Supplementary File

Effect of postruminal supply of linseed oil in dairy cows: 2. Milk fatty acid profile and oxidative stability

Daniel E. Rico, Rachel Gervais, Sara M. Peňa-Cotrino, Yolaine Lebeuf, and
P. Yvan Chouinard

Material and methods

Milk fatty acid analysis

Prior to analysis, milk samples were thawed in a water bath at 40°C and pooled by cow and period, proportionally to milk yield. Lipid extraction and methylation were performed according to the method of Rico *et al*. (2021). Milk FA composition was carried out by gas chromatography (Agilent 7890; Agilent Technologies, Santa Clara, CA, USA) using a CPSil-88 capillary column (100 m × 0.25 mm inside diameter × 0.20 µm film; Agilent Technologies Canada Inc., Mississauga, ON, Canada) and a flame ionization detector. Three different temperature programs were used, as described by Boivin *et al*. (2013). The split ratio was 100:1. Most FA peaks were identified and quantified using either a quantitative mixture or pure methyl ester standards (Larodan Fine Chemicals, Malmö, Sweden; Sigma-Aldrich, Oakville, ON, Canada; Matreya LLC, Pleasant Gap, PA, USA; and Nu Chek Prep, Elysian, MN, USA). Fatty acids for which standards were not available commercially were identified by order of elution according to Kramer *et al*. (2008).

Redox potential and conjugated diene and triene hydroperoxides

Redox potential of milk samples was measured with an Orion epoxy refillable ORP/ATC triode (No. 9180BNMD; Thermo Fisher Scientific, Waltham, MA, USA) filled with oxidation-reduction potential solution (No. 900011; Thermo Fisher Scientific). The electrode was connected to a portable pH/mV meter (Orion STAR A321; Thermo Fisher Scientific). The reading was recorded after 10 min of stable measurements and verified with a standard solution of potassium iodide (+220 mV at 25 °C; No. 967901; Thermo Fisher Scientific). Conjugated diene and triene hydroperoxides were determined as described by Kiokias *et al*. (2006) with modifications (Fauteux *et al*., 2016), except that conjugated triene hydroperoxides were measured against an isooctane:2-propanaol blank at 268 nm.

References

Boivin M, Gervais R and Chouinard PY (2013) Effect of grain and forage fractions of corn silage on milk production and composition in dairy cows. *Animal* **7,** 245-254.

Fauteux MC, Gervais R, Rico DE, Lebeuf Y and Chouinard PY (2016) Production, composition, and oxidative stability of milk highly enriched in polyunsaturated fatty acids from dairy cows fed alfalfa protein concentrate or supplemental vitamin E. *Journal of Dairy Science* **99,** 4411-4426.

Kiokias SN, Dimakou CP, Tsaprouni IV and Oreopoulou V (2006) Effect of compositional factors against the thermal oxidative deterioration of novel food emulsions. *Food Biophysics* **1,** 115-123.

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

Rico DR, Gervais R, Schwebel L, Lebeuf Y and Chouinard PY (2021) Production performance and oxidative stability of milk enriched with n-3 fatty acids in Holstein cows fed flaxseed meal. *Canadian Journal of Animal Science* **101**, 329-341.

Table S1. Milk fat composition in dairy cows abomasally infused with increasing levels of linseed oil

	Linseed oil infusion mL/d						
Item, g/100 g fat	0	75	150	300	600	SEM	Contrast
4:0	2.355	2.402	2.297	2.228	2.140	0.100	L*
6:0	1.702	1.799	1.765	1.725	1.677	0.071	
8:0	1.065	1.156	1.196	1.160	1.151	0.058	
10:0	2.694	2.970	3.206	3.058	3.080	0.235	
10:1	0.250	0.253	0.267	0.234	0.231	0.022	
11:0	0.080	0.085	0.089	0.073	0.065	0.012	L*
12:0	3.491	3.700	4.036	3.796	3.775	0.316	
cis-9 12:1	0.099	0.096	0.110	0.096	0.092	0.012	
iso 13:0	0.023	0.022	0.025	0.029	0.025	0.002	Q*
anteiso 13:0	0.020	0.022	0.021	0.016	0.017	0.002	L**
13:0	0.128	0.134	0.134	0.109	0.096	0.012	L**
iso 14:0	0.152	0.139	0.132	0.124	0.113	0.014	L*
14:0	10.661	10.821	10.874	10.192	9.447	0.373	L**
cis-9 14:1	0.919	0.851	0.820	0.687	0.602	0.096	L**
cis-11 14:1	0.059	0.062	0.074	0.069	0.064	0.010	
iso 15:0	0.174	0.175	0.167	0.144	0.122	0.008	L**C*
anteiso 15:0	0.401	0.415	0.380	0.357	0.284	0.018	L**
15:0	1.189	1.194	1.101	0.961	0.802	0.056	L**
iso 16:0	0.280	0.299	0.300	0.290	0.215	0.023	L**Q*
16:0	31.045	29.031	26.116	24.499	21.835	1.089	L**Q**
trans-9 16:1	0.043	0.040	0.039	0.038	0.030	0.004	L**
iso 17:0[2]	0.213	0.207	0.202	0.186	0.154	0.007	L**
cis-9 16:1	1.304	1.075	0.917	0.768	0.590	0.076	L**Q**
anteiso 17:0[3]	0.368	0.381	0.348	0.341	0.307	0.025	L*
cis-11 16:1	0.034	0.026	0.026	0.028	0.038	0.008	
cis-13 16:1	0.207	0.205	0.212	0.195	0.166	0.019	L**
17:0	0.489	0.481	0.447	0.419	0.358	0.012	L**

Item, g/100 g fat	Linseed oil infusion mL/d 0	75	150	300	600	SEM	Contrast
cis-7 17:1	0.026	0.033	0.035	0.040	0.052	0.005	L**
cis-8 17:1	0.018	0.019	0.015	0.013	0.009	0.003	L*
cis-9 17:1	0.165	0.147	0.130	0.108	0.095	0.007	L**Q**
iso 18:0	0.057	0.049	0.052	0.056	0.042	0.004	L*
18:0	7.223	7.582	7.335	7.608	7.088	0.488	
trans-4 18:1	0.024	0.029	0.026	0.028	0.027	0.003	
trans-5 18:1	0.017	0.021	0.019	0.017	0.019	0.002	
trans-6-8 18:1	0.177	0.179	0.164	0.167	0.142	0.009	L**
trans-9 18:1	0.137	0.133	0.126	0.128	0.112	0.008	L**
trans-10 18:1	0.229	0.235	0.233	0.240	0.225	0.011	
trans-11 18:1	0.567	0.569	0.541	0.572	0.494	0.040	
trans-12 18:1	0.205	0.196	0.192	0.188	0.170	0.017	L**
trans-13/14 18:1	0.333	0.313	0.286	0.284	0.238	0.030	L**
trans-15 18:1	0.259	0.266	0.256	0.268	0.261	0.017	
trans-16 18:1	0.207	0.207	0.198	0.193	0.182	0.013	L*
cis-6-8 18:1	0.098	0.090	0.067	0.074	0.095	0.009	Q**
cis-9 18:1[4]	12.759	12.559	12.949	13.382	13.608	0.504	
cis-11 18:1	0.414	0.391	0.414	0.406	0.437	0.027	
cis-12 18:1	0.293	0.312	0.279	0.295	0.268	0.022	
cis-13 18:1	0.052	0.039	0.034	0.031	0.037	0.007	
cis-14 18:1	0.043	0.040	0.037	0.038	0.034	0.003	
cis-15 18:1	0.052	0.047	0.039	0.040	0.046	0.007	
cis-9, *cis*-12 18:2[5]	2.011	2.328	3.013	3.691	4.543	0.141	L**Q*
cis-9, *trans*-11 18:2[6]	0.301	0.282	0.263	0.251	0.209	0.020	L**
cis-9, *trans*-12 18:2	0.049	0.049	0.046	0.044	0.038	0.004	L**
cis-9, *trans*-13 18:2	0.211	0.207	0.181	0.171	0.145	0.015	L**
trans-8, *cis*-13 18:2	0.094	0.085	0.081	0.077	0.064	0.009	L**
trans-9, *trans*-12 18:2	0.015	0.016	0.018	0.020	0.016	0.002	Q*
trans-9, *cis*-12 18:2	0.023	0.022	0.021	0.026	0.019	0.003	

Item, g/100 g fat	Linseed oil infusion mL/d 0	75	150	300	600	SEM	Contrast
trans-10, *cis*-12 18:2	0.021	0.022	0.023	0.028	0.020	0.002	Q^{**}
trans-11, *cis*-15 18:2	0.045	0.068	0.053	0.087	0.112	0.024	L^{*}
cis-6, *cis*-9, *cis*-12 18:3[5]	0.030	0.027	0.033	0.056	0.079	0.013	L^{**}
cis-9, *cis*-12, *cis*-15 18:3[7]	0.581	1.452	3.537	5.608	9.501	0.328	L^{**}
cis-9, *trans*-11, *cis*-15 18:3	0.021	0.040	0.068	0.094	0.115	0.013	$L^{**}Q^{**}$
cis-6, *cis*-9, *cis*-12, *cis*-15 18:4[7]	0.019	0.024	0.021	0.020	0.022	0.003	
19:0	0.082	0.077	0.069	0.056	0.056	0.005	$L^{**}Q^{*}$
20:0	0.109	0.113	0.113	0.115	0.103	0.006	
cis-9 20:1	0.074	0.077	0.072	0.070	0.063	0.004	L^{**}
cis-11, *cis*-14 20:2[5]	0.036	0.034	0.040	0.040	0.043	0.002	L^{**}
cis-8, *cis*-11, *cis*-14 20:3[5]	0.080	0.085	0.073	0.053	0.050	0.008	$L^{**}Q^{*}$
cis-11, *cis*-14, *cis*-17 20:3[7]	0.016	0.023	0.050	0.080	0.117	0.009	L^{**}
cis-5, *cis*-8, *cis*-11, *cis*-14 20:4[5]	0.109	0.120	0.113	0.098	0.087	0.009	L^{**}
cis-8, *cis*-11, *cis*-14, *cis*-17 20:4[7]	0.021	0.021	0.025	0.024	0.020	0.002	Q^{*}
cis-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 20:5[7]	0.069	0.067	0.084	0.092	0.134	0.015	L^{**}
22:0	0.040	0.043	0.042	0.043	0.040	0.003	
cis-13 22:1	0.015	0.015	0.020	0.021	0.027	0.001	L^{**}
cis-13, *cis*-16 22:2[5]	0.014	0.016	0.015	0.018	0.017	0.002	
cis-13, *cis*-16, *cis*-19 22:3[7]	0.015	0.018	0.020	0.017	0.016	0.002	
cis-7, *cis*-10, *cis*-13, *cis*-16 22:4[5]	0.022	0.029	0.026	0.024	0.016	0.003	L^{*}
cis-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:5[7]	0.080	0.073	0.086	0.092	0.081	0.009	
cis-4, *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 22:6[7]	0.017	0.017	0.020	0.020	0.018	0.002	
24:0	0.027	0.024	0.027	0.029	0.030	0.002	
cis-15 24:1	0.014	0.016	0.021	0.016	0.015	0.002	
Others	0.757	0.782	0.802	0.812	1.008	0.061	L^{**}
Glycerol	12.186	12.234	12.205	12.108	12.021	0.060	L^{*}
Sum of n-6 fatty acids	2.301	2.639	3.313	3.980	4.836	0.140	$L^{**}Q^{**}$
Sum of n-3 fatty acids	0.817	1.697	3.842	5.954	9.909	0.334	$L^{**}Q^{*}$
Δ-9 Desaturase index[8]	0.079	0.073	0.070	0.063	0.060	0.008	L^{**}

[1]L = linear effect, Q = quadratic effect, $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$.

[2]Coelution with minor concentration of *trans*-10 16:1.

[3]Coelution with minor concentration of *cis*-10 16:1.

[4]Coelution with minor concentration of *cis*-10 18:1.

[5]n-6 family.

[6]Coelution with minor concentration of *trans*-7, *cis*-9 18:2.

[7]n-3 family.

[8]*cis*-9 14:1/(14:0 + *cis*-9 14:1)

Table S2. Oxidative stability parameters of fresh milk from dairy cows abomasally infused with increasing levels of linseed oil

	Linseed oil infusion mL/d						
Item	0	75	150	300	600	SEM	Contrast[1]
Peroxidability index, mg/g	1.95	2.81	4.49	6.47	10.82	0.39	L**
Redox potential, mV	120.1	125.0	124.9	124.6	129.0	3.8	L*
Volatile oxidation products, µg/kg							
Propanal	1.70	4.23	7.32	10.57	24.55	2.45	L**
Hexanal	23.5	28.6	63.0	51.3	102.9	19.2	L**
trans-2+*cis*-3-Hexenals	0.313	0.313	1.430	1.107	2.894	0.463	L**
cis-4-Heptenal	0.000	0.006	0.049	0.068	0.149	0.024	L**
Conjugated hydroperoxide, mmol/L							
Diene	1.02	1.10	1.10	1.17	1.33	0.06	L**
Triene	0.224	0.253	0.232	0.243	0.246	0.015	

[1]L = linear effect, *$P \leq 0.05$, **$P \leq 0.01$.

Table S3. Variations in redox potential and concentrations of volatile oxidation products during storage of homogenized milk from dairy cows abomasally infused with increasing levels of linseed oil[1]

	Linseed oil infusion, mL/d						
Item	0	75	150	300	600	SEM	Contrast[1]
Redox potential, mV	136	140	142	144	144	51	
Volatile oxidation products, µg/kg							
1-Octen-3-one	132	237	334	529	590	78	$L^{**}Q^{*}$
Propanal	0.6	2.7	6.9	16.7	23.6	3.2	L^{**}
Hexanal	2.5	4.2	5.9	10.6	11.5	1.5	$L^{**}Q^{*}$
trans-2 + *cis*-3-Hexenals	242	614	824	2292	3474	339	L^{**}
cis-4-Heptenal	4.9	16.9	25.5	62.0	86.0	8.2	L^{**}
trans-2, *cis*-6-Nonadienal	53	145	389	884	1263	207	L^{**}
trans-2, *trans*-4-Nonadienal	303	707	1293	1809	1947	542	L^{**}
Conjugated hydroperoxide, mmol/L							
Diene	0.25	0.44	0.64	1.24	1.82	0.11	L^{**}
Triene	-0.02	0.01	0.07	0.18	0.31	0.03	L^{**}

[1]Measured as the difference between final (day 11) and initial (day 0) redox potential and concentrations of each component following storage at 4°C under fluorescent light.

[2]L = linear effect, Q = quadratic effect, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$.