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

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## Research Article

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## Abstract

Our objective was to study the effect of increasing postruminal supply of linseed oil (L-oil), as a source of *cis*-9, *cis*-12, *cis*-15 18:3, on milk fatty acid profile and to assess the resulting impact on the development of volatile degradation products during the storage of homogenized milk. Five Holstein dairy cows fitted with a rumen cannula were randomly distributed in a 5 × 5 Latin square design. Abomasal infusion of L-oil was performed at the rate of 0, 75, 150, 300, and 600 ml/d during periods of 14 d. The concentration of *cis*-9, *cis*-12, *cis*-15 18:3 in milk fat increased linearly with L-oil dose. Concentrations of primary (conjugated diene and triene hydroperoxides) and secondary oxidation products (1-octen-3-one, propanal, hexanal, *trans*-2 + *cis*-3-hexenals, *cis*-4-heptenal, *trans*-2, *cis*-6-nonadienal *trans*-2, *trans*-4-nonadienal) increased during 11 d of storage at 4°C of homogenized milk under fluorescent light. The magnitude of the increase (difference between final and initial measurements) was linearly greater for all nine lipid oxidation products evaluated in response to increasing level of infusion. Results of the current experiment have shown that milk enriched in *cis*-9, *cis*-12, *cis*-15 18:3 *via* postruminal supply of L-oil is highly prone to oxidative degradation. This low oxidative stability, exposed under controlled experimental conditions, would represent a major obstacle to those who aim to market milk enriched in polyunsaturated fatty acids.

The adequate intake of α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 18:3) has been established at 1.6 g/d for adult men and 1.1 g/d for adult women (Flock *et al.*, 2013). Dairy products may contribute to a low proportion of this recommendation, as the consumption of two servings of regular whole milk (3.25% fat) containing 4.1 mg *cis*-9, *cis*-12, *cis*-15 18:3/g of fatty acids (FA; Heck *et al.*, 2012) brings only 33 mg of this essential FA.

Linseed (also called flaxseed; *Linum usitatissimum*) is a rich source of *cis*-9, *cis*-12, *cis*-15 18:3 (INRA-AFZ, 2004). This oilseed has been evaluated for its potential to increase the concentration of n-3 FA in milk fat. In this regard, a meta-analysis by Leduc *et al.* (2017) has shown that the transfer efficiency of dietary *cis*-9, *cis*-12, *cis*-15 18:3 varied from 1.95%, with diets supplemented with linseed oil (L-oil), to 5.84% with diets based on mechanically treated whole linseed. As a result of this transfer, consumption of food products (including milk) from animals fed linseed has been associated with positive effects on blood lipid profile in humans (Weill *et al.*, 2002).

Unfortunately, experiments have shown that milk enriched in *cis*-9, *cis*-12, *cis*-15 18:3 is highly prone to oxidative deterioration (Fauteux *et al.*, 2016; Rico *et al.*, 2021). Oxidation of polyunsaturated FA has been associated with the development of undesirable flavours (eg rancidity) and potentially toxic chemicals (Arab-Tehrany *et al.*, 2012), which can impair the nutritional and sensory properties of dairy products. Liu *et al.* (2010) studied the effects of graded amounts of *cis*-9, *cis*-12, *cis*-15 18:3 infused into the duodenum, from 0 to 132 g/d, resulting in a linear increase of milk fat concentration of this FA from 0.6 to 25.4%. This modification of milk FA profile quadratically decreased the activity of enzymatic radical scavenging systems, such as superoxide dismutase, glutathione peroxidase and catalase (Liu *et al.*, 2010). However, the subsequent consequences on the development of volatile degradation products such as aldehydes and ketones responsible for the development of milk off-odours and off-flavours have not been assessed.

Our objective was to study the effect of increasing postruminal supply of L-oil as a source of *cis*-9, *cis*-12, *cis*-15 18:3 on milk FA profile, and to assess the resulting impact on the development of volatile degradation products during the storage of homogenized milk.

## Materials and methods

### Animals and treatments

The experimental procedures involving dairy cows followed the guidelines of the Canadian Council on Animal Care (2009) and were approved by the Université Laval Animal Care

Committee (Protocol # 2015001). Information about cows, feeding, treatments, and experimental design is reported in a companion paper (Gervais *et al.*, 2023, In press). Briefly, 5 Holstein dairy cows (36 ± 2 d postpartum; mean ± SD) were randomly distributed in a 5 × 5 Latin square design with periods of 21 d. All cows were fed the same total mixed ration. During the first 14 d of each period, L-oil (Pokonobe Industries Inc., Westmount, QC; containing 5.6% 16:0, 3.4% 18:0, 18.4% *cis*-9 18:1, 0.7% *cis*-11 18:1, 14.9% *cis*-9, *cis*-12 18:2, 55.9% *cis*-9, *cis*-12, *cis*-15 18:3, and 0.2% 20:0) was abomasally infused at 0, 75, 150, 300, and 600 mL/d using peristaltic pumps. Infusions were followed by a 7-d washout interval.

### Sampling, measurements, and analyses

Dry matter intake and milk yield were recorded, and samples of feed and milk were harvested during the last 3 d of each infusion period. Data on dry matter intake, milk production, as well as concentration and yield of major milk constituents were reported previously (Gervais *et al.*, 2023, In press). An additional set of milk samples without preservative was harvested during the last 3 d of each infusion and stored at −20°C for later determination of the FA profile following the procedure described by Boivin *et al.* (2013), and reported in the online Supplementary File, material and methods. Glycerol in milk fat was calculated as described by Stamey *et al.* (2010).

A last set of milk samples for oxidative stability analyses was collected during the morning milking on day 11 of each infusion period. These samples were immediately transported to Université Laval pilot plant in 1-L stainless-steel cans, while being kept on ice until processed as described by Fauteux *et al.* (2016). Briefly, milk samples were heated at 50°C, homogenized at 24 MPa (EmulsiFlex-C50, Avestin, Ottawa, ON, Canada), and then cooled at 4°C.

Oxidation was induced by the addition of 0.001% Fe (as $FeSO_4$). Samples were stored horizontally for 0, 2, 4, 7, and 11 d in two sets of glass tubes at 4°C in a cabinet under fluorescent light (warm white, linear T12, 40 W; Lumisolution Inc., Québec, QC, Canada). Sodium azide (0.02%) was added to prevent microbial growth. Samples in the first set of tubes were analysed for 1-octen-3-one, propanal, hexanal, *trans*-2 + *cis*-3-hexenals, *cis*-4-heptenal, *trans*-2, *cis*-6-nonadienal, and *trans*-2, *trans*-4-nonadienal. The second set of tubes was analysed for redox potential, as well as conjugated diene and triene hydroperoxides. The same analyses were performed on fresh non-homogenized milk on day 0. A second subsample of fresh milk was stored at −20°C, without preservative, until analysed for FA profile as described above. In order to assess susceptibility of milk fat to oxidation as affected by unsaturated FA content, a peroxidability index (PI) was calculated for each sample based on milk concentrations of monoenoic (Mono), dienoic (Di), trienoic (Tri), tetraenoic (Tetra), pentaenoic (Penta), and hexaenoic (Hexa) FA (Witting and Horwitt, 1964), as follows:

$$\mathrm{PI} = (0.025 \times \mathrm{Mono}) + (1 \times \mathrm{Di}) + (2 \times \mathrm{Tri}) + (4 \times \mathrm{Tetra}) + (6 \times \mathrm{Penta}) + (8 \times \mathrm{Hexa})$$

to account for individual oxidation sensitivity of FA. The use of concentrations of these FA groups as a proportion of milk constituents, rather than as a proportion of total fat, was intended to account for the variation in substrate availability for peroxidation resulting from differences in milk fat concentration among samples.

Analyses of secondary lipid oxidation products of fresh and stored milk were conducted using the solid-phase microextraction technique with a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) attached to an Agilent 6890N gas chromatograph with a 5973 inert mass spectrometry detection (Agilent Technologies Canada Inc.) as previously described by Fauteux *et al.* (2016). Finally, redox potential and conjugated diene and triene hydroperoxides were analysed as reported in the online Supplementary File, Material and methods.

### Statistical analysis

Data were analysed using the MIXED procedure of SAS 9.4 (SAS Institute Inc, Cary, NC, USA). For variables where repeated measures were not performed, the following model was fit:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + C_l(S_k) + \varepsilon_{ijkl}$$

where $Y_{ijkl}$ is the individual observation, μ the overall mean, $T_i$ the fixed effect of treatment ($i = 1–5$), $P_j$ the random effect of period ($j = 1–5$), $S_k$ the random effect of sequence ($k = 1–5$), $C_l(S_k)$ the random effect of cow ($l = 1–5$) nested in sequence, and $\varepsilon_{ijkl}$ the residual error terms. Linear and quadratic contrasts for treatment effect were performed.

For variables submitted to repeated measures, data were analysed according to the following model:

$$Y_{ijklm} = \mu + T_i + P_j + S_k + C_l(S_k) + D_m + \mathrm{TD}_{im} + \varepsilon_{ijklm}$$

where $Y_{ijklm}$ is the individual observation, μ the overall mean, $T_i$ the fixed effect of treatment ($i = 1–5$), $P_j$ the random effect of period ($j = 1–5$), $S_k$ the random effect of sequence ($k = 1–5$), $C_l(S_k)$ the random effect of cow ($l = 1–5$) nested in sequence, $D_m$ the effect of day ($m = 1–5$) + $\mathrm{TD}_{im}$ the interaction of treatment and day, and $\varepsilon_{ijklm}$ the residual error terms. Linear and quadratic contrasts for the effects of treatment and day were performed. Differences between treatments were declared at $P \leq 0.05$.

## Results

Complete data are provided in the online Supplementary File for milk fat composition (Table S1), oxidative stability parameters (Table S2) and changes in redox potential and volatile products during storage (Table S3). The concentration of *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3 in milk fat increased linearly with L-oil dose (Fig. 1). Conversely, the concentration of 14:0 decreased linearly, and the concentration 16:0 decreased linearly and quadratically with the level of L-oil. Proportions of 6:0, 8:0, 10:0, 12:0, 18:0, and *cis*-9 18:1 were not affected by L-oil infusion (Fig. 1). The PI increased linearly with the level of L-oil (Fig. 2).

In fresh milk, redox potential as well as concentrations of propanal, hexanal, *trans*-2 *cis*-3-hexenals, *cis*-4-heptenal, and conjugated diene hydroperoxide increased linearly with the dose of L-oil (Fig. 2). The concentration of conjugated triene hydroperoxides was not affected, whereas *trans*-2, *cis*-6-nonadienal and *trans*-2, *trans*-4-nonadienal were not detected in fresh milk.

Redox potential and concentrations of all nine lipid oxidation products evaluated increased during the storage of homogenized milk under light exposure (Fig. 3). The magnitude of the

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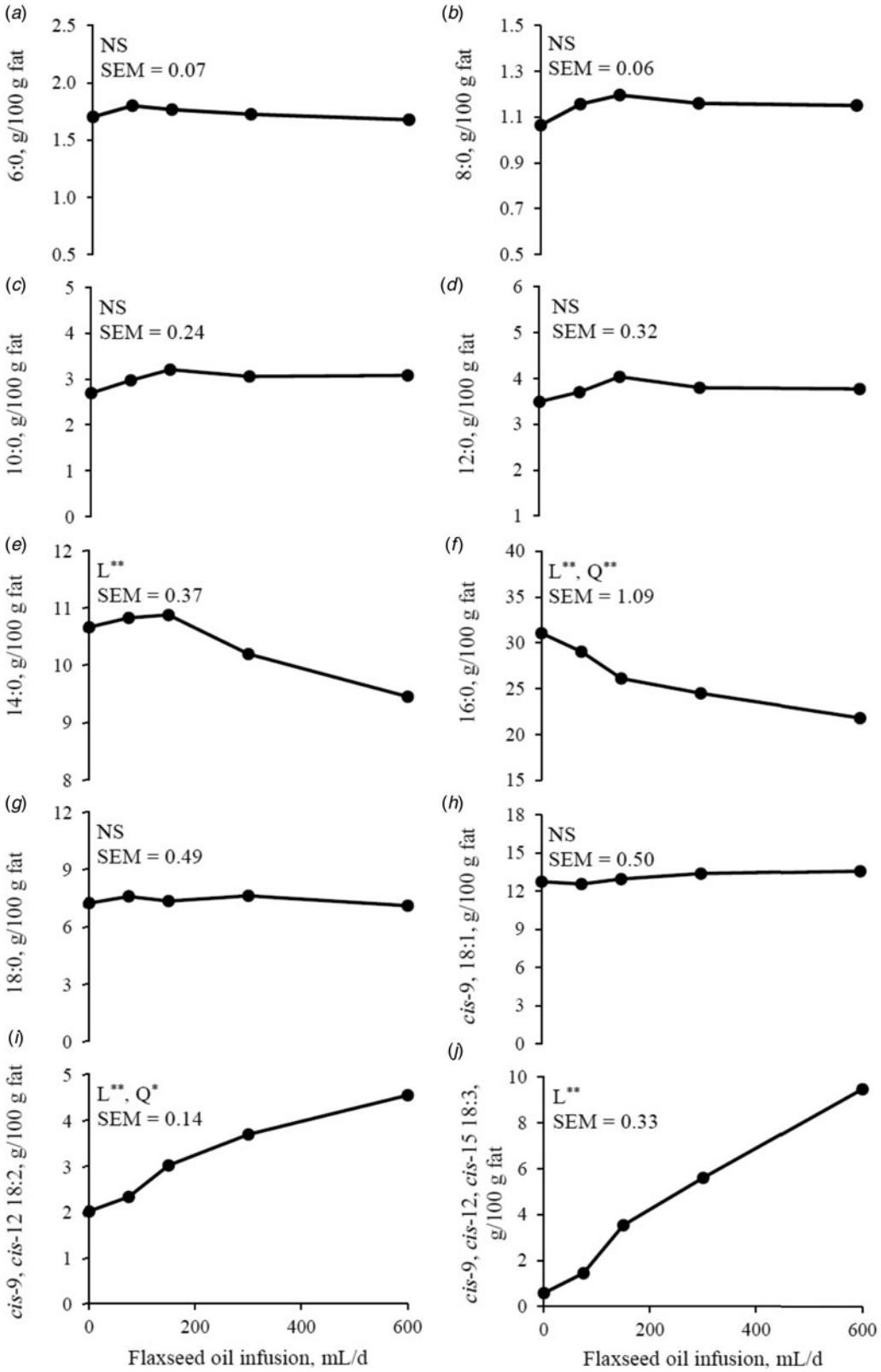


**Figure. 1.** Milk fat concentrations of 6:0 (a), 8:0 (b), 10:0 (c), 12:0 (d), 14:0 (e), 16:0 (f), 18:0 (g), *cis*-9 18:1 (h), *cis*-9, *cis*-12 18:2 (i), and *cis*-9, *cis*-12, *cis*-15 18:3 (j) in dairy cows abomasally infused with increasing levels of linseed oil. SEM = standard error of the mean. L, linear, and Q, quadratic effect of the level of linseed oil infusion. $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. NS, not significantly affected ($P > 0.10$). See online Supplementary File, Table S1 for complete fatty acid profiles.

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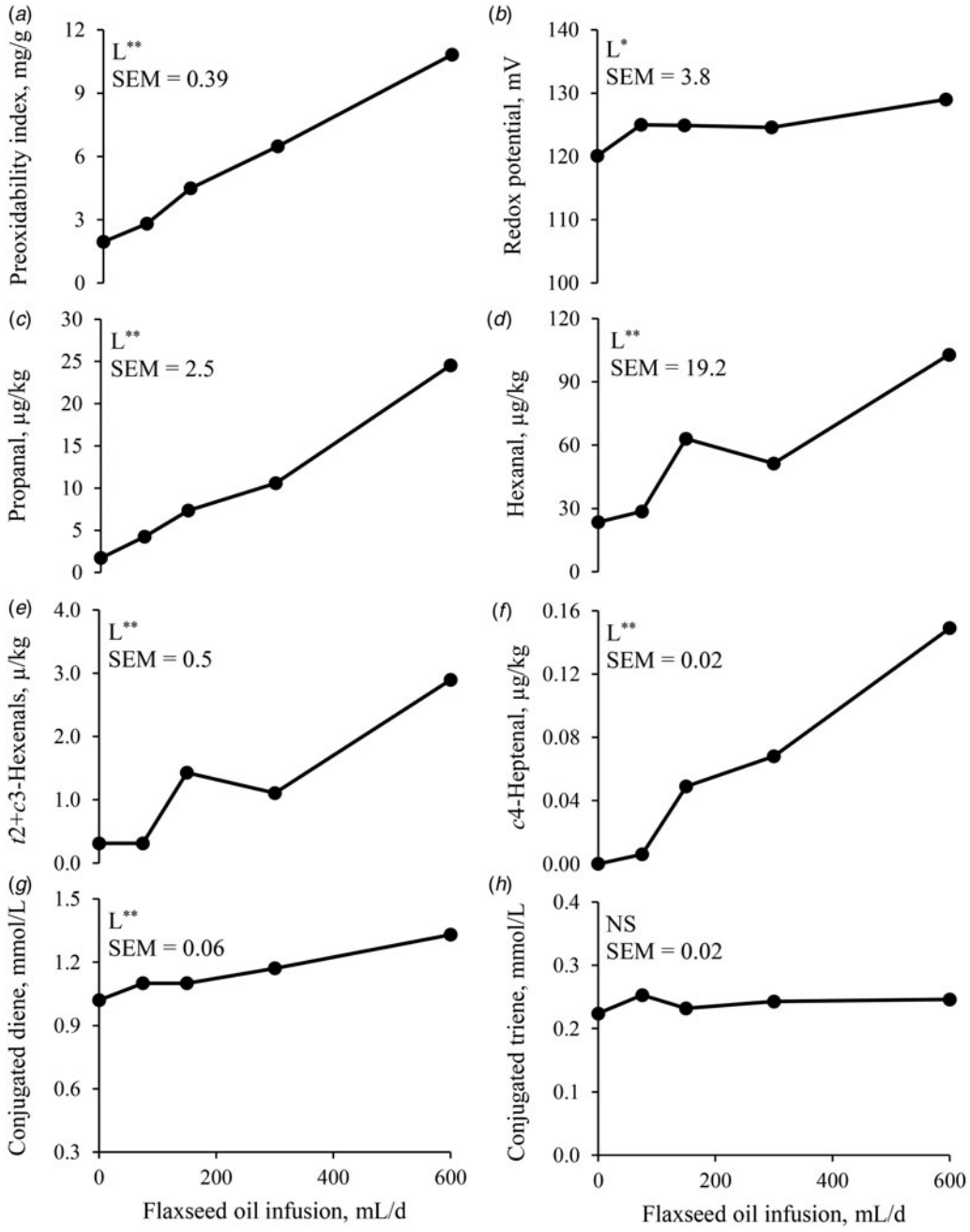


**Figure. 2.** Peroxidability index (a), redox potential (b), and concentrations of propanal (c), hexanal (d), *trans*-2 + *cis*-3-hexenals (e), *cis*-4-heptenal (f), and conjugated diene (g) and triene (h) hydroperoxides in fresh milk of dairy cows abomasally infused with increasing levels of linseed oil. SEM, standard error of the mean. L, linear and Q, quadratic effect of the level of linseed oil infusion. *$P \leq 0.05$ and **$P \leq 0.01$. NS, not significantly affected ($P > 0.05$). Table values can be found in online Supplementary File, Table S2.

difference between final and initial measurements increased linearly with the level of infusion for the concentrations of 1-octen-3-one, propanal, hexanal, *trans*-2 + *cis*-3-hexenals, *cis*-4-heptenal, *trans*-2, *cis*-6-nonadienal *trans*-2, *trans*-4-nonadienal, and conjugated diene and triene hydroperoxides (Fig. 4).

## Discussion

Here, abomasal infusion of L-oil has been effective in increasing milk fat concentration of polyunsaturated FA. In particular, the proportions of *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3 increased by 2.3- and 16.4-fold, respectively, when infusing 600 ml/d of L-oil as compared with control (no infusion). The greatest level of *cis*-9, *cis*-12, *cis*-15 18:3 (9.0 g/100 g fat) is equivalent to 772 mg in a glass of whole milk (3.25% fat). At this concentration, adequate intake of *cis*-9, *cis*-12, *cis*-15 18:3 for adult women (1.1 g/d) and men (1.6 g/d; Flock *et al.*, 2013) could be achieved by the consumption of 2 servings of milk per day. These increases were mainly compensated by decreased concentrations of 14:0 and 16:0. Similar effects on milk FA profile were reported by Lima *et al.* (2014) in cows abomasally infused with L-oil.

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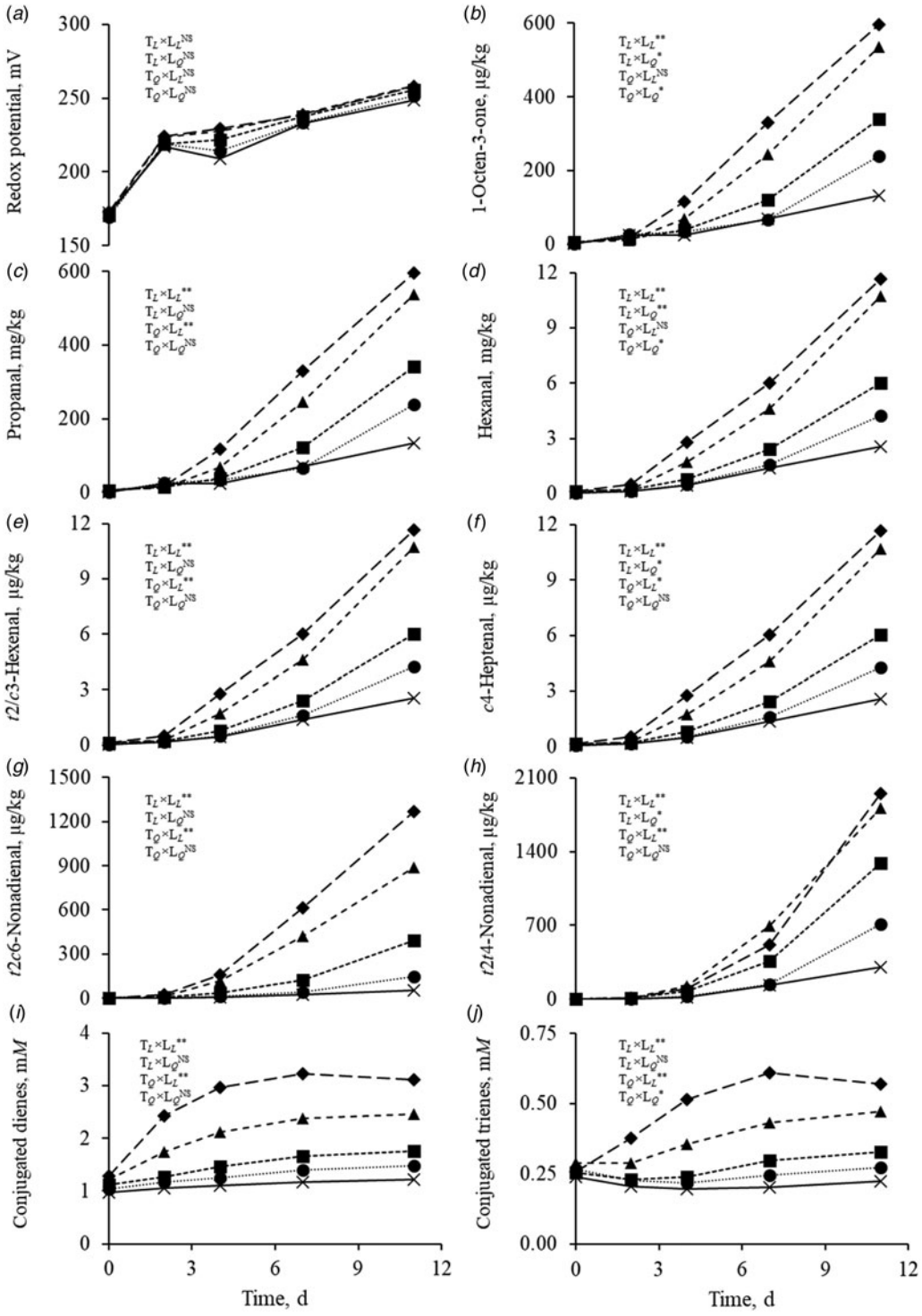


**Figure. 3.** Effect of time of storage under light exposure on redox potential (a) and on concentrations of 1-octen-3-one, (b) propanal (c), hexanal (d), *trans*-2 + *cis*-3-hexenals, (e) *cis*-4-heptenal (f), *trans*-2, *cis*-6-nonadienal (g), *trans*-2, *trans*-4-nonadienal (h), and conjugated diene (i) and triene (j) hydroperoxides in homogenized milk from cows abomasally infused with linseed oil at the rate of 0 (×), 75 (●), 150 (■), 300 (▲), and 600 (◆) ml/d. $T_L$, Linear effect of treatment; $T_Q$, Quadratic effect of treatment; $L_L$, Linear effect of infusion level; $L_Q$, Quadratic effect of infusion level, $^*P \leq 0.05$ and $^{**}P \leq 0.01$. NS, not significantly affected ($P > 0.05$).

Fatty acids are not distributed randomly in milk TAG (Jensen, 2002). By feeding formaldehyde treated sunflower seeds as a source of ruminally protected unsaturated FA, Morrison and Hawke (1977a) observed an increase in the concentration of *cis*-9, *cis*-12 18:2 from 1.8 to 15.5% of milk FA, on a molar basis, mostly at the expense of 14:0 and 16:0. Bovine milk fat can be divided into high- and low-molecular-weight TAG. Morrison and Hawke (1977b) reported the stereospecific distribution of FA in the high molecular weight fractions and showed that increases in *cis*-9, *cis*-12 18:2 in each of the three positions of TAG

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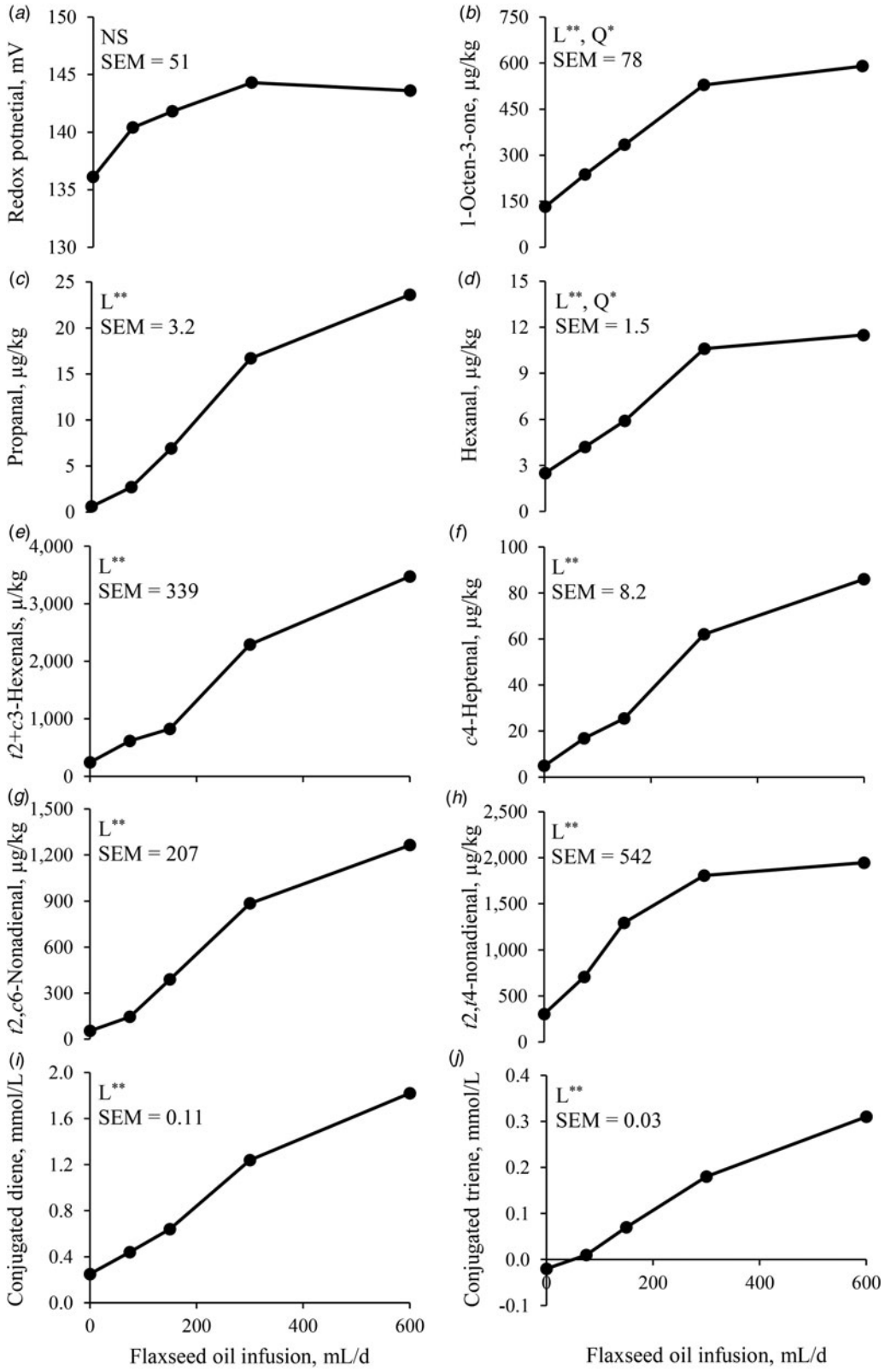


**Figure. 4.** Variations of redox potential (a) and in concentrations of 1-octen-3-one, (b) propanal (c), hexanal (d), *trans*-2 + *cis*-3-hexenals, (e) *cis*-4-heptenal (f), *trans*-2, *cis*-6-nonadienal (g), *trans*-2, *trans*-4-nonadienal (h), and conjugated diene (i) and triene (j) hydroperoxides during storage of homogenized milk from dairy cows abomasally infused with increasing levels of linseed oil. Data represent 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. SEM, standard error of the mean; L, linear and Q, quadratic effect of the level of linseed oil infusion. $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. NS, not significantly affected ($P > 0.05$). Table values can be found in online Supplementary File, Table S3.

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were paralleled by decreases in the levels of 14:0 and 16:0. More specifically, the preference of *cis*-9, *cis*-12 18:2 for position *sn*-3 over position *sn*-1 was shown to divert the available 14:0 and 16:0 into position *sn*-1 at the expense of position *sn*-3. In our research, a similar phenomenon could explain lower proportions of 14:0 and 16:0 in milk fat in response to increasing levels of *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3 from abomasally infused L-oil. Such disruption of TAG synthesis, due to increase availability of polyunsaturated FA for esterification, could also potentially explain the lower milk fat concentration and yield observed in response to increasing levels of infusion (Gervais *et al*., 2023, In press). The substitution of polyunsaturated for saturated FA linearly increased the PI of milk fat, from 2.0 mg/g milk in the control to 10.8 mg/g milk at the highest dose. These milk samples, with increasing PI, were submitted to oxidative conditions with the addition of $FeSO_4$ and storage at 4°C under fluorescent light. Conjugated diene and triene hydroperoxides were determined as primary lipid oxidation products. Both *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3 are known to form conjugated diene hydroperoxides. Conjugated trienes are produced from *cis*-9, *cis*-12, *cis*-15 18:3 when two positions of the carbon chain are attacked (Patterson, 1989). Alternatively, conjugated trienes may be produced by dehydration of conjugated diene hydroperoxides (Fishwick and Swoboda, 1977).

We observed that the concentration of conjugated dienes in fresh milk was about 4.8 times greater compared with conjugated trienes. These concentrations increased quadratically over time, reaching a plateau at 7 d of storage in milk from cows receiving the highest level of L-oil infusion (600 ml/d). Hydroperoxides eventually break down to secondary lipid oxidation products (Patterson, 1989). In this regard, hexanal (Frankel, 1982) and 1-octen-3-one (Ullrich and Grosch, 1987) are volatile products expected from *cis*-9, *cis*-12 18:2 oxidation. Propanal, *cis*-4 heptenal, *cis*-3-hexenal, *trans*-2-hexenal, *trans*-2, *cis*-6-nonadienal, and *trans*-2, *trans*-4-nonadienal arise from oxidation of *cis*-9, *cis*-12, *cis*-15 18:3 (Frankel, 1982; Josephson and Lindsay, 1987; Ullrich and Grosch, 1988). Concentrations of secondary lipid oxidation products increased exponentially during storage in our experiments. This observation is consistent with the fact that oxidative progression is autocatalytic and needs only one initiating radical to begin the production of hydroperoxides (Timmons *et al*., 2001). Such phenomena may explain why the concentrations of secondary lipid oxidation products intensified as storage time increases. After 11 d under fluorescent light, the overall increase (d 11 minus d 0) in the concentrations of these secondary products was enhanced linearly with the level of L-oil infusion.

In conclusion, our results have shown that milk enriched in *cis*-9, *cis*-12 18:2 and *cis*-9, *cis*-12, *cis*-15 18:3 *via* postruminal supply of L-oil is highly prone to oxidative degradation. Attempts have been made in the past to prevent oxidation of milk containing high levels of polyunsaturated FA (Fauteux *et al*., 2016; Rico *et al*., 2021) using dietary treatments aimed to increase levels of vitamin E, carotenoids or enterolactones. None of these interventions have been efficient in significantly reducing the production of primary and secondary volatile oxidation products known for their impacts on organoleptic properties of milk (Bendall, 2001). This low oxidative stability, exposed under controlled experimental conditions, would represent a major obstacle to commercial initiatives to market milk enriched in polyunsaturated FA.

**Supplementary material.** The supplementary material for this article can be found at https://doi.org/10.1017/S0022029923000262

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