RELATIONSHIPS OF *TRANS*-18:1 ISOMERS BETWEEN RED BLOOD CELLS AND BEEF TISSUES IN STEERS FED RED CLOVER SILAGE WITH/WITHOUT FLAXSEED

C. Mapiye[1], M.E.R. Dugan[1*], T.D. Turner[1], D.C. Rolland[1], J.A. Basarab[2], V.S. Baron[1], T.A. McAllister[3], H.C. Block[4], B. Uttaro[1] and J.L. Aalhus[1]

[1]Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, Alberta, Canada; [2]Alberta Agriculture and Rural Development, Lacombe Research Centre, Lacombe, Alberta, Canada; [3]Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, Canada; [4]Agriculture and Agri-Food Canada, Morden Research Centre, Morden, Manitoba, Canada.
[*]Corresponding author: email: duganm@agr.gc.ca

Abstract- The current study evaluated the effects of flaxseed supplementation in a red clover silage based diet on steer red blood cell (RBC) *trans(t)*18:1 isomers over time and their relationships to adipose tissue and muscle profiles at slaughter. Steers were fed a red clover diet with or without flaxseed (6 steers/diet). Blood (RBC) samples were collected once a month for 6 months while kidney fat, subcutaneous fat and *longissimus thoracis* muscle were sampled *post-mortem*. Most *t*18:1 isomers exhibited both linear (*P*<0.001) and quadratic (*P*<0.05) patterns within diets over time, except for *t*6*t*/7/*t*8-, *t*9- and *t*10-. There were low (*P*<0.05) to high (*P*<0.001) correlations of *t*6*t*/7/*t*8-, *t*12- and *t*/13/*t*14-18:1 between RBCs and beef tissues but the correlations were inconsistent over time. Moderate (*P*<0.01) to high (*P*<0.001) correlations of *t*9-, *t*10- and *t*11-18:1 isomers were observed between RBCs from month 2 to 6 and beef tissues, with *t*11-18:1 having the strongest correlations of the isomers. Results indicate RBCs could be sampled as early as 2 months into the feeding period and used to estimate final *t*9-, *t*10- and *t*11-18:1 concentrations in beef.

Key words: intramuscular fat, time on feed, vaccenic acid.

I. INTRODUCTION

Cattle fed grain-based diets have been reported to produce beef with more *tran*s (*t*) 10-18:1 than *t*11-18:1 (vaccenic acid) [1]. High intakes of *t*10-18:1 have been shown to negatively impact plasma cholesterol profiles in animal models [2], while *t*11-18:1 may serve to reduce plasma triglyceride levels [3]. Although research on the effects of individual *t*18:1 isomers is limited, there are on-going efforts to reduce *t*10-18:1 and increase *t*11-18:1 in beef. Currently, one of the promising strategies includes long-term supplementation of grass hay with flaxseed, which has yielded 2.5% *t*11-18:1 in subcutaneous fat versus 0.9% when feeding barley silage as a roughage source [4]. Large inter-animal variation in concentrations of biohydrogenation products have been seen when trying to increase these levels. Producers may, therefore, benefit from being able to predict the levels of *t*18:1 isomers in beef early in the feeding period. A recent study [5] reported strong correlations of some *t*18:1 isomers between red blood cells (RBCs) collected at slaughter and beef tissues indicating the potential of RBCs to predict levels *t*18:1 isomers in beef. How early in the feeding period RBC *trans* profiles can be used to predict final adipose tissue and muscle concentrations is unknown. The current study aimed to evaluate the effects of flaxseed supplementation in a red clover silage based diet on steer RBCs *t*18:1 isomers over time and determine their relationships to adipose tissue and muscle profiles at slaughter. In this study, red clover silage was chosen as the roughage source as it has been shown to have positive effects on rumen lipolysis and biohydrogenation [6]. Consequently, it might produce higher levels of *t*11-18:1 when supplemented with flaxseed compared to grass hay.

II. MATERIALS AND METHODS

Twelve British × Continental crossbred steers (16-18 months of age) with an initial mean body weight of 363 ± 26.5 kg were randomly assigned to 2 pens of 6 animals and fed experimental diets from December 2010 to July 2011 at Lacombe Research Centre, Alberta, Canada. Diets contained 70% red clover silage, 15% steam

rolled barley/vitamin-mineral supplement and either additional 15% barley or 15% triple rolled flaxseed (all DM basis). Animals were cared for in accordance with guidelines established by the CACC [7].

Feed samples were collected weekly and stored at -40°C, then pooled monthly before determination of DM, minerals, crude fat, crude protein and acid detergent fibre [8] (Table1). Fatty acids from the finishing total mixed ration were extracted and methylated as described by Sukhija & Palmquist [9] and analysed according to Dugan *et al*. [1] (Table1).

Table 1 Nutritional composition of the experimental diets and their fatty acid profiles

Nutrient (%)	Control diet	Flaxseed diet
Dry matter (DM)	0.53	0.52
Crude protein	13.65	15.66
Crude fat	2.66	7.97
Acid detergent fiber (ADF)	33.53	39.11
Calcium	0.90	0.89
Phosphorus	0.27	0.29
Magnesium	0.29	0.31
Fatty acids (% of total fatty acids)		
16:0	19.69	8.32
18:0	1.86	3.81
*cis*9-18:1	9.85	15.07
*cis*11-18:1	0.82	0.64
18:2*n*-6	33.51	19.08
18:3*n*-3	27.82	50.97
Total fatty acids (mg/g DM)	14.62	70.78

[1]vitamin-mineral premix per kg diet DM provided 0.08% potassium, 0.03% sulphur, 0.2 mg/kg cobalt, 6.69 mg/kg copper, 0.39 mg/kg iodine, 19.9 mg/kg iron, 13.6 mg/kg manganese, 21.6 mg/kg zinc, 0.19 mg/kg cobalt added, 5.63 mg/kg copper added, 0.39 mg/kg iodine added, 11.3 mg/kg manganese added, 0.11 mg/kg selenium added, 18.7 mg/kg zinc added, 1.88 KIU/kg vitamin A added, 0.38 KIU/kg vitamin D_3 added, 52.5 IU/kg vitamin E added.

Blood was collected from the jugular vein using an 18-gauge needle into Vacutainer® blood tubes containing EDTA anticoagulant once every month for 6 months. After collection the blood was centrifuged at 800 g for 18 min, plasma and white cells discarded and RBCs were methylated according to Rise *et al.* [10] and fatty acid methyl esters (FAMEs) were extracted and stored in hexane at -20°C pending analysis. Steers were slaughtered at target ultrasound subcutaneous fat of 6-8 mm between the 12th and 13th rib over the *longissimus thoracis* (LT) muscle on the right side. At slaughter, animals were stunned, exsanguinated and dressed in a commercial manner at the Lacombe Research Centre abattoir. At approximately 20 min *post-mortem*, during evisceration, samples of kidney (perirenal) fat and subcutaneous fat adjacent to the 12th rib were collected and stored at -80°C until analysed. At 24 h *post mortem*, a left LT steak 2.5 cm thick was dissected from the 12th rib, comminuted and frozen at -80°C for subsequent fatty acid analyses. Subcutaneous and kidney fatty acid samples (50 mg) were freeze-dried and directly methylated with sodium methoxide [1]. Intramuscular fatty acids were extracted with 2:1 chloroform: methanol using a 20:1 solvent to sample ratio [11]. The extracts were then methylated using both 5% methanolic HCl and 0.5 N sodium methoxide. The *t*18:1 FAMEs were analyzed using the 175^{0}C plateau temperature program [1].

Concentrations of RBC *t*18:1 isomers (% of total FAME) were subjected to repeated measures analysis using the PROC MIXED procedure of the SAS Institute [12]. Assuming a first-order autoregressive covariance structure, the model incorporated the fixed effects of diet, time on feed, diet × time on feed, with RBC measurements repeated over time. The polynomial CONTRAST statement was included in the model to test for linear and quadratic effects within diets over time. Pearson correlation coefficients between *t*18:1 isomer concentrations in RBC and beef tissues were generated using the PROC CORR procedure of SAS with the partial correlation option to adjust for the fixed effects of diet [12].

III. RESULTS AND DISCUSSION

The concentrations of *t*18:1 isomers in RBCs were influenced by both diet and time on feed (P<0.01; Table 2). Although most *t*18:1 isomers exhibited both linear (P<0.001) and quadratic (P<0.05) patterns within diets over time, their concentrations tended to increase and decrease quadratically in steers fed flaxseed and control diets, respectively (Table 2). The concentration of *t*11-18:1 in steers supplemented with flaxseed increased markedly in the first month before

increasing gradually to month 6. These findings may in part be attributed to the presence of polyphenol oxidase in red clover [6] and a slight decrease in stearoyl-CoA desaturase activity in beef cattle aged between 18 and 30 months [13]. Polyphenol oxidase might have interfered with ruminal biohydrogenation of α-linolenic acid in flaxseed in a cumulative manner resulting in increased recovery of *t*18:1 isomers in RBCs, especially *t*11-18:1 over time [6].

Table 2 Effects of flaxseed supplementation and time on feed on *trans (t)*18:1 isomers in red blood cells of steers fed a red clover silage based diet

*t*18:1 isomer[1]		Time on feed (months)								P-values		
	Diet	0	1	2	3	4	5	6	s.e.m[2]	ANOVA	Linear	Quadratic
*t*6/*t*7/*t*8-	Control	0.08	0.05	0.05	0.04	0.05	0.05	0.05	0.01	0.004	0.001	0.01
	Flaxseed	0.07	0.09	0.11	0.08	0.10	0.10	0.10			0.20	0.21
*t*9-	Control	0.09	0.08	0.07	0.06	0.09	0.09	0.05	0.01	0.001	0.001	0.95
	Flaxseed	0.11	0.15	0.14	0.13	0.15	0.16	0.12			0.51	0.03
*t*10-	Control	0.06	0.06	0.06	0.05	0.07	0.07	0.04	0.01	0.001	0.69	0.20
	Flaxseed	0.06	0.10	0.10	0.09	0.11	0.11	0.10			0.09	0.03
*t*11-	Control	0.63	0.52	0.50	0.32	0.34	0.41	0.40	0.21	0.001	0.001	0.01
	Flaxseed	0.61	1.79	1.79	1.93	1.88	1.86	2.09			0.001	0.03
*t*12-	Control	0.12	0.12	0.11	0.10	0.11	0.12	0.10	0.02	0.001	0.001	0.01
	Flaxseed	0.13	0.30	0.34	0.34	0.38	0.37	0.45			0.001	0.01
*t*13/*t*14-	Control	0.42	0.24	0.23	0.21	0.24	0.22	0.22	0.03	0.001	0.001	0.01
	Flaxseed	0.42	0.46	0.54	0.53	0.69	0.72	0.70			0.001	0.01

[1]*Trans*-18:1 isomers are expressed as percent (%) of total fatty acids. [2]Standard error of mean

Generally, low (P<0.05) to high (P<0.001) correlations of *t*6*t*/7/*t*8-, *t*12- and *t*/13/*t*14-18:1 isomers were observed between RBCs and beef tissues but were not consistent over time (Table 3). Moderate (P<0.01) to high (P<0.001) correlations of *t*9- and *t*10-18:1 isomers were observed between RBCs from month 2 to 6 and beef tissues, with some minor and inconsistent variations across tissues (Table 3). Similar correlations of these *t*18:1 isomers between RBCs and beef tissues were found previously [5] using RBC samples collected at slaughter.

Table 3 Pearson correlation coefficients of *trans*-18:1 isomers between red blood cells (RBC) and three beef tissues from steers fed a red clover silage based diet

		Time on feed (months)						
*t*18:1 isomer[1]	Tissues	0	1	2	3	4	5	6
*t*6/*t*7/*t*8-	RBC-SF[1]	0.07	0.11	0.56	0.69**	0.48	0.71**	0.72**
	RBC-KF[2]	0.10	0.03	0.65*	0.86***	0.52	0.71**	0.76**
	RBC-IMF[3]	0.17	0.10	0.72**	0.70**	0.39	0.83***	0.78**
*t*9-	RBC-SF	0.20	0.68*	0.89***	0.79**	0.66*	0.77**	0.71**
	RBC-KF	0.26	061*	0.78**	0.91***	0.85***	0.73**	0.78**
	RBC-IMF	0.28	0.71**	0.89***	0.76**	0.77**	0.75**	0.75**
*t*10-	RBC-SF	0.31	0.61*	0.82***	0.72**	0.83***	0.72**	0.75**
	RBC-KF	0.21	0.66*	0.80**	0.78**	0.86***	0.79**	0.70**
	RBC-IMF	0.38	0.59*	0.82***	0.71**	0.79**	0.79**	0.85***
*t*11-	RBC-SF	0.01	0.60*	0.82***	0.86***	0.85***	0.84***	0.90***
	RBC-KF	0.03	0.60*	0.95***	0.96***	0.95***	0.98***	0.90***
	RBC-IMF	0.04	0.70**	0.98***	0.96***	0.94***	0.99***	0.90***
*t*12-	RBC-SF	0.33	0.55	0.44	0.64*	0.83***	0.93***	0.83***
	BC-KF	0.25	0.17	0.37	0.47	0.40	0.83***	0.72**
	RBC-IMF	0.03	0.18	0.70**	0.58	0.34	0.70**	0.84***
*t*13/*t*14-	RBC-SF	0.39	0.20	0.06	0.40	0.51	0.90***	0.17
	RBC-KF	0.16	0.33	0.14	0.58	0.57	0.90***	0.11
	RBC-IMF	0.38	0.05	0.23	0.40	0.34	0.72**	0.10

[1]Subcutaneous fat; [2]kidney fat; [3]intramuscular fat. *P<0.05= low; P<0.01=moderate; ***P<0.001=high. *t= trans.*

Trans 11-18:1concentrations in RBCs from month 2 to 6 were strongly (P<0.001) correlated with those in beef tissues. Contrary to current findings, Aldai [5] found weak and non significant correlations for *t*11-18:1 between RBCs and subcutaneous fat, and RBCs and intramuscular fat, respectively. The variation could be partly due to basal diet-related differences in concentrations of *t*11-18:1 in RBCs and beef tissues between the two studies.

IV. CONCLUSIONS

Including flaxseed in the red clover diet had a positive effect (increased *t*11- more than *t*10-18:1) on *t*18:1 isomeric profile of RBCs. The levels of *t*9-, *t*10- and *t*11-18:1 isomers in RBCs measured early (2 months) in the feeding period have the potential to predict final concentrations in beef. This could be used to produce beef with consistent and high levels of *t*11-18:1, and reduce feed costs by sorting and only feeding oilseeds to animals accumulating high levels of *t*11-18:1.

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