SHORT COMMUNICATION: Erythrocytes assayed early ante mortem can predict adipose tissue and muscle *trans*-18:1 isomeric profiles of steers fed red clover silage supplemented with flaxseed

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Mapiye, C., Dugan, M. E. R., Turner, T. D., Rolland, D. C., Basarab, J. A., Baron, V. S., McAllister, T. A., Block, H. C., Uttaro, B. and Aalhus, J. L. 2013. **SHORT COMMUNICATION: Erythrocytes assayed early ante mortem can predict adipose tissue and muscle *trans*-18:1 isomeric profiles of steers fed red clover silage supplemented with flaxseed**. Can. J. Anim. Sci. **93**: 149–153. Steers were fed a red clover silage-based diet with or without flaxseed to evaluate over time the effects of flaxseed supplementation on erythrocytes (ERC) *trans(t)*18:1 isomers composition and their relationships to adipose tissue and muscle *t*18:1 profiles at slaughter. Concentrations of most ERC *t*18:1 isomers in steers fed flaxseed increased ($P<0.01$) markedly in the first 2 mo and increased gradually thereafter. Strong ($P<0.01$) correlations of *t*9-, *t*10- and *t*11-18:1 isomers were observed from month 2 to 6 between ERC and beef tissues collected at slaughter from steers fed flaxseed. Findings suggest that ERC sampled as early as 2 mo into the feeding period can be indicative of variation in beef *t*18:1 isomeric profile at a later slaughter date when feeding red clover silage with flaxseed.

Key words: Beef tissues, fat, feeding period, *t*18:1 isomers, vaccenic acid

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Mots clés: Tissus du bæuf, matière grasse, période d'engrais, isomères *t*18:1, acide vaccénique

Feeding high levels of grain to cattle can elevate *trans* (*t*) 10-18:1 more than *t*11-18:1 (vaccenic acid) in beef (Dugan et al. 2007; Aldai et al. 2011). Increased consumption of *t*10-18:1 has been shown to increase the plasma ratio of very low density lipoprotein + low density lipoprotein to high density lipoprotein ratio in rabbits (Bauchart et al. 2007), while *t*11-18:1 may serve to reduce plasma triglyceride levels (Wang et al. 2012). Although research on the effects of individual *t*18:1 isomers on human health is limited, there are on-going commercial efforts to lower *t*10-18:1 and raise *t*11-18:1 levels in beef. Current potential nutritional strategies include 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

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Abbreviations: **ERC**, erythrocyte; **FAME**, fatty acid methyl ester; **LT**, longissimus thoracis roughage source (Nassu et al. 2011). Interestingly, large inter-animal variation in concentrations of *t*11-18:1 isomers has been observed when dietary approaches are used to try to increase muscle and adipose tissue levels of this isomer. Producers and researchers may, therefore, benefit from being able to predict the concentrations of *t*18:1 isomers in beef early in the feeding period.

A recent study (Aldai et al. 2012) reported strong correlations of some *t*18:1 isomers between erythrocytes (ERC) and beef tissues collected at slaughter, indicating the potential of ERC to predict levels of *t*18:1 isomers in beef. How early in the feeding period ERC *t*18:1 profiles can be used to predict final adipose tissue and muscle concentrations is, however, unknown. The current study, therefore, aimed to evaluate the effects of flaxseed supplementation in a red clover silage-based diet on steer ERC *t*18:1 isomers over time and determine their relationships to adipose tissue and muscle *t*18:1 profiles at slaughter. In this study, red clover silage was chosen as the roughage source as it has been reported to reduce the extent of rumen lipolysis and biohydrogenation (Van Ranst et al. 2011). Consequently, red

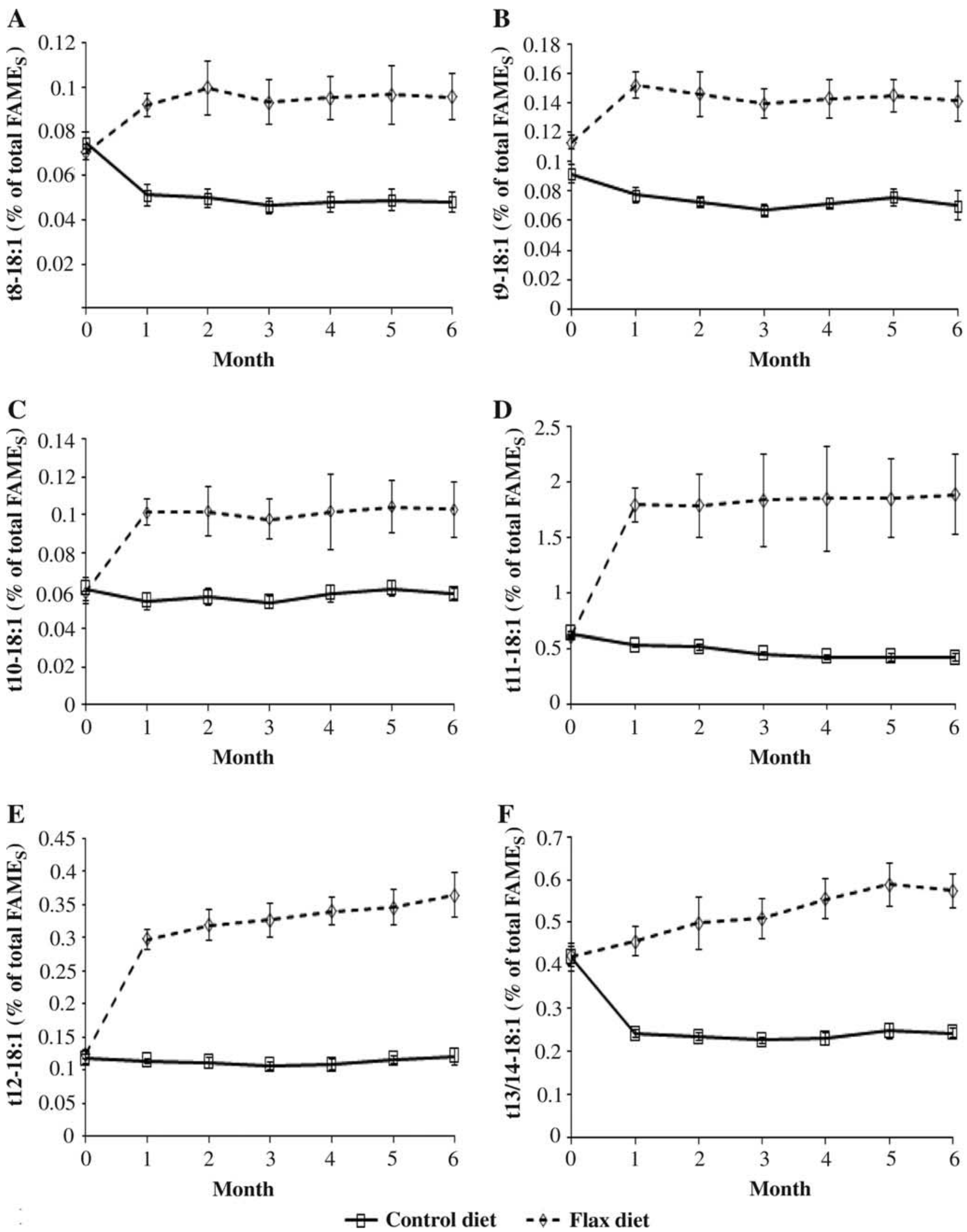

Fig. 1. Effects of flaxseed supplementation and time on feed on *t*6/*t*7/*t*8- (A), *t*9- (B), *t*10- (C), *t*11- (D), *t*12- (E) and *t*13/*t*14-18:1 (F) isomers in erythrocytes from steers fed a red clover silage based diet.

clover silage might yield higher levels of *t*11-18:1 in beef when supplemented with flaxseed. It was hypothesized that erythrocytes assayed early ante-mortem can be indicative of variation in beef *trans*-18:1 isomeric profiles when feeding red clover silage supplemented with flaxseed.

Twelve British × Continental crossbred steers (15–16 mo; 363 ± 26.5 kg) were randomly assigned to two pens of six animals and fed experimental diets for 215 d from December 2010 to July 2011 at the Lacombe Research Centre, Alberta, Canada. Diets contained 70% red clover silage, 15% steam rolled barley/vitamin-mineral supplement and either additional 15% barley or 15% triple rolled flaxseed (all dry matter basis). The flaxseed diet provided (on% dry matter basis) 14.6 crude protein, 8.0 fat, 36.1 acid detergent fibre, 1.1 Ca, 0.34 P and 0.27 Mg while the control diet provided 11.9 crude protein, 3.3 fat, 33.9 acid detergent fibre, 1.0 Ca, 0.30 P and 0.22 Mg. The major fatty acids (% of total fatty acids) in the control and flaxseed diets were α-linolenic acid (27.8 and 51%, respectively), linoleic acid (33.5 and 19.1%) and oleic acid (9.85 and 15.1%). Animals were cared for in accordance with guidelines established by the Canadian Council on Animal Care (1993).

Blood was collected from the jugular vein using an 18-gauge needle into Vacutainer® (Becton, Dickinson and Company, Franklin Lakes, NJ) blood tubes containing EDTA anticoagulant once every month at 0900 in the morning before feeding. After collection the blood was centrifuged at 800 × *g* for 18 min, plasma and white cells were discarded and ERC were methylated according to Risé et al. (2005) and fatty acid methyl esters (FAMEs) were extracted and stored in hexane at −20°C pending analysis.

Table 1. Pearson correlation coefficients of *trans (t)*-18:1 isomers between erythrocytes (ERC) and three beef tissues from steers fed a red clover silage with or without flaxseed

			Time on feed (months)						
Isomer	Tissues	Diet	0	1	2	3	4	5	6
*t*6/*t*7/*t*8-	ERC-SF[z]	Flaxseed	−0.11	0.18	0.60	0.80*	0.53	0.79*	0.75
		Control	−0.09	−0.14	0.10	−0.60	0.16	−0.08	0.61
	ERC-KF[y]	Flaxseed	0.05	0.20	0.71	0.98***	0.59	0.75	0.84*
		Control	0.13	−0.72	−0.02	−0.42	0.01	0.26	0.20
	ERC-IMF[x]	Flaxseed	−0.14	0.30	0.87*	0.79*	0.44	0.92**	0.84*
		Control	−0.45	−0.73	−0.84*	−0.24	0.03	0.01	0.42
*t*9-	ERC-SF	Flaxseed	0.38	0.78	0.92**	0.92**	0.77	0.90**	0.94**
		Control	−0.11	0.20	0.49	−0.50	−0.71	0.06	−0.11
	ERC-KF	Flaxseed	0.49	0.73	0.81*	0.98***	0.96***	0.79*	0.89*
		Control	−0.21	−0.10	0.07	−0.04	−0.84*	0.54	0.57
	ERC-IMF	Flaxseed	0.53	0.89**	0.95**	0.90**	0.90**	0.93**	0.91**
		Control	−0.13	−0.41	−0.37	−0.78	−0.30	−0.36	0.25
*t*10-	ERC-SF	Flaxseed	0.41	0.73	0.85*	0.80*	0.86*	0.76	0.75
		Control	0.19	0.08	0.46	−0.53	0.03	0.02	0.52
	ERC-KF	Flaxseed	0.24	0.79*	0.88*	0.89**	0.93**	0.79*	0.67
		Control	0.28	0.08	−0.08	−0.75	−0.51	0.75	−0.08
	ERC-IMF	Flaxseed	0.53	0.76	0.88*	0.74	0.82*	0.81*	0.87*
		Control	0.17	−0.54	−0.05	−0.70	−0.29	0.49	0.54
*t*11-	ERC-SF	Flaxseed	0.01	0.49	0.82*	0.86*	0.85*	0.84*	0.90**
		Control	−0.16	−0.66	0.17	−0.20	−0.38	0.26	−0.10
	ERC-KF	Flaxseed	0.01	0.53	0.96***	0.97***	0.96**	0.99***	0.91**
		Control	0.17	−0.40	0.63	0.11	0.10	0.70	0.23
	ERC-IMF	Flaxseed	0.04	0.70	0.99***	0.96***	0.94**	0.99***	0.90**
		Control	0.44	−0.05	0.66	0.69	0.40	0.83*	0.79
*t*12-	ERC-SF	Flaxseed	−0.57	0.62	0.45	0.65	0.88*	0.97***	0.86*
		Control	0.53	0.24	0.34	0.43	0.37	0.00	0.63
	ERC-KF	Flaxseed	−0.52	0.20	0.50	0.61	0.65	0.88*	0.77
		Control	0.15	0.05	−0.21	−0.31	−0.64	0.86	0.55
	ERC-IMF	Flaxseed	−0.24	0.18	0.80*	0.59	0.42	0.74	0.88*
		Control	0.86*	0.09	−0.60	0.23	−0.50	−0.04	0.51
*t*13/*t*14-	ERC-SF	Flaxseed	−0.69	0.20	0.06	0.40	0.53	0.95**	0.51
		Control	0.15	0.19	0.33	0.55	0.04	0.32	−0.14
	ERC-KF	Flaxseed	−0.63	0.45	0.74	0.63	0.73	0.94**	0.06
		Control	0.31	−0.20	0.18	0.68	−0.05	0.91**	0.46
	ERC-IMF	Flaxseed	−0.69	−0.04	0.65	0.42	0.37	0.80*	−0.12
		Control	0.12	−0.28	−0.03	−0.50	−0.19	−0.41	0.46

[z]Subcutaneous fat.
[y]Kidney fat.
[x]Intramuscular fat.
*$P<0.05$ = low; **$P<0.01$ = moderate; ***$P<0.001$ = high.

Steers were slaughtered at target ultrasound subcutaneous fat depths of 6–8 mm between the 12th and 13th rib over the right longissimus thoracis (LT) muscle. At slaughter, animals were stunned, exsanguinated and dressed following commercial procedures at the Lacombe Research Centre abattoir. At approximately 20 min postmortem, 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 postmortem, 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 fat samples (50 mg) were freeze-dried and direct methylated with sodium methoxide (Dugan et al. 2007). Intramuscular fatty acids were extracted with 2:1 chloroform: methanol using a 20:1 solvent to sample ratio (Folch et al. 1957). The LT extracts were then methylated using both 5% methanolic HCl and 0.5 N sodium methoxide. The *t*18:1 FAMEs were analyzed using a 175°C plateau temperature program (Dugan et al. 2007).

Rolling monthly averages of the pecentages of ERC *t*18:1 isomers in total FAMEs were subjected to repeated measures analysis using the PROC MIXED

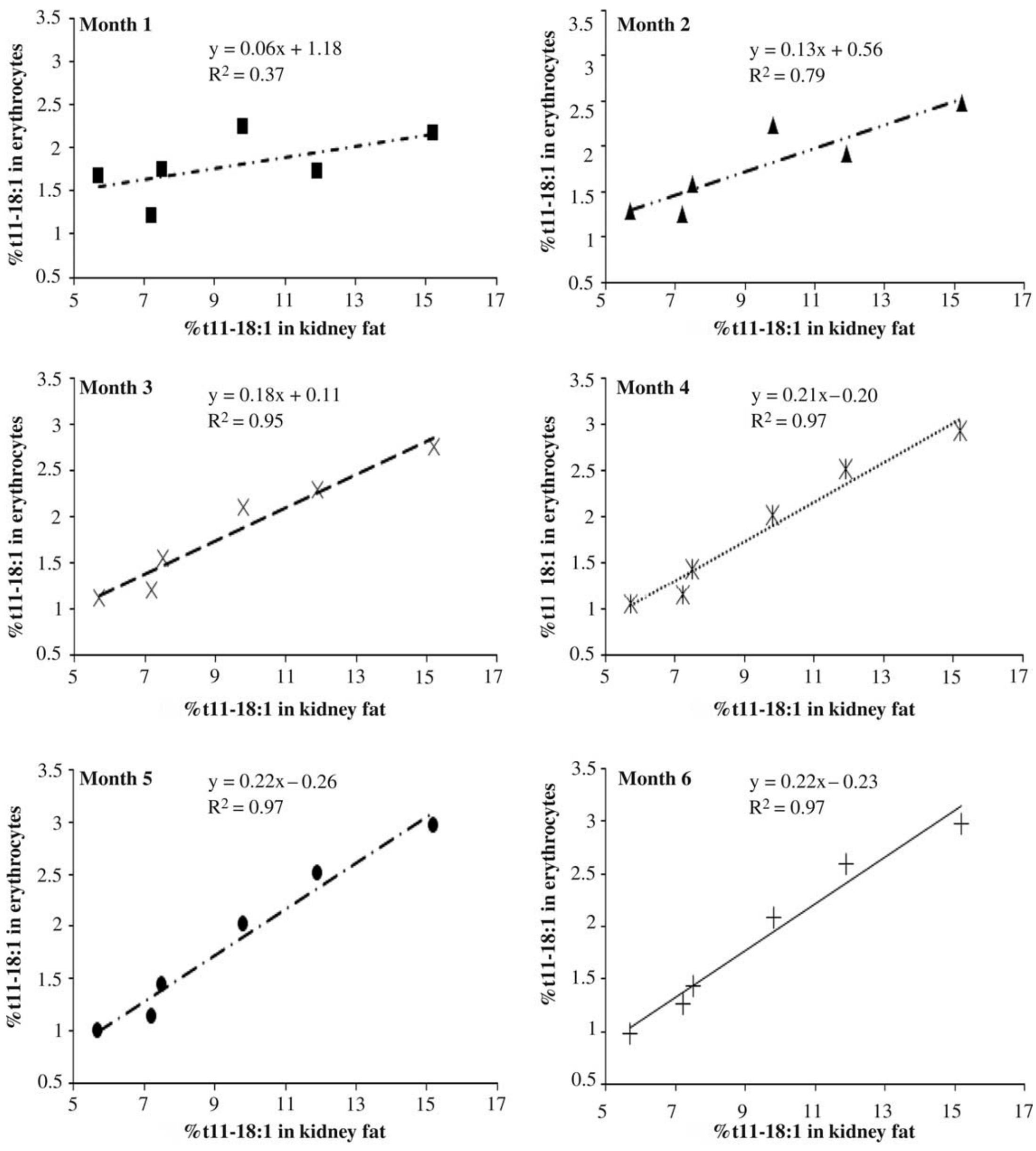

Fig. 2. Relationships between *t*11-18:1 concentration in kidney fat collected at slaughter and rolling pre-slaughter monthly averages in erythrocytes from steers fed red clover silage supplemented with flaxseed.

procedure of SAS software (SAS Institute, Inc. 2009). Using a first-order autoregressive covariance structure, the model incorporated the fixed effects of diet, time on feed, diet × time on feed, with ERC measurements repeated over time. The polynomial CONTRAST statement was also included in the model to test for linear and quadratic effects within diets over time. Individual animal was considered the experimental unit for all analyses. Pearson correlation coefficients between *t*18:1 isomer concentrations in ERC and beef tissues from steers fed red clover silage with or without flaxseeed were generated using the PROC CORR procedure of SAS software (SAS Institute, Inc. 2009).

The average daily gains for steers fed flaxseed and control diets were 0.88 ± 0.066 kg d^{-1} $steer^{-1}$ (mean ± standard deviation) and 0.86 ± 0.066 kg d^{-1} $steer^{-1}$, respectively. Most of *t*18:1 isomers (*t*6*t*/7/*t*8-, Fig. 1A; *t*9-, Fig. 1B; *t*11-, Fig. 1D and *t*13/*t*14-, Fig. 1F) exhibited both linear ($P < 0.01$) and quadratic ($P < 0.05$) trends within diets over time except for *t*10- (Fig. 1C) and *t*12- (Fig. 1E), which remained relatively constant ($P > 0.05$) for the control diet throughout the trial. Overall, the concentrations (percent of total fatty acids) of *t*18:1 isomers in steers fed flaxseed increased markedly in month 1 (*t*10-, *t*11- and *t*12-) and 2 (*t*6*t*/7/*t*8-, and *t*13/*t*14-) and increased gradually thereafter. Similarly, He et al. (2012) and Nassu et al. (2011) reported increases in tissue biohydrogenation intermediates over time when feeding forage diets supplemented with flaxseed. These findings could also be partly explained by a slight decrease in stearoyl-CoA desaturase activity observed over time in beef cattle aged between 18 and 30 mo (Niwińska 2010).

Generally, highly significant ($P < 0.01$) positive correlations of *t*9-, *t*10- and *t*11-18:1 were observed between ERC from month 2 to 6 and beef tissues collected at slaughter from steers fed red clover silage with flaxseed (Table 1). In support of these findings, concentrations of *t*11-18:1 in kidney fat of steers fed red clover silage with flaxseed, for example, were directly related to rolling pre-slaughter monthly averages in erythrocytes from month 2 to 6 (Fig. 2). Similar correlations of *t*9- and *t*10-18:1 isomers between ERC and beef tissues were found in our previous study (Aldai et al. 2012) using ERC samples collected at slaughter. However, in that study weak and non-significant correlations for *t*11-18:1 between ERC and subcutaneous fat, and ERC and intramuscular fat were found, respectively. This difference could be partly due to differences in the levels of flaxseed (15 vs. 10%) fed in the two studies and the limited range and amounts of *t*11-18:1 found in ERC and tissues in the former study.

The current results suggest that concentrations of *t*9-, *t*10- and *t*11-18:1 isomers in ERC assayed as early as 2 mo from steers fed red clover silage with flaxseed can be indicative of variation seen in beef tissues composition at a later slaughter date. This could be used to select individual cattle that consistently deposit higher levels of *t*11-18:1 in muscle and adipose tissue when fed red clover silage with flaxseed. Such an approach could dramatically lower feed costs, as feeding strategies could be specifically targeted to only those individuals that would consistently accumulate high levels of *t*11-18:1 in tissues.

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