen XJ, Mao HL, Ma XN and Liu JX 2010. Effects of dietary corn oil and vitamin E supplementation on fatty acid profiles and expression of acetyl CoA carboxylase and stearoyl-CoA desaturase gene in Hu sheep. Animal Science Journal 81, 165–171.

Cruz-Hernandez C, Deng Z, Zhou J, Hill AR, Yurawecz MP, Delmonte P, Mossoba MM, Dugan MER and Kramer JKG 2004. Methods to analyze conjugated linoleic acids (CLA) and *trans*-18:1 isomers in dairy fats using a combination of GC, silver ion TLC-GC, and silver ion HPLC. Journal of AOAC International 87, 545–560.

Daley CA, Abbot A, Doyle PS, Nader GA and Larson S 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal 9, 1–12.

Dugan MER, Rolland DC, Aalhus JL, Aldai N and Kramer JKG 2008. Subcutaneous fat composition of youthful and mature Canadian beef: emphasis on individual conjugated linoleic acid and *trans*-18:1 isomers. Canadian Journal of Animal Science 88, 591–599.

Dugan MER, Kramer JKG, Robertson WM, Meadus WJ, Aldai N and Rolland DC 2007. Comparing subcutaneous fat in beef and muskox with emphasis on *trans*-18:1 and conjugated linoleic acids. Lipids 42, 509–518.

Dugan MER, Aldai N, Kramer JKG, Gibb DJ, Juárez M and McAllister TA 2010. Feeding wheat dried distillers grains with solubles improves beef *trans* and conjugated linoleic acid profiles. Journal of Animal Science 88, 1842–1847.

Field CJ, Blewett HH, Proctor S and Vine D 2009. Human health benefits of vaccenic acid. Applied Physiology Nutrition and Metabolism 34, 979–991.

Folch J, Lees M and Stanley GHS 1957. A simple method for the isolation and purification of total lipids from animal tissue. The Journal of Biological Chemistry 226, 497–509.

Fritsche S, Rumsey TS, Yurawecz MP, Ku Y and Fritsche J 2001. Influence of growth promoting implants on fatty acid composition including conjugated linoleic acid isomers in beef fat. European Food Research and Technology 212, 621–629.

Gabryszuk M, Czauderna M, Baranowski A, Strzałkowska N, Jóźwik A and Krzyżewski J 2007. The effect of diet supplementation with Se, Zn and vitamin E on cholesterol, CLA and fatty acid contents of meat and liver of lambs. Animal Science Papers and Reports 25, 25–33.

Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV and Bauman DE 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by $^{\Delta}$9-desaturase. Journal of Nutrition 130, 2285–2291.

Härtig C, Loffhagen N and Harms H 2005. Formation of *trans* fatty acids is not involved in growth-linked membrane adaptation of *Pseudomonas putida*. Applied Environmental Microbiology 71, 1915–1922.

He ML, Chung Y-H, McAllister TA, Beauchemin KA, Mir PS, Aalhus JL and Dugan MER 2011. Inclusion of flaxseed in hay- and barley silage diets increases alpha-linolenic acid in cow plasma independent of forage type. Lipids 46, 577–585.

Health Canada 2006. Food and nutrition. Transforming the food supply. Report of the *trans* fat task force submitted to the minister of health. Retrieved August 10, 2011, from http://www.hcsc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/nutrition/tf-gt_reprap-eng.pdf

Hristov AN, Kennington LR, McGuire MA and Hunt CW 2005. Effect of diets containing linoleic acid- or oleic acid-rich oils on ruminal fermentation and nutrient digestibility, and performance and fatty acid composition of fat and muscle tissues of finishing cattle. Journal of Animal Science 83, 1312–1321.

Hughes PE and Tove SB 1980a. Identification of an endogenous electron donor for biohydrogenation as α-tocopherolquinol. Journal of Biology and Chemistry 255, 4447–4452.

Hughes PE and Tove SB 1980b. Identification of deoxy-α-tocopherolquinol as another endogenous electron donor for biohydrogenation. Journal of Biology and Chemistry 255, 11802–11806.

Juárez M, Dugan MER, Aalhus JL, Aldai N, Basarab JA, Baron VS and McAllister TA 2011. Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. Meat Science 88, 434–440.

Juárez M, Dugan MER, Aldai N, Aalhus JL, Basarab JA, Baron VS and McAllister TA 2010. Dietary vitamin E inhibits the *trans* 10–18:1 shift in beef backfat. Canadian Journal of Animal Science 90, 9–12.

Kay JK, Roche JR, Kolver ES, Thomson NA and Baumgard LH 2005. A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows. Journal of Dairy Research 72, 322–332.

Kong YH, He ML, McAlister TA, Seviour R and Forster R 2010. Quantitative fluorescence in situ hybridization of microbial communities in the rumen of cattle fed different diets. Applied Environmental Microbiology 76, 6933–6938.

Kraft J, Kramer JKG, Schoene F, Chambers JR and Jahreis G 2008. Extensive analysis of long-chain polyunsaturated fatty acids, CLA, *trans*-18:1 isomers, and plasmalogenic lipids in different retail beef types. Journal of Agricultural and Food Chemistry 56, 4775–4782.

Kramer JKG, Hernandez M, Cruz-Hernandez C, Kraft J and Dugan MER 2008. Combining results of two GC separations partly achieves determination of all *cis* and *trans* 16:1, 18:1, 18:2, 18:3 and CLA isomers of milk fat as demonstrated using Ag-ion SPE fractionation. Lipids 43, 259–273.

Lee S, Panjono K, Kang SM, Kim TS and Park YS 2008. The effects of dietary sulphur and vitamin E supplementation on the quality of beef from the *longissimus* muscle of Hanwoo bulls. Asian–Australian Journal of Animal Science 21, 1059–1066.

Leheska JM, Thompson LD, Howe JC, Hentges E, Boyce J, Brooks JC, Shriver B, Hoover L and Miller MF 2008. Effects of conventional and grass feeding systems on the nutrient composition of beef. Journal of Animal Science 88, 3575–3585.

Liao G, Xu X and Zhou G 2009. Effects of cooked temperatures and addition of antioxidants on formation of heterocyclic aromatic amines in pork floss. Journal of Food Processing and Preservation 33, 159–175.

Martin SA and Jenkins TC 2002. Factors affecting conjugated linoleic acid and *trans*-C18:1 fatty acid production by mixed ruminal bacteria. Journal of Animal Science 80, 3347–3352.

Morrissey PA, Quinn PB and Sheehy PJA 1994. Newer aspects of micronutrients in chronic disease: vitamin E. Proceedings of the Nutrition Society 53, 571–582.

Nassu RT, Dugan MER, Juárez M, Basarab JA, Baron VS and Aalhus JL 2011a. Effect of α-tocopherol tissue levels on beef quality. Animal 5, 2010–2018.

Nassu RT, Dugan MER, He ML, McAllister TA, Aalhus JL, Aldai N and Kramer JKG 2011b. The effects of feeding flaxseed to beef cows given forage based diets on fatty acids of *longissimus thoracis* muscle and backfat. Meat Science 89, 469–477.

NRC – National Research Council 1996. Nutrient requirements of beef cattle, 7th edition. National Academy Press, Washington, DC.

Nuernberg K, Nuernberg G, Ender K, Dannenberger D, Schabbel W, Grumbach S, Zupp W and Steinhart H 2005. Effect of grass vs. concentrate feeding on the fatty acid profile of different fat depots in lambs. European Journal of Lipid Science and Technology 107, 737–745.

Oh K, Hu FB, Manson JE, Stampfer MJ and Willett WC 2005. Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. American Journal of Epidemiology 161, 672–679.

Özkan Y, Yilmaz Ö, Öztürk A and Ersan Y 2005. Effects of triple antioxidant combination (vitamin E, vitamin C and α-lipoic acid) with insulin on lipid and cholesterol levels and fatty acid composition of brain tissue in experimental diabetic and non-diabetic rats. Cell Biology International 29, 754–760.

Pottier J, Focant M, Debier C, De Buysser G, Goffe C, Mignolet E, Froidmont E and Larondelle Y 2006. Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. Journal of Dairy Science 89, 685–692.

Roy A, Chardigny JM, Bauchart D, Ferlay D, Lorenz S, Durand D, Gruffat D, Faulconnier Y, Sébédio JL and Chilliard Y 2007. Butters rich either in *trans*-10-C18:1 or in *trans*-11-C18:1 plus *cis*-9, *trans*-11 CLA differentially affect plasma lipids and aortic fatty streak in experimental atherosclerosis in rabbits. Animal 1, 467–476.

Sofi F, Buccioni A, Cesari F, Gori AM, Minieri S, Mannini L, Casini A, Gensini GF, Abbate R and Antongiovanni M 2010. Effects of a dairy product (pecorino cheese) naturally rich in *cis*-9, *trans*-11 conjugated linoleic acid on lipid, inflammatory and haemorheological variables: a dietary intervention study. Nutrition, Metabolism and Cardiovascular Diseases 20, 117–124.

Statistical Analysis Systems Institute (SAS) 2009. SAS user's guide: statistics, SAS for windows, release 9.2. SAS Institute Inc., Cary, NC.

Sukhija PS and Palmquist DL 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. Journal of Agricultural and Food Chemistry 36, 1202–1206.

Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E, Hall WL, Gerry AB, Leake DS, Grimble RF, Williams CM, Calder PC and Yaqoob P 2006. Effects of dairy products naturally enriched with *cis*-9,*trans*-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. American Journal of Clinical Nutrition 83, 744–753.

Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597.

Vasta V, Makkar HPS, Mele M and Priolo A 2009. Ruminal bio-hydrogenation as affected by tannins *in vitro*. British Journal of Nutrition 102, 82–92.

Wang Y, Jacome-Sosa MM and Proctor SD 2012. The role of ruminant *trans* fat as a potential nutraceutical in the prevention of cardiovascular disease. Food Research International 46, 460–468.