Effect of dietary flax seed and oil on milk yield, gross composition, and fatty acid profile in dairy cows: A meta-analysis and meta-regression

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ABSTRACT

Several experiments were conducted over the past few years to evaluate the feeding value of flax seed and oil in dairy cow diets. The current meta-analysis and meta-regression was undertaken to assess the overall effect of different forms of flax, as a source of trienoic (*cis*-9,*cis*-12,*cis*-15 18:3) fatty acids (FA), on lactation performance and on transfer efficiency of its constituent n-3 FA from diet to milk fat. Comparisons were first conducted with nonsupplemented controls or with diets containing either saturated (mainly 16:0 or 18:0 or both), monoenoic (mainly *cis*-9 18:1), or dienoic (mainly *cis*-9,*cis*-12 18:2) FA. Results indicate that supplementing flax seed and oil decreased dry matter intake, as well as actual and energy-corrected milk yield without affecting the efficiency of utilization of dietary dry matter or energy as compared with nonsupplemented iso-energetic controls. When compared with the other 3 types of dietary fat evaluated, flax rich in trienoic FA supported a yield of energy-corrected milk similar to supplements rich in saturated, monoenoic, or dienoic FA. Greater milk fat concentration and feed efficiency were observed with saturated supplements. However, milk fat concentration and yield were lower with dienoic FA than with flax supplements. Further analyses were conducted to compare the effect of different forms of flax oil, seed, or fractions of seed. Among the 6 categories evaluated, mechanically processed whole seed (rolled or ground) allowed the greatest yield of energy-corrected milk and the best feed efficiency when compared with free oil, intact or extruded whole seed, protected flax, and flax hulls. Feeding protected flax and flax hulls allowed the greatest milk fat concentration of *cis*-9,*cis*-12,*cis*-15 18:3. Moreover, the greatest transfer efficiencies of this fatty acid from diet to milk were recorded with the same 2 treatments, plus the mechanically processed whole seed. These results make this last category the most suitable treatment,

among the 6 flax forms evaluated, to combine optimum lactation performance and protection of flax constituent *cis*-9,*cis*-12,*cis*-15 18:3.

Key words: linseed, dietary fat, milk composition, fatty acid, α -linolenic acid

INTRODUCTION

Consumption of food products from animals fed flax seed (also called linseed; *Linum usitatissimum*) as a source of n-3 fatty acids (FA), including milk, has been associated with positive effects on blood lipid profile in humans (Weill et al., 2002; Malpuech-Brugère et al., 2010). Flax oil contains 54 to 55% α -linolenic acid (*cis*-9,*cis*-12,*cis*-15 18:3, INRA-AFZ, 2002; Glasser et al., 2008a) and is therefore a rich source of n-3 PUFA. Intake of flax seed or oil by dairy ruminants has also been shown to mitigate their methane production (Chilliard et al., 2009), improve reproductive performance (Santos et al., 2008; Zachut et al., 2010b), and modulate immune functions (Caroprese et al., 2009, 2016). For these reasons, several experiments were conducted in dairy cows to study the effect of different forms of flax seed and oil, and also to compare their effects with other lipid sources on milk yield and composition. Among several factors evaluated, a specific objective of these experiments was to study the effects on milk FA profile, with a special emphasis on the transfer efficiency of *cis*-9,*cis*-12,*cis*-15 18:3 from diet to milk secretion.

In a meta-analysis, Glasser et al. (2008a) individually compared different oilseeds (flax seed, rapeseed, soybean, and sunflower seed) with nonsupplemented control treatments. However, no direct comparison between these different types of oilseeds was conducted. Similarly, Brunschwig et al. (2010) in a literature review and Sterk et al. (2012) in a meta-analysis compared different forms of technologically processed flax seed and oil to control treatments, but the difference between these varying flax forms was not determined. More recently, Meignan et al. (2017) conducted a meta-analysis to study the effect of extruded flax seed on milk yield and composition. Finally, Petit (2010) published a literature review on the effects of flax seed on feed intake, milk production, and milk composition of dairy cows,

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but differences between processing technics were not assessed statistically.

Strategies to design nonsupplemented control diets may differ in feeding trials conducted to study the effect of different lipid sources, such as flax seed or oil, on animal performance. In several experiments, supplemental fats are added to dairy cow diets at the expense of forage, concentrate, or both on a kilogram-for-kilogram basis, without adjustment for the extra energy provided by the lipid source. Another strategy is to modify the ingredient composition of the control diet, in particular the forage-to-concentrate ratio, with the objective to adjust its energy concentration as compared with the fat-supplemented ration. These different approaches, related to dietary energy supply, may affect the outcomes of the experiment.

Consequently, the current meta-analysis and meta-regression was undertaken to assess the effect of flax seed and oil when comparison is made with low-fat control treatments or with diets containing different lipid sources presenting varying levels of unsaturation. A second objective was to compare the effects of different forms of flax seed and oil on milk yield, milk composition, milk FA profile, and on the transfer efficiency of *cis*-9,*cis*-12,*cis*-15 18:3 from diet to milk secretion.

MATERIALS AND METHODS

Data Collection

The research of articles was conducted using Google Scholar (Google, Mountain View, CA) for which literature coverage has been assessed by Gehanno et al. (2013). The following key words were used: dairy cows, flaxseed, linseed, FA profile, milk yield, and milk composition. The research was conducted from September 2015 to January 2016, included articles published until December 31, 2015, and was restricted to French and English languages. With each positive result, the “cited by” function was used to find articles that did not contain our key words, but which could possibly be included in the database. One hundred thirteen articles were recorded. Among them, a total of 78 research papers reporting 84 experiments published between 1998 and 2015 (Table 1) met the predetermined selection criteria. These criteria included evidence that (1) paper was peer-reviewed; (2) at least one treatment was supplemented with flax oil, seed, or fractions of seed; (3) the study was conducted on dairy cattle breeds; and (4) milk yield, milk fat percentage, and concentration of *cis*-9,*cis*-12,*cis*-15 18:3 in milk fat were reported. The database contained 294 treatments among which 160 included flax oil, seed, or fractions of seed.

Number of observations, diet composition, as well as production performance and milk composition for 5 different comparisons evaluated in experiments 1 and 2 are reported in Supplemental Tables S1 and S2 (<https://doi.org/10.3168/jds.2017-12637>). The composition of experimental rations containing 6 different categories of flax forms is presented along with their respective number of observations in Supplemental Table S3 (<https://doi.org/10.3168/jds.2017-12637>).

When ether extract (EE) concentration of the ration was the only value available to establish lipid intake, the FA intake was estimated by multiplying the EE concentration by the average FA to EE ratio (0.760 ± 0.095 ; mean $\pm$ SD), calculated from research articles included in the original database where both values were reported ($n = 33$).

The feed library of the Cornell Net Carbohydrate and Protein System (version 6.6.5, Cornell University, Ithaca, NY) and the INRA-AFZ (2002) tables of feed composition were used to complete the missing FA profile of experimental feed ingredients, as suggested by Mannai et al. (2016). The yield of ECM was calculated as described by Madsen et al. (2008). Metabolizable energy was converted to NE_L according to NRC (2001).

Total odd-chain FA (Σ OCFA), *trans* octadecenoic acids (Σ t 18:1), *cis* octadecenoic acids (Σ c 18:1), and CLA (Σ CLA) were calculated based on the FA in these categories reported within each article. Total de novo synthesized FA (Σ DNFA) included 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, and 14:1. Articles for which major FA of the respective categories were not reported or out of biologically valid ranges were excluded from the analysis.

Data Investigation and Statistical Analysis

Four different series of statistical analyses were conducted using sub-data sets from the original database. In a first experiment, rations containing dietary supplements of flax oil, seed, or fractions of seed rich in *cis*-9,*cis*-12,*cis*-15 18:3, a trienoic FA (flax-TRI), were compared with nonsupplemented control treatments. In a second experiment, flax-TRI supplements were compared with lipid sources varying in FA composition. In a third experiment, comparisons were established between different forms of flax-TRI supplementations. The fourth experiment was a factorial analysis with the objective to compare the interrelationships within a set of variables of our original database with established theoretical meaningfulness with regards to lipid supplementation and its effect on milk production and composition, as well as milk FA profile.

Table 1. List of references used in statistical analyses including the type of comparisons reported and the information available on diet composition¹

Reference	Flax-TRF vs.					Flax form									
	No-fat control		Fat supplement			Ration chemical analysis					Whole seed				
	LOW-E	ISO-E	SFA	MONO	DI	NE _L	CP	NDF	ADF	EE	FA	Oil	Intact	Mechanical	Extruded
AbuGhazaleh et al. (2003)			x	x	x	x	x	x	x		x			x	
Akram et al. (2007)															
Ambrose et al. (2006)			x				x	x	x		x			x	
Angulo et al. (2012)					x	x	x	x	x						
Bell et al. (2006)					x	x	x	x	x						
Benchaa et al. (2012)						x	x	x	x						
Benchaa et al. (2014)						x	x	x	x						
Brzóska (2006)						x	x	x	x						
Bu et al. (2007)						x	x	x	x						
Caroprese et al. (2010)						x	x	x	x						
Cavallieri et al. (2005)						x	x	x	x						
Chilliard et al. (2009)						x	x	x	x						
Chouinard et al. (1998)						x	x	x	x						
Collomb et al. (2004)															
Cortés et al. (2010)						x	x	x	x						
Cortés et al. (2011)						x	x	x	x						
Cortés et al. (2015)						x	x	x	x						
da Silva et al. (2007)						x	x	x	x						
da Silva-Kazama et al. (2011)						x	x	x	x						
Danesh Mesgaran et al. (2012)						x	x	x	x						
Dang Van et al. (2008)															
Dang Van et al. (2011)															
Deville et al. (2004)															
Egger et al. (2007)						x	x	x	x						
Ferlay et al. (2010)						x	x	x	x						
Ferlay et al. (2013)						x	x	x	x						
Flowers et al. (2008)															
Fuentes et al. (2008)															
Gonthier et al. (2005)															
Goodridge et al. (2001)															
Hawkins et al. (2013)															
He and Armentano (2011)															
Hurtud et al. (2010)															
Hurtud et al. (2013a)															
Hurtud et al. (2013b)															
Kelly et al. (1998)															
Kliem et al. (2009)															
Kudrna and Marounek (2008)															
Lerch et al. (2012)															
Liu et al. (2008)															
Livingstone et al. (2015)															
Loor et al. (2005a)															

Continued

Table 1 (Continued). List of references used in statistical analyses including the type of comparisons reported and the information available on diet composition¹

Reference	Flax-TRI vs.					Flax form												
	No-fat control		Fat supplement			Ration chemical analysis						Whole seed						
	LOW-E	ISO-E	SFA	MONO	DI	NE _L	CP	NDF	ADF	EE	FA	Oil	Intact	Mechanical	Extruded	Protected	Hulls	Other
Loor et al. (2005b)					x		x	x	x	x	x	x						
Mach et al. (2013)			x			x	x	x	x	x	x				x			
Moallem (2009)		x				x	x	x	x	x					x			
Mohammed et al. (2011)				x			x	x	x	x	x							
Mustafa et al. (2003)						x	x	x	x	x	x							
Mutsvangwa et al. (2012)							x	x	x	x	x							
Neveu et al. (2013)				x		x	x	x	x	x	x				x			
Neveu et al. (2014)	x		x			x	x	x	x	x	x				x			
Oba et al. (2009)							x	x	x	x	x							
Oeffner et al. (2013)															x			
Palin et al. (2014)		x				x	x	x	x	x	x						x	
Petit and Benchaar (2007)			x															
Petit and Côrtes (2010)							x	x	x	x	x							
Petit and Gagnon (2009)		x				x	x	x	x	x	x							
Petit and Gagnon (2011)		x				x	x	x	x	x	x							
Petit et al. (2002)			(x)				x	x	x	x	x							
Petit et al. (2004)	x		x				x	x	x	x	x							
Petit et al. (2005)						x	x	x	x	x	x							
Petit et al. (2007)		x				x	x	x	x	x	x							
Petit et al. (2009)		x				x	x	x	x	x	x							
Petit (2002)						x	x	x	x	x	x							
Petit (2003)						x	x	x	x	x	x							
Petit (2015)						x	x	x	x	x	x							
Rego et al. (2009)	(x)			(x)	(x)													
Resende et al. (2015)	x					x	x	x	x	x	x							
Saliba et al. (2014)	x					x	x	x	x	x	x							
Secchiari et al. (2003)			x			x	x	x	x	x	x							
Stergiadis et al. (2014)				x		x	x	x	x	x	x							
Sterk et al. (2011)						x	x	x	x	x	x							
Sterk et al. (2012)						x	x	x	x	x	x							
Sultana et al. (2008)		x				x	x	x	x	x	x							
Thanh and Sukombat (2015)						x	x	x	x	x	x							
Ward et al. (2002)	x			x			x	x	x	x	x							
Ye et al. (2009)	x					x	x	x	x	x	x							
Zachut et al. (2010a)			x			x	x	x	x	x	x							
Zachut et al. (2010b)			x			x	x	x	x	x	x							

¹Flax-TRI = diets supplemented with flax seed and oil rich in trienoic fatty acids (*cis*-9, *cis*-12, *cis*-15 18:3); LOW-E = nonsupplemented control diets used without adjustment for the extra energy provided by flax seed and oil; ISO-E = nonsupplemented control diets where ingredient composition was modified to adjust energy concentration as compared with flax-supplemented treatments; SFA = diets containing fat supplement rich in SFA (mainly 16:0 or 18:0 or both); MONO = diets containing fat supplement rich in monoenoic fatty acids (mainly *cis*-9 18:1); DI = diets containing fat supplement rich in dienoic fatty acids (mainly *cis*-9, *cis*-12 18:2); EE = ether extract; FA = fatty acids; other = micronized or roasted whole seed.

²An x in parentheses indicates that treatment meets the criterion but was not included in statistical analyses of experiments 1 and 2 because processing included formaldehyde protection, or the proportion of EE from experimental fat supplement could not be calculated for cows on pasture.

Experiment 1—Flax-TRI vs. Nonsupplemented Control

To assess the effect of flax-TRI supplements on milk production and composition, a sub database was constituted with the fat-supplemented diets for which the main lipid source was flax oil, seed, or fractions of seed. This sub database also included the nonsupplemented diets, for which treatments were further categorized as low-energy (**LOW-E**) or iso-energetic (**ISO-E**) controls. Experiments with a LOW-E control were those where flax lipids were added in the diet at the expense of forages, concentrates, or both, on a kilogram-for-kilogram basis. Experiments with an ISO-E control were those where dietary modifications were made to adjust the concentration of NE_L between treatments by changing the proportions of dietary ingredients with different energy density (e.g., forage vs. concentrate, starchy vs. fibrous concentrate). The objective of obtaining ISO-E control was considered to be reached when the difference between diets was less than 35 kcal/kg of DM, which corresponds to the substitution of 1% vegetable oil for 1% ground corn, according to NRC (2001). Dietary oil or oilseeds treated with formaldehyde were removed from the model due to superior protection

or inertness in the rumen, as compared with all other treatments, leading to aberrant results.

A random regression was performed, using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC), where the dependent variable was the natural logarithm of the response ratio (**RR**) when flax-TRI was included in the diet, as compared with LOW-E or ISO-E, for all the variables tested. Independent variables were study, as well as linear and quadratic effects of the level of EE coming from the lipid supplementation. Study was included as a random effect according to Sauvant et al. (2008). In each model, the intercept was forced to zero, considering the absence of difference between treatments at a zero level of lipid supplementation. The percentage of change in a variable was estimated by $(e^{RR} - 1) \times 100\%$.

Additionally, the proportion of corn silage in the basal diet was included as an independent variable in the model and its interaction with flax-TRI supplement on milk fat concentration and yield was assessed, using the RR as the independent variable.

Data for which the absolute value of conditional studentized residual was >3.0 were considered as outliers and discarded. The normal distribution of residues and the homogeneity of variance were confirmed (Sauvant et al., 2008).

Table 2. Dietary lipid sources evaluated and number of treatments in each category of supplementation (experiments 1 and 2)

Lipid source	Category ¹			
	Flax-TRI	Fat supplement		
		SFA	MONO	DI
Flax seed or oil	148	—	—	—
Corn	—	—	—	1
Cottonseed	—	—	—	1
Linola	—	—	—	1
Safflower	—	—	—	2
Soybean	—	—	—	10
Sunflower	—	—	—	14
High-oleic safflower	—	—	1	—
High-oleic sunflower	—	—	1	—
Olive	—	—	1	—
Peanut	—	—	2	—
Rapeseed	—	—	15	—
Energy Booster 100 ²	—	1	—	—
Ca salt of palm	—	13	—	—
Palm	—	2	1	—
Rumo-fat ³	—	1	—	—
Tallow	—	1	—	—
Total	148	18	21	29

¹Flax-TRI = diets supplemented with flax seed and oil rich in tri-enoic fatty acids (*cis*-9,*cis*-12,*cis*-15 18:3); SFA = diets containing fat supplement rich in SFA (mainly 16:0 or 18:0 or both); MONO = diets containing fat supplement rich in monoenoic fatty acids (mainly *cis*-9 18:1); DI = diets containing fat supplement rich in dienoic fatty acids (mainly *cis*-9,*cis*-12 18:2).

²Milk Specialties Global, Eden Prairie, Minnesota.

³Robt Morgan Inc., Paris, Illinois.

Experiment 2—Flax-TRI vs. Varying Lipid Sources

To compare the effect of different lipid sources on production performance and milk composition, including milk FA profile, lipid-supplemented diets were extracted from the database (except for treatments involving dietary oil or oilseed treated with formaldehyde). Treatments were regrouped according to the main FA of the lipid source as follows: flax-TRI vs. fat supplements rich in SFA (mainly 16:0 or 18:0 or both), monoenoic FA (mainly *cis*-9 18:1; **MONO**), or dienoic FA (mainly *cis*-9,*cis*-12 18:2; **DI**). A list of specific dietary lipid sources and the number of treatments in each category of supplementation is presented in Table 2.

Comparisons between treatments on production performance, concentration and yield of milk components, and milk FA profile were performed using a mixed model (SAS 9.4, SAS Institute Inc.) including the effects of study (random) and source of dietary lipid supplementation, the general linear and quadratic effects of the level of lipid supplementation, and the effect of interactions between lipid source and the level of lipid supplementation. Variables were kept in the final model when $P \leq 0.05$, except for the source of dietary lipid supplementation, which was automatically included.

The presence of outliers as well as the normality and homogeneity of the residuals were assessed according to the procedures mentioned previously.

Experiment 3—Effects of Flax Forms

To compare the effect of different forms of flax on lactation performance and transfer efficiency of *cis*-9,*cis*-12,*cis*-15 18:3 from diet to milk fat, treatments for which the lipid source was flax-TRI were extracted from the original database and classified into 6 categories, according to the form of supplementation. The flax-TRI supplements were offered as free oil ($n = 22$), fraction of the seed (flax hulls; $n = 8$), or whole seed. The whole seed category was further divided into 3 subclasses, consisting of diets in which flax was included: (1) as intact untreated seed ($n = 32$), (2) after a mechanical processing (rolling, $n = 20$; or grinding, $n = 15$), or (3) following a heat treatment using the extrusion procedure ($n = 43$). The sixth category included the treatments for which flax seed and oil were submitted to different technologies to protect PUFA against ruminal biohydrogenation, including encapsulation in a SFA matrix ($n = 1$), treatments with xylose ($n = 3$) or formaldehyde ($n = 6$), or the formation of Ca salts of FA ($n = 6$). Diets supplemented with either roasted ($n = 2$) or micronized ($n = 2$) flax seed were not analyzed as a category due to the limited number of treatments recorded in the original database.

The Wilcoxon nonparametric test (JMP 11, SAS Institute Inc.) was used to compare the 6 different categories of flax-TRI supplementation without study effect.

Experiment 4—Multivariate Factor Analysis

A sub database was created with variables related to milk production and composition, including FA profile, from all treatments ($n = 294$) included in the original database. First, missing values of the database were replaced by a multiple ($n = 25$) imputed value using the Monte Carlo Markov chain method of the MI procedure (SAS 9.4, SAS Institute Inc.). Then, this database was submitted to a factorial analysis, based on the procedure described by Conte et al. (2016). The number of rotated factors included was selected based on an intrinsic value superior to 1 (Kaiser, 1960). Variables with a loading ≥ 0.12 or ≤ -0.12 were considered for the interpretation of the factor pattern. This limit value was established based on the following equation (Sokal and Rohlf, 1995):

$$l = \tanh \left[\frac{1.96}{\sqrt{n-3}} \right],$$

where l = limit loading value, $\tanh$ = hyperbolic tangent, and n = number of observations ($n = 282$). The presence of outliers was assessed using the ROBUST-REG procedure and the least trimmed squares option (SAS 9.4, SAS Institute Inc.). Outliers were identified by visual observation of the robust minimum covariance determinant distances.

RESULTS

Experiment 1—Flax-TRI vs. Nonsupplemented Control

Flax-TRI vs. LOW-E Control. Increasing the level of EE supplementation from flax-TRI in dairy rations, as compared with LOW-E control (Figure 1A), decreased DMI by 0.8% per percentage unit (PU) of increase of the EE (DM basis) from the lipid supplement, without affecting milk yield (both actual and energy-corrected) and the production of major milk constituents (fat, protein, and lactose). Feed efficiency was not affected when calculated on a DMI basis, but decreased by 1.0% per PU of dietary EE supplementation from flax-TRI when calculated on NE_L basis. Milk fat and protein concentrations were decreased by 0.9 and 0.8% per PU of dietary EE supplementation from flax-TRI, respectively, whereas lactose concentration was not affected by the level of EE supplementation from flax-TRI to dairy cows as compared with the LOW-E control. Moreover, the effect of flax-TRI supplementation on milk fat concentration ($R^2 = 0.64$; $P < 0.01$) and yield ($R^2 = 0.16$; $P = 0.07$) was affected by the composition of basal rations (Figure 2). Indeed, the quadratic response of flax-TRI supplementation on milk fat (concentration and yield) observed, as a proportion of forage represented by corn silage increased, revealed that flax-TRI supplementation has a limited effect on milk fat when corn silage represents less than 50% of forage. Concentrations of $\Sigma DNFA$, 16:0, and $\Sigma OCFA$ in milk fat were decreased by 7, 9, and 6% per PU of dietary EE from flax-TRI (Figure 1B). In contrast, the proportions of 18:0 (+10%), Σc 18:1 (+8%), Σt 18:1 (+22%), *cis*-9,*cis*-12,*cis*-15 18:3 (+19%), and ΣCLA (+14%) increased with each PU of EE supplementation from flax-TRI, whereas milk fat *cis*-9,*cis*-12 18:2 was not affected.

Flax-TRI vs. ISO-E Control. When flax-TRI supplementation was compared with ISO-E control rations, DMI, milk yield (both actual and energy-corrected), and the production of fat, protein, and lactose decreased by 1.2 to 2.1% per PU of dietary EE from the flax-TRI supplement (Figure 1C). Feed efficiency (DMI and NE_L bases) and milk fat concentration were not affected. Supplemental EE from flax-TRI decreased

A. LOW-E **B. LOW-E** **C. ISO-E** **D. ISO-E**

Figure 1. Percent change per unit of increase in dietary ether extract from lipid supplements, expressed as a percentage of DM, in production performance and concentration and yield of milk components (A and C) and individual milk fatty acid concentrations (B and D) when flax seed and oil rich in trienoic fatty acids were included in the diet, as compared with nonsupplemented control treatments adjusted (ISO-E) or not (LOW-E) for dietary energy concentration. Percent change is represented as the mean $\pm$ 95% CI. Means represented by solid symbols are statistically different from 0 at $P \leq 0.05$. Σ DNFA = total de novo synthesized fatty acids (4:0 + 6:0 + 8:0 + 10:0 + 12:0 + 14:0 + 14:1); Σ c 18:1 = total *cis* octadecenoic acids; Σ t 18:1 = total *trans* octadecenoic acids; Σ CLA = total CLA; Σ OCFA = total odd-chain fatty acids; *c* = *cis*; *t* = *trans*.

protein (-0.5%), but increased lactose concentrations in milk ($+0.2\%$). Regarding milk FA profile (Figure 1D), concentrations of Σ DNFA, 16:0, *cis*-9,*cis*-12 18:2, and Σ OCFA were decreased by 5.9, 7.4, 1.1, and 2.7%, respectively, per PU of dietary EE from flax-TRI supplementation. Increases in milk fat concentrations of 18:0 ($+9.6\%$), Σ c 18:1 ($+7.0\%$), Σ t 18:1 ($+19.7\%$), *cis*-9,*cis*-12,*cis*-15 18:3 ($+17.7\%$), and Σ CLA ($+15.9\%$) were observed with every PU of increase in dietary EE from flax-TRI supplementation.

Experiment 2—Flax-TRI vs. Varying Lipid Sources

Flax-TRI vs. SFA Supplements. No difference was observed between flax-TRI and SFA for DMI; the yields of milk, fat, protein, and lactose; feed efficiency

(on DMI and NE_L bases); and the proportions of *cis*-9,*cis*-12 18:2 and Σ OCFA in milk fat (Table 3). As compared with SFA supplements, feeding flax-TRI decreased feed efficiency (DMI basis), milk fat concentration, and proportion of 16:0 in milk fat. On the contrary, greater concentrations of milk protein and lactose, and greater proportions of Σ c 18:1, Σ t 18:1, *cis*-9,*cis*-12,*cis*-15 18:3, and Σ CLA in milk fat were observed with flax-TRI than with SFA supplements. Regardless of source, lipid supplementation increased milk fat concentration of Σ c 18:1 (linearly), whereas milk fat concentration varied quadratically. Feeding both lipid sources linearly increased the proportion of 18:0 in milk fat, and the increase was of a greater magnitude with flax-TRI than with SFA (Table 3 and Supplemental Figure S1; <https://doi.org/10.3168/jds.2017>

Figure 2. Response ratios for (A) milk fat concentration and (B) milk fat yield in relation to the proportions of corn silage in basal rations when flax seed and oil rich in trienoic fatty acids (*cis*-9,*cis*-12,*cis*-15 18:3) were included in the diet, as compared with nonsupplemented control diets used without adjustment for the extra energy provided by ether extract from flax seed and oil. * $P < 0.05$. RMSE = root mean squared error.

-12637). Finally, an interaction between lipid source and the quadratic effect of the level of supplementary EE was observed on the feed efficiency (NE_L basis)

and the proportion of Σ DNFA in milk fat (Table 3 and Supplemental Figure S1; <https://doi.org/10.3168/jds.2017-12637>).

Table 3. Estimates ± SE¹ of the models for production performance, concentration and yield of milk components, and milk fatty acid profile when flax rich in trienoic fatty acids (flax-TRI, *cis*-9, *cis*-12, *cis*-15 18:3) was compared with lipid supplements rich in SFA (mainly 16:0 or 18:0 or both; experiment 2)

Item	Model		P-value				
	SFA	Flax-TRI	Lipid source	Level L ²	Level Q ³	Lipid source × level L	Lipid source × level Q
Performance (kg/d)							
DMI	19.661 ± 0.996		0.10				0.97
Milk yield	30.630 ± 2.697		0.36				0.99
ECM	29.472 ± 2.483		0.55				0.99
Feed efficiency							
ECM/DMI (kg/kg)	1.521 ± 0.081	1.443 ± 0.081	0.02				0.96
ECM/NE _L intake (kg/Mcal)	1.126 ± 0.193 – 0.183 ± 0.150 x + 0.034 ± 0.029 x ²	0.812 ± 0.138 + 0.087 ± 0.108 x – 0.024 ± 0.022 x ²	0.04	0.69	0.83	0.04	0.98
Milk component concentration (%)							
Fat	3.366 ± 0.422 + 0.770 ± 0.294 x – 0.194 ± 0.058 x ²	3.262 ± 0.422 + 0.770 ± 0.294 x – 0.194 ± 0.058 x ²	0.03	0.02	<0.01		0.99
Protein	3.229 ± 0.104	3.281 ± 0.104	0.01				0.99
Lactose	4.642 ± 0.055	4.697 ± 0.055	0.01				0.97
Milk component yield (kg/d)							
Fat	1.137 ± 0.091		0.10				0.97
Protein	0.985 ± 0.079		0.13				0.99
Lactose	1.456 ± 0.169		0.12				0.99
Milk fatty acid profile (g/100 g of total fatty acids)							
ΣDNFA ⁴	23.568 ± 5.161 – 0.423 ± 3.924 x – 0.274 ± 0.729 x ²	31.564 ± 3.774 – 8.199 ± 2.931 x + 1.539 ± 0.595 x ²	0.05	0.18	0.30	0.03	0.98
16:0	33.850 ± 0.772	26.858 ± 0.750	<0.01				0.83
18:0	5.895 ± 1.313 + 1.594 ± 0.464 x	4.295 ± 1.298 + 3.267 ± 0.451 x	0.09	<0.01		<0.01	0.97
Σ <i>c</i> 18:1 ⁵	14.120 ± 2.206 + 3.638 ± 0.758 x	15.426 ± 2.223 + 3.638 ± 0.758 x	0.01	<0.01			0.96
Σ <i>t</i> 18:1 ⁶	2.185 ± 0.354	2.636 ± 0.355	0.04				0.91
<i>cis</i> -9, <i>cis</i> -12 18:2			0.78				0.88
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.513 ± 0.081	1.051 ± 0.080	<0.01				0.15
ΣCLA ⁷	0.641 ± 0.101	0.911 ± 0.102	<0.01				0.95
ΣOCFA ⁸	1.692 ± 0.174		0.14				0.95

¹Root mean squared error.

²Linear effect of ether extract from lipid supplements, expressed as % of DM.

³Quadratic effect of ether extract from lipid supplements, expressed as % of DM.

⁴Total de novo synthesized fatty acids (4:0 + 6:0 + 8:0 + 10:0 + 12:0 + 14:0 + 14:1).

⁵Total *cis* octadecenoic acids.

⁶Total *trans* octadecenoic acids.

⁷Total CLA.

⁸Total odd-chain fatty acids.

Flax-TRI vs. MONO Supplements. Dietary flax-TRI, as compared with MONO supplements, did not affect DMI, concentrations and yields of milk fat and protein, and proportions of Σ OCFA in milk fat (Table 4). Similarly, milk yield, ECM, feed efficiency (NE_L basis), as well as the proportions of Σ DNFA, 18:0, and Σc 18:1 in milk fat, were not affected by lipid source. However, these variables were affected quadratically with the level of dietary EE from both lipid supplements. In milk fat, greater proportions of *cis*-9,*cis*-12 18:2 and *cis*-9,*cis*-12,*cis*-15 18:3, but lower proportion of 16:0 were observed with flax-TRI than with MONO supplements. Milk fat concentration of 16:0 also varied quadratically with an increase in dietary lipid supplementation, regardless of source. Lactose yield was not different between lipid supplements, but varied quadratically with the level of lipid supplementation. The concentration of this milk constituent also decreased with the level of EE, the decrease being of greater magnitude with MONO supplements (Table 4 and Supplemental Figure S2; <https://doi.org/10.3168/jds.2017-12637>). Significant interactions between lipid source and the level of EE supplementation were also observed with an increase of feed efficiency (DMI basis), and greater increments in the proportions of Σt 18:1 and Σ CLA in milk fat with flax-TRI than with MONO (Table 4 and Supplemental Figure S2; <https://doi.org/10.3168/jds.2017-12637>).

Flax-TRI vs. DI Supplements. No difference was observed between flax-TRI and DI supplements for DMI, milk yield, ECM, feed efficiency (DMI and NE_L bases), milk protein concentration and yield, lactose yield, and proportion of 16:0, Σc 18:1, Σ CLA, and Σ OCFA in milk fat (Table 5). Milk fat concentration and yield were increased by feeding flax-TRI as compared with DI supplements. Cows fed flax-TRI produced milk fat with greater proportions of *cis*-9,*cis*-12,*cis*-15 18:3 and Σ DNFA, but lower proportions of Σt 18:1 and *cis*-9,*cis*-12 18:2 as compared with DI supplements. With both lipid sources, milk fat concentrations of Σc 18:1 and Σ CLA increased linearly with an increase in supplemental dietary EE. The opposite was observed for milk fat concentrations of Σ DNFA and 16:0, whereas Σ OCFA varied quadratically. Finally, a significant interaction was observed between lipid source and the quadratic effect of supplementation level on lactose concentration and proportions of 18:0 in milk fat (Table 5 and Supplemental Figure S3; <https://doi.org/10.3168/jds.2017-12637>).

Experiment 3—Effects of Flax Forms

Dry matter intake was similar among the different forms of flax oil, seed, or fractions of seed studied

(Table 6). Actual milk yield was greater for mechanically processed whole flax seed as compared with flax oil, whereas intermediate milk yields were observed with the other 4 flax categories. Feeding mechanically processed flax seed increased the yield of ECM as compared with free oil and flax hulls, as well as intact and extruded whole seed. Intermediate value was observed with protected flax, and the numerically lowest ECM was obtained with free oil. As a result of the effect of treatments on production, the efficiency of ECM yield, expressed both on DMI and NE_L bases, was improved by feeding mechanically processed whole seed as compared with all other flax forms. Among these remaining categories, feed efficiency was greater for extruded whole seed, lower with flax hulls, and intermediate with free oil, intact whole seed, and protected flax.

Greater milk fat concentration was obtained with mechanically processed as compared with extruded whole seed. Feeding free oil further decreased milk fat concentration as compared with these 2 treatments. Intermediate milk fat concentrations were obtained with intact whole seed, protected flax, and flax hulls. Similar results were obtained for milk fat yield, except that no difference was observed between intact or extruded whole seed. Milk protein concentration was greater with free oil, protected flax, and flax hulls when compared with extruded whole seed, whereas intermediate values were observed with intact or mechanically processed whole seed. Feeding mechanically processed seed increased milk protein yield as compared with free oil or intact whole seed, whereas intermediate values were associated with extruded whole seed, protected flax, or flax hulls.

Milk fat concentration of 16:0 was lower with free oil, intermediate with flax hulls, and greater with intact, mechanically treated, or extruded whole seed, as well as protected flax. Greater milk fat concentration of Σt 18:1 was obtained with free oil followed by extruded whole seed then mechanically processed whole seed. Feeding intact whole seed and protected flax further decreased the concentration of Σt 18:1 as compared with these former 3 treatments. Average concentration of Σt 18:1 observed with flax hulls only differed statistically ($P < 0.05$) with free oil treatment. The proportion of Σ CLA in milk fat was greater with free oil as compared with extruded whole seed and protected flax, whereas intermediate values between these treatments were observed with flax hulls. Feeding mechanically processed and then intact whole seed further decreased the concentration of Σ CLA as compared with these 4 treatment categories.

Protected seed and flax hulls are the forms of lipid supplement associated with the highest milk fat concentrations of *cis*-9,*cis*-12,*cis*-15 18:3. In contrast, the

Table 4. Estimates $\pm$ SE¹ of the models for production performance, concentration and yield of milk components, and milk fatty acid profile when flax rich in trienoic fatty acids (flax-TRI, *cis*-9,*cis*-12,*cis*-15 18:3) was compared with lipid supplements rich in monoenoic fatty acids (MONO, mainly *cis*-9 18:1; experiment 2)

Item	Model		P-value				
	MONO	Flax-TRI	Lipid source	Level L ²	Level Q ³	Lipid source $\times$ level L	Lipid source $\times$ level Q
Performance (kg/d)							
DMI	20.474 $\pm$ 0.562		0.11				
Milk yield	24.741 $\pm$ 3.361 + 3.914 $\pm$ 1.701 x - 0.672 $\pm$ 0.215 x ²		0.66	0.05	<0.01		0.84
ECM	23.178 $\pm$ 3.196 + 1.726 x - 0.447 $\pm$ 0.222 x ²		0.21	0.06	0.03		0.83
Feed efficiency							0.90
ECM/DMI (kg/kg)	1.338 $\pm$ 0.106 - 0.023 $\pm$ 0.030 x	1.212 $\pm$ 0.101 + 0.034 $\pm$ 0.031 x	0.16	0.85		0.04	0.06
ECM/NE _L intake (kg/Mcal)	0.582 $\pm$ 0.131 + 0.146 $\pm$ 0.074 x - 0.022 $\pm$ 0.009 x ²		0.17	0.03	0.01		0.04
Milk component concentration (%)							
Fat	3.388 $\pm$ 0.140		0.46				0.20
Protein	3.144 $\pm$ 0.070		0.79				0.05
Lactose	4.972 $\pm$ 0.101 - 0.052 $\pm$ 0.020 x	4.888 $\pm$ 0.099 - 0.022 $\pm$ 0.020 x	0.06	0.07		0.03	0.03
Milk component yield (kg/d)							
Fat	0.980 $\pm$ 0.047		0.76				0.06
Protein	0.925 $\pm$ 0.049		0.91				0.03
Lactose	1.251 $\pm$ 0.182 + 0.180 $\pm$ 0.092 x - 0.035 $\pm$ 0.011 x ²		0.60	0.11	0.01		0.04
Milk fatty acid profile (g/100 g of total fatty acids)							
Σ DNFA ⁴	32.882 $\pm$ 2.904 - 7.632 $\pm$ 1.573 x + 1.000 $\pm$ 0.204 x ²		0.63	<0.01	<0.01		0.86
16:0	35.469 $\pm$ 2.718 - 7.478 $\pm$ 1.506 x + 1.025 $\pm$ 0.200 x ²	34.603 $\pm$ 2.616 - 7.478 $\pm$ 1.506 x + 1.025 $\pm$ 0.200 x ²	0.05	<0.01	<0.01		0.90
18:0	3.675 $\pm$ 2.340 + 5.807 $\pm$ 1.379 x - 0.798 $\pm$ 0.191 x ²		0.93	<0.01	<0.01		1.03
Σc 18:1 ⁵	9.180 $\pm$ 3.091 + 8.735 $\pm$ 1.838 x - 1.064 $\pm$ 0.258 x ²		0.10	<0.01	<0.01		1.33
Σt 18:1 ⁶	5.398 $\pm$ 1.496 - 0.325 $\pm$ 0.412 x	3.538 $\pm$ 1.463 + 0.453 $\pm$ 0.435 x	0.09	0.87		0.03	0.82
<i>cis</i> -9, <i>cis</i> -12 18:2	2.025 $\pm$ 0.142	2.223 $\pm$ 0.144	0.03				0.20
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.506 $\pm$ 0.093	1.026 $\pm$ 0.096	<0.01				0.22
Σ CLA ⁷	0.865 $\pm$ 0.243 + 0.024 $\pm$ 0.066 x	0.583 $\pm$ 0.234 + 0.178 $\pm$ 0.068 x	0.13	0.11		<0.01	0.15
Σ OCFA ⁸	1.533 $\pm$ 0.188		0.86				0.10

¹Root mean squared error.

²Linear effect of ether extract from lipid supplements, expressed as % of DM.

³Quadratic effect of ether extract from lipid supplements, expressed as % of DM.

⁴Total de novo synthesized fatty acids (4:0 + 6:0 + 8:0 + 10:0 + 12:0 + 14:0 + 14:1).

⁵Total *cis* octadecenoic acids.

⁶Total *trans* octadecenoic acids.

⁷Total CLA.

⁸Total odd-chain fatty acids.

Table 5. Estimates $\pm$ SE of the models for production performance, concentration and yield of milk components, and milk fatty acid profile when flax rich in trienoic fatty acids (flax-TRI, *cis*-9, *cis*-12, *cis*-15 18:3) was compared with lipid supplements rich in dienoic fatty acids (DI, mainly *cis*-9, *cis*-12 18:2; experiment 2)

Item	Model		P-value				
	DI	Flax-TRI	Lipid source	Level L^2	Level Q^3	Lipid source $\times$ level L	Lipid source $\times$ level Q
Performance (kg/d)							
DMI		19.536 $\pm$ 0.658	0.81				0.97
Milk yield		29.093 $\pm$ 1.469	0.86				0.99
ECM		25.686 $\pm$ 1.273	0.26				0.98
Feed efficiency		1.292 $\pm$ 0.045	0.15				0.94
ECM/DMI (kg/kg)		0.756 $\pm$ 0.034	0.24				0.95
ECM/NE _L intake (kg/Mcal)							0.05
Milk component concentration (%)							0.03
Fat	3.176 $\pm$ 0.103	3.341 $\pm$ 0.103	<0.01				0.94
Protein		3.163 $\pm$ 0.047	0.84				0.93
Lactose	4.868 $\pm$ 0.072 – 0.091 $\pm$ 0.029 x + 0.016 $\pm$ 0.004 x ²	4.758 $\pm$ 0.074 – 0.005 $\pm$ 0.032 x + 0.004 $\pm$ 0.005 x ²	0.05	0.08	0.03	0.02	0.99
Milk component yield (kg/d)							0.13
Fat	0.919 $\pm$ 0.049	0.960 $\pm$ 0.049	0.03				0.96
Protein		0.907 $\pm$ 0.047	0.54				0.99
Lactose		1.385 $\pm$ 0.087	0.60				0.99
Milk fatty acid profile (g/100 g of total fatty acids)							0.04
Σ DNFA ⁴	22.495 $\pm$ 1.443 – 1.121 $\pm$ 0.343 x	23.609 $\pm$ 1.355 – 1.121 $\pm$ 0.343 x	<0.01	<0.01			0.94
16:0	29.623 $\pm$ 1.482 – 1.421 $\pm$ 0.379 x		0.85	<0.01			0.88
18:0	11.263 $\pm$ 1.720 – 0.493 $\pm$ 0.936 x + 0.205 $\pm$ 0.135 x ²	7.499 $\pm$ 1.826 + 2.190 $\pm$ 1.025 x – 0.172 $\pm$ 0.152 x ²	0.04	0.31	0.89	0.02	0.97
Σ c 18:1 ⁵	20.402 $\pm$ 1.827 + 1.375 $\pm$ 0.465 x		0.43	<0.01			0.92
Σ t 18:1 ⁶	7.106 $\pm$ 1.029	5.353 $\pm$ 1.031	<0.01				1.48
<i>cis</i> -9, <i>cis</i> -12 18:2	3.102 $\pm$ 0.196	2.519 $\pm$ 0.197	<0.01				0.97
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.444 $\pm$ 0.058	0.866 $\pm$ 0.059	<0.01				0.85
Σ CLA ⁷	0.473 $\pm$ 0.312 + 0.339 $\pm$ 0.086 x		0.06	<0.01			0.83
Σ OCFA ⁸	2.455 $\pm$ 0.242 – 0.470 $\pm$ 0.114 x + 0.051 $\pm$ 0.018 x ²		0.57	<0.01	0.02		0.89
							0.99

¹Root mean squared error.²Linear effect of ether extract from lipid supplements, expressed as % of DM.³Quadratic effect of ether extract from lipid supplements, expressed as % of DM.⁴Total de novo synthesized fatty acids (4:0 + 6:0 + 8:0 + 10:0 + 12:0 + 14:0 + 14:1).⁵Total *cis* octadecenoic acids.⁶Total *trans* octadecenoic acids.⁷Total CLA.⁸Total odd-chain fatty acids.

Table 6. Means $\pm$ SD (no. of observations) for production performance, concentration and yield of milk components, milk fatty acid profile, and transfer efficiency of dietary fatty acids to milk fat with 6 different categories of flax forms (experiment 3)

Item	Form					
	Whole seed			Protected		
	Oil	Intact	Mechanical	Extruded	Protected	Hulls
Performance (kg/d)						
DMI	19.8 $\pm$ 3.04 (16)	20.3 $\pm$ 2.68 (32)	21.2 $\pm$ 3.17 (27)	20.3 $\pm$ 3.44 (38)	20.0 $\pm$ 2.46 (15)	20.4 $\pm$ 1.24 (7)
Milk yield	27.5 $\pm$ 8.30 (22) ^b	28.6 $\pm$ 5.89 (31) ^{ab}	31.8 $\pm$ 7.12 (35) ^a	29.3 $\pm$ 7.13 (41) ^{ab}	29.8 $\pm$ 5.08 (16) ^{ab}	26.4 $\pm$ 1.78 (7) ^{ab}
ECM	24.5 $\pm$ 3.55 (19) ^c	26.0 $\pm$ 5.82 (32) ^{bc}	31.7 $\pm$ 6.97 (34) ^a	27.2 $\pm$ 6.00 (41) ^b	27.9 $\pm$ 4.02 (16) ^{ab}	25.5 $\pm$ 1.71 (8) ^{bc}
Feed efficiency	1.32 $\pm$ 0.181 (17) ^{bc}	1.28 $\pm$ 0.229 (32) ^{bc}	1.54 $\pm$ 0.221 (26) ^a	1.35 $\pm$ 0.165 (37) ^b	1.39 $\pm$ 0.157 (15) ^{bc}	1.24 $\pm$ 0.0520 (7) ^c
ECM/DMI (kg/kg)	0.800 $\pm$ 0.0569 (12) ^{bc}	0.784 $\pm$ 0.149 (27) ^{bc}	0.937 $\pm$ 0.109 (21) ^a	0.843 $\pm$ 0.123 (28) ^b	0.807 $\pm$ 0.0925 (6) ^c	0.765 $\pm$ 0.0224 (6) ^c
ECM/NE _L intake (kg/Mcal)						
Milk component concentration (%)						
Fat	3.25 $\pm$ 0.502 (22) ^c	3.78 $\pm$ 0.556 (32) ^{ab}	3.92 $\pm$ 0.429 (35) ^a	3.59 $\pm$ 0.516 (42) ^b	3.87 $\pm$ 0.514 (15) ^{ab}	3.64 $\pm$ 0.618 (8) ^{abc}
Protein	3.20 $\pm$ 0.147 (21) ^a	3.17 $\pm$ 0.210 (31) ^{ab}	3.15 $\pm$ 0.158 (34) ^{ab}	3.10 $\pm$ 0.144 (40) ^b	3.25 $\pm$ 0.167 (16) ^a	3.31 $\pm$ 0.181 (8) ^a
Lactose	4.74 $\pm$ 0.202 (20) ^{ab}	4.57 $\pm$ 0.159 (30) ^c	4.79 $\pm$ 0.131 (27) ^a	4.76 $\pm$ 0.159 (33) ^{ab}	4.74 $\pm$ 0.134 (11) ^{ab}	4.68 $\pm$ 0.104 (8) ^{bc}
Milk component yield (kg/d)						
Fat	0.885 $\pm$ 0.269 (22) ^c	1.03 $\pm$ 0.225 (32) ^b	1.26 $\pm$ 0.268 (35) ^a	1.08 $\pm$ 0.284 (43) ^b	1.09 $\pm$ 0.226 (16) ^{ab}	0.979 $\pm$ 0.135 (8) ^{bc}
Protein	0.835 $\pm$ 0.219 (21) ^b	0.880 $\pm$ 0.228 (32) ^b	1.03 $\pm$ 0.254 (34) ^a	0.939 $\pm$ 0.235 (42) ^{ab}	0.959 $\pm$ 0.160 (16) ^{ab}	0.893 $\pm$ 0.0841 (8) ^{ab}
Lactose	1.30 $\pm$ 0.411 (20) ^b	1.33 $\pm$ 0.309 (30) ^b	1.56 $\pm$ 0.377 (27) ^a	1.40 $\pm$ 0.318 (30) ^{ab}	1.43 $\pm$ 0.287 (11) ^{ab}	1.23 $\pm$ 0.0675 (7) ^b
Milk fatty acid profile (g/100 g of total fatty acids)						
Σ DNFA ¹	18.7 $\pm$ 3.64 (22) ^c	21.7 $\pm$ 5.20 (31) ^{ab}	20.5 $\pm$ 4.76 (33) ^{abc}	21.8 $\pm$ 3.53 (43) ^a	22.2 $\pm$ 7.30 (16) ^{ab}	18.8 $\pm$ 2.90 (8) ^{bc}
16:0	19.5 $\pm$ 2.10 (19) ^b	26.1 $\pm$ 2.97 (31) ^a	25.6 $\pm$ 3.91 (33) ^a	24.3 $\pm$ 4.07 (43) ^a	24.9 $\pm$ 4.21 (16) ^a	23.1 $\pm$ 4.87 (8) ^{ab}
18:0	11.0 $\pm$ 1.52 (19) ^c	14.4 $\pm$ 3.05 (31) ^a	13.3 $\pm$ 3.21 (33) ^{ab}	12.3 $\pm$ 2.73 (43) ^{bc}	12.3 $\pm$ 2.01 (12) ^{bc}	14.6 $\pm$ 1.69 (8) ^a
Σc 18:1 ²	23.7 $\pm$ 4.82 (21) ^{abc}	25.5 $\pm$ 5.02 (31) ^{ac}	22.8 $\pm$ 4.15 (33) ^b	22.9 $\pm$ 2.84 (42) ^{bc}	22.9 $\pm$ 2.92 (12) ^c	26.1 $\pm$ 3.29 (8) ^a
Σt 18:1 ³	8.39 $\pm$ 3.40 (21) ^a	2.51 $\pm$ 0.875 (29) ^d	4.44 $\pm$ 1.86 (33) ^c	5.95 $\pm$ 3.10 (39) ^b	2.50 $\pm$ 1.02 (10) ^d	4.68 $\pm$ 3.37 (8) ^{bcd}
<i>cis</i> -9, <i>cis</i> -12 18:2	1.81 $\pm$ 0.390 (21) ^b	1.85 $\pm$ 0.461 (29) ^b	1.79 $\pm$ 0.382 (33) ^b	1.83 $\pm$ 0.440 (37) ^b	3.07 $\pm$ 1.57 (16) ^a	1.89 $\pm$ 0.245 (8) ^{ab}
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.770 $\pm$ 0.326 (22) ^d	0.842 $\pm$ 0.220 (31) ^{cd}	0.996 $\pm$ 0.286 (35) ^{bc}	1.13 $\pm$ 0.367 (43) ^b	1.67 $\pm$ 0.916 (15) ^a	1.62 $\pm$ 0.379 (8) ^a
Σ CLA ⁴	1.61 $\pm$ 0.751 (20) ^a	0.629 $\pm$ 0.179 (18) ^d	0.722 $\pm$ 0.193 (32) ^c	1.15 $\pm$ 0.513 (40) ^b	1.08 $\pm$ 0.416 (12) ^b	1.23 $\pm$ 0.643 (8) ^{ab}
Σ OCFA ⁵	1.83 $\pm$ 0.503 (19) ^{bc}	2.06 $\pm$ 0.526 (26) ^b	1.47 $\pm$ 0.160 (23) ^d	1.60 $\pm$ 0.279 (26) ^c	2.02 $\pm$ 0.468 (6) ^{ab}	2.52 $\pm$ 0.510 (8) ^a
Transfer efficiency (g secreted in milk/100 g consumed)						
<i>cis</i> -9, <i>cis</i> -12 18:2	6.15 $\pm$ 1.08 (16) ^d	8.37 $\pm$ 3.18 (30) ^{bc}	10.9 $\pm$ 3.36 (25) ^a	8.19 $\pm$ 3.89 (28) ^{cd}	12.4 $\pm$ 3.19 (4) ^{ac}	9.60 $\pm$ 2.79 (7) ^{abc}
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	1.95 $\pm$ 0.968 (16) ^d	3.09 $\pm$ 1.85 (29) ^c	5.84 $\pm$ 2.50 (27) ^a	4.12 $\pm$ 1.99 (30) ^b	6.49 $\pm$ 4.75 (5) ^{abc}	4.80 $\pm$ 1.27 (7) ^{ab}

^{a-d}Within the same row, means followed by different letters are significantly different, using a nonparametric analysis, without study effects ($P < 0.05$).

¹Total de novo synthesized fatty acids (4:0 + 6:0 + 8:0 + 10:0 + 12:0 + 14:0 + 14:1).

²Total *cis* octadecenoic acids.

³Total *trans* octadecenoic acids.

⁴Total CLA.

⁵Total odd-chain fatty acids.

lowest concentration of this milk FA was observed when cows received free oil. Among the other 3 categories in between, lower milk fat concentration of *cis*-9,*cis*-12,*cis*-15 18:3 was obtained with intact as compared with extruded whole seed, whereas intermediate value was observed with mechanically processed whole seed. The least efficient transfer of *cis*-9,*cis*-12,*cis*-15 18:3 from diet to milk fat was observed when feeding free oil. Among the remaining categories, the transfer efficiency of *cis*-9,*cis*-12,*cis*-15 18:3 was lower for intact and then extruded whole seed as compared with mechanically processed whole seed. Intermediary efficiencies between these latter 2 treatments were obtained with flax hulls. Average efficiency calculated with protected flax ($n = 5$) did not statistically differ ($P > 0.05$) from any of the latter 4 categories.

Experiment 4—Multivariate Factor Analysis

Five principal components (PC) were identified, which explained 75.5% of the variation (Table 7). Milk fat concentration and yield, milk protein concentration, as well as short- and medium-chain FA (8:0, 10:0, 12:0, and 14:0, 14:1, and 16:0) were positively correlated

with the PC 1, which explained 32.3% of the variability (Table 7 and Supplemental Figure S4; <https://doi.org/10.3168/jds.2017-12637>). Negative associations were observed for the same PC with proportions of milk 18:0, Σc 18:1, Σt 18:1, *cis*-9,*cis*-12 18:2, and ΣCLA . The second PC (15.9% of the variability) was related in its positive part with the yields of milk, fat, and protein, as well as proportions of milk 12:0 and *cis*-9,*cis*-12 18:2; but in its negative part with concentrations of milk fat and protein, and proportions of milk Σc 18:1 and ΣCLA . The PC 3 (12.5% of the variability) revealed positive associations with proportions of milk Σt 18:1, *cis*-9,*cis*-12,*cis*-15 18:3, and ΣCLA , but negative associations with milk fat concentration and yield, as well as proportions of milk 8:0, 10:0, 12:0, 14:0, 16:0 18:0, and Σc 18:1. Milk yield, milk protein concentration and yield, and proportions of milk 14:1, 16:0, 16:1, Σc 18:1, and *cis*-9,*cis*-12 18:2 were positively correlated with PC 4 (8.0% of the variability), whereas milk fat concentration and proportions of milk 18:0 and Σt 18:1 were negatively correlated. Finally, the PC5 (6.8% of the variability) consisted of milk fat concentration and proportions of milk 8:0, 10:0, 18:0 Σc 18:1, *cis*-9,*cis*-12 18:2, *cis*-9,*cis*-12,*cis*-15 18:3, and ΣCLA with positive loading values, whereas proportions of milk

Table 7. Common factor loading and communalities for milk yield and composition and milk fatty acid profile (experiment 4)

Item	Rotated factor ^{1,2}					Communality
	PC1	PC2	PC3	PC4	PC5	
Milk yield and composition						
Milk yield (kg/d)	0.09	0.95‡	0.07	0.25‡	−0.05	0.98
Fat (%)	0.07	−0.13§	−0.62§	−0.46§	0.20‡	0.66
Protein (%)	0.37‡	−0.44§	0.00	0.22‡	−0.05	0.39
Fat (kg/d)	0.13‡	0.86‡	−0.34§	−0.09	0.08	0.89
Protein (kg/d)	0.21‡	0.86‡	0.07	0.32‡	−0.08	0.90
Milk fatty acid profile (g/100 g of total fatty acids)						
8:0	0.72‡	0.03	−0.39§	0.01	0.28‡	0.75
10:0	0.86‡	0.06	−0.38§	0.05	0.12‡	0.91
12:0	0.89‡	0.12‡	−0.30§	0.10	−0.05	0.91
14:0	0.82‡	0.06	−0.35§	0.10	−0.17§	0.83
14:1	0.35‡	−0.04	0.07	0.54‡	−0.42§	0.59
16:0	0.47‡	0.07	−0.56§	0.19‡	−0.45§	0.78
16:1	0.09	0.09	−0.04	0.69‡	−0.29§	0.57
18:0	−0.61§	−0.09	−0.27§	−0.16§	0.60‡	0.84
Σcis 18:1 ³	−0.79§	−0.12§	−0.17§	0.21	0.27‡	0.79
$\Sigma trans$ 18:1 ⁴	−0.25§	0.02	0.87‡	−0.13§	0.09	0.85
<i>cis</i> -9, <i>cis</i> -12 18:2	−0.13§	0.18‡	0.02	0.78‡	0.31‡	0.75
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.08	0.02	0.21‡	−0.04	0.71‡	0.56
ΣCLA ⁵	−0.22§	−0.25§	0.70‡	0.09	0.20‡	0.65
Eigenvalue	5.8	2.9	2.3	1.4	1.2	
Proportion	32.3	15.9	12.5	8.0	6.8	
Cumulative	32.3	48.2	60.8	68.8	75.5	

¹PC: principal component.

²Values with a loading ≥ 0.12 (‡) or ≤ -0.12 (§) were considered for the interpretation of the factor pattern ($n = 282$).

³Total *cis* octadecenoic acids.

⁴Total *trans* octadecenoic acids.

⁵Total CLA.

14:0, 14:1, 16:0, and 16:1 were associated with negative loading values.

DISCUSSION

Experiments 1 and 2—Flax-TRI vs. Nonsupplemented Control or Varying Lipid Sources

Dietary fat supplements have been shown to reduce DMI through different mechanisms (Allen, 2000). The negative effect of flax-TRI supplementation exposed by the current meta-analysis when compared with LOW-E and ISO-E controls is in line with previous experiments where fat supplementation resulted in a decrease in DMI by reducing feed fermentability and digestibility (Palmquist and Jenkins, 1980). In addition to the effects of dietary lipids on rumen fermentation, Drackley et al. (1992) demonstrated, using postruminal infusion of UFA, a hypophagic effect of lipids. Moreover, Glasser et al. (2008b), in a meta-analysis, observed a linear within-experiment decrease in DMI with increasing duodenal flow of C18 FA (mean slope of -1.7 ± 0.5 g of DMI per gram of duodenal C18 increase). It was also proposed by Bradford et al. (2008) that an increase in plasma glucagon-like peptide-1 and cholecystokinin, with a possible decrease in pre-meal ghrelin, could be involved in the reduction of DMI when PUFA are added to the diets of dairy cows. It is not known, however, whether postruminal supplies of PUFA were enough to cause a hypophagic effect in cows receiving flax seed or oil for experiments included in the current meta-analysis.

One of the main differences observed when flax-TRI supplements were studied in relation to nonsupplemented diets was a decrease in milk production as well as fat, protein, and lactose yields when compared with ISO-E, which was not apparent when the comparison was made with LOW-E controls. Importantly, feed efficiency, calculated on a NE_L basis, was decreased by flax-TRI diets as compared with the LOW-E control, which means that the extra energy provided by supplemental EE was used less efficiently than other dietary components to produce milk.

Assessing the effect of dietary treatments on lactation performance requires considering the variations in concentrations of major milk constituents. In the current analysis, cows receiving SFA produced milk with greater fat concentrations as compared with flax-TRI supplements. Diets in the SFA category contained greater levels of 16:0 (Supplemental Table S1; <https://doi.org/10.3168/jds.2017-12637>). In this regard, Mannai et al. (2016) have shown that the level of dietary 16:0 is positively associated with milk fat concentra-

tion. Moreover, SFA are known to have a minimal effect on the rumen fermentation (Jenkins, 1993) and to be more efficiently used by animal tissues, including the mammary gland (Moore and Christie, 1979). This may explain the increased efficiency of milk ECM production, on a DMI basis, in cows fed SFA as compared with flax-TRI supplements.

On the contrary, milk fat depression (MFD) is often associated with feeding unsaturated lipid supplements in dairy cows (He and Armentano, 2011; Mannai et al., 2016). Accordingly, in the current analysis, cows receiving flax-TRI produced milk with lower fat concentrations compared with the LOW-E control. Under these conditions, lower milk fat concentration can be explained by a disturbance in ruminal fermentation causing a shift from the *trans*-11 to the *trans*-10 pathway of *cis*-9,*cis*-12 18:2 biohydrogenation, leading to the production of *trans*-10,*cis*-12 18:2. This CLA isomer has been shown to decrease the expression of lipogenic enzymes and reduce milk fat synthesis in the mammary gland (Bauman and Grinari, 2003). Moreover, other FA isomers are known to induce an anti-lipogenic effect in the mammary gland. For example, *trans*-9,*cis*-11 18:2, which is a ruminal biohydrogenation intermediate produced from *cis*-9,*cis*-12,*cis*-15 18:3, seems to be also involved in MFD (Shingfield et al., 2010). Ruminal disturbance caused by dietary PUFA could be exacerbated by increasing the proportions of corn silage in rations. In this regard, a meta-analysis by Meignan et al. (2017) revealed that milk fat concentration and yield decreased when extruded flax seed was supplemented to diets with high proportions of corn silage in the forage but did not decrease when the diet contained alfalfa hay. In the current meta-analysis, the quadratic decrease in milk fat concentration and yield observed when supplementing flax-TRI in diets containing increasing proportions of corn silage confirms this interaction between forage type and fat supplementation.

Also, in the present analysis, the decrease in milk fat concentration associated with lipid supplementation was affected by the degree of unsaturation of dietary 18-carbon FA. Indeed, milk fat concentrations were similar between flax-TRI and MONO supplements, but were decreased by feeding DI lipid sources as compared with flax-TRI. The production of FA intermediates of the *trans*-10 pathway has been reported during the biohydrogenation of *cis*-9 18:1 (main FA in MONO; Mosley et al., 2002) and *cis*-9,*cis*-12,*cis*-15 18:3 (main FA in flax-TRI; Lee and Jenkins, 2011). However, as opposed to dietary *cis*-9,*cis*-12 18:2, no significant amounts of *trans*-10,*cis*-12 18:2 appear to be directly produced during ruminal biohydrogenation of *cis*-9 18:1 and *cis*-9,*cis*-12,*cis*-15 18:3. Although milk fat

concentration of *trans*-10,*cis*-12 18:2 was not assessed in the current meta-analysis, these previous observations could explain the similar milk fat concentration and yield observed with MONO and flax-TRI supplements, but the lower milk fat concentration and yield observed with DI supplements.

In the current analysis, a decrease in milk protein percentage was observed when flax-TRI was compared with LOW-E and ISO-E nonsupplemented controls. Several mechanisms were proposed to explain the protein-depressing effects of dietary fat (DePeters and Cant, 1992). Among them is a dilution effect due to an increased milk yield following fat supplementation. This mode of action is not likely to be applicable in the current analysis as actual milk yield was either not affected (vs. LOW-E) or decreased (vs. ISO-E) with flax-TRI. Another mechanism is that inclusion of lipids in the ration tends to reduce rumen bacterial protein synthesis through a direct inhibiting effect on microbial growth or a reduction of fermentable substrate when fat replaces starch in the diet (Palmquist et al., 1993b). The supply of AA for protein synthesis in the mammary gland would consequently be limited. Other authors also suggested that postabsorption hormonal regulations involving insulin or growth hormone might also be implicated (Doreau and Chilliard, 1992). Wu and Huber (1994) further reported that milk protein depression occurs regardless of the type of fat and at different levels of supplementation. This observation is in line with the lack of difference in milk protein concentration and yield when flax-TRI was compared with MONO and DI supplements. The greater milk protein content observed when cows received flax-TRI as compared with SFA supplements, which are less likely to affect ruminal fermentation, constitutes an exception in this regard.

As expected, feeding flax-TRI increased milk fat concentration of *cis*-9,*cis*-12,*cis*-15 18:3 for all comparisons reported (i.e., vs. LOW-E, ISO-E, SFA, MONO, or DI). Several health benefits are potentially attributed to the consumption of this FA (Cunnane, 2003). As milk fat is a common component of the human diet, enrichment of dairy products with *cis*-9,*cis*-12,*cis*-15 18:3 provides the opportunity to increase intake of this essential FA by the general population.

As also expected, feeding DI supplements increased the concentration of *cis*-9,*cis*-12 18:2 in milk fat as compared with flax-TRI. Less expectedly, the increase in the level of 18:0 was of greater magnitude with flax-TRI than with SFA lipid sources, despite the fact that the concentration of this FA was 2.7 times greater in SFA than in flax-TRI supplements (Supplemental Table S1; <https://doi.org/10.3168/jds.2017-12637>). It appears

from this result that flax *cis*-9,*cis*-12,*cis*-15 18:3 was efficiently biohydrogenated to 18:0 in the rumen. After absorption, 18:0 is converted to *cis*-9 18:1 through the action of Δ^9 -desaturase (Palmquist et al., 1993a). This reaction may explain the lack of difference in milk fat proportions of Σc 18:1 between flax-TRI and MONO supplements.

The concentrations of Σ DNFA and 16:0 in milk fat were decreased by lipid supplementation. It has been known for a while that increasing the uptake of preformed FA from dietary origin by the mammary gland is reducing de novo synthesis of FA, and consequently the concentration of Σ DNFA and 16:0 in milk fat (Palmquist and Jenkins, 1980). An exception is observed with the SFA category, which contained several supplements of mainly 16:0, leading to greater concentrations of this FA in milk fat as compared with flax-TRI treatments.

Most of the odd-chain fatty acids (OCFA) secreted in milk fat are known to be of microbial origin (Vlaeminck et al., 2006). Monitoring the concentrations of these FA in milk secretion has then been suggested as a noninvasive tool to evaluate the conditions of ruminal fermentation (Vlaeminck et al., 2006). A negative effect of lipid supplementation on milk Σ OCFA was observed when flax-TRI was compared with either LOW-E or ISO-E controls, implicating adverse effects on rumen conditions, and more specifically on the growth of ruminal microorganisms responsible for the synthesis of these FA. Considering that ruminal concentration of propionate, the major precursor of OCFA, is not a limiting factor (French et al., 2012), dietary lipids seem to exert their effects through a reduced bacterial growth in the rumen either by a direct toxicity or by decreasing nutrient availability (Bauchart et al., 1990), leading to a lower concentration of OCFA in milk.

Experiment 3—Effects of Flax Forms

Current literature comparing different forms of flax seed and oil is limited to a few animal trials (comparison of 2 forms: $n = 11$, or 3 forms: $n = 3$, out of a total of 84 experiments in the database). Considering the paucity of direct within-experiment comparisons, a nonparametric analysis was conducted, in which the effect of study was not considered. In the current analysis, the choice of an optimal technological processing applied to flax seed and oil was based on lactation performance and milk FA profile, with a focus on the transfer efficiency of *cis*-9,*cis*-12,*cis*-15 18:3 from diet to milk fat.

Concerning animal performance, the greatest yield of ECM and the best feed efficiency were obtained with

mechanically processed whole seed. Lower than what was observed with mechanically processed whole seed, milk yield and feed efficiency were similar among the 5 other form categories. Regarding milk FA profile, the greatest concentrations of *cis*-9,*cis*-12,*cis*-15 18:3 were observed when feeding protected flax and flax hulls. Moreover, the greatest transfer efficiencies of this FA from diet to milk were recorded with the same 2 treatments, plus the mechanically processed whole seed. These values were expected for protected flax, as the category was created based on this specific objective. It should be pointed out that the category of protected flax is heterogeneous, as it regroups several technologies. Among them, 6 experimental diets in the database were designed to test formaldehyde-treated flax seed or oil, which is known to provide a superior protection. The low numbers of treatment diets testing different protection technologies did not allow individual assessment of their effects on milk yield and composition in the current data analysis. The high level of *cis*-9,*cis*-12,*cis*-15 18:3 in milk fat, obtained when feeding flax hulls, suggests that residual PUFA in this flax by-product are partially protected against ruminal biohydrogenation. Indeed, different methods are used in the separation of flax hulls from the whole seed, and even if oil is concentrated in the kernel and embryo fraction, flax hulls still contain an appreciable amount of fat (>25%; Hosseini and Beta, 2009). However, it should be underlined that this observation is based on the evaluation of a lower number of treatment means ($n = 8$) as compared with other form categories. In contrast, low transfer efficiencies and milk concentrations of *cis*-9,*cis*-12,*cis*-15 18:3 were observed when cows received free oil and intact whole seed. On the one hand, feeding free oil offers no protection of dietary PUFA against ruminal biohydrogenation, which reduces their transfer to milk secretion. These observations are in accordance with Glasser et al. (2008a). On the other hand, dietary PUFA, as part of intact whole seed, although protected in the rumen, appears to be less available for absorption in the small intestine, thus leading to low transfer efficiency from diet to milk. Mechanical processing seems to offer a compromise between these 2 treatments, with a better balance between ruminal protection, and availability for intestinal absorption of dietary PUFA. In line with these observations, intermediate levels of Σt 18:1 were observed in milk when cows were fed mechanically processed whole seed (4.44 g/100 g) as compared with free oil (8.39 g/100 g) and intact whole seed (2.51 g/100 g), which suggests that mechanical processing increased the extent of exposure to microbial enzymes. A similar phenomenon has been observed by Kennelly (1996). Moreover, da Silva et al. (2007) reported that

EE digestibility was 17% lower when cows were fed diets based on intact whole flax seed (73.0%) as compared with ground flax seed (88.4%). Finally, Oba et al. (2009) observed that apparent total-tract digestibility of *cis*-9,*cis*-12,*cis*-15 18:3 was about twice greater with rolled (67.2%) as compared with intact whole flax seed (35.2%).

Experiment 4—Multivariate Factor Analysis

The first latent factor (PC 1) is focused on milk FA profile and is more clearly associated with the equilibrium between de novo synthesis and uptake of pre-formed FA by the mammary gland during lipogenesis (Supplemental Figure S4; <https://doi.org/10.3168/jds.2017-12637>). Positive loadings were observed for short- and medium-chain FA (from 8:0 to 16:0) in association with negative loadings for long-chain C18 FA (18:0, Σc 18:1, Σt 18:1, *cis*-9,*cis*-12 18:2, and ΣCLA). These results are in agreement with observations made in previous sections concerning the negative effect of dietary long-chain FA on de novo synthesis in the mammary gland (Palmquist et al., 1993a), a situation frequently observed in the current database. The PC 1 also appears to be related to milk protein depression often associated with dietary fat supplementation (see discussion above; DePeters and Cant, 1992; Doreau and Chilliard, 1992; Wu and Huber, 1994). In this scenario, lower milk concentrations of preformed long-chain FA (negative loadings for 18:0 and Σc 18:1) could be associated with increased de novo synthesis in the mammary gland (positive loadings for FA from 8:0 to 16:0) when supplemental lipids are removed from the diet, these effects being associated with a greater milk protein synthesis (positive loadings for both milk protein concentration and yield). These results are also consistent with more recent observations reported by Woolpert et al. (2016) showing that greater de novo FA is associated with increased milk protein concentration and yield in bulk tank milk from commercial dairy farms.

The PC 2 is associated with an overall increase in lactation performance (positive loadings for the yields of actual milk, milk fat, and milk protein). Negative loadings for the concentrations of milk fat and milk protein suggest a potential dilution effect associated with increased milk yield. The improved lactation performance appears to be weakly dependent on milk FA profile, when affected by dietary lipid supplements, as highlighted in PC 1 (Supplemental Figure S4; <https://doi.org/10.3168/jds.2017-12637>). Strong positive loadings for milk, protein, and fat yields in PC 2 could then be related to nondietary factors such as breeds (Jersey vs. Holstein) or lactation stage. The PC 3 is associ-

ated with the symptoms of MFD (negative loadings for milk fat concentration and yield), a situation that could be attributed to the effects of intermediates such as *trans*-10 18:1 and *trans*-10,*cis*-12 18:2 from incomplete biohydrogenation of dietary PUFA (potentially associated with strong positive loadings for Σt 18:1 and ΣCLA ; Supplemental Figure S4; <https://doi.org/10.3168/jds.2017-12637>).

The PC 4 is associated with the activity of Δ^9 -desaturase, as positive loading factors were calculated for the FA products of this enzyme in milk fat [i.e., 14:1, 16:1, and Σc 18:1 (mainly *cis*-9 18:1)]. It should be pointed out that this PC only explains 8% of the variability. Nevertheless, a positive association was concomitantly observed with milk fat concentration of *cis*-9,*cis*-12 18:2 suggesting that this situation is specific to dietary treatments with DI supplements, or to diets containing high levels of cereal concentrates, also rich in this n-6 PUFA (e.g., corn and barley; INRA-AFZ, 2002). Furthermore, cereal grains contain high levels of starch, which has been shown to increase plasma insulin in dairy cows (Oba and Allen, 2003). Ward et al. (1998) observed that insulin significantly increased the expression of the enzyme Δ^9 -desaturase in ruminants. These putative metabolic events were associated with negative loading values for milk fat concentration. In accordance with these results, Rico and Harvatine (2013) reported an increase of Δ^9 -desaturation index during the induction of MFD in dairy cows. In this context, the positive loading value for milk yield could be explained by a sparing effect of energy during MFD (Bauman et al., 2008).

The PC 5 revealed the strongest loading for milk proportion of *cis*-9,*cis*-12,*cis*-15 18:3, which is of particular interest from the current database built to study the effects of flax seed and oil. This positive loading could be associated with flax treatments showing the greatest efficiency of transfer of *cis*-9,*cis*-12,*cis*-15 18:3 from diet to milk fat. A negative loading was observed for the proportions of milk C14 (14:0 and 14:1) and C16 (16:0 and 16:1) FA. A similar phenomenon has been observed by Fauteux et al. (2016) who reported that the increase in milk *cis*-9,*cis*-12,*cis*-15 18:3 (+13.9 PU) observed as a result of abomasal infusion of flax oil was almost solely at the expense of 14:0 (−2.9 PU) and 16:0 (−10.7 PU).

CONCLUSIONS

In conclusion, this meta-analysis and meta-regression revealed that supplementing flax-TRI decreased DMI and the yields of milk, fat, protein, and lactose without affecting the efficiency of utilization of dietary DM or

energy as compared with nonsupplemented ISO-E diets. Of particular interest, the decrease in milk fat in response to flax-TRI supplementation was of a greater magnitude with increasing levels of corn silage in the basal diet. This information should be taken into account when formulating diets containing flax seed or oil, and perhaps other types of dietary PUFA supplements. Feeding flax-TRI increased milk fat concentration of *cis*-9,*cis*-12,*cis*-15 18:3 for all comparisons reported (i.e., vs. LOW-E, ISO-E, SFA, MONO, or DI). Flax-TRI also supported similar ECM as SFA, MONO, or DI supplements. As compared with flax-TRI supplements, greater milk fat concentration and feed efficiency were observed with SFA, but lower milk fat concentration and yield were obtained with DI supplements. The comparison of different forms of flax seed and oil was based on the criteria of maximizing both production performance and transfer efficiency of *cis*-9,*cis*-12,*cis*-15 18:3 from diet to milk fat. The analysis revealed that among the 6 categories evaluated, mechanical processing (rolling or grinding) allowed the greatest yield of ECM and the best feed efficiency. Feeding protected flax and flax hulls allowed the greatest milk fat concentrations of *cis*-9,*cis*-12,*cis*-15 18:3. Moreover, the greatest transfer efficiencies of this FA from diet to milk were recorded with the same 2 treatments, plus the mechanically processed whole seed. These results make this last category the most suitable treatment, among the 6 flax forms evaluated, to combine lactation performance and protection of flax constituent n-3 FA. Finally, the PC analysis was an opportunity to assess several concepts in relation with the metabolic response to dietary fat supplementation. These notions include the overall effect of dietary lipid supplements on the balance between de novo synthesis and uptake of preformed FA, and their potential negative effects on milk fat and protein concentrations.

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