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Effect of Feeding Canola or Sunflower Seeds on Conjugated Linoleic Acid Enrichment in Cow's Milk Fat

Research Article

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ABSTRACT

Two experiments studied the effects of canola or sunflower seed supplementation on conjugated linoleic acid (CLA) concentration in milk fat of lactating cows. In each experiment, only eight cows in early lactation were available to assign to one of two treatments in a completely randomized design and were fed individually for 12 wk. Experiment 1 compared similar diets with or without the addition of ground canola seed [12.1 g/100 g dry matter (DM)]. Cows fed canola had higher *cis*-9, *trans*-11 CLA in milk fat at wk 9 and 12 ($P<0.05$). In experiment 2, treatments were two levels of rolled sunflower seed: low (2.0 g/100 g DM) and high (11.4 g/100 g DM). In experiment 2, cows on the high sunflower diet had greater *cis*-9, *trans*-11 CLA in milk fat compared with that of the low sunflower treatment at wk 3 and 12; however, significant individual cow variation existed within the high sunflower treatment. Milk yield, milk composition, and dry matter intake (DMI) were not altered in either experiment. Even given this experiment was not robust, the oilseeds of canola and sunflower increased CLA content in milk fat of lactating cows without negatively affecting milk yield or milk fat concentration.

KEY WORDS canola, conjugated linoleic acid, dairy cow, milk fat, sunflower.

INTRODUCTION

Conjugated linoleic acids are positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12 octadecadienoic acid) and are present in high levels in ruminant-derived food products compared with those of non-ruminant origin (Kelly and Bauman, 1996). Certain isomers of CLA are associated with a wide range of positive health benefits in animal models including inhibition of chemically-induced carcinogenesis (Ip *et al.* 1991; Majumder *et al.* 2002), improvement of immune response and growth rate, and reduction of atherosclerosis and body fat gain (Pariza *et al.* 2001). Milk fat is a major natural source of CLA, and *cis*-9, *trans*-11 CLA is the most abundant isomer present in fluid milk and cheese (Parodi, 1977; Chin *et al.* 1992). Further, *cis*-9, *trans*-11 CLA is the isomer associated with the anti-carcinogenic effects observed in animal models (Ip *et al.* 1991; Ip *et al.* 2002).

In ruminants, *cis*-9, *trans*-11 CLA occurs through incomplete biohydrogenation of dietary linoleic acid (Kepler and Tove, 1967), as well as through desaturation of *trans*-11 octadecenoic acid (vaccenic acid), an intermediate of linoleic and linolenic acid biohydrogenation (Harfoot and Hazlewood, 1988), via the mammalian enzyme Δ^9-desaturase (Griinari *et al.* 2000). Several plant oils and oilseeds differing in fatty acid composition have been shown to increase CLA concentration in milk of dairy cows (Kelly *et al.* 1998; Abu Ghazaleh *et al.* 2002; Mustafa *et al.* 2003; Mir *et al.* 2004; Petit *et al.* 2004; Dhiman *et al.* 2005). Oilseeds such as canola and sunflower are attractive feedstuffs for lactating dairy cattle because they provide protein and fiber, in addition to being concentrated energy

sources that are less likely to negatively affect milk fat concentration compared with free oils (Kelly *et al.* 1998; Dhiman *et al.* 2000).

The objectives of this research were to determine whether feeding canola or sunflower seed to lactating dairy cows enhances *cis*-9, *trans*-11 CLA and *trans*-11 18:1 content of milk fat and to measure the effects of oilseeds on milk yield, milk composition, and feed intake.

MATERIALS AND METHODS

All animal procedures and protocols were approved by the North Dakota State University Institutional Animal Care and Use Committee. Two preliminary experiments were conducted in succession and in a similar manner to study the effects of canola (Experiment 1) or sunflower seed (including the hull; Experiment 2) supplementation on CLA concentration in milk fat of lactating dairy cows. Cows were housed in individual tie-stalls at the North Dakota State University Dairy Research and Teaching Center. For each experiment, eight early lactating Holstein cows were stratified by days in milk (DIM) and milk production, and assigned randomly to 1 of 2 treatments. Fresh water was available at all times, and feed was mixed at 1500 h and offered twice daily to ensure a 5 to 10% refusal on an as-fed basis.

Cows were milked twice per day at 0400 and 1500 h and immediately returned to tie-stalls. Milk yield was measured and recorded daily.

Experiment 1

Treatments of four cows each were fed a diet without canola (control) or with ground canola seed (canola; 12.1 g/100 g DM).

All cows were offered the control diet for 1 wk prior to initiation of the experiment. Canola seeds were blended into the concentrate mix, which was then added to the total mixed ration (TMR). The ingredient composition of the experimental diets is detailed in Table 1.Total mixed rations, forages, concentrate mixes, and canola seeds were sampled once every 3 wk, dried in a forced-air oven for 48 h at 55 ˚C, and ground through a Wiley Mill (2 mm screen; Arthur H. Thomas, Philadelphia, PA). Refused feed was sampled at the same interval for determination of DM. Feed ingredients and TMR samples were analyzed for acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Robertson and Van Soest, 1981), crude protein (CP), ether extract, Ca, and P AOAC (1990). Chemical composition of the TMR samples is found in Table 1. Net energy for lactation for both diets was estimated using NRC, (2001) equations. Milk was collected every 3 wk from two consecutive milkings and mixed.

An aliquot was preserved (Broad Spectrum Micro tabs II; D and F Control Systems, Dublin, CA) and analyzed for fat and protein (Heart of America Dairy Herd Association Laboratory, Manhattan, KS). The remaining sample (40 mL) was frozen at -20 ˚C until analysis of fatty acid composition.

Experiment 2

Treatments consisted of two levels of rolled sunflower seeds: Low (2.0 g/100 g DM) and High (11.4 g/100 g DM). Whole sunflower seeds were rolled and stored separately until being added to the TMR. All cows were offered the Low sunflower seed diet for 1 week prior to the beginning of the 12-wk feeding period. The ingredients of each ration are presented in Table 1.

Feed offered and feed refused were weighed daily and recorded. Refusals from each cow were sampled weekly and mixed every 3 weeks for determination of DM; corn silage and alfalfa were sampled weekly for DM determination and ration adjustment. Corn silage, alfalfa, rolled sunflower seed, and each TMR were sampled every 3 weeks period.

Procedures used and nutrients analyzed are the same as those described in Experiment 1. Net energy of lactation was estimated using the NRC (2001) Nutrient Requirements for Dairy Cattle analyzer.

The chemical composition each diet is presented in Table 1. Sampling of milk and analysis of composition occurred at the same intervals and involved the same procedures as Experiment 1.

Fatty acid analysis

Feed samples were dried and ground and 100 g of each TMR and oilseed sample were mixed and stored at -20 ˚C until fatty acid determination. Milk fat was separated by centrifugation (8000 g; 45 min, room temperature), and whey was removed by vacuum aspiration before collection of the fat layer. Feed sample particle size was further reduced in a Wiley Mill (1 mm screen; Arthur H. Thomas, Philadelphia, PA). Lipids were extracted from the feed with chloroform:methanol (2:1 vol/vol) (Folch *et al.* 1957). Methyl esters of fatty acids from feed and milk were prepared by the transesterification procedure of Park and Goins (1994).

10 undecenoate was used as an internal standard (Nu-Check Prep, Elysian, MN). Methyl esters of fatty acids from feed and milk were injected by auto sampler into an Agilent 6890 N gas chromatograph fitted with a flame ionization detector (Agilent Technologies, Palo Alto, CA). A fused silica column (CP Sil 88, 100 m × 0.25 mm i.d., 0.2 µm film thickness; Varian, Inc., Palo Alto, CA) was used to separate methyl esters of fatty acids.

Table 1 Ingredient and chemical composition of total mixed diets containing canola or sunflower seed fed to lactating dairy cows

Composition	Canola study		Sunflower study	
	Control	Canola	Low	High
Ingredient, % dry matter				
Corn silage	18.2	40.0	29.6	28.5
Alfalfa, chopped	7.8	17.6	17.5	19.7
Barley, rolled	16.8	1.0	10.4	-
Corn, ground	16.8	1.0	12.7	9.0
Beet pulp, pelleted	12.4	4.7	5.9	9.8
Soybean meal	23.2	19.1	17.7	16.3
Canola seed, ground	-	12.1	-	-
Sunflower seed, rolled	-	-	2.0	11.4
Sodium bicarbonate	0.9	0.8	1.0	1.0
Magnesium oxide	0.4	0.4	0.3	0.3
Salt	-	-	0.2	0.2
Trace-mineralized salt[1]	0.9	0.8	-	-
Limestone	1.0	0.8	0.2	-
Dicalcium phosphate	1.1	1.2	0.7	1.6
Blood meal	-	-	0.8	1.0
Corn gluten meal	-	-	0.8	1.0
Zinc methionine[2]	0.4	0.4	-	-
Vitamin premix[3]	0.06	0.03	-	-
Vitamin and mineral[4] premix	-	-	0.2	0.2
Chemical				
Net energy for lactation, Mcal/kg	1.61	1.68	1.61	1.74
Crude protein, %	19.6	19.9	19.6	20.2
Acid detergent fiber, %	14.7	23.4	18.5	21.4
Neutral detergent fiber, %	35.0	40.5	33.1	34.2
Ether extract, %	1.6	5.2	2.9	6.1

[1] Contained per kg: NaCl: 930 g; Co (cobalt carbonate): 0.08 g; Cu (copper sulfate): 0.39 g; I (calcium iodate): 0.08 g; Fe (iron oxide): 2 g; Mn (manganese sulfate): 1.9 g; Zn (zinc sulfate): 3.8 g and Se (sodium selenite): 0.05 g.
[2] Contained per kg: Zn: 40 g; Methionine: 80 g; Protein: 54 g; Fiber: 139 g; Ca: 85 g and NaCl: 28 g; (Zinpro 40; Zinpro Corporation, Eden Prairie, MN).
[3] Contained per kg: vitamin A: 3.3 MIU; vitamin D: 1.1 MIU and vitamin E: 1.1 kIU.
[4] Contained per kg: Ca (calcium carbonate): 210 g; Mg (magnesium oxide): 50 g; K (potassium chloride): 50 g; Co (cobalt carbonate): 0.14 g; Cu (copper sulfate): 5.1 g; I (calcium iodate): 0.38 g; Fe (iron sulfate): 12 g; Mn (manganese proteinate): 27 g; Se (sodium selenite): 0.13 g; Zn (zinc proteinate): 27 g; vitamin A: 2.7 MIU; vitamin D: 771 kIU and vitamin E: 13 kIU.

Gas chromatography conditions were as follows: the injection volume was 0.5 µL, a split injection was used (70:1 vol/vol), ultra pure hydrogen was the carrier gas, and the injector and detector temperatures were 250 and 300 ˚C, respectively. The initial temperature was 70 ˚C (held for 1 min), increased by 5 ˚C/min to 100 ˚C (held for 3 min), increased by 10 ˚C/min to 175 ˚C (held for 40 min), then increased by 5 ˚C/min to 220 ˚C (held for 19 min) for a total time of 86.5 min. Peak area was measured, and data was integrated with an Agilent 3365 ChemStation.

Statistical analysis

Data is presented as least squares means ± SEM. Data were analyzed using MIXED procedures of Statistical Analysis Software.

Fixed effects of treatment, week, and the interaction of treatment and week, in addition to random effects of cow nested within treatment were included in the model. Week was used as a repeated measurement in the model, and the covariance structure that yielded the lowest Akaike's Information Criterion value was used.

All data were adjusted for covariance by using the initial measurement obtained at d 0 following 2 weeks of adaption to diet and non significant covariables were removed from the final model.

Treatment differences were considered significant at $P<0.05$ unless otherwise noted. When treatments by week interactions were significant ($P<0.05$), means were separated by Fisher's *t*-test ("lsmeans/pdiff" statement in SAS) and considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The authors used this experiment for gathering preliminary information and acknowledge the limitations of small-scale experiments and inherent variation.

Experiment 1

The fatty acid profiles of canola seed, the control, and canola diets are detailed in Table 2; the major fatty acids in ground canola seed were 18:1 (oleic; 55.6 g/100 g fatty acids) and 18:2 (linoleic; 28.1 g/100 g fatty acids).

Table 2 Fatty acid composition of total mixed diets, canola seed, and sunflower seed

	Canola study[2]			Sunflower study[3]		
	Diet		Oilseed	Diet		Oilseed
Fatty acid[1]	Control	Canola	Canola seed	Low	High	Sunflower seed
			(g/100 g fatty acids)			
14:0	0.26	0.13	0.06	0.18	0.11	0.05
16:0	10.44	6.29	3.78	9.88	6.89	5.25
18:0	2.63	2.47	2.57	3.58	4.27	3.98
18:1	17.11	45.97	55.55	18.06	17.15	15.82
18:2	58.17	34.35	28.07	63.10	68.83	74.18
18:3	8.77	9.00	8.55	3.56	1.56	0.16

[1] Expressed as number of carbon: number of double bonds.
[2] Control: no canola seed (n=4) and Canola: 12.1 g/100 g dry matter (n=4) as ground canola seed.
[3] Low: 2.0 g/100 g dry matter (n=4) and High: 11.4 g/100 g dry matter (n=4) as rolled sunflower seed.

Linoleic acid was the major fatty acid in the control diet (58.2 g/100 g fatty acids); while oleic acid was the predominant fatty acid in the canola diet (46.0 g/100 g fatty acids). The canola diet contained greater total fat compared with the control diet (5.2 vs. 1.6% of DM, respectively; Table 1). The fat content of the canola diet was lower than expected because canola contained less fat (28.8% of DM) than anticipated (40.0 to 45.0% of DM).Supplementation of ground canola seed altered the fatty acid profile of milk from dairy cows (Table 3). Short (4:0 to 12:0) and medium chain (14:0 to 17:0) fatty acids were lower (P<0.05), whereas long chain (18:0 to 22:6) fatty acids were higher (P<0.01) in milk from cows fed canola. *Cis*-9, *trans*-11 CLA was higher at wk 9 and 12 (treatment × week; P<0.05) in canola cows compared with that of control cows (Figure 1 A); likewise, canola feeding caused an increase in *trans*-11 18:1 in milk fat at wk 9 and 12 (treatment × week; P<0.05; Figure 1 B). At week 3 and 6, there were no differences between treatments for *cis*-9, *trans*-11 CLA or *trans*-11 18:1.

Daily milk yield (37.9 vs. 36.9±1.2 kg; P=0.62) as well as milk fat (3.41 vs. 3.71±0.16%; P=0.26) and milk protein concentrations (2.80 vs. 2.73±0.04%; P=0.29) were not different between control and canola treatments, respectively (Table 4). Consequently, milk fat and protein yields were not altered by treatment. Dry matter intake (22.7 vs. 23.6±1.0 kg/d; P=0.51) was not affected by canola supplementation.

Experiment 2

Linoleic acid (18:2) was the major fatty acid in rolled sunflower seed (74.2 g/100 g fatty acids; Table 2) and in the Low and High sunflower diets (63.1 vs. 68.8 g/100 g fatty acids, respectively; Table 2).

The concentration of 18:1 was also comparable between Low and High sunflower diets (18.1 vs. 17.2 g/100 g fatty acids, respectively).

Total dietary fat content (Table 1) was substantially greater in the High sunflower diet (6.1% of DM) compared with the Low sunflower diet (2.9% of DM). Fatty acid composition of milk from cows fed either Low or High sunflower seed is detailed in Table 5. The changes in milk fatty acid composition induced by the High sunflower diet were potent but transient over the 12 weeks experiment. The High sunflower diet reduced total short-chain fatty acids at wk 3, 9, and 12 and medium-chain fatty acids at wk 3 and 12 (treatment × week; P<0.05), (Figure 2 A). Long chain fatty acids were higher in milk fat from cows fed the High sunflower diet (treatment × week; P<0.01) at week 3 and 12 compared with milk fat from cows fed the Low sunflower diet (Figure 2 B). Cows fed the High sunflower diet had higher *cis*-9, *trans*-11 CLA and *trans*-11 18:1 concentrations in milk at wk 3 and 12 of the experiment (treatment × week; P<0.05) compared with those fed the Low sunflower diet (Figure 3). There were no differences between sunflower treatments at wk 6 or 9. Overall, *cis*-9, *trans*-11 CLA concentration was 31% greater in milk fat from the High sunflower treatment compared with that from the Low sunflower treatment (0.67 vs. 0.51±0.06 g/100 g fatty acids, respectively). The changes in *trans*-11 18:1 concentration over the 12 weeks experiment followed a similar trend as *cis*-9, *trans*-11 CLA and long-chain fatty acids in both treatment groups. Dry matter intake (23.1 vs. 23.2±1.4 kg/d; P=0.68) and milk yield (38.3 vs. 40.9±1.8 kg/d; P=0.36) were not different between the Low and High sunflower treatments, respectively (Table 4). Similarly, concentrations and yields of milk fat and protein were not affected by dietary treatment.

Fatty acid composition of milk

It is well-documented that linoleic and linolenic acid provided in the diet of dairy cattle can lead to greater CLA in milk (Kelly *et al.* 1998; Dhiman *et al.* 2000; Abu Ghazaleh *et al.* 2002; Abu Ghazaleh *et al.* 2003).

Table 3 Composition of fatty acids in milk from cows fed a control or canola-containing diet

	Treatment[2]		SEM[3]	*P*[4]	
Fatty acid[1]	Control	Canola		Treatment	Treatment × week
			(g/100 g fatty acids)		
4:0	4.58	4.58	0.15	0.99	0.80
6:0	2.44	2.25	0.06	0.08	0.48
8:0	1.46	1.28	0.06	0.07	0.02
10:0	3.25	2.62	0.18	0.05	< 0.01
12:0	3.62	2.81	0.21	0.03	< 0.01
14:0	11.59	9.82	0.49	0.04	< 0.01
14:1	0.98	0.76	0.07	0.07	< 0.01
15:0	1.43	0.91	0.07	<0.01	0.07
16:0	32.20	22.43	0.91	<0.01	< 0.01
16:1	2.19	1.72	0.14	0.06	0.01
17:0	0.71	0.55	0.01	< 0.01	< 0.01
18:0	8.64	14.68	0.56	< 0.01	< 0.01
18:1	21.43	30.42	0.92	< 0.01	< 0.01
trans-11 18:1[5]	0.65	0.91	0.08	0.06	0.02
18:2	4.45	3.78	0.45	0.33	0.01
cis-9, *trans*-11 18:2[6]	0.47	0.54	0.04	0.22	< 0.01
18:3	0.24	0.41	0.03	< 0.01	< 0.01
20:0	0.11	0.26	0.01	< 0.01	< 0.01
20:3	0.18	0.14	0.02	0.15	0.03
20:4	0.25	0.17	0.02	0.05	< 0.01
20:5	0.02	0.02	0.01	0.63	0.65
22:4	0.05	0.04	0.01	0.01	0.07
22:5	0.07	0.08	0.01	0.25	0.06
Short[7]	15.36	13.54	0.47	0.03	0.05
Medium[8]	49.00	36.20	1.11	< 0.01	< 0.01
Long[9]	35.64	49.97	1.47	< 0.01	< 0.01

[1] Expressed as number of carbon: number of double bonds.
[2] Control, no canola seed (n=4); Canola, 12.1 g/100 g DM (n=4) as ground canola seed.
[3] Standard error of the mean (n=4).
[4] Probability, the significance level of F-test for equality.
[5] Transvaccenic acids.
[6] Conjugated linoleic acid.
[7] Short-chain fatty acids (C4:0 to C12:0).
[8] Medium-chain fatty acids (C14:0 to C17:0).
[9] Long-chain fatty acids (≥C18:0).

Specifically, *cis*-9, *trans*-11 CLA, the CLA isomer of highest concentration in dairy products, is formed as an intermediate in the ruminal biohydrogenation of dietary *cis*-9, *cis*-12 linoleic acid (Harfoot and Hazlewood, 1988; Kelly *et al.* 1998). *Trans*-11 18:1, a product of the biohydrogenation of both linoleic and linolenic acids (Harfoot and Hazlewood, 1988), also leads to higher *cis*-9, *trans*-11 CLA in milk fat through post absorptive enzymatic conversion by Δ^9-desaturase (Griinari *et al.* 2000; Shingfield *et al.* 2002).

Piperova *et al.* (2002) reported that in cows fed a diet containing soybean oil (high in 18:2), duodenal flow of *cis*-9, *trans*-11 CLA is low compared with levels found in milk fat, therefore post absorptive desaturation of *trans*-11 18:1 by Δ^9-desaturase is the primary source of *cis*-9, *trans*-11 CLA in milk fat.

Current evidence also suggests that *cis*-9 18:1 is converted to *trans*-11 18:1 *in vitro* (Mosley *et al.* 2002); dietary oleic acid may potentially lead to elevated CLA in milk fat as well.

In humans, dietary intake of *trans*-11 18:1 increases serum *cis*-9, *trans*-11 CLA (Turpeinen *et al.* 2002); therefore, increasing both *cis*-9, *trans*-11 CLA and *trans*-11 18:1 in dairy products would presumably be beneficial to human health.

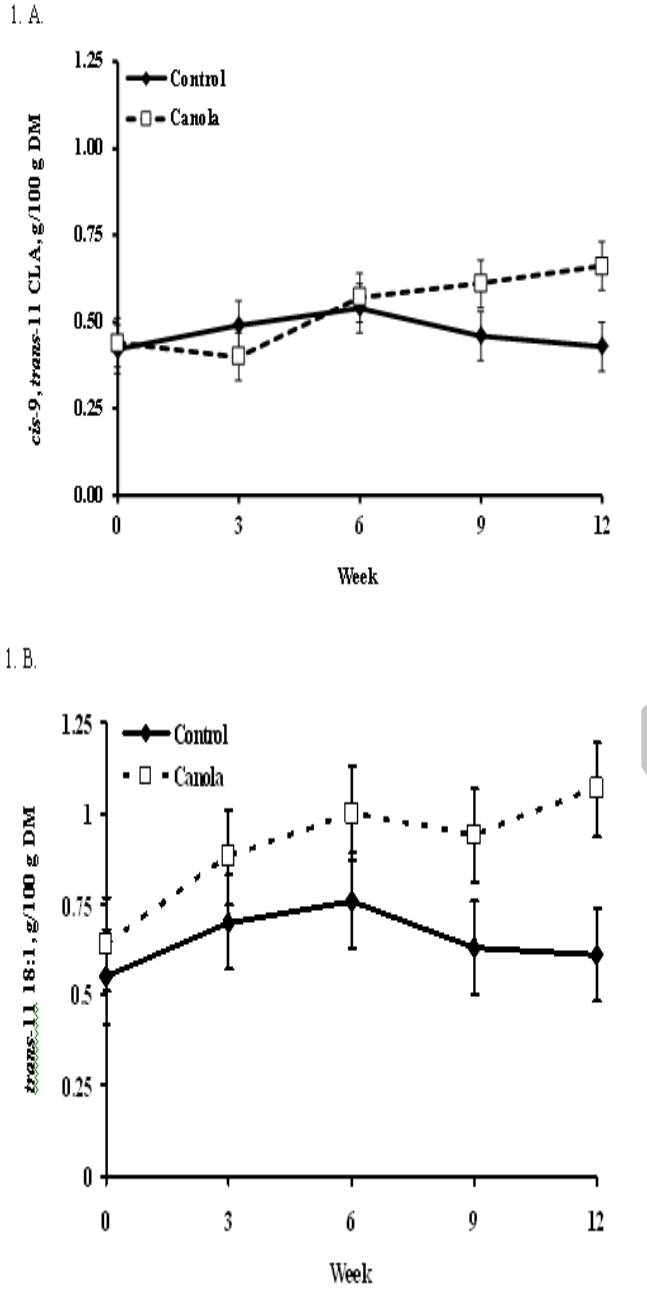

Figure 1 *Cis*-9, *trans*-11 CLA (A) and *trans*-11 18:1 (B) in milk fat from cows (n=4) fed either control (no canola) or canola (12.1 g/100 g DM). Values are least squares means ±SEM. (A) Effects in model: treatment (P=0.22), week (P=0.001), and treatment × week (P=0.001). Treatment × week effects: week 0 (P=0.78), week 3 (P=0.20), week 6 (P=0.63), wk 9 (P=0.03), and week 12 (P=0.002). (B) Effects in model: treatment (P=0.06), week (P=0.0002), and treatment × week (P=0.02). Treatment × wk effects: week 0 (P=0.50), week 3 (P=0.17), week 6 (P=0.07), week 9 (P=0.02) and week 12 (P=0.001)

In ruminants, nearly all milk fatty acids containing 4 to 14 carbons and half of those containing 16 carbons in length are the products of *de novo* fat synthesis, while half of the 16-carbon and nearly all long-chain fatty acids (18 carbons and greater) are absorbed intact (Palmquist *et al.* 1969). In Experiment 1, supplementation of canola decreased short and medium-chain fatty acids in milk fat, while long-chain fatty acids were increased. This response has been observed in other studies where canola was supplemented (Khorasani *et al.* 1991; Aldrich *et al.* 1997). The reduction in *de novo* synthesized fatty acids in milk is driven by increased intake and ruminal passage of long-chain fatty acids, which compete with shorter chain fatty acids during esterification to triglycerides in the mammary gland (Palmquist *et al.* 1993). In fact, specific long-chain fatty acids have been shown to potently inhibit *de novo* fat synthesis in the mammary gland leading to a significant reduction in total milk fat content (Dhiman *et al.* 2005; Collomb *et al.* 2006; Bauman *et al.* 2011).

The major fatty acid in the canola diet is oleic acid, which is biohydrogenated primarily to stearic acid in the rumen (Harfoot and Hazlewood, 1988). Stearic acid (18:0) in milk fat from canola fed cows was lower at wk 9 (15.22 g/100 g fatty acids) and week 12 (13.77 g/100 g fatty acids) vs. week 3 (16.97 g/100 g fatty acids) and week 6 (17.34 g/100 g fatty acids). Perhaps the amount or activity of Δ^9-desaturase influenced this value and the conversion of *trans*-11 18:1 to *cis*-9. Studies confirm that the endogenous synthesis of CLA occurs in the mammary gland by Δ^9-desaturase (Dhiman *et al.* 2005), and further investigation explores factors that influence and regulate Δ^9-desaturase activity in the tissues of ruminants. A likely scenario is that the extent of biohydrogenation of dietary unsaturated fatty acids and the amount of postruminal *trans*-11 18:1 influenced the concentration of both *cis*-9, *trans*-11 CLA and *trans*-11 18:1 in milk fat. Therefore, *cis*-9, *trans*-11 CLA and *trans*-11 18:1 in milk fat of cows fed canola were higher than the control only when 18:0 concentration decreased, indicating that perhaps the extent of ruminal biohydrogenation of oleic, linoleic, and linolenic acids to stearic acid was greater at wk 3 and 6 than at wk 9 and 12. Mosley *et al.* (2002) found that oleic acid is biohydrogenated to several *trans* 18:1 isomers in addition to stearic acid *in vitro*. In the present study, it is possible that dietary oleic acid from canola lead to higher *trans* 18:1 isomers in the rumen which was, in part, the source of higher *cis*-9, *trans*-11 CLA and *trans*-11 18:1 in milk fat.

Other studies have shown that processed oilseeds (i.e., extruded or roasted full fat soybeans) can increase the CLA content of milk fat (Dhiman *et al.* 2000; Abu Ghazaleh *et al.* 2002; Dhiman *et al.* 2005), whereas, raw soybeans have no effect (Dhiman *et al.* 2000).

Table 4 Dry matter intake, milk yield, and milk composition of cows fed diets containing canola or sunflower seed

	Canola study[1]				Sunflower study[2]			
Item	Control	Canola	SEM[3]	*P*[4]	Low	High	SEM[3]	*P*[4]
Milk composition								
Fat, %	3.41	3.71	0.16	0.26	4.03	4.07	0.14	0.83
Fat, kg/d	1.28	1.38	0.07	0.34	1.55	1.67	0.13	0.54
Protein, %	2.80	2.73	0.04	0.29	2.65	2.57	0.05	0.30
Protein, kg/d	1.05	1.01	0.04	0.51	1.02	1.05	0.03	0.46
Milk yield, kg/d	37.9	36.9	1.2	0.62	38.3	40.9	1.8	0.36
3.5% FCM[5], kg/d	37.8	37.5	2.6	0.95	41.9	44.7	2.4	0.46
DMI, kg/d	22.7	23.6	1.0	0.51	23.1	23.2	1.4	0.68

[1] Control: no canola seed (n=4) and Canola: 12.1 g/100 g DM (n=4) as ground canola seed.
[2] Low: 2.0 g/100 g DM (n=4) and High: 11.4 g/100 g DM (n=4) as rolled sunflower seed.
[3] Standard error of the mean (n=4).
[4] Probability, the significance level of F-test for equality.
[5] Fat-corrected milk= (0.4225 × milk yield) + (16.425 × fat yield).

Table 5 Composition of fatty acids in milk from cows fed different levels of sunflower seed

	Treatment[2]		SEM[3]	*P*[4]	
Fatty acid[1]	Low	High		Treatment	Treatment × week
(g/100 g fatty acids)					
4:0	5.61	5.90	0.09	0.07	0.13
6:0	2.67	2.40	0.09	0.09	0.20
8:0	1.47	1.16	0.07	0.02	0.13
10:0	3.27	2.27	0.19	0.01	0.02
12:0	3.14	2.13	0.20	0.01	0.01
14:0	10.75	8.61	0.61	0.05	0.01
14:1	0.84	0.71	0.06	0.20	0.20
15:0	1.10	0.92	0.07	0.11	0.01
16:0	26.68	24.32	1.43	0.29	< 0.01
16:1	1.47	1.45	0.04	0.76	0.96
17:0	0.60	0.52	0.03	0.08	0.01
18:0	13.31	14.91	0.79	0.21	0.06
18:1	23.48	28.26	1.66	0.09	< 0.01
trans-11 18:1[5]	0.92	1.42	0.15	0.06	< 0.01
18:2	4.55	5.44	0.28	0.07	< 0.01
cis-9, *trans*-11 18:2[6]	0.51	0.67	0.06	0.11	< 0.01
18:3	0.34	0.34	0.02	0.88	0.37
20:0	0.16	0.15	0.01	0.65	0.17
20:3	0.16	0.15	0.01	0.44	0.11
20:4	0.22	0.20	0.01	0.36	0.79
20:5	0.05	0.04	0.01	0.22	0.17
22:4	0.05	0.05	0.01	0.84	0.81
22:5	0.07	0.07	0.01	0.86	0.69
Short[7]	16.16	13.86	0.53	0.02	0.03
Medium[8]	41.44	36.53	2.13	0.15	< 0.01
Long[9]	42.39	49.60	2.56	0.09	< 0.01

[1] Control: no canola seed (n=4) and Canola: 12.1 g/100 g DM (n=4) as ground canola seed.
[2] Low: 2.0 g/100 g DM (n=4) and High: 11.4 g/100 g DM (n=4) as rolled sunflower seed.
[3] Standard error of the mean (n=4).
[4] Probability, the significance level of F-test for equality.
[5] Fat-corrected milk= (0.4225 × milk yield) + (16.425 × fat yield).
[6] Conjugated linoleic acids.
[7] Short-chain fatty acids (C4:0 to C12:0).
[8] Medium-chain fatty acids (C14:0 to C17:0).
[9] Long-chain fatty acids (≥C18:0).

These findings and those of the present study suggest a need for some type of processing to make the oil available to rumen microbes for biohydrogenation. Dhiman *et al.* (2000) reported that free oil causes greater increases in CLA compared with oilseeds, likely an effect of the oil being immediately available for biohydrogenation by rumen microbes, thereby influencing the flow of intermediates to the duodenum.

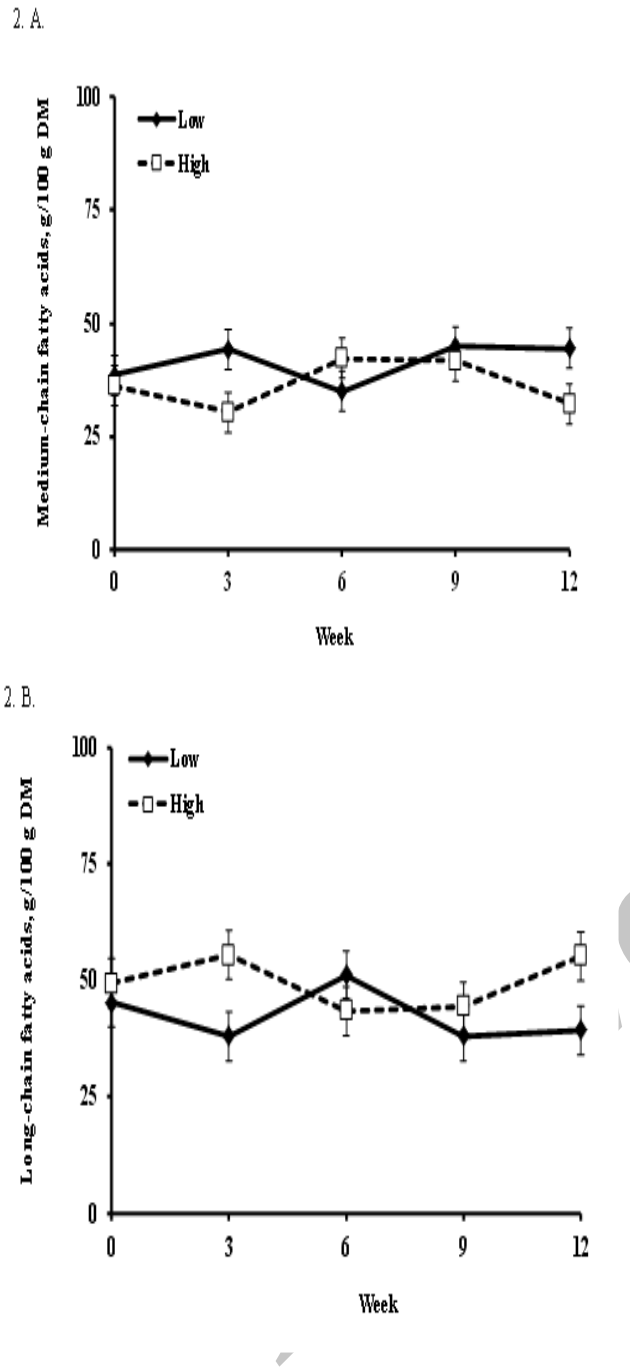

Figure 2 Medium-chain fatty acids (14:0 to 17:0) (A) and long-chain fatty acids (18:0 to 22:6) (B) in milk fat from cows (n=4) fed either Low (2.0 g/100 g DM) or High (11.4 g/100 g DM) sunflower seed. Values are least squares means ±SEM. (A) Effects in model: treatment (P=0.15), week (P=0.14), and treatment × week (P=0.001). Treatment × week effects: week 0 (P=0.59), week 3 (P=0.005), week 6 (P=0.12), week 9 (P=0.47), and week 12 (P=0.01). (B) Effects in model: treatment (P=0.09), week (P=0.15), and treatment × week (P=0.001). Treatment × week effects: week 0 (P=0.44), week 3 (P=0.003), week 6 (P=0.16), week 9 (P=0.24), and week 12 (P=0.006)

The magnitude of CLA increase in the present study was not as great as anticipated, primarily due to the low oil content of the ground canola seed (28.8 g/100 g DM) compared with the typical content (40.0 to 45.0 g/100 g DM). Nonetheless, canola did increase CLA in milk fat.

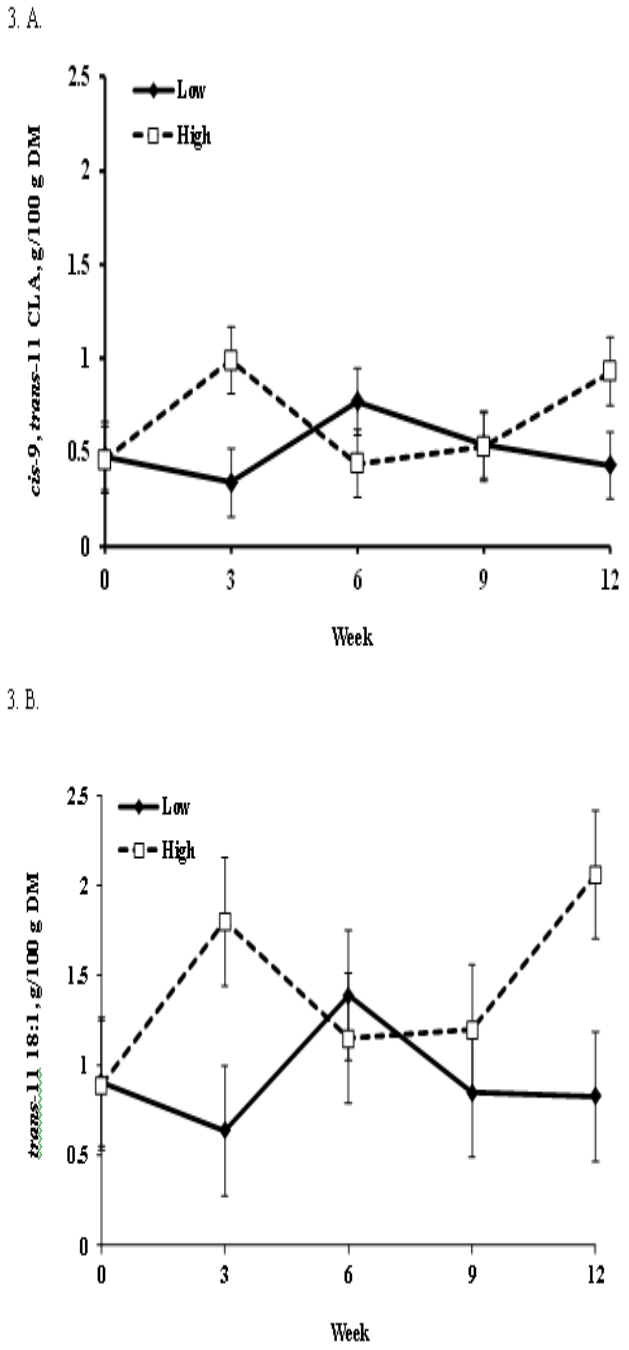

Figure 3 *Cis*-9, *trans*-11 CLA (A) and *trans*-11 18:1 (B) in milk fat from cows (n=4) fed either Low (2.0 g/100 g DM) or High (11.4 g/100 g DM) sunflower. Values are least squares means ±SEM. (A) Effects in model: treatment (P=0.11), week (P=0.36), and treatment × week (P=0.001). Treatment × week effects: week 0 (P=0.92), week 3 (P=0.001), week 6 (P=0.52), week 9 (P=0.33), and week 12 (P=0.002). (B) Effects in model: treatment (P=0.06), week (P=0.14), and treatment × week (P=0.004). Treatment × week effects: week 0 (P=0.96), week 3 (P=0.004), week 6 (P=0.08), week 9 (P=0.95), and week 12 (P=0.009)

In Experiment 2, the High sunflower group had higher concentrations of *cis*-9, *trans*-11 CLA and *trans*-11 18:1 in

milk fat compared with the Low sunflower group at week 3 and week 12, but levels were similar between treatment groups at wk 6 and 9 of the experiment.

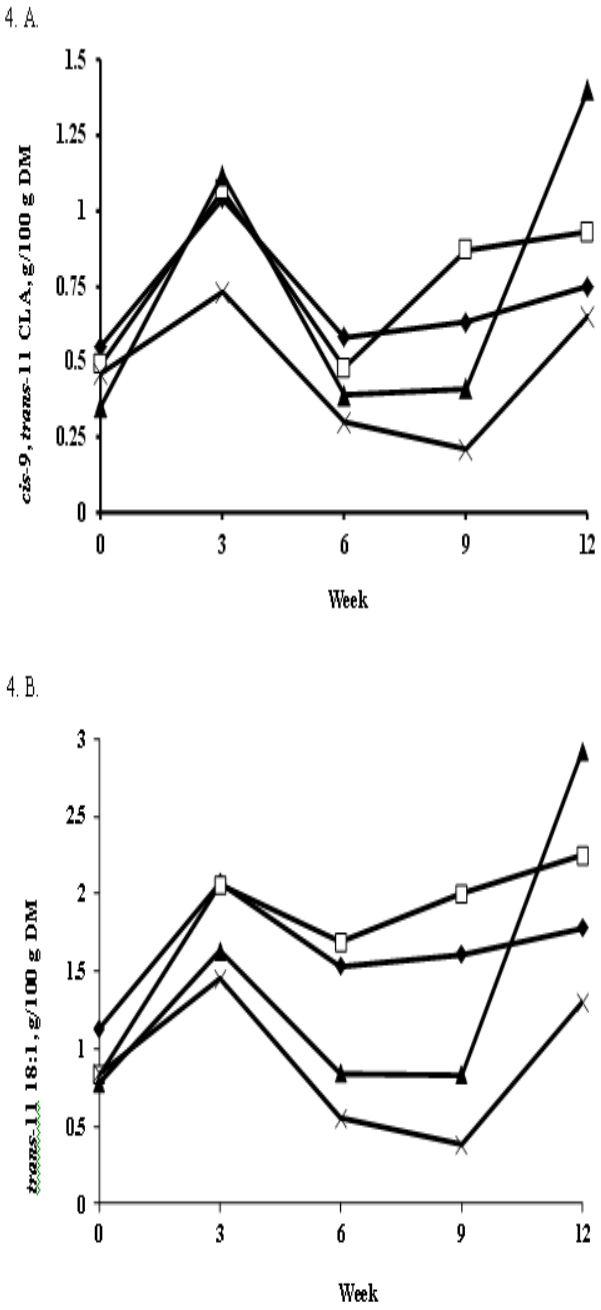

Figure 4 *Cis*-9, *trans*-11 CLA (A) and *trans*-11 18:1 (B) in milk fat from cows (n=4) fed High (11.4 g/100 g DM) sunflower. Each symbol represents an individual cow, and results are representative of samples taken from 2 consecutive milkings every 3 week

Dayani *et al.* (2004) reported that CLA yield is highly variable in cows consuming a diet containing sunflower seeds; yield increased substantially over the first wk, but declined over the subsequent 2 weeks. Abu Ghazaleh *et al.* (2004) determined that *cis*-9, *trans*-11 CLA and *trans*-11 18:1 in milk fat is promptly elevated over the first 3 wk of a 10 weeks experiment by supplementing fish meal and extruded soybeans to dairy cows. The concentrations decline at wk 4 but remain stable from week 5 to 10 (Abu Ghazaleh *et al.* 2004).

Abu Ghazaleh *et al.* (2004) hypothesized that their results were possibly due to ruminal adaptation to fat supplementation.

Our findings support this hypothesis, but it is unclear why concentrations of *cis*-9, *trans*-11 CLA and *trans*-11 18:1 were once again elevated at wk 12. Conjugated linoleic acid content of milk fat is highly variable among individual cows consuming the same diet (Kelly *et al.* 1998; Peterson *et al.* 2002).

In the present study, individual cow variation and limited observations influenced the change in *cis*-9, *trans*-11 CLA content of milk fat. When examining the changes in *cis*-9, *trans*-11 CLA and *trans*-11 18:1 over the 12 weeks study, there were substantial differences among the four cows within the High sunflower treatment (Figure 4). *Cis*-9, *trans*-11 CLA concentration increased in all cows at wk 3 followed by a decrease at week 6.

Conversely, *trans*-11 18:1 concentration was consistently elevated in milk fat from all cows at week 3, but only two cows showed a sharp decline at week 6. Peterson *et al.* (2002) reported that when a standard lactation diet (no supplemental fat) is alternated with a diet containing extruded full fat soybeans over 12 wk, there is 2 to 3 fold difference in milk fat content of *cis*-9, *trans*-11 CLA among individual cows within the extruded full fat soybean treatment, which contributes to variability in weekly treatment averages (Peterson *et al.* 2002).

In addition, individual cows respond differently in terms of the absolute increase and consistency of *cis*-9, *trans*-11 CLA content in milk fat, potentially due to differences in Δ^9-desaturase activity among individuals consuming the same diet (Peterson *et al.* 2002).

A plausible explanation for the difference between patterns of *cis*-9, *trans*-11 CLA and *trans*-11 18:1 concentrations over time in cows consuming the High sunflower diet could be differences in Δ^9-desaturase amount or activity in the mammary gland. Our results are consistent with other studies where processed oilseeds were fed (Dhiman *et al.* 2000; Abu Ghazaleh *et al.* 2002; Peterson *et al.* 2002).

Abu Ghazaleh *et al.* (2003) reported that high linoleic sunflower (59.6 g linoleic acid/100 g fatty acids) in combination with fish oil (1.0 g/100 g DM) causes the greatest increase in *cis*-9, *trans*-11 CLA in milk fat when compared with diets containing high oleic or linolenic acid. Although variability existed, *cis*-9, *trans*-11 CLA and *trans*-11 18:1 in milk fat were enhanced by adding high levels of rolled sunflower seed to the diet.

Milk fat synthesis

Recommendations for the maximum fat content of lactating dairy cow diets are approximately 7.0 g/100 g DM (NRC 2001), and the form in which the fat is supplemented (i.e., oil vs. oilseed) seems to have different effects upon fat synthesis in mammary tissue.

Dhiman *et al.* (2000) found that plant oils such as soybean and linseed, when fed at high levels, markedly depress milk fat in lactating dairy cows. Oil fed in oilseed form has no effect on milk fat concentration, even though total dietary fat is similar between free oil and oilseed treatments (Dhiman *et al.* 2000), which agrees with the findings of Mohamed *et al.* (1988) who compared soybean and cottonseed fed as free oil or whole oilseed. Indeed, whole or processed oilseeds can cause milk fat depression (Finn *et al.* 1985; Abu Ghazaleh *et al.* 2002; Abu Ghazaleh *et al.* 2003); however, other research has reported no difference in milk fat concentration from cows consuming oilseeds (Rafalowski and Park, 1982; Markus *et al.* 1996; Dhiman *et al.* 2000).

The role of specific unsaturated fatty acids in milk fat depression has been studied; and an intermediate of linoleic acid biohydrogenation, *trans*-10, *cis*-12 CLA, has been identified as having a potent effect on milk fat synthesis in lactating cows (Baumgard *et al.* 2001). Abomasal infusion of this isomer causes a 42% decrease in milk fat percentage and a 44% reduction in milk fat yield, while the *cis*-9, *trans*-11 isomer does not reduce fat synthesis (Baumgard *et al.* 2001; Loor and Herbein, 2003). Diet induced milk fat depression is caused by specific bioactive fatty acids produced during ruminal biohydrogenation under some dietary conditions.

Multiple CLA isomers have been observed to reduce milk fat synthesis in the cow (Bauman *et al.* 2011), but most mechanistic research has focused on *trans*-10, *cis*-12 CLA.

Trans-10, *cis*-12 CLA was not detectable in milk fat from cows in either study, which agrees with findings where *trans*-10, *cis*-12 CLA is not measurable in cows fed a conventional diet unless it is infused into the abomasum (Loor and Herbein, 2003).

The potential detrimental effects of fat supplementation reported in other studies (Finn *et al.* 1985) were alleviated in this study.

Furthermore, total dietary fat content did not exceed recommended levels (NRC, 2001). During milk fat depression, mammary lipid synthesis capacity is decreased due to a coordinated down-regulation of lipid synthesis enzymes (Bauman *et al.* 2011).

The maintenance of milk fat synthesis was likely due to the slow release of oil in the rumen, the presence of sufficient dietary fiber, and the reasonable level of total fat in the experimental rations.

CONCLUSION

Despite biological variation inherent to these data, they provide an indication of effects of dietary canola and sunflower seed supplementation on milk composition. Ground canola and rolled sunflower seed added to the diets of lactating dairy cattle can effectively increase *cis*-9, *trans*-11 CLA and *trans*-11 18:1 content of milk fat. Altering the fatty acid content of milk fat can have implications in the nutritional value of milk.

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