INTERPRETIVE SUMMARY: **Effect of Incremental Amounts of Camelina Oil on Milk Fatty Acid Composition in Lactating Cows Fed Diets Based on a Mixture of Grass and Red Clover Silage and Concentrates Containing Camelina Expeller** *By Halmemies-Beauchet-Filleau et al.*

Effects of incremental amounts of camelina oil (CO) in concentrates containing camelina expeller on animal performance and milk fat composition were examined. Supplements of CO progressively lowered silage intake and milk yield. Camelina oil enriched 18-carbon biohydrogenation intermediates in milk fat, including *trans*-11 18:1 and *cis*-9,*trans*-11 18:2 in the absence of milk fat depression. Supplements of CO decreased the secretion of medium-chain saturates in milk, but had no effect on 18:0, *cis*-9 18:1, *cis*-9,*cis*-12 18:2, and *cis*-9,*cis*-12,*cis*-15 18:3 output. Milk fat composition on all treatments suggested that one or more components in camelinaseeds may inhibit complete ruminal biohydrogenation of unsaturates.

RUNNING SHORT TITLE: CAMELINA OIL ON BOVINE MILK FAT

Effect of Incremental Amounts of Camelina Oil on Milk Fatty Acid Composition in Lactating Cows Fed Diets Based on a Mixture of Grass and Red Clover Silage and Concentrates Containing Camelina Expeller

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ABSTRACT

Camelina is an ancient oilseed crop that produces an oil rich in *cis*-9,*cis*-12 18:2 (LA) and *cis*-9,*cis*-12,*cis*-15 18:3 (ALA), but reports on the use of camelina oil (CO) for ruminants are limited. The present study investigated the effects of incremental CO supplementation on animal performance, milk fatty acid (FA) composition, and milk sensory quality. Eight Finnish Ayrshire cows (91 d in milk) were used in replicated 4 × 4 Latin squares with 21 d periods. Treatments comprised 4 concentrates (12 kg/d on an air-dry basis) based on cereals and camelina expeller containing 0 (control), 2, 4, or 6% of CO on an air-dry basis. Cows were offered a mixture of grass and red clover silage (RCS; 1:1 on a dry matter basis) ad libitum. Incremental CO supplementation lowered linearly silage and total dry matter intake, and increased linearly LA, ALA, and total FA intake. Treatments had no effect on whole tract apparent organic matter or fiber digestibility, or a major influence on rumen fermentation. Supplements of CO decreased quadratically daily milk and lactose yields and lowered linearly milk protein yield and milk taste panel score from 4.2 to 3.6 [on a scale of 1 (poor) to 5 (excellent)], without altering milk fat yield. Inclusion of CO decreased linearly the proportions of saturated FA synthesised *de novo* (4:0 to 16:0), without altering milk fat 18:0, *cis*-9 18:1, LA, and ALA concentrations. Milk fat 18:0 was low (< 5 g/100 g FA) across all treatments. Increases in CO decreased linearly the proportions of total saturates from 58 to 45 g/100 g FA and enriched linearly *trans*-11 18:1, *cis*-9,*trans*-11 18:2, and *trans*-11,*cis*-15 18:2 from 5.2, 2.6, and 1.7 to 11, 4.3, and 5.8 g/100 g FA, respectively. Furthermore, CO decreased quadratically milk fat *trans*-10 18:1 and linearly *trans*-10,*cis*-12 18:2 concentration. Overall, milk FA composition on all treatments suggests that one or more components in camelinaseeds may inhibit the complete reduction of 18-carbon unsaturates in the rumen. In conclusion, CO decreased the secretions of saturated FA in milk and increased those of the *trans*-11 biohydrogenation pathway or their desaturation products. Despite increasing the intake of 18-carbon unsaturated FA, CO had no effect on the secretions of 18:0, *cis*-9 18:1, LA, and ALA in milk. Concentrates containing camelina expeller and 2% CO could

be used for the commercial production of low-saturated milk from grass and RCS based diets without major adverse effects on animal performance.

Keywords: camelina, saturated fatty acid, *trans* fatty acid, conjugated linoleic acid

INTRODUCTION

Public health policies recommend a population wide decrease in the consumption of SFA and an increase in PUFA, specifically n-3 fatty acids (**FA**) to lower the incidence of cardiovascular and metabolic diseases (USDA and HHS, 2010; FAO, 2010; Perk et al., 2012). Milk and dairy products contribute to total fat intake and are typically the major source of SFA in the human diet (Kliem and Shingfield, 2016). Ruminant milk fat also contains several FA with anti-mutagenic properties including 4:0, odd- and branched-chain FA (**OBCFA**), *trans*-11 18:1, and *cis*-9,*trans*-11 CLA (Parodi, 2001; Shingfield et al., 2008b). Altering milk fat composition offers the opportunity to lower SFA intake and increase the consumption of PUFA and bioactive lipids without requiring a change in eating habits.

Diet is the major environmental factor influencing milk fat composition. Forage species, forage conservation methods, and dietary lipid supplements are known to affect milk FA composition (Dewhurst et al., 2006; Shingfield et al., 2013). Several studies have examined the potential to lower SFA and enrich *trans*-11 18:1, *cis*-9,*trans*-11 CLA, and *cis*-9,*cis*-12,*cis*-15 18:3 (α-linolenic acid, **ALA)** in milk fat using oils or processed seeds rich in ALA principally from linseed (Chilliard et al., 2009; Kliem and Shingfield, 2016). Interest in cultivating Camelina (*Camelina sativa*) as an alternative to linseed has been increasing due to low input requirements, a high drought tolerance, and an ability to adapt to changes in climatic and soil conditions (Zubr, 2003a). Camelina oil (**CO**) is a rich source of 18 carbon n-6 PUFA in the form of *cis*-9,*cis*-12 18:2 [linoleic acid (**LA**); 15 to 16 g/100 g FA] and n-3 PUFA (ALA; 37 to 38 g/100 g FA; Zubr, 2003b;

Bayat et al., 2015). Camelinaseeds are also relatively abundant in essential AA (Zubr, 2003b), highlighting the potential of camelina as a high quality feed for ruminants.

It is well established that the amount of supplemental lipid and the composition of the basal diet are important determinants of milk FA responses to plant oils and oilseeds in lactating cows (Roy et al., 2006; Shingfield et al., 2013). Both factors also influence DMI, milk yield, and milk fat content (Roy et al., 2006; Drackley et al., 2007; Steinshamn, 2010). Milk fat from cows fed red clover silage (**RCS**; *Trifolium pratense*) contains higher concentrations of ALA compared with grass silage (Dewhurst et al., 2006; Steinshamn, 2010; Halmemies-Beauchet-Filleau et al., 2014). However, owing to inconsistencies in annual herbage yield, red clover is typically cultivated as a mixed sward with grasses rather than as a monoculture. Feeding grass silage and RCS as a mixture often increases DMI and milk yield compared with feeding grass or RCS as sole forages (Vanhatalo et al., 2009; Halmemies-Beauchet-Filleau et al., 2014). Several studies have shown that camelinaseeds or CO can be used to alter milk fat composition in cows fed diets based on maize silage (Hurtaud and Peyraud, 2007), RCS (Halmemies-Beauchet-Filleau et al., 2011) or grass silage (Mihhejev et al., 2007; Bayat et al., 2015) and in goats (Pikul et al., 2014) and in sheep (Szumacher-Strabel et al., 2011) based on a mixture of alfalfa silage, grass silage, and meadow hay. However, no single experiment has examined the effect of CO inclusion rate in cows fed diets containing a mixture of grass silage and RCS.

The aim of this study was to determine the optimal amount of CO for altering milk FA composition without causing adverse effects on DMI or milk production in cows fed diets based on a mixture of grass silage and RCS supplemented with concentrates containing camelina expeller as the main protein source. Eight cows were used in a replicated 4 × 4 Latin square to test the hypotheses that i) incremental inclusion of CO progressively lowers DMI and milk fat medium-chain SFA secretion and ii) supplements of CO increase milk fat *cis*-9,*trans*-11 CLA and ALA concentrations in a dose dependent manner.

MATERIALS AND METHODS

Animals, Experimental Design, and Experimental Diets

All experimental procedures were approved by the National Animal Ethics Committee (Hämeenlinna, Finland) in accordance with guidelines established by the European Union (1986). The experiment was performed at the University of Helsinki research farm in Viikki, Finland. Eight multiparous Finnish Ayrshire cows of mean ± SD 634 ± 68.5 kg of BW, 91 ± 16.5 DIM, and producing 37.7 ± 2.60 kg milk/d were used. Four cows were fitted with rumen cannulae (100 mm i.d.; Bar Diamond Inc., Parma, ID). Cows were allocated at random to experimental diets according to a replicated 4 × 4 Latin square design with 21-d periods (Supplemental Table 1; available on line at www.journalofdairyscience.org). At the end of the experiment cows weighed on average 630 kg (SD 72.2 kg). Treatments comprised 4 pelleted concentrate supplements based on cereals and camelina expeller containing 0 (control), 2, 4, or 6% of CO on an air-dry basis. Cows received 12 kg/d of concentrates on an air-dry basis (Table 1) fed as equal meals at 0615, 1000, 1300, 1645, and 2000 h with ad libitum access to a mixture of grass silage and RCS (1:1 on a DM basis). Fresh silage was made available 4 times daily at 0700, 1200, 1500, and 1800 h. Experimental diets were designed to meet ME and MP requirements and to support maintenance and 40 kg ECM/d (Luke, 2015). All experimental animals were housed in individual tie stalls equipped with forage intake control feeding stations (Insentec BV, Marknesse, the Netherlands) that were fitted with separate concentrate troughs. Cows had continuous access to water and were milked twice daily at 0615 and 1700 h.

Grass silage was prepared on June 16, 2008, from primary growths of a 2-yr ley of mixed timothy (*Phleum pratense*, 83% of DM) and meadow fescue (*Festuca pratensis*, 13% of DM) containing small amounts of cocksfoot (*Dactylis glomerata*), weeds, and dead plant material (1, 2, and 1% of DM, respectively). On April 25, grass swards used for silage production were fertilized

with N, P, and K at a rate of 97, 16, and 16 kg/ha, respectively. On reaching the heading stage, grass was cut with a mower-conditioner, wilted for approximately 5 h to a DM content of 27.7%, and ensiled using a formic acid based additive (760 g formic acid and 55 g of ammonium formate, AIV 2 Plus, Kemira Ltd., Helsinki, Finland) applied at a rate of 6 L/t fresh herbage and roundbaled. Primary growths of 2-yr red clover leys were cut at an early flowering stage on July 1, 2008 with a mower-conditioner, wilted for 48 h during inclement weather to a final DM content of 14.8%, harvested with a self-loading wagon, and ensiled using formic acid based additive (6 L/t fresh herbage, AIV 2 Plus) in a clamp silo. Prior to harvesting, red clover swards were fertilized on April 28 with N, P, and K at a rate of 4.1, 10, and 42 kg/ha, respectively.

Sampling and Chemical Analysis

Individual cow intakes were recorded throughout the experiment, but only measurements for the penultimate 6 d of each period (d 15 to 20) were used for statistical analysis. Representative samples of feeds (d 15 to 21) and spot fecal samples (at 0700 and 1500 h on d 17 to 21) were collected, composited, and stored at -20°C prior to chemical analysis (Kokkonen et al., 2000). The concentration of indigestible NDF (**iNDF**) of silages and concentrates was determined in duplicate by incubating samples (from 0.5 to 1.0 g) within polyester bags (60 × 120 mm, pore size 17 μm) in the rumen for 12 d (Ahvenjärvi et al., 2000). The OM content of the indigestible residue was determined by ashing at 600ºC for 18 h (Heraeus K1253, Heraeus GmbH, Hanau, Germany). Ethanol was analyzed using a commercially available kit (Cat. No 10 176 290 035, Boehringer-Mannheim, Darmstadt, Germany) in accordance with the instructions of the manufacturer with a UV-spectrophotometer (Shimadzu UV-VIS mini 1240, Shimadzu Europa GmbH, Duisburg, Germany). Nutrient digestibility was estimated using acid-insoluble ash as an internal marker (Kokkonen et al., 2000). Cows were weighed (CV 9600 Scale, Solotop Ltd., Helsinki, Finland) over 2 consecutive days at the beginning and the end of the experiment. Body condition scores were

158 recorded on a scale of 1 (thin) to 5 (fat) at the start of the experiment and at the end of each
159 experimental period (Edmonson et al., 1989).

160 Samples of ruminal fluid were collected from cows fitted with a rumen cannula on 8 occasions
161 over 1.5 h intervals starting at 0600 h on d 19 of each period, filtered through a single layer of
162 cheesecloth, and analyzed for pH, ammonia N, and VFA (Koivunen et al., 2015). To assess rumen
163 protozoal numbers, a 10-mL sub-sample of filtered rumen fluid was taken and preserved with 30
164 mL of aqueous NaCl (0.9% wt/vol) containing 10% (vol/vol) CH_2O. Samples collected at each time
165 point were composited and replicate (n = 6) measurements of protozoal numbers were made using
166 a counting chamber (Fuchs-Rosenthal, Fortuna, Germany).

167 On d 21 of each period, blood samples were obtained from the coccygeal vessels at 0530,
168 0830, and 1130 h into evacuated collection tubes (Venoject, Terumo Europe Ltd., Leuven, Belgium)
169 containing potassium ethylene diamine tetra-acetic acid and placed on ice. Once collected, blood
170 samples were centrifuged (15 min at 870 *g* at room temperature) and plasma was stored at -20°C
171 pending analysis for BHB, glucose, insulin, NEFA (Selim et al., 2014), and acetic acid.
172 Concentrations of acetic acid were determined by UPLC (Waters Acquity UPLC, Waters, Milford,
173 MA). After thawing at room temperature, plasma (0.1 ml) was deproteinised following the addition
174 of 0.1 ml of acetonitrile. The mixture was centrifuged (10 min at 15,000 *g*), and 40 µl of the
175 supernatant was used in a conjugation reaction with 40 µl 1-Ethyl-3-(3-
176 dimethylaminopropyl)carbodiimide (100 mM) in ethanol containing 3% of pyridine and 40 µl
177 pentafluorobenzylhydroxylamine (50 mM) in 50%/50% acetonitrile/300 mM KH_2PO_4 solution.
178 Solutions were combined, mixed thoroughly and incubated for 30 min at +60^{0}C. Chromatography
179 was achieved using 0.35% formic acid in mQ water and 0.1% formic acid in acetonitrile (Eluents A
180 and B, respectively), using a MassTrak AAA 2.1 × 150 mm column (Waters) maintained at + 60^{0}C,
181 and a gradient method (initial to 1 min = 75% A, 5.2 min = 54% A, 5.5 min to 6 min = 10% A, 6.5
182 min to 7.5 min=75% A). Initial solvent flow rate was 0.45 ml/min that was increased to 0.5 ml/min

183 from 1 min to 5.2 min and reversed to 0.45 ml/min from 5.2 min to 5.5 min. Concentrations of
184 acetate were determined for a sample volume of 1 µl and monitoring column effluent at 269 nm.
185 Standard solutions of an authentic standard (A6283, Sigma-Aldrich, Helsinki, Finland) over a range
186 in concentrations from 46 µM to 1.15 mM were used to check the linearity of responses and to
187 develop a calibration curve.

188 Milk yield was recorded daily throughout the experiment, but only measurements made from
189 d 15 through to d 20 of each period were used for statistical analysis. Samples of milk were collected
190 from each cow over 4 consecutive milkings starting at 1700 h on d 19. Milk samples (20 ml) treated
191 with preservative (Bronopol, Valio Ltd., Helsinki, Finland) were analyzed for milk fat, CP, urea,
192 and lactose (Milko-Scan605 analyser, Foss Electric, Hillerød, Denmark). Additional samples of
193 unpreserved milk (500 ml) were also collected, composited according to yield, and stored at -20°C
194 until analysed for FA composition. Sensory analysis was performed on milk samples (750 ml)
195 collected over 2 consecutive milkings, starting at 1700 h on d 18, that had been placed in an ice-bath
196 immediately after collection, and stored at +4°C thereafter. Assessment of aroma and flavor of
197 unpasteurized milk were made on d 19 by an experienced 6-member taste panel. Milk samples were
198 presented to the panel at +15°C and evaluated using a numerical interval scale from 1 (poor) to 5
199 (excellent) as outlined previously (Halmemies-Beauchet-Filleau et al., 2011). For the determination
200 of butter storage properties, all milk from both cows fed the same treatment within each period was
201 pooled over 3 consecutive milkings starting at 0615 h on d 18. Immediately after milking, milk was
202 transferred into stainless steel 50 L containers and placed in a water bath containing ice to facilitate
203 rapid cooling. Milk was stored at +4°C until butter manufacture in the dairy pilot-plant of the
204 Department of Food Technology, University of Helsinki (Helsinki, Finland). Milk was passed
205 through a Seital SE 02 separator (Seital Separatori Italia, Santorso, Italy) and the cream obtained
206 was pasteurized at 90°C for 25 sec (Fisher E 5 FHG plate heat exchanger, Fischer AG, Ebreichsdorf,
207 Austria). Pasteurized cream was ripened and the cream was churned (Elba 30, Elecrem, Chatillon,

France). Sub-samples of butter (from 1 to 2 g) were used to determine water content by drying to a constant weight loss at 102˚C. Concentrations of peroxides (Halmemies-Beauchet-Filleau et al., 2011) and NEFA in butter were determined immediately after manufacture and at 2 wk intervals until 12 wk of storage. Butterfat acidity (NEFA %) was determined with an automatic titrator (DL-58, Mettler-Toledo, Greifensee, Switzerland) equipped with a photoelectrode (DP550, Mettler-Toledo) according to International Dairy Federation Standard 6 (IDF, 2004) with minor modifications. A quality assurance sample was prepared by adding a known amount of *cis*-9 18:1 in refined rapeseed oil and benzoic acid was used instead of potassium hydrogen phthalate to validate the tetra-n-butylammonium hydroxide titer. Results are expressed as a weight percentage of *cis*-9 18:1 in butterfat. Butters were tasted and a verbal description of flavor chracteristics was provided by trained panelists.

Lipid Analysis

Lipid in 1 mL milk samples was extracted in triplicate with a mixture of ammonia, methanol, diethylether, and hexane (0.2:1:2.5:2.5, vol/vol, respectively). Organic extracts were combined and converted to FAME using methanolic sodium methoxide as a catalyst (Halmemies-Beauchet-Filleau et al., 2011).

Samples of FAME were quantified using a gas chromatograph (model 6890, Hewlett-Packard, Wilmington, DE) equipped with a flame-ionization detector, automatic injector, split injection port and a 100-m fused silica capillary column (i.d. 0.25 mm) coated with a 0.2 μm film of cyanopropyl polysiloxane (CP-Sil 88, Chromopack 7489, Middelburg, The Netherlands). Total FAME profile in a 2-μL sample at a split ratio of 1:50 was determined using a temperature gradient program and hydrogen as a carrier gas operated at constant pressure (137.9 kPa) at a flow rate of 0.5 mL/min. Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170ºC. Peaks were identified by comparison of retention times with authentic FAME standards. Fatty acid

methyl esters not available as commercial standards were identified based on GC-MS analysis of 4,4-dimethyloxoline (**DMOX**) derivatives prepared from FAME. Preparation of DMOX derivatives, parameters used for GC-MS analysis, and the interpretation of mass spectra were in accordance with earlier reports (Halmemies-Beauchet-Filleau et al., 2011). Relative retention time and order of elution was used to differentiate between diastereomers of 3,7,11,15-tetramethyl-16:0 (Schröder and Vetter, 2011).

The distribution of CLA isomers in milk samples was determined using a HPLC system (Model 1090; Hewlett-Packard, Wilmington, DE) equipped with four silver-impregnated silica columns (Chrom-Spher 5 Lipids, 250 × 4.6 mm, 5 μm particle size; Varian Ltd., Walton-on-Thames, UK) coupled in series. Methyl esters of CLA were separated under isothermal conditions at 22ºC using 0.1% (vol/vol) acetonitrile in heptane at a flow rate of 1 mL/min and monitoring column effluent at 233 and 210 nm (Halmemies-Beauchet-Filleau et al., 2011).

Milk FA composition was expressed as a weight percentage of total FA using theoretical relative response factors to account for the carbonyl deficiency in the flame ionization detector response for 4- to 10-carbon containing FAME (Halmemies-Beauchet-Filleau et al., 2011). Concentrations of specific conjugated isomers were calculated based on proportionate peak area responses determined by HPLC and the sum of *trans*-7,*cis*-9 CLA, *trans*-8,*cis*-10 CLA, and *cis*-9,*trans*-11 CLA weight percentage determined by GC.

Calculations

Digestible organic matter content in silage DM (DOMD) was calculated from measurements of pepsin-cellulase solubility (Koivunen et al., 2015). Metabolizable energy content of experimental concentrates was calculated as the weighted sum of published energy concentrations of individual ingredients (Luke, 2015). The ME content of silage was calculated based on DOMD content (Luke, 2015). Metabolizable energy intakes were corrected for associative effects according to Luke

(2015). Energy requirements (MJ/d) for maintenance and milk production was calculated as $BW^{0.75}$ (kg) × 0.515 + ECM yield (kg/d) × 5.15 (Luke, 2015). Energy-corrected milk yield was calculated as milk yield (kg) × [383 × fat (%) + 242 × protein (%) + 165.4 × lactose (%) + 20.7] / 3140 (Sjaunja et al., 1990).

Statistical Analysis

Data were analysed by ANOVA with a model that included the random effect of cow within square and fixed effects of square, period within square, carry over, and treatment using the PROC MIXED procedure of SAS (SAS Version 9.3. SAS Institute, Inc., NC). For all parameters, the effect due to carryover was $P > 0.10$, and therefore this term was removed from the final statistical model. Sums of squares for treatment effects were further separated using orthogonal contrasts into single degree of freedom comparisons to test for the significance of linear, quadratic, and cubic components of response to incremental amounts of CO in the diet. Least square means are reported with treatment effects declared significant at $P \leq 0.05$, with P values between 0.05 and 0.10 considered a trend towards significance.

Unfortunately 4 out of 32 cow period observations could not be made (Supplemental Table 1) due to a leg injury (cow number 3 during period 1) or digestive disorders (cow number 6 during periods 3 and 4). Furthermore, cow number 5 was replaced due to digestive disorders after period 1 with a different cow.

Measurements of rumen pH and fermentation characteristics were analysed by ANOVA for repeated measures with a model that included the fixed effects of treatment, period, sampling time, the interaction of sampling time and period and that of sampling time with treatment and the random effects of cow, the interaction of cow, treatment, and period and the interaction of cow and sampling time using the Satterthwaite correction. The covarariance structure AR(1) was applied with the interaction of cow and period as the subject for repeated measures. Time by treatment interactions

were not significant. Therefore daily averages are reported. Changes in the concentration of peroxides and NEFA during butter storage were analysed by ANOVA for repeated measures using a model that included the fixed effect of treatment, period, time, the interactions of time and period and time and treatment, and the random effect of the interaction of treatment and period with the Satterthwaite correction. The AR(1) covarariance structure was applied with diet within period as the subject for repeated measures.

RESULTS

Chemical Composition of Experimental Feeds

The chemical composition of concentrate treatments and experimental silages is presented in Table 2. Due to a prolonged period of rain around harvesting, the DM content of red clover was lower than targeted. Red clover silage was of moderate fermentation quality and nutritive value as indicated by the relatively high pH, VFA and ammonia N concentrations and low DOMD, whereas grass silage was of high fermentation quality and digestibility. Compared with grass silage, RCS had a higher ash and CP and lower NDF content. However, total FA content and FA composition were similar between grass silage and RCS. For both silages, ALA was the major FA (from 45 to 49 g/100 g total FA), but RCS and grass silage also contained relatively high proportions of 16:0 (from 15 to 17 g/100 g total FA) and LA (from 17 to 19 g/100 g total FA). By design, experimental concentrates contained different amounts of FA varying 44 to 91 g/kg DM, with CO inclusion being associated with incremental changes in the relative proportions of 16:0, *cis*-9 18:1, LA, ALA, and *cis*-11 20:1 in total lipid from 12, 16, 32, 20, and 7 to 8, 14, 23, 25, and 15 g/100 g total FA, respectively.

Nutrient Intake

Cows consumed all concentrates on the 0 and 2% CO treatments, but on occasion small

amounts of the 4% CO and 6% CO concentrates were refused. Increasing levels of dietary CO linearly decreased ($P < 0.01$) silage and diet DM intakes (Table 3). Overall, the proportion of concentrate in the diet DM increased linearly ($P < 0.04$) from 50 to 55% in response to incremental CO supplementation. Furthermore, CO inclusion linearly decreased ($P < 0.01$) ME, OM, NDF, and N intake, but linearly increased ($P < 0.05$) the intake of 16- to 22-carbon FA (Table 3).

Rumen Fermentation and Nutrient Digestibility

Dietary CO supplementation had no effect ($P > 0.10$) on rumen pH, ammonia N, and total VFA concentrations or on rumen protozoal numbers (Table 3, Supplemental Figure 1). Rumen pH varied between 5.93 to 7.13 across sampling times (Supplemental Figure 1), averaging 6.50 across all treatments and sampling times (Table 3). Incremental CO supplementation had no major effect ($P \geq 0.09$) on the molar proportions of individual VFA in rumen fluid (Table 3). Increasing amounts of CO had no effect ($P > 0.10$) on whole tract apparent nutrient digestion, except for a linear increase ($P < 0.01$) in crude fat digestibility (Table 3).

Plasma Metabolites

Plasma BHB tended to be higher ($P = 0.07$, cubic effect) on the 4% CO treatment compared with the other diets (Table 3). Plasma acetic acid concentration linearly decreased ($P < 0.02$) in response to CO. Incremental CO supplementation linearly increased ($P < 0.01$) plasma NEFA concentration with a numerical linear decrease ($P = 0.11$) in plasma glucose and insulin.

Milk Production, Milk Sensory Quality, and Butter Storage Properties

The effect of CO treatments on milk production and milk sensory quality is shown in Table 4. Incremental CO supplementation decreased ($P < 0.03$) milk yield in a quadratic manner and linearly decreased ($P < 0.01$) ECM yield. Inclusion of CO had no effect ($P > 0.10$) on milk fat output, but

linearly increased ($P < 0.02$) milk fat concentration. Supplements of CO linearly decreased ($P < 0.04$) milk protein yield and milk protein and urea concentrations. Increases in CO had no effect on milk lactose concentration ($P > 0.10$), but decreased ($P < 0.02$) milk lactose yield in a quadratic manner. Furthermore, incremental CO supplementation linearly decreased ($P < 0.02$) milk taste panel score. Treatment had no effect ($P > 0.10$) on butter peroxide and NEFA concentrations over a 12 weeks storage period, which were consistently below 0.15 mmol O_2/kg milk fat and 0.3%, respectively (Supplemental Figures 2 and 3). Butter water content averaged 13.9, 13.4, 13.8, and 12.8% for 0%, 2%, 4%, and 6% CO, respectively (data not presented). Overall, butter flavor was assessed as good or satisfactory for up to 6 wk of storage, but off-flavors were detected thereafter (data not shown).

Milk FA Composition and Secretion

The effect of CO supplementation on the major FA in milk fat, 18:1 and 18:2 composition, and FA secretion in milk is shown in Tables 5, 6, and 7, respectively. Treatment effects on the relative proportions of OBCFA, 16:1 and 16:2, and 20:1 isomers in milk fat are reported in Supplemental Tables 2, 3, and 4, respectively. Supplements of CO linearly decreased ($P < 0.01$) the proportions of FA synthesized de novo (4- to 14-carbon and 16:0) in milk fat with an overall decrease ($P < 0.01$) in total SFA concentration from 58 to 45 g/100 g total FA (Table 5).

Inclusion of CO supplementation had no effect ($P \geq 0.10$) on *cis*-9 18:1, LA, ALA or total FA output in milk (Table 7). Supplements of CO linearly decreased ($P < 0.01$) the apparent transfer of LA and ALA from the diet into milk (Table 5). Increases in CO linearly enriched ($P < 0.01$) several geometric Δ9,11,15 18:3 isomers in milk fat (Table 5) and increased linearly ($P < 0.01$) the relative abundance of Δ8,15 18:2 and Δ11,15 18:2 (Table 6). Inclusion of CO linearly increased ($P < 0.01$) milk fat total CLA content (Table 5) and altered the distribution of milk fat CLA isomers characterised as linear increases ($P < 0.01$) in *cis*-9,*trans*-11 CLA, *trans*-8,*cis*-10 CLA, *trans*-11,*cis*-

13 CLA and concominant linear decreases ($P < 0.03$) in *trans*-9,*trans*-11 CLA and Δ10,12 CLA concentrations (Table 6). Furthermore, CO elevated milk fat *trans*-7,*cis*-9 CLA, *trans*-11,*trans*-13 CLA, and *trans*-12,*trans*-14 CLA concentrations reaching a maxium on the 2% and 4% CO treatments that declined in response to further oil addition ($P < 0.04$ for quadratic effect; Table 6). (Incremental amounts of CO linearly increased ($P < 0.03$) milk fat proportions of *trans*-4 18:1, *trans*-5 18:1, and *trans*-11 18:1, but quadratically decreased ($P < 0.01$) *trans*-10 18:1 concentration (Table 6). Overall, CO supplementation increased linearly ($P < 0.01$) total *trans* FA concentration in milk fat (Table 5) and the secretion of *trans* FA in milk (Table 7), without altering ($P > 0.10$) the relative proportions of 18:0 in milk fat (Table 5) or secretion of 18:0 in milk (Table 7).

Supplements of CO altered milk fat OBCFA concentrations (Supplemental Table 2), changes characterised by linear decreases ($P < 0.05$) in milk fat concentration of numerous 5- to 25-carbon OBCFA. Inclusion of CO altered the relative distribution of 16-carbon FA in milk resulting in linear decreases in ($P < 0.04$) *cis* 16:1 (Δ9 to 13) and *trans*-13 16:1 and linear increases ($P < 0.01$) in *trans*-9 16:1, *trans*-9,*trans*-12 16:2, and Δ9,13 16:2 concentrations. (Supplemental Table 3).

Incremental amounts of CO linearly increased ($P < 0.02$) milk fat *cis* 20:1 (Δ11 and 13) and *trans* 20:1 (Δ6+7+8 and 11) concentrations (Supplemental Table 4), but had no major effect ($P > 0.07$) on the daily yields of 20:0 and *cis*-9 20:1 (Table 7). Inclusion of CO elevated ($P < 0.01$) also the proportion of *cis*-13 22:1 in milk fat in a linear manner (Table 5). Camelina oil supplementation altered the relative abundance of 20- to 22-carbon n-3 PUFA, increasing linearly ($P < 0.01$) *cis*-11,*cis*-14,*cis*-17 20:3 and *cis*-13,*cis*-16,*cis*-19 22:3 and decreasing linearly ($P < 0.03$) *cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17 20:5 [eicosapentaenoic acid (**EPA**)], *cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19 22:5, and *cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19 22:6 [docosahexaenoic acid (**DHA**)] concentrations. Furthermore, inclusion of CO decreased linearly ($P < 0.05$) several product to substrate concentration ratios for stearoyl-CoA desaturase (SCD1) in milk fat (Table 5).

DISCUSSION

Dry Matter Intake

Supplements of plant oils and oilseeds typically lower DMI when included in amounts above 50 g of oil/kg DM, a response often attributed to the adverse effect of unsaturated FA on rumen microbial communities, lowered ruminal OM and NDF digestion (Allen, 2000; Lock and Shingfield, 2004), and an increase in gut peptide secretion (Litherland et al., 2005; Relling and Reynolds, 2007). By design, incremental inclusion of CO in concentrate supplements increased the FA content of the total diet from 33 to 60 g/kg DM. Increases in CO decreased silage and total DMI by 27 and 18%, respectively, in the absence of changes in total tract NDF digestibility. The magnitude of decreases in intake were higher compared with a recent report of a 12% decrease in DMI in response to inclusion of 60 g CO/kg DM that increased the FA content of a TMR based on grass silage from 22 to 77 g/kg DM (Bayat et al., 2015). However, both the proportion of concentrate in diet DM (from 50 to 55%) and apparent total tract OM digestibility (from 74 to 75%) were similar in this and the earlier report (50 and from 69 to 72%, respectively; Bayat et al., 2015). It is possible that the larger decrease in DMI to CO in the present experiment was related to the inclusion in concentrates fed separately at specified time points leading to a more rapid release of unsaturated FA in the rumen compared with adding CO as part of a TMR (Bayat et al., 2015). Decreases in DMI to linseed oil supplementation (from 4 to 6% of diet DM) have been shown to be much more pronounced when concentrates were fed separately (-26%; Chilliard et al., 2009), rather than as part of a TMR (from no change to -9%; Bell et al., 2006; Benchaar et al., 2015).

There are relatively few reports on the effect of CO on DMI, but indirect comparisons do not provide substantive evidence to suggest that CO has more adverse effects on intake (Hurtaud and Peyraud, 2007; Halmemies-Beauchet-Filleau et al., 2011; Bayat et al., 2015) compared with other plant oils of similar FA composition (Bell et al., 2006; Chilliard et al., 2009). Nevertheless, camelina expeller or meal has been reported to result in marginally greater decreases in DMI compared with

408 provision of the same amount of lipid as camelina oil (Halmemies-Beauchet-Filleau et al., 2011) or
409 whole seeds (Hurtaud and Peyraud, 2007). Furthermore, the intake of diets based on RCS has been
410 shown to be unaffected or marginally decreased in response to plant oil (rapeseed, sunflower-seed,
411 camelinaseed or linseed) supplements (Halmemies-Beauchet-Filleau et al., 2011; Benchaar et al.,
412 2015), with the implication that feeding diets containing a mixture of grass silage and RCS is not
413 the sole explanation for the relatively high decrease in DMI on the 6% CO treatment.
414
415 ***Rumen Fermentation and Nutrient Digestibility***
416 Supplements of PUFA typically modify rumen fermentation characterized by a shift towards
417 propionate at the expense of acetate, butyrate or both lipogenic VFA (Ueda et al., 2003; Hurtaud
418 and Peyraud, 2007; Shingfield et al., 2008a). Changes in molar VFA proportions to plant oils may
419 be related to the toxic effects of LA and ALA on specific cellulolytic and butyrate-producing
420 bacteria (Maia et al., 2007; Yang et al., 2009). There were no indications from this or an earlier
421 study (Bayat et al., 2015) to suggest that CO alters rumen fermentation characteristics or depresses
422 fiber digestion. However, the associated decrease in DMI in response to CO supplements may have
423 resulted in a higher ruminal retention time compensating for possible adverse effects of PUFA on
424 NDF digestion. It has been suggested that soluble Ca may alleviate possible negative effects of
425 unsaturated FA on fiber digestion due to formation of Ca salts (Doreau and Chilliard, 1997).
426 Conversely, a shortage of Ca may compromise the attachment and colonization of cellulolytic
427 bacteria to feed particles in the rumen (Doreau and Chilliard, 1997). Typically, Ca concentrations
428 are higher in RCS compared with grass silage or maize silage (Luke, 2015).
429 Increases in CO supplementation were associated with an increase in total tract apparent crude
430 fat digestibility from 71 to 81%, but the increases from 4% CO to 6% CO were marginal. Such
431 findings are consistent with a finite capacity for FA absorption in the small intestine of lactating
432 cows (Schmidely et al., 2008) that may be related to the saturation of 18:0 intestinal absorption at

high postruminal flows (Glasser et al., 2008). In cows fed grass silage or RCS diets, 18:0 is typically the major FA leaving the rumen (Shingfield et al., 2008a; 2012; Halmemies-Beauchet-Filleau et al., 2013b).

Plasma Metabolites

Incremental CO supplementation increased plasma NEFA and lowered acetic acid concentration and led to numerical decreases in plasma glucose and insulin concentrations, changes that can be explained by the decreases in DMI. Such responses suggest that a higher proportion of energy requirements for milk production was met through the mobilization of body energy reserves consistent with the loss of BCS at high CO inclusion rates. Increases in FA supply at intestine may also elevate circulating NEFA concentrations (Drackley et al., 1992; Gagliostro et al., 1991), due to the release of FA into the plasma NEFA pool following the action of lipoprotein lipase on triacylglycerols transported in chylomicrons. Earlier studies have also reported that camelinaseeds and meal lower plasma glucose concentration and DMI in lactating cows (Hurtaud and Peyraud, 2007).

Milk Production and Sensory Quality

Incremental CO supplementation progressively lowered milk and lactose yields reflecting the decrease in DMI. No direct measurements of energy status were made, but inclusion of CO in concentrates was associated with numerical decreases in calculated ME balance and plasma glucose concentrations consistent with changes in BCS. A linear decline in milk protein yield, and milk protein and urea concentrations in response to CO can be attributed to lowered ME and nitrogen intakes due to the decrease in both total DMI and concentrate CP content. Milk protein concentration is often decreased by lipid supplementation due to the effects on energy intake, and limitations in glucose supply and microbial protein synthesis (Lock and Shingfield, 2004). In the present study,

458 milk protein concentration was rather low across all diets (29 to 31 g/kg milk) compared with typical
459 values of 34 to 36 g/kg in milk from Finnish Ayrshire cows in mid-lactation (Korhonen at al., 2002;
460 Halmemies-Beauchet-Filleau et al., 2014). Earlier studies have shown that camelina expeller or meal
461 lowers milk protein concentrations (Hurtaud and Peyraud, 2007; Halmemies-Beauchet-Filleau et
462 al., 2011). In addition to the effects attributable to increases in lipid intake, the low milk protein
463 concentration across all treatments may also be explained by the lower histidine concentration of
464 camelina protein compared with soybean or rapeseed protein (Zubr, 2003b). Histidine is considered
465 the first-limiting AA for milk and protein synthesis in grass silage and cereal based diets (Vanhatalo
466 et al., 1999). Rumen ammonia concentrations did not differ due to treatment, but varied between
467 4.37 and 10.9 mmol/L across sampling times (data not shown), averaging 8.58 mmol/L which
468 suggests that there was sufficient RDP on all treatments to support microbial growth (Schwab et al.,
469 2005).

470 Even though CO resulted in dose dependent changes in milk FA composition, milk fat
471 secretion was unaffected. Feeding low fiber-high starch diets, rations containing relatively high
472 amounts of PUFA or high concentrate-high oil diets often lower milk fat content in lactating cows,
473 a phenomenon referred to as diet-induced milk fat depression (**MFD**; Shingfield et al. 2010). Earlier
474 reports have shown that dietary supplements of camelinaseeds or CO depress milk fat yield when
475 fed in relatively high amounts (Mihhejev et al., 2007; Hurtaud and Peyraud, 2007; Bayat et al.,
476 2015). The relative proportion of concentrate in diet DM were rather similar in the present (from 50
477 to 55%) and earlier experiments (42%; Hurtaud and Peyraud, 2007; 50% Bayat et al., 2015), but
478 forage species and composition differed. Previous reports have examined responses in cows fed
479 diets based on corn silage (Hurtaud and Peyraud, 2007), or grass silage as the sole forage (Mihhejev
480 et al., 2007; Bayat et al., 2015), wherein decreases in milk fat secretion have been accompanied by
481 enrichment of intermediates formed by the *trans*-10 biohydrogenation pathway (Shingfield et al.,
482 2010). In contrast, the relative abundance of these intermediates in milk fat was decreased by CO

483 treatments, possibly due to the inclusion of RCS in the diet that has an inherently higher buffering
484 capacity (Koivunen et al., 2015), which may have prevented shifts in biohydrogenation pathways
485 despite the increase in PUFA availability in the rumen. Nevertheless, this does not appear to be the
486 sole explanation as earlier study showed that camelina expeller, and to a lesser extent CO, slightly
487 increased milk fat *trans*-10 18:1 and *trans*-10,*cis*-12 CLA concentrations also when RCS was the
488 only forage in the diet (Halmemies-Beauchet-Filleau et al., 2011). In the present experiment, CO
489 elevated milk fat concentration due to a decrease in milk yield rather than higher fat synthesis in the
490 mammary gland.

491 Incremental amounts of CO in concentrates resulted in a progressive decrease in the secretion
492 in milk of FA synthesized de novo in the mammary gland, that may at least in part, be explained by
493 a decrease in the mammary supply of acetate, as inferred from the decline in plasma acetate
494 concentrations. Despite the decrease in FA de novo synthesis in the mammary gland, CO treatments
495 did not induce MFD. Measurements of milk FA output indicated that the decrease in short- and
496 medium-chain FA to CO treatments was compensated for by higher uptake and incorporation of
497 preformed FA. During MFD, the secretion of all FA is depressed, but the decrease is proportionately
498 greater for FA synthesized de novo (Shingfield et al., 2010). Several theories have been proposed to
499 explain diet-induced MFD that include i) a decrease in the supply of acetate and BHB for FA
500 synthesis de novo, ii) elevated insulin secretion causing the partitioning of FA towards adipose tissue
501 at the expense of mammary gland or iii) direct inhibition of mammary lipogenesis by specific *trans*
502 FA intermediates formed by alterations in the major biohydrogenation pathways in the rumen
503 (Shingfield et al., 2010). Experimental treatments had no effect on milk fat output consistent with
504 numerical decreases in plasma insulin concentrations and an absence of shifts in ruminal
505 biohydrogenation favoring the formation of *trans*-10 containing intermediates, including *trans*-
506 10,*cis*-12 CLA known to inhibit milk fat synthesis in lactating cows (Baumgard et al., 2001).
507 Typically, supplementing fiber-rich diets with plant oils increase the output of long-chain FA in

508 milk, whereas inclusion of plant oils in starch-rich diets causing MFD has no effect or decreases the
509 secretion of ≥ 16 carbon FA (Shingfield et al., 2010).

510 Incremental CO supplementation compromised the sensory attributes of milk that may reflect
511 the alterations in milk fat composition. There is increasing evidence that FA interact with human
512 taste cells playing important roles in gustation, olfaction, and somatosensation, factors that
513 contribute to the overall flavor perception of foods (Tucker et al., 2014). Furthermore, NEFA
514 receptors may react differently or have a different affinity for FA varying in chain length, degree of
515 saturation or both (Tucker et al., 2014). Relative to sunflower-seed oil or rapeseed oil, dietary
516 supplements of camelina expeller result in higher enrichment of PUFA and *trans* FA, lower milk fat
517 SFA concentration, and a numerical decrease in taste panel score, changes that were not associated
518 with changes in milk fat peroxides (Halmemies-Beauchet-Filleau et al., 2011), formed during the
519 initial stages of lipid peroxidation (Halliwell and Chirico, 1993). In butter, oxidative off-flavors
520 become detectable at 0.6 mmol O_2/kg and a maximum level of 0.4 mmol O_2/kg is recommended
521 (Early, 1998). In the present study, the peroxide value of all experimental butters did not exceed 0.3
522 mmol O_2/kg. Camelina oil contains α- and γ-tocopherols (28.1 and 742 ppm, respectively; Zubr and
523 Matthaus, 2002) that may account for the stable peroxide concentrations during prolonged storage
524 of butter despite a higher concentration of PUFA in milk fat from cows fed CO treatments. Milk α-
525 and γ-tocopherol concentrations can be modified by diet (Kanno et al., 1968; Havemose et al., 2004),
526 but the efficiency of transfer of α-tocopherol from the diet into milk gradually declines at high
527 inclusion rates (Weiss and Wyatt, 2003).

528

529 ***Milk FA Composition and Secretion***

530 ***SFA.*** Bovine milk fat typically contains SFA between 67 and 75 g/100 g total FA (Lock and
531 Shingfield, 2004; Lindmark-Månsson, 2008). Inclusion of CO in concentrate supplements
532 progressively decreased milk fat SFA concentration from 58 to 45 g/100 g total FA due to lowered

533 secretion of 4- to 16-carbon FA. All 4- to 12-carbon FA, most of 14:0 and a high proportion of 16:0
534 in milk fat are synthesised de novo in the bovine mammary gland using acetate and BHB as
535 substrates (Chilliard et al., 2000; Halmemies-Beauchet-Filleau et al., 2013a; Shingfield et al., 2013).
536 Incremental CO supplementation had no major effect on plasma BHB concentration, but the
537 circulating level of acetic acid was decreased by 27%. The mammary gland extracts from 51 to 77%
538 of acetic acid in arterial blood (Bickerstaffe et al., 1974; Korhonen et al., 2002) and there is a direct
539 positive relation between acetic acid in plasma and mammary uptake (Cant et al., 1993). It is
540 therefore possible that a decrease in the supply of short-chain FA precursors for mammary FA
541 synthesis may, at least in part, explain the lowered secretion of de novo SFA in response to CO
542 treatments.

543 Increases in the availability of 16 carbon atoms or longer chain FA are known to inhibit
544 mammary acetyl-CoA carboxylase activity (Chilliard et al., 2000) that may also have contributed to
545 lowered de novo FA synthesis in response to CO. *Trans*-10,*cis*-12 CLA is known to inhibit milk fat
546 synthesis in cows (Baumgard et al., 2001) and there is some evidence to suggest that other
547 biohydrogenation intermediates including *trans*-10 18:1 and *trans*-9,*cis*-11 CLA may also exert
548 antilipogenic effects (Shingfield et al., 2010). A lack of increase in the abundance of these FA in
549 milk fat would tend to suggest that these had no major role in contributing to the decreases in
550 mammary de novo FA synthesis in cows fed CO in the present study. Overall, CO supplementation
551 decreased milk fat total SFA by 0.03 percentage units per g of additional FA intake that is within
552 the range of responses (from 0.02 to 0.04) reported for rapeseed, sunflower-seed or linseed oil
553 (Chilliard et al., 2009; Halmemies-Beauchet-Filleau et al., 2011). On all diets, milk fat SFA
554 concentrations were much lower than typical for cows fed diets based on grass silage or RCS or a
555 mixture of both forages (Halmemies-Beauchet-Filleau et al., 2013a; 2014) that appears to be
556 explained by the use of camelina expeller as a protein source in concentrate supplements. Camelina
557 expeller is relatively rich in lipid (Mihhejev et al., 2007; Halmemies-Beauchet-Filleau et al., 2011)

and is known to decrease milk SFA concentrations (Mihhejev et al., 2007; Halmemies-Beauchet-Filleau et al., 2011).

LA and ALA. Despite being relatively rich in PUFA incremental CO supplementation did not increase LA and ALA secretion in milk. A lack of enrichment in milk can be explained by extensive biohydrogenation of dietary PUFA in the rumen as indicated by the progressive decrease in the efficiency of LA and ALA transfer from the diet into milk in response to incremental inclusion of CO in concentrates. These findings are consistent with previous reports indicating that CO supplementation in lactating cows is not an effective means for enriching LA and ALA in milk (Bayat et al., 2015).

Milk 18-carbon Biohydrogenation Intermediate and 18:0 concentrations. Inclusion of CO linearly increased the abundance of FA in milk fat formed during the incomplete biohydrogenation of *cis*-9 18:1, LA, and ALA in the rumen, with a relatively high enrichment of intermediates synthesized by the *trans*-11 pathway, including *cis*-9,*trans*-11,*cis*-15 18:3, *trans*-11,*cis*-15 18:2, *cis*-9,*trans*-11 CLA, and *trans*-11 18:1 (Shingfield et al., 2010). Even though concentrations of *cis*-9,*trans*-11 CLA were increased by CO, the majority of *cis*-9, *trans*-11 CLA in milk is known to originate from the desaturation of *trans*-11 18:1 in the mammary glands (Mosley et al., 2006; Halmemies-Beauchet-Filleau et al., 2013a). Consistent with a product-substrate for SCD1, CO elevated milk fat *cis*-9,*trans*-11 CLA concentrations from 2.6 up to 4.3 g/100 g total FA that were accompanied by increases in milk *trans*-11 18:1 concentrations from 5.2 to 11 g/100 g total FA. Earlier studies have reported *cis*-9,*trans*-11 CLA and *trans*-11 18:1 concentrations in milk of 5.0 and 8.3 g/100 g total FA in milk from cows fed camelina expeller (Mihhejev et al., 2007). In the present study, the increase in milk fat *cis*-9,*trans*-11 CLA to CO (0.0046 percentage unit per g of additional FA intake) was several-fold higher than reported previously for CO (from 0.00047 to 0.00054; Halmemies-Beauchet-Filleau et al., 2011; Bayat et al., 2015) or rapeseed, sunflower-seed or linseed oil (from 0 to 0.00084; Rego et al., 2009; Halmemies-Beauchet-Filleau et al., 2011), but

583 similar to the responses to camelina meal or camelina expeller (from 0.0033 to 0.0086; Hurtaud and
584 Peyraud, 2007; Mihhejev et al., 2007; Halmemies-Beauchet-Filleau et al., 2011) or fish oil (0.0076;
585 Shingfield et al., 2003).

586 Increases in the availability and absorption of *trans*-11 18:1 in the small intestine are
587 preferentially incorporated into plasma triacylglycerols (Tyburczy et al., 2008; Halmemies-
588 Beauchet-Filleau et al., 2013a) that serve as a substrate for milk fat synthesis (Shingfield et al.,
589 2010). Such a mechanism explains the relatively high apparent transfer from the gut into milk and
590 increases in milk fat *trans*-11 18:1 and *cis*-9,*trans*-11 CLA concentrations to increases in
591 postruminal *trans*-11 18:1 supply (Tyburczy et al., 2008; Halmemies-Beauchet-Filleau et al.,
592 2013a). Milk fat total CLA concentration typically varies between 0.3 and 0.5 g/100 g total FA
593 (Lindmark-Månsson, 2008), but on diets supplemented with fish oil or high amounts of plant oil,
594 enrichment of CLA can approach 3.6 g/100 g total FA (Dewhurst et al., 2006). In the present study,
595 the 6% CO treatment elevated milk fat total CLA to a concentration of 4.3 g/100 g total FA which
596 when considered in conjunction with the increase in *trans*-11 18:1 would be expected to increase
597 the CLA status of human consumers (Shingfield and Wallace, 2014).

598 Dietary plant oil supplements often elevate milk fat *trans*-10 18:1 concentration (Chilliard et
599 al., 2009; Shingfield et al., 2013). However, milk fat *trans*-10 18:1 concentration decreased
600 quadratically in response to CO treatments from 1.2 to 0.8 g/100 g total FA. In earlier studies, CO
601 supplements have been shown to cause a marginal enrichment in milk fat *trans*-10 18:1
602 concentration in cows fed grass silage or RCS based diets of between 0.06 to 0.14 percentage units
603 (Halmemies-Beauchet-Filleau et al., 2011; Bayat et al., 2015). However, camelina expeller or
604 camelina meal have resulted in higher increases in milk fat *trans*-10 18:1 concentration in cows fed
605 diets based on grass silage or RCS (from 0.60 to 0.89 percentage units; Mihhejev et al., 2007;
606 Halmemies-Beauchet-Filleau et al., 2011) and corn silage based diets (10 percentage units; Hurtaud
607 and Peyraud, 2007). The use of camelina expeller as a protein source probably accounts for the

608 elevated *trans*-10 18:1 concentration on the control diet, whereas lipid from CO appears to have a
609 rather minor influence on milk fat *trans*-10 18:1 concentration in cows fed diets based on grass
610 silage or RCS.

611 The secretion of 18:0, the end-product of the biohydrogenation of 18-carbon unsaturated FA
612 (Shingfield et al., 2010) in milk was similar across all diets despite the rather high intake of 18-
613 carbon unsaturated FA (from 477 to 676 g/d) across diets. For all treatments, the concentration of
614 18:0 of 4 to 5 g/100 g total FA was lower than typical for bovine milk fat (8 to 14 g/100 g total FA;
615 Jensen, 2002; Lindmark-Månsson, 2008; Chilliard et al., 2009). Nevertheless, the relative
616 proportions of 18:0 in milk fat are similar to earlier reports for milk from cows fed diets containing
617 camelinaseeds, meal or expeller (from 3 to 7 g/100 g FA; Mihhejev et al., 2007; Hurtaud and
618 Peyraud, 2007; Halmemies-Beauchet-Filleau et al., 2011) or fish oil (from 3 to 9 g/100 g FA;
619 Shingfield et al., 2013). Consistent with a high proportion (between 44% and 69%) of 18:0 taken up
620 by mammary gland being desaturated to *cis*-9 18:1 (Shingfield et al., 2010; Halmemies-Beauchet-
621 Filleau et al., 2013a), treatments had no effect on *cis*-9 18:1 output in milk. For all treatments, the
622 proportion of *cis*-9 18:1 in milk fat was relatively low, ca. 10 g/100 g total FA compared with
623 concentrations of 12 to 31 g/100 g total FA reported for milk from cows fed diets containing plant
624 oils (Shingfield et al., 2013). Earlier investigations reported relatively low milk fat *cis*-9 18:1
625 concentrations of between 14 to 15 g/100 g FA in cows fed dietary supplements of camelina meal
626 and camelina expeller (Mihhejev et al., 2007; Hurtaud and Peyraud, 2007; Halmemies-Beauchet-
627 Filleau et al., 2011), but not in response to camelina oil (17 to 24 g/100 g FA; Halmemies-Beauchet-
628 Filleau et al., 2011; Bayat et al., 2015). In cows fed diets supplemented with fish oil or marine algae
629 milk fat *cis*-9 18:1 concentrations can be as low as 6 g/100 g FA (Shingfield et al., 2013).

630 The lack of increase in milk 18:0 and *cis*-9 18:1 secretion, enrichment of *trans* 18:1 and 18:2
631 intermediates in milk fat, and the absence of alterations in the main biohydrogenation pathways in
632 response to CO treatments, suggest that ruminal metabolism of unsaturated 18-carbon FA in

633 camelina may differ compared with lipid from other plant sources. Direct comparisons suggest that
634 camelina oilseeds, but not CO, contain additional components that may interfere with the complete
635 biohydrogenation of 18-carbon unsaturates to 18:0 in the rumen (Halmemies-Beauchet-Filleau et
636 al., 2011). Milk fat concentrations of 18:0 and *cis*-9 18:1 were lower and the relative proportions of
637 *trans*-11 containing biohydrogenation intermediates were higher from cows fed RCS based diets
638 supplemented with similar amounts of lipid as camelina expeller compared with camelina oil
639 (Halmemies-Beauchet-Filleau et al., 2011). Similar changes in milk 18-carbon FA have been
640 reported to camelina expeller relative to rapeseed or linseed expeller in cows fed grass silage based
641 diets (Mihhejev et al., 2007). Supplementing corn silage based diets with camelinaseed or meal has
642 also been shown to markedly decrease 18:0 and *cis*-9 18:1 secretion in milk with a concominant
643 increase in milk fat *trans*-11 18:1 and *cis*-9,*trans*-11 CLA concentrations (Hurtaud and Peyraud,
644 2007). Furthermore, replacing rapeseed meal with camelina expeller in rations based on a mixture
645 of alfalfa silage, grass silage, and meadow hay has been reported to cause similar changes in milk
646 18-carbon FA composition in lactating sheep (Szumacher-Strabel et al., 2011).
647 Ruminal bacteria capable of biohydrogenation are classified into group A and B. Group A
648 bacteria reduce LA and ALA to *trans*-18:1, but only group B bacteria are able to reduce *trans*-18:1
649 to 18:0 (Harfoot and Hazlewood, 1988). Due to the presence of camelina expeller in all experimental
650 diets, it is plausible that the biohydrogenating activity of group B bacteria in the rumen was
651 potentially suppressed. Milk fat *trans*-11,*cis*-15 18:2 concentration was also relatively high on all
652 treatments (from 1.7 to 5.8 g/100 g total FA) consistent with a partial inhibition of the major pathway
653 of ALA biohydrogenation in the rumen that involves the initial formation of *cis*-9,*trans*-11,*cis*-15
654 18:3 that is sequentially reduced to yield *trans*-11,*cis*-15 18:2 and *trans*-11 18:1 as intermediates
655 (Shingfield et al., 2010; Honkanen et al., 2016). A relatively high abundance of *trans*-11,*cis*-15 18:2
656 has also been detected in milk from cows fed intact or processed camelinaseeds (0.6 to 2.5 g/100 g
657 total FA; Hurtaud and Peyraud, 2007; Mihhejev et al., 2007; Halmemies-Beauchet-Filleau et al.,

2011). In contrast, the concentrations of *trans*-11,*cis*-15 18:2 in milk from diets containing CO have been much lower (from 0.2 to 0.4 g/100 g total FA; Halmemies-Beauchet-Filleau et al., 2011; Bayat et al., 2015). Direct measurements of ruminal lipid metabolism of camelina oilseeds and camelina oil in vitro and confirmation in vivo are required to confirm the potential bioactivity in the rumen and to identify one or more active components in camelinaseeds.

OBCFA. The appearance of OBCFA in milk originate primarily from the digestion of microbial OBCFA synthesized de novo in the rumen (Vlaeminck et al., 2006). Changes in the relative proportions of OBCFA in milk may, at least in part, be associated with alterations in microbial counts or in the relative abundance of specific populations of bacteria and protozoa in the rumen. Inclusion of CO in concentrates linearly decreased the concentration of all saturated 14- to 18-carbon OBCFA. Such changes may indicate that CO alters the rumen microbial community due to the inhibitory effects of PUFA on microbial growth (Ivan et al., 2001; Maia et al., 2007). However, CO supplementation was found to have no effect on ruminal protozoal counts in this or an earlier investigation (Bayat et al., 2015). It is also possible that the higher availability of 18-carbon NEFA in the rumen from CO promoted direct incorporation of dietary FA into microbial lipid at expense of bacterial FA synthesis de novo (Sauvant and Bas, 2001), such that the outflow of OBCFA may be altered without affecting total microbial numbers.

16-carbon trans FA. Milk fat 16-carbon *trans* FA are thought to originate from incomplete biohydrogenation of dietary 16-carbon unsaturated FA in the rumen (Shingfield and Wallace, 2014), and β-oxidation of 18-carbon FA (Destaillats et al., 2000). Small amounts of *trans* 16:1 (Δ6 to 13) are known to escape the rumen (Shingfield et al., 2012; Halmemies-Beauchet-Filleau et al., 2013b). However, silage and concentrates did not contain high proportions of 16-carbon unsaturated FA (less than 3.5 and 0.96 g/100 g of total FA, respectively) suggesting that the appearance of 16 carbon *trans* FA in milk in the present study may have originated primarily from β-oxidation of 18-carbon precursors. Both *trans*-9 16:1 and *trans*-11 18:1 were increased ca. 2.0-fold in milk fat in response

683 to incremental CO supplementation. Recent reports suggest that *trans*-9 16:1 can be elongated to
684 *trans*-11 18:1 and subsequently desaturated to *cis*-9,*trans*-11 CLA in bovine adipocytes (Shingfield
685 and Wallace, 2014). It is notable that the magnitude of Δ9,13 16:2 and Δ11,15 18:2 enrichment in
686 milk fat to CO were also similar.

687 ***20:0 and 20-carbon MUFA.*** Camelina lipid is relatively abundant in *cis*-11 20:1 (ca. 15 g/100
688 g total FA; Zubr, 2003b; Bayat et al., 2015). In the present study, CO increased the intake and
689 secretion of *cis*-11 20:1 in milk. However, the increase in *cis*-11 20:1 intake from 34 to 132 g/d to
690 CO treatments was not accompanied by an increase in 20:0 and *cis*-9 20:1 output in milk, whereas
691 that of *trans*-11 20:1 was higher. These findings suggest that the inclusion of camelina expeller in
692 all experimental diets may have inhibited the complete biohydrogenation of 20-carbon unsaturated
693 FA in the rumen.

694 ***Desaturation.*** Milk fat concentration ratios of product to substrate for SCD1 were marginally
695 decreased in response to CO. Part of the decrease may reflect a higher availability of PUFA at the
696 mammary gland inhibiting SCD1 activity (Chilliard et al., 2000). Several biohydrogenation
697 intermediates including *trans*-10,*cis*-12 CLA, *trans*-10,*trans*-12 CLA, and *trans*-9,*trans*-11 CLA in
698 addition to 20- and 22-carbon n-3 FA are thought to lower SCD1 desaturase activity in the bovine
699 mammary gland (Angulo et al., 2012). In the present study, the concentration of these CLA isomers
700 in milk fat declined linearly in response to CO supplementation, but a 3.2- and 2.7-fold linear
701 increase in milk fat *cis*-11,*cis*-14,*cis*-17 20:3 and *cis*-13,*cis*-16,*cis*-19 22:3 concentrations,
702 respectively was detected.

703 In the present study, the ratio of *cis*-9,*trans*-11 CLA to *trans*-11 18:1 concentration in milk
704 ranged between 0.41 and 0.49 that is in accordance with earlier reports (between 0.35 and 0.60;
705 Chilliard et al., 2009; Halmemies-Beauchet-Filleau et al., 2011; 2013a). In cows, mammary SCD1
706 gene expression and the concentration ratio of *cis*-9,*trans*-11 CLA to *trans*-11 18:1 in milk has been
707 found to lower in response to linseed oil (mean ratio 0.31) or linseed and DHA enriched algae (0.16)

compared with the same diet containing saturated FA from palm oil (0.41; Angulo et al., 2012). It therefore appears unlikely that dietary supplements of CO have a substantive negative effect on mammary SCD1 activity, with the implication that the majority of *cis*-9,*trans*-11 CLA in milk on all experimental diets originated from the desaturation of *trans*-11 18:1 in the mammary gland.

CONCLUSIONS

Incremental amounts of CO in concentrate supplements lowered silage and total diet DM intake that together with a decrease in plasma glucose and insulin concentrations, and lower yields of ECM, milk protein, and lactose suggests that high levels of CO in the diet may result in an inadequacy of energy supply to meet the requirements of high yielding dairy cows. Incremental CO supplementation compromised the sensory attributes of milk possibly due to marked changes in milk fat composition. Inclusion of CO in concentrates containing oil-rich camelina expeller as a protein source altered milk FA composition characterised by a relatively high enrichment of 18-carbon biohydrogenation intermediates of *trans*-11 pathway, including *trans*-11 18:1 and *cis*-9,*trans*-11 CLA, and a decrease in short- and medium-chain SFA and biohydrogenation intermediates of *trans*-10 pathway, in the absence of changes in the relative proportions of 18:0, *cis*-9 18:1, LA, and ALA. Indirect comparisons of milk fat composition measured in the present experiment and earlier investigations suggest that one or more components in camelina expeller may inhibit the complete biohydrogenation of 18-carbon unsaturated FA in the rumen. Dietary CO supplements can be used to alter milk FA composition, but in high amounts may depress DMI and milk yield. Concentrates containing camelina expeller and 2% CO supplying an additional 240 g oil/d could be used for the commercial production of low-saturated milk from grass silage and RCS based diets without major adverse effects on animal performance compared with an unsupplemented control diet.

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Table 1. Formulation and ingredient composition of experimental concentrates (% on an air-dry basis)

	Experimental concentrates (% camelina oil)			
Item	0%	2%	4%	6%
Barley	24.3	23.5	23.0	22.0
Wheat	24.0	23.5	23.0	22.2
Camelina expeller	20.0	20.0	20.0	20.0
Molassed sugar-beet pulp	24.0	23.2	22.2	22.0
Sugar-beet molasses	5.0	5.0	5.0	5.0
Camelinaseed oil	-	2.0	4.0	6.0
Calcium carbonate	1.0	1.1	1.1	1.1
Sodium bicarbonate	0.4	0.5	0.5	0.5
Sodium chloride	0.3	0.3	0.3	0.3
Magnesium oxide	0.4	0.4	0.4	0.4
Biotin	0.2	0.2	0.2	0.2
Mineral premix [1]	0.3	0.3	0.3	0.3
Vitamin premix [2]	0.2	0.2	0.2	0.2

[1] Declared as containing (g/kg DM) Zn (37), Cu (6.3), Mn (5.0), I (1.9), Co (0.37), and Se (0.14).
[2] Declared as containing (mg/kg DM) retinyl acetate (1350), cholecalciferol (23), dl-α-tocopheryl acetate (4500), and choline (800).

Table 2. Chemical composition of experimental feeds

Item	Experimental concentrates (% camelina oil) 0	2	4	6	Red clover silage	Grass silage
pH					4.17	4.37
DM	883	881	880	883	194	289
In DM, g/kg						
Ash	63.8	59.8	62.2	62.7	86.6	69.2
Crude fat	54.6	76.0	85.9	107	38.8	39.7
CP	171	164	161	156	181	135
NDF	205	209	200	194	461	562
Indigestible NDF	50.4	51.4	49.2	47.7	159	87.9
Water soluble carbohydrates					36.7	106
Ethanol					1.28	2.51
Lactic acid					44.3	30.9
Acetic acid					20.0	4.46
Propionic acid					nd[1]	nd
Butyric acid					11.3	nd
In total nitrogen, g/kg						
Ammonia nitrogen					68.5	58.6
Soluble nitrogen					320	693
DOMD, g/kg DM [2]					588	700
FA, g/kg DM [3]	44.2	60.1	73.1	91.4	21.6	21.9
FA composition, g/100 g FA						
16:0	12.1	10.1	9.04	8.46	17.0	15.1
18:0	1.89	2.03	2.09	2.17	2.48	1.28
cis-9 18:1	15.9	14.4	14.5	14.2	2.55	3.38
cis-11 18:1	1.69	1.33	1.33	1.12	0.46	0.53
cis-9,*cis*-12 18:2	31.7	27.4	25.3	23.0	19.2	16.7
cis-9,*cis*-12,*cis*-15 18:3	20.4	24.1	25.5	25.4	44.9	49.4
20:0	0.91	1.07	1.14	1.19	1.07	0.79
cis-11 20:1	6.98	10.4	11.8	14.9	nd	nd
cis-13 22:1	1.69	2.14	2.32	2.47	nd	0.03
Others [4]	6.74	7.03	6.98	7.09	12.3	12.8

[1] Not detected.

[2] Digestible OM content in silage DM.

[3] Fatty acid.

[4] Contains 12:0, *anteiso* 13:0, *iso* 13:0, 14:0, *iso* 14:0, 15:0, *anteiso* 15:0, *iso* 15:0, *cis*-6+7+8 16:1, *cis*-9 16:1, *trans*-3 16:1, *trans*-5 16:1, *trans*-6 16:1, *trans*-9 16:1, 17:0, *anteiso* 17:0, *iso* 17:0, *iso* 18:0, 10-O-18:0, *cis*-10 18:1, *trans*-9 18:1, *trans*-10 18:1, *trans*-11 18:1, *trans*-12 18:1, *cis*-9,*trans*-11 18:2, *cis*-9,*trans*-12 18:2, *trans*-9,*cis*-12 18:2, *trans*-9,*trans*-12 18:2, *cis*-9,*cis*-12,*trans*-15 18:3 + *trans*-9,*trans*-12,*cis*-15 18:3, *cis*-9,*trans*-12,*cis*-15 18:3, *cis*-9,*trans*-12,*trans*-15 18:3, *cis*-6,*cis*-9,*cis*-12,*cis*-15 18:4, 19:0, *cis*-10 19:1, S3,R7,R11,15-tetramethyl-16:0, *cis*-8 20:1, *cis*-9 20:1, *cis*-13 20:1, *trans*-11 20:1, *cis*-11,*cis*-14 20:2, *cis*-11,*cis*-14,*cis*-17 20:3, *cis*-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17 20:5, 21:0, 22:0, *cis*-13,*cis*-16 22:2, *cis*-13,*cis*-16,*cis*-19 20:3, *cis*-9,*cis*-12,*cis*-15,*cis*-18 22:4, 23:0, *cis*-14 23:1, 24:0, *cis*-14 24:1, *cis*-15 24:1, 25:0, 26:0, 27:0, 28:0, 29:0, 30:0, and 26 unidentified FA.

Table 3. Effect of incremental amounts of camelina oil on nutrient intake, rumen fermentation, whole tract apparent nutrient digestibility, and plasma metabolite concentrations

	Camelina oil in concentrate (%)				SEM [2]	*P* [1]		
Item	0	2	4	6		LIN	QUAD	CUB
Intake								
Silage, kg DM/d	10.9	9.78	9.68	7.98	0.797	0.002	0.477	0.197
Total DM, kg DM/d	21.5	20.3	20.0	17.7	0.89	0.001	0.321	0.248
ME, MJ/d	235	226	225	205	8.8	0.005	0.329	0.256
OM, kg/d	20.0	18.6	18.5	16.6	0.95	0.006	0.712	0.267
NDF, kg/d	7.75	7.00	6.96	6.00	0.477	0.003	0.733	0.246
Nitrogen, g/d	567	515	509	448	27.3	0.002	0.822	0.244
16:0, g/d	95.0	96.8	102	104	4.01	0.049	0.984	0.619
18:0, g/d	13.2	16.7	19.7	22.6	0.65	<0.001	0.681	0.912
cis-9 18:1, g/d	81.2	97.4	115	131	3.15	<0.001	0.903	0.787
cis-9,*cis*-12 18:2, g/d	191	210	229	236	6.6	<0.001	0.304	0.718
cis-9,*cis*-12,*cis*-15 18:3, g/d	205	249	292	309	11.4	<0.001	0.174	0.587
20:0, g/d	6.43	8.68	10.5	12.3	0.317	<0.001	0.427	0.807
cis-11 20:1, g/d	33.8	66.0	88.7	132	4.48	<0.001	0.187	0.111
cis-13 22:1	7.88	13.6	17.3	22.0	0.564	<0.001	0.311	0.253
∑ Fatty acid, g/d	703	838	965	1,066	30.5	<0.001	0.533	0.878
Concentrate in diet DM, %	50.0	52.4	51.8	54.9	2.13	0.032	0.788	0.241
Calculated ME balance, MJ/d	16.2	9.97	8.30	4.95	8.356	0.194	0.800	0.805
Change in BCS	0.07	0.05	-0.02	-0.08	0.068	0.090	0.713	0.810
Rumen fermentation								
pH	6.52	6.43	6.56	6.50	0.106	0.852	0.777	0.221
Ammonia nitrogen, mmol/l	8.16	9.17	8.67	8.31	0.641	0.984	0.283	0.528
VFA total, mmol/l	138	139	137	134	4.0	0.435	0.590	0.847
VFA mmol/mol								
Acetic acid (A)	639	631	644	639	8.8	0.698	0.830	0.207
Propionic acid (P)	189	193	176	188	9.0	0.586	0.479	0.090
Butyric acid (B)	133	137	142	137	7.4	0.773	0.682	0.820
Isobutyric acid	8.27	8.56	9.25	8.05	0.558	0.988	0.218	0.363
Valeric acid	13.5	13.7	12.9	12.7	0.55	0.215	0.681	0.518
Isovaleric acid	9.30	9.81	9.21	9.79	1.493	0.857	0.968	0.540
Caproic acid	7.97	7.69	8.14	7.18	0.800	0.603	0.655	0.510
Molar ratio of lipogenic to glucogenic VFA								
A / P	3.45	3.32	3.70	3.42	0.200	0.698	0.576	0.090
(A + B) / P	4.17	4.04	4.51	4.15	0.246	0.656	0.476	0.086
Protozoa, × 10^5 counts/ml	10.5	10.5	11.7	13.4	1.90	0.210	0.485	0.894
Apparent digestibility, %								
DM	72.9	73.5	73.8	73.4	0.58	0.381	0.257	0.783
OM	74.2	74.7	75.1	74.8	0.57	0.212	0.350	0.764
NDF	62.1	61.5	62.4	60.6	7.22	0.249	0.355	0.171
Nitrogen	65.4	66.4	67.3	66.6	0.86	0.185	0.270	0.619
Crude fat	71.3	76.1	79.2	81.4	0.96	<0.001	0.084	0.742
Plasma								
NEFA, mmol/l	0.13	0.16	0.18	0.21	0.014	<0.001	0.864	0.366
Glucose, mmol/l	3.96	3.99	3.68	3.82	0.124	0.102	0.508	0.074
Insulin, µIU/ml	11.8	13.0	9.66	8.94	2.310	0.105	0.513	0.286
Acetic acid, mmol/l	2.14	1.84	1.89	1.56	0.183	0.017	0.922	0.236
BHB, mmol/l	1.13	1.00	1.42	0.96	0.201	0.923	0.322	0.066

[1] Significance of linear (LIN), quadratic (QUAD), and cubic (CUB) components of the response to incremental inclusion of camelina oil in concentrates fed at 12 kg/d on an air-dry basis to cows receiving a mixture (1:1 on a DM basis) of grass silage and red clover silage.

[2] SEM for the 4% camelina oil treatment. SEM for 0, 2, and 6% camelina oil treatments are proportionately 0.954, 0.921, and 0.955 of the reported value, respectively. For rumen fermentation characteristics SEM for the 0% camelina oil treatment. SEM for 2, 4, and 6% camelina oil treatments are proportionately 0.886, 0.998, and 0.998 of the reported value, respectively.

Table 4. Effect of incremental amounts of camelina oil on milk production, milk composition, and milk sensory quality

Item	Camelina oil in concentrate (%)				SEM [2]	*P* [1]		
	0	2	4	6		LIN	QUAD	CUB
Yield								
Milk, kg/d	33.5	32.5	32.3	28.0	1.38	<0.001	0.022	0.108
ECM[3], kg/d	29.9	29.3	29.4	26.1	1.82	0.007	0.094	0.210
Fat, g/d	1,117	1,123	1,148	1,055	99.5	0.438	0.261	0.479
Protein, g/d	1,027	962	946	815	48.6	<0.001	0.140	0.107
Lactose, g/d	1,554	1,507	1,499	1,287	71.9	<0.001	0.014	0.081
Concentration								
Fat, g/kg	32.7	34.7	36.1	37.4	2.73	0.014	0.762	0.938
Protein, g/kg	30.7	29.6	29.3	29.1	0.54	0.032	0.313	0.650
Lactose, g/kg	46.3	46.4	46.6	45.8	0.61	0.287	0.168	0.428
Urea, mmol/l	6.20	5.20	4.35	3.62	0.505	<0.001	0.663	0.994
Milk taste panel score [4]	4.15	3.80	3.78	3.56	0.156	0.018	0.675	0.416

[1] Significance of linear (LIN), quadratic (QUAD), and cubic (CUB) components of the response to incremental inclusion of camelina oil in concentrates fed at 12 kg/d on an air-dry basis to cows receiving a mixture (1:1 on a DM basis) of grass silage and red clover silage.

[2] SEM for the 4% camelina oil treatment. SEM for 0, 2, and 6% camelina oil treatments are proportionately 0.954, 0.921, and 0.955 of the reported value, respectively.

[3] Calculated as milk yield (kg) × [383 × fat (%) + 242 × protein (%) + 165.4 × lactose (%) + 20.7] / 3140 (Luke, 2015).

[4] Evaluated by 6 trained panellists using a numerical interval scale from 1 (poor) to 5 (excellent).

1010
1011 **Table 5.** Effect of incremental amounts of camelina oil on milk fatty acid composition
1012

	Camelina oil in concentrate (%)				SEM [2]	*P* [1]		
Fatty acid, g/100 g	0	2	4	6		LIN	QUAD	CUB
4:0	2.87	2.88	2.92	2.61	0.112	0.132	0.142	0.433
6:0	1.69	1.67	1.62	1.45	0.076	0.028	0.285	0.794
8:0	1.05	1.00	0.92	0.84	0.056	0.004	0.694	0.894
10:0	2.40	2.23	2.00	1.82	0.150	0.002	0.967	0.769
cis-9 10:1	0.26	0.24	0.22	0.17	0.017	0.001	0.189	0.566
12:0	3.01	2.76	2.47	2.20	0.161	<0.001	0.932	0.922
cis-9 12:1	0.08	0.07	0.06	0.05	0.005	<0.001	0.127	0.176
trans-9 12:1	0.08	0.07	0.07	0.05	0.005	<0.001	0.148	0.488
14:0	11.6	10.7	9.89	8.94	0.430	<0.001	0.980	0.741
cis-9 14:1	1.31	1.19	1.11	0.81	0.096	<0.001	0.053	0.230
cis-13 14:1	0.03	0.02	0.02	0.01	0.003	0.006	0.796	0.688
trans-9 14:1	0.02	0.02	0.02	0.01	0.001	<0.001	0.138	0.069
16:0	26.2	25.3	23.1	20.1	1.38	0.001	0.262	0.917
∑ *cis* 16:1	2.13	2.00	1.82	1.45	0.117	<0.001	0.069	0.553
∑ *trans* 16:1	0.82	0.96	0.99	1.05	0.060	0.007	0.427	0.503
∑ 16:1	2.94	2.96	2.82	2.52	0.129	0.004	0.084	0.964
18:0	4.73	3.75	4.33	3.78	0.523	0.265	0.620	0.182
9-O-18:0	0.02	0.02	0.02	0.05	0.007	0.006	0.076	0.243
10-O-18:0	0.05	0.07	0.06	0.20	0.029	0.004	0.057	0.188
13-O-18:0	0.01	0.01	0.01	0.02	0.001	0.005	0.412	0.294
15-O-18:0	0.01	0.01	0.01	0.01	0.001	0.962	0.825	0.802
∑ *cis* 18:1	12.7	11.3	12.7	12.2	0.75	0.991	0.419	0.067
∑ *trans* 18:1	11.7	13.9	14.5	15.9	0.67	<0.001	0.434	0.280
∑ 18:1	24.4	25.2	27.2	28.1	1.06	0.002	0.907	0.454
∑ 18:2 [3]	5.10	6.37	6.53	9.35	0.679	<0.001	0.242	0.203
∑ CLA	2.92	3.68	3.83	4.60	0.415	0.008	0.983	0.457
cis-6,*cis*-9,*cis*-12 18:3	0.02	0.02	0.02	0.01	0.002	0.023	0.753	0.385
cis-9,*cis*-12,*cis*-15 18:3	0.66	0.63	0.64	0.64	0.049	0.843	0.752	0.718
cis-9,*trans*-11,*cis*-15 18:3	0.05	0.06	0.06	0.07	0.005	0.005	0.992	0.662
Δ9,11,15 18:3 [4]	0.18	0.26	0.29	0.41	0.032	<0.001	0.586	0.295
Δ9,11,15 18:3	0.01	0.01	0.01	0.02	0.001	<0.001	0.483	0.545
Δ9,11,15 18:3	0.01	0.01	0.01	0.01	0.001	0.233	0.956	0.773
20:0	0.36	0.35	0.44	0.46	0.044	0.026	0.623	0.286
∑ *cis* 20:1	1.96	2.72	3.17	4.61	0.371	<0.001	0.323	0.392
∑ *trans* 20:1	0.34	0.37	0.46	0.43	0.054	0.116	0.464	0.332
∑ 20:1	2.31	3.10	3.65	5.04	0.345	<0.001	0.337	0.445
cis-11,*cis*-14 20:2	0.17	0.24	0.26	0.43	0.040	<0.001	0.220	0.215
trans-9,*trans*-15 20:2	0.01	0.01	0.02	0.01	0.002	0.953	0.434	0.217
Δ9,16 20:2	0.03	0.04	0.05	0.05	0.003	<0.001	0.301	0.437
cis-8,*cis*-11,*cis*-14 20:3	0.05	0.04	0.04	0.04	0.006	0.006	0.215	0.920
cis-11,*cis*-14,*cis*-17 20:3	0.06	0.09	0.10	0.19	0.020	<0.001	0.127	0.188
cis-5,*cis*-8,*cis*-11,*cis*-14 20:4	0.050	0.048	0.046	0.047	0.0035	0.070	0.345	0.919
cis-8,*cis*-11,*cis*-14,*cis*-17 20:4	0.05	0.05	0.05	0.04	0.005	0.015	0.593	0.030
Δ5,11,14,17 20:4	0.01	0.02	0.02	0.03	0.003	0.002	0.520	0.519
cis-5,*cis*-8,*cis*-11,*cis*-14,*cis*-17 20:5	0.04	0.03	0.03	0.03	0.003	0.003	0.368	0.459
22:0	0.09	0.09	0.08	0.08	0.005	0.260	0.991	0.557
cis-9 22:1	0.020	0.016	0.018	0.016	0.0011	0.126	0.743	0.071
cis-13 22:1	0.24	0.33	0.38	0.53	0.036	<0.001	0.348	0.391
cis-15 22:1	0.03	0.04	0.04	0.04	0.004	0.050	0.173	0.166
cis-13,*cis*-16 22:2	0.02	0.03	0.03	0.03	0.002	<0.001	0.470	0.332
cis-13,*cis*-16,*cis*-19 22:3	0.03	0.05	0.05	0.08	0.006	<0.001	0.489	0.241
cis-9,*cis*-12,*cis*-15,*cis*-18 22:4	0.01	0.01	0.01	0.01	0.001	0.152	0.756	0.887
cis-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16 22:5	0.01	0.01	0.01	0.02	0.003	0.028	0.058	0.389
cis-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19 22:5	0.05	0.04	0.04	0.04	0.006	0.025	0.208	0.771

cis-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19 22:6	0.004	0.004	0.003	0.003	0.0004	0.017	0.822	0.890
24:0	0.04	0.03	0.03	0.03	0.001	<0.001	0.059	0.073
cis-15 24:1	0.04	0.04	0.04	0.05	0.003	0.012	0.621	0.841
26:0	0.03	0.03	0.03	0.03	0.004	0.172	0.227	0.513
cis-17 26:1	0.004	0.004	0.004	0.004	0.0004	0.680	0.841	0.691
28:0	0.006	0.006	0.006	0.005	0.0005	0.166	0.755	0.733
∑ Unidentified	0.29	0.29	0.28	0.22	0.025	0.035	0.114	0.735
Summary								
∑ 4- to 14-carbon	24.8	23.1	21.6	19.2	0.89	<0.001	0.518	0.714
∑ *Trans* fatty acids	19.5	24.1	25.2	30.2	1.53	<0.001	0.868	0.234
∑ Saturates	57.7	53.9	50.9	45.3	2.14	<0.001	0.595	0.672
∑ Monounsaturates	32.3	33.8	36.2	37.9	1.23	<0.001	0.887	0.698
∑ Polyunsaturates	9.61	11.9	12.3	16.4	1.190	0.002	0.411	0.275
Concentration ratios								
cis-9 10:1 / 10:0	0.110	0.108	0.112	0.095	0.0071	0.031	0.073	0.126
cis-9 12:1 / 12:0	0.027	0.025	0.026	0.022	0.0015	0.002	0.086	0.063
cis-9 14:1 / 14:0	0.115	0.113	0.113	0.090	0.0100	0.004	0.034	0.208
cis-9 16:1 / 16:0	0.074	0.072	0.072	0.067	0.0038	0.116	0.648	0.602
cis-9 18:1 / 18:0	2.361	2.581	2.539	3.002	0.2820	0.100	0.596	0.458
cis-9,*trans*-11 CLA / *trans*-11 18:1	0.487	0.453	0.461	0.414	0.0220	0.032	0.725	0.287
cis-9 20:1 / 20:0	1.098	1.032	1.049	0.912	0.0544	0.018	0.427	0.241
Transfer from the diet in milk, %								
cis-9,*cis*-12 18:2	8.16	6.81	6.34	5.57	0.655	0.008	0.619	0.643
cis-9,*cis*-12,*cis*-15 18:3	3.42	2.53	3.05	1.88	0.305	0.005	0.437	0.814

[1] Significance of linear (LIN), quadratic (QUAD), and cubic (CUB) components of the response to incremental inclusion of camelina oil in concentrates fed at 12 kg/d on an air-dry basis to cows receiving a mixture (1:1 on a DM basis) of grass silage and red clover silage.

[2] SEM for the 4% camelina oil treatment. SEM for 0, 2, and 6% camelina oil treatments are proportionately 0.954, 0.921, and 0.955 of the reported value, respectively.

[3] Does not include isomers of CLA.

[4] Co-elutes with Δ13,17 20:2.

Table 6. Effect of incremental amounts of camelina oil on milk 18:1 and 18:2 composition

Item	Camelina oil in concentrate (%)				SEM [2]	*P* [1]		
	0	2	4	6		LIN	QUAD	CUB
18:1, g/100 g fatty acids								
cis-9 18:1	10.7	9.46	10.7	10.16	0.616	0.988	0.428	0.075
cis-11 18:1	0.66	0.68	0.70	0.76	0.051	0.079	0.567	0.840
cis-12 18:1	0.51	0.38	0.46	0.29	0.074	0.016	0.675	0.039
cis-13 18:1	0.19	0.17	0.20	0.18	0.012	0.760	0.961	0.047
cis-15 18:1 [3]	0.68	0.64	0.72	0.75	0.098	0.347	0.543	0.547
trans-4 18:1	0.05	0.05	0.06	0.06	0.005	0.026	0.860	0.100
trans-5 18:1	0.05	0.05	0.06	0.06	0.004	0.015	0.727	0.081
trans-6+7+8 18:1	0.58	0.61	0.67	0.59	0.042	0.642	0.151	0.273
trans-9 18:1	0.58	0.57	0.62	0.56	0.042	0.566	0.331	0.282
trans-10 18:1	1.18	1.34	1.13	0.77	0.100	0.003	0.009	0.533
trans-11 18:1	5.15	7.48	7.81	10.7	0.785	<0.001	0.688	0.132
trans-12 18:1	0.85	0.76	0.88	0.67	0.073	0.148	0.367	0.071
trans-13+14 18:1	2.11	2.17	2.17	1.73	0.173	0.073	0.075	0.514
trans-15 18:1	0.73	0.59	0.69	0.54	0.089	0.151	0.947	0.111
trans-16 18:1 [4]	0.39	0.26	0.35	0.22	0.055	0.057	0.948	0.046
Non methylene-interrupted 18:2, mg/100 g fatty acids								
cis-9,*cis*-12 18:2	1,488	1,368	1,382	1,310	87.7	0.070	0.676	0.396
cis-11,*cis*-14 18:2	4.17	5.27	5.68	6.17	1.100	0.166	0.739	0.864
cis-12,*cis*-15 18:2	37.7	33.5	48.6	43.9	6.22	0.123	0.955	0.071
cis-9,*trans*-12 18:2	107	91.7	104	73.3	7.49	0.006	0.186	0.017
cis-9,*trans*-13 18:2	839	847	901	746	58.1	0.325	0.117	0.264
cis-9, *trans*-14 18:2	284	205	255	172	31.0	0.020	0.930	0.025
trans-11,*cis*-15 18:2	1,680	2,908	2,860	5,783	618.1	<0.001	0.164	0.123
trans-12,*cis*-15 18:2 [5]	124	137	156	147	12.4	0.072	0.257	0.448
trans-9,*trans*-12 18:2 [6]	77.3	93.0	94.1	82.0	6.91	0.578	0.038	0.963
trans-9,*trans*-13 18:2	89.2	94.2	100	90.7	4.59	0.531	0.068	0.339
trans-11,*trans*-15 18:2	293	459	504	760	54.2	<0.001	0.349	0.137
Δ8,15 18:2	89.9	130	142	175	14.60	<0.001	0.790	0.455
CLA, mg/100 g fatty acids								
cis-9, *trans*-11 CLA	2,591	3,332	3,480	4,276	403.4	0.007	0.939	0.443
cis-11,*trans*-13 CLA	4.47	3.55	2.93	3.82	0.853	0.438	0.231	0.711
trans-7,*cis*-9 CLA	124	130	143	113	8.2	0.555	0.039	0.167
trans-8,*cis*-10 CLA	29.1	35.7	38.5	55.7	5.34	0.002	0.229	0.348
trans-9,*cis*-11 CLA	48.1	44.7	44.7	42.9	4.78	0.438	0.866	0.801
trans-10,*cis*-12 CLA	4.42	4.52	3.22	2.31	0.552	<0.001	0.126	0.212
trans-11,*cis*-13 CLA	11.0	15.0	17.2	21.6	2.18	<0.001	0.892	0.492
trans-12,*cis*-14 CLA [7]	8.91	7.23	7.94	7.00	1.120	0.082	0.515	0.129
trans-7,*trans*-9 CLA	3.32	3.23	2.73	2.64	0.284	0.052	0.993	0.501
trans-8,*trans*-10 CLA	2.80	2.48	2.48	2.61	0.269	0.598	0.336	0.848
trans-9,*trans*-11 CLA	28.7	28.7	22.6	22.9	1.99	0.012	0.922	0.126
trans-10,*trans*-12 CLA	5.06	4.49	4.29	3.44	0.642	0.026	0.740	0.602
trans-11,*trans*-13 CLA	32.7	37.4	33.4	26.1	3.69	0.052	0.026	0.617
trans-12,*trans*-14 CLA	22.4	28.9	28.4	25.7	2.27	0.317	0.038	0.590
trans-13,*trans*-15 CLA	0.72	0.52	0.52	0.30	0.174	0.074	0.952	0.501

[1] Significance of linear (LIN), quadratic (QUAD), and cubic (CUB) components of the response to incremental inclusion of camelina oil in concentrates fed at 12 kg/d on an air-dry basis to cows receiving a mixture (1:1 on a DM basis) of grass silage and red clover silage.

[2] SEM for the 4% camelina oil treatment. SEM for 0, 2, and 6% camelina oil treatments are proportionately 0.954, 0.921, and 0.955 of the reported value, respectively.

[3] Co-elutes with 19:0.

[4] Co-elutes with *cis*-14 18:1.

[5] Co-elutes with *cis*-11 19:1.

[6] Co-elutes with *cis*-11,*trans*-15 18:2.

[7] Co-elutes with *cis*-13,*trans*-15 CLA.

Table 7. Effect of incremental amounts of camelina oil on milk fatty acid secretion

	Camelina oil in concentrate (%)				SEM [2]	*P* [1]		
Fatty acid, g/d	0	2	4	6		LIN	QUAD	CUB
∑4- to 14-carbon	255	241	230	189	23.3	0.002	0.207	0.473
16:0	278	274	256	204	32.5	0.005	0.117	0.775
18:0	49.5	40.2	46.8	39.3	6.30	0.211	0.604	0.446
cis-9 18:1	112	100	115	104	8.5	0.768	0.957	0.102
trans-10 18:1	11.9	13.9	12.0	7.80	1.190	0.012	0.013	0.742
trans-11 18:1	55.7	79.4	88.4	109	12.06	<0.001	0.847	0.457
cis-9,*cis*-12 18:2	15.7	14.3	14.6	13.0	1.23	0.100	0.900	0.386
trans-11,*cis*-15 18:2	19.0	30.5	35.2	58.1	7.92	<0.001	0.363	0.375
cis-9,*trans*-11 CLA	28.4	35.5	39.0	43.2	6.01	0.041	0.757	0.840
cis-9,*cis*-12,*cis*-15 18:3	7.12	6.54	6.86	6.36	0.645	0.434	0.942	0.487
20:0	3.86	3.77	4.73	4.71	0.531	0.093	0.925	0.297
cis-9 20:1	4.21	3.87	5.10	4.22	0.473	0.504	0.533	0.074
cis-11 20:1	16.0	22.8	28.1	39.8	4.77	<0.001	0.532	0.657
trans-11 20:1	0.88	1.02	1.34	1.22	0.170	0.054	0.372	0.328
∑ 18:0 + *cis*-9 18:1	161	140	162	143	13.5	0.511	0.900	0.115
∑ 20:0 + *cis*-9 20:1	8.07	7.63	9.81	8.92	0.970	0.220	0.782	0.141
∑ *Trans* fatty acid	210	254	278	304	28.6	0.004	0.606	0.798
∑ Saturates	602	574	550	452	59.3	0.005	0.250	0.544
∑ Monounsaturates	343	359	393	382	31.2	0.120	0.486	0.466
∑ Polyunsaturates	105	125	139	165	18.1	0.008	0.840	0.765
∑ Fatty acid	1,052	1,058	1,082	995	93.8	0.458	0.270	0.482

[1] Significance of linear (LIN), quadratic (QUAD), and cubic (CUB) components of the response to incremental inclusion of camelina oil in concentrates fed at 12 kg/d on an air-dry basis to cows receiving a mixture (1:1 on a DM basis) of grass silage and red clover silage.

[2] SEM for the 4% camelina oil treatment. SEM for 0, 2, and 6% camelina oil treatments are proportionately 0.954, 0.921, and 0.955 of the reported value, respectively.

SUPPLEMENTARY MATERIALS

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Supplemental Table 1. Allocation of experimental animals to experimental treatments comprising concentrates containing camelina expeller and 0, 2, 4 or 6% of camelina oil (CO) on an air-dry basis and the distribution of 4 missing observations

	Latin Square 1				Latin Square 2 (rumen fistulated animals)			
	Cow number				Cow number			
Period	1	2	3	4	5	6	7	8
I	0 CO	2 CO	4 CO missing	6 CO	0 CO missing	2 CO	4 CO	6 CO
II	2 CO	4 CO	6 CO	0 CO	6 CO	0 CO	2 CO	4 CO
III	6 CO	0 CO	2 CO	4 CO	2 CO	4 CO missing	6 CO	0 CO
IV	4 CO	6 CO	0 CO	2 CO	4 CO	6 CO missing	0 CO	2 CO

Supplemental Table 2. Effect of incremental amounts of camelina oil on milk odd- and branched-chain fatty acid composition

	Camelina oil in concentrate (%)				SEM [2]	*P* [1]		
Fatty acid, mg/100 g	0	2	4	6		LIN	QUAD	CUB
5:0	15.8	14.6	12.1	12.5	1.12	0.007	0.688	0.036
7:0	11.7	10.7	9.58	9.04	1.353	0.059	0.796	0.881
9:0	15.4	13.3	9.32	11.3	1.487	0.004	0.052	0.092
11:0	29.1	25.7	19.7	19.8	2.87	0.002	0.319	0.262
anteiso 13:0	10.3	8.31	7.27	7.10	0.758	0.005	0.205	0.980
iso 13:0	21.5	24.6	22.0	20.6	2.26	0.484	0.168	0.348
iso 14:0	8.46	7.44	7.16	5.34	0.563	0.002	0.460	0.351
15:0	1,010	920	857	768	34.4	<0.001	0.987	0.588
anteiso 15:0	461	411	377	336	18.6	<0.001	0.581	0.578
iso 15:0	181	173	171	140	9.4	0.007	0.196	0.374
cis-9 15:1	17.5	16.5	15.2	11.6	1.28	<0.001	0.168	0.626
trans-5 15:1	80.4	77.9	69.3	60.1	4.11	<0.001	0.204	0.632
trans-10 15:1	24.6	19.1	16.3	15.9	2.68	0.002	0.113	0.952
iso 16:0	232	209	196	173	16.1	0.009	0.972	0.707
17:0	471	429	400	361	19.7	<0.001	0.893	0.696
iso 17:0	367	315	309	284	15.3	<0.001	0.307	0.262
cis-7 17:1	29.8	24.8	22.4	18.1	1.65	<0.001	0.775	0.445
cis-8 17:1	69.2	70.9	68.7	63.5	2.42	0.051	0.097	0.914
cis-9 17:1	172	157	146	130	4.9	<0.001	0.896	0.554
cis-11 17:1	9.67	10.1	8.06	10.3	1.310	0.987	0.466	0.228
iso 18:0 [3]	56.3	52.7	50.4	49.9	2.54	0.003	0.228	0.940
11-*cyclohexyl*-11:0	143	124	138	105	9.3	0.011	0.360	0.032
cis-10 19:1	51.2	60.1	60.2	65.5	3.86	0.015	0.597	0.363
cis-12 19:1	32.9	38.6	39.1	45.3	8.09	0.241	0.970	0.730
S3,R7,R11,15-tetramethyl-16:0 [4]	187	164	146	137	11.9	0.002	0.469	0.941
R3,R7,R11,15-tetramethyl-16:0	8.63	7.86	6.51	4.81	0.768	<0.001	0.445	0.929
21:0	35.7	31.9	31.2	26.8	1.74	0.002	0.856	0.296
cis-12 21:1	12.7	12.0	13.0	12.5	1.10	0.908	0.888	0.427
23:0 [5]	29.0	36.5	36.4	42.8	3.70	0.016	0.860	0.349
cis-14 23:1	42.8	40.0	39.5	36.1	1.38	<0.001	0.741	0.242
25:0	6.13	4.80	5.17	3.44	0.442	<0.001	0.603	0.043

[1] Significance of linear (LIN), quadratic (QUAD), and cubic (CUB) components of the response to incremental inclusion of camelina oil in concentrates fed at 12 kg/d on an air-dry basis to cows receiving a mixture (1:1 on a DM basis) of grass silage and red clover silage.

[2] SEM for the 4% camelina oil treatment. SEM for 0, 2, and 6% camelina oil treatments are proportionately 0.954, 0.921, and 0.955 of the reported value, respectively.

[3] Co-elutes with Δ11,15 16:2.

[4] Co-elutes with *cis*-11 16:1.

[5] Co-elutes with a FA that could not be identified.

Supplemental Table 3. Effect of incremental amounts of camelina oil on milk 16:1 and 16:2 composition

	Camelina oil in concentrate (%)				SEM [2]	*P* [1]		
Fatty acid, mg/100 g	0	2	4	6		LIN	QUAD	CUB
cis-9 16:1 [3]	1,940	1,830	1,671	1,329	116.8	<0.001	0.066	0.610
cis-10 16:1	39.0	35.3	36.4	27.5	3.24	0.007	0.254	0.146
cis-12 16:1	33.7	27.0	29.0	21.7	2.72	0.013	0.744	0.123
cis-13 16:1	118	104	89.5	73.0	11.75	<0.001	0.830	0.970
trans-4 16:1	22.8	21.0	22.6	22.5	1.77	0.917	0.628	0.479
trans-5 16:1	38.2	21.8	24.4	23.5	7.70	0.215	0.295	0.490
trans-6 16:1	70.0	70.7	69.2	63.6	4.17	0.178	0.324	0.886
trans-8 16:1	49.9	56.1	52.6	40.5	4.56	0.108	0.041	0.949
trans-9 16:1	293	458	475	597	45.1	<0.001	0.579	0.148
trans-10 16:1	32.1	33.4	37.8	24.8	2.54	0.104	0.007	0.060
trans-11 16:1	93.5	91.1	101	65.8	10.50	0.081	0.073	0.150
trans-12 16:1 [4]	164	165	157	181	96.8	0.212	0.124	0.213
trans-13 16:1	52.5	44.0	50.2	35.4	5.40	0.037	0.464	0.081
trans-9,*trans*-12 16:2	13.7	18.0	19.4	25.9	1.79	<0.001	0.450	0.242
Δ9,13 16:2	61.3	102	107	196	19.01	<0.001	0.162	0.131

[1] Significance of linear (LIN), quadratic (QUAD), and cubic (CUB) components of the response to incremental inclusion of camelina oil in concentrates fed at 12 kg/d on an air-dry basis to cows receiving a mixture (1:1 on a DM basis) of grass silage and red clover silage.

[2] SEM for the 4% camelina oil treatment. SEM for 0, 2, and 6% camelina oil treatments are proportionately 0.954, 0.921, and 0.955 of the reported value, respectively.

[3] Co-elutes with *anteiso* 17:0.

[4] Co-elutes with *cis*-7 16:1.

Supplemental Table 4. Effect of incremental amounts of camelina oil on milk 20:1 composition

	Camelina oil in concentrate (%)				SEM [2]	*P* [1]		
Fatty acid, mg/100 g	0	2	4	6		LIN	QUAD	CUB
cis-9 20:1	398	359	479	413	40.7	0.231	0.652	0.049
cis-11 20:1	1,410	2,171	2,487	3,949	374.3	<0.001	0.311	0.305
cis-13 20:1	123	154	159	205	13.4	<0.001	0.513	0.211
cis-14 20:1	37.1	39.0	45.8	46.0	6.49	0.188	0.875	0.632
trans-6+7+8 20:1	14.9	15.9	19.3	19.8	1.46	0.008	0.851	0.350
trans-9 20:1	70.0	72.9	95.4	91.7	13.50	0.104	0.767	0.368
trans-10 20:1 [3]	40.8	45.0	49.9	49.5	8.20	0.314	0.725	0.838
trans-11 20:1	82.0	93.7	123	118	14.10	0.019	0.438	0.292
trans-12 20:1	80.2	88.7	104	89.2	10.40	0.286	0.183	0.330
trans-13 20:1	57.0	55.8	72.1	58.3	8.63	0.550	0.405	0.170

[1] Significance of linear (LIN), quadratic (QUAD), and cubic (CUB) components of the response to incremental inclusion of camelina oil in concentrates fed at 12 kg/d on an air-dry basis to cows receiving a mixture (1:1 on a DM basis) of grass silage and red clover silage.

[2] SEM for the 4% camelina oil treatment. SEM for 0, 2, and 6% camelina oil treatments are proportionately 0.954, 0.921, and 0.955 of the reported value, respectively.

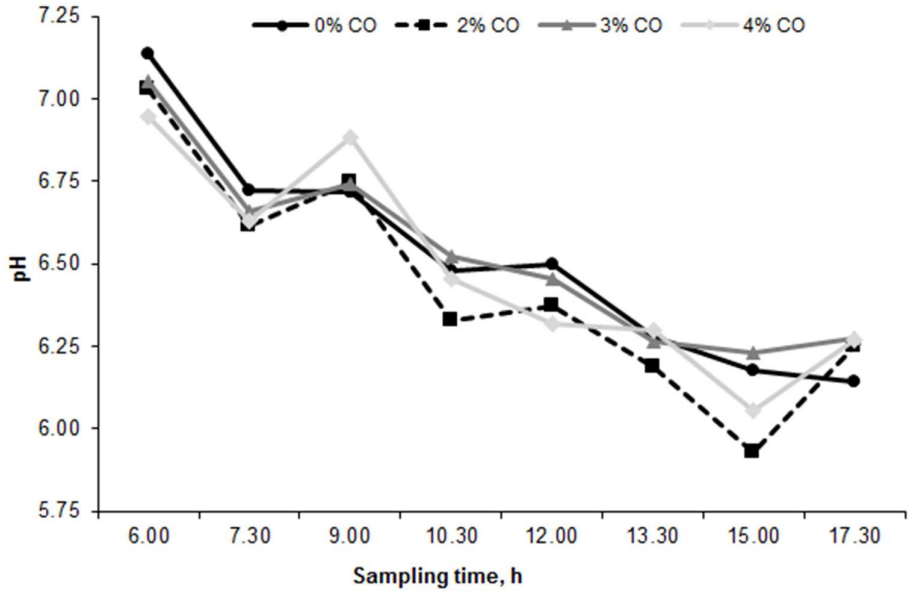
[3] Co-elutes with *cis*-5 20:1.

Supplemental Figure 1. Effect of incremental amounts of camelina oil (CO) in concentrate supplements containing camelina expeller on diurnal variation in rumen pH of lactating cows fed diets based on a mixture of grass and red clover silage. $P > 0.10$ for treatment by time interactions (SEM 0.135) as well as linear, quadratic, and cubic effects on treatment averages (6.52, 6.43, 6.56, and 6.50 for 0, 2, 4, and 6% CO respectively, SEM 0.109).

Supplemental Figure 2. Effect of incremental amounts of camelina oil (CO) in concentrate supplements containing camelina expeller on peroxide concentrations during storage of butter prepared from milk of cows fed a mixture of grass and red clover silage. $P > 0.10$ for treatment by time interactions (SEM 0.014) as well as linear, quadratic, and cubic effects on treatment averages (0.08, 0.08, 0.08, and 0.09 mmol O_2/kg milk fat for 0, 2, 4, and 6% CO respectively, SEM 0.010).

Supplemental Figure 3. Effect of incremental amounts of camelina oil (CO) in concentrate supplements containing camelina expeller on NEFA concentrations during storage of butter prepared from milk of cows fed a mixture of grass and red clover silage. Results are expressed as a weight percentage of free oleic acid (*cis*-9 18:1) in butterfat. $P > 0.10$ for treatment by time interactions (SEM 0.066) as well as linear, quadratic and cubic effects on treatment averages (0.17, 0.22, 0.18 and 0.22 % for 0, 2, 4 and 6% CO respectively, SEM 0.051).

1102 HALMEMIES-BEAUCHET-FILLEAU ET AL. SUPPLEMENTAL FIGURE 1



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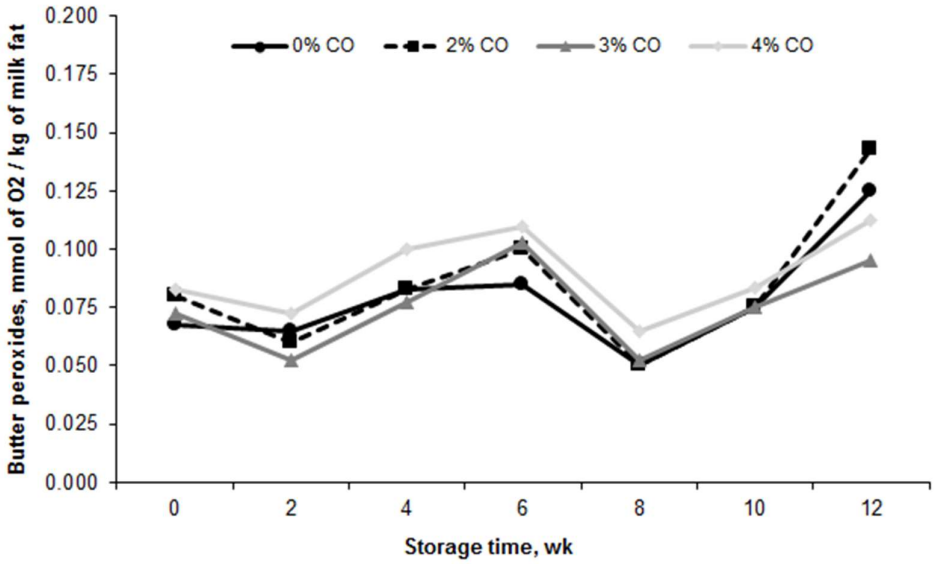
1105 HALMEMIES-BEAUCHET-FILLEAU ET AL. SUPPLEMENTAL FIGURE 2



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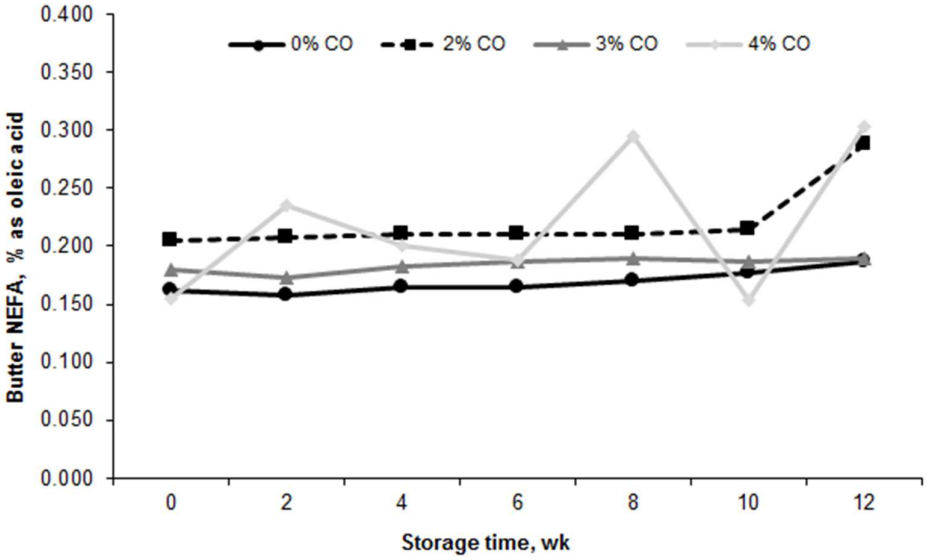
1108 HALMEMIES-BEAUCHET-FILLEAU ET AL. SUPPLEMENTAL FIGURE 3



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