Suranaree Journal of Science and Technology

AGRICUTURE

THE EFFECT OF SOYBEAN OIL OR SUNFLOWER OIL SUPPLEMENTATION ON DAIRY COW PERFORMANC E AND CONJUGATED LINOLEIC ACID (CLA) IN MILK

Pipat Lounglawan*

Received: Nov 14, 2005; Revised: May 23, 2006; Accepted: May 23, 2006

Abstract

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. I has been reported to have a wide range of beneficial effects, including; anticarcinogenic, antiatherogenic, antidiabetic and immune stimulatory. The objective of the present experiment was aimed at studying the increase of CLA in milk and performance of dairy cows through supplementation of high linoleic acid plant oils in dairy cattle feeds. Twenty four crossbred Holstein Friesian lactating dairy cows, averaging 22.9 ± 4.6 kg milk/d, 97 ± 41 days in milk and 451 ± 45 kg body weight, were blocked into 3 groups of 8 cows each. The first group was fed the control diet, the second and the third groups were fed the control diet together with 200 g of soybean and sunflower oils per day respectively. The experimental design was a Randomized Complete Block Design (RCBD). Dry matter and protein intakes, milk yield, milk composition and body weight change were similar ($p > 0.05$) in all treatment groups; however, net energy intake of both supplemented groups were higher than that of the control group. The $C_{6:0}$, $C_{8:0}$ and $C_{16:0}$ fatty acids in the milk of cows supplemented with plant oils were reduced ($p < 0.05$) while the $C_{18:0}$, $C_{18:1n9t}$, $C_{18:1n9c}$ and $C_{18:2n6t}$ fatty acids were significantly increased ($p < 0.05$) compared to the control cows. In conclusion, supplementation of plant oils significantly increased CLA (*cis*-9, *trans*-11 octadecadienoic) however; there was no significant difference between sunflower oil and soybean oil on CLA in milk.

Keywords: Soybean oil, sunflower oil, conjugated linoleic acid, milk production, fatty acids, dairy cows

Introduction

Recently, conjugated linoleic acid (CLA), a group of isomers of octadecadienes, has received much attention. Several beneficial effects are attributed to these isomers, e.g. anticarcinogenic and antatherosclerotic effects, influencing both fat metabolism and protein deposition (Pariza *et al*., 1987; Ha *et al*., 1989; Ip *et al*., 1991). Consumption of CLA by humans has been shown to elicit many favorable health benefits such as modulating immune functions, weight reduction, and providing protection against diseases such as cancer and arteriosclerosis (Chin *et al*., 1992; Lee *et al*., 1994; Nicolosi *et al*., 1997). CLA is naturally presented in

School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, 111 University Avenue, Amphur Muang, Nakhon Ratchasima, Thailand. 30000 Tel: 0-4422-4372; Fax: 0-4422-4150; E-mail: pipat_l2000@yahoo.com+

products originating from ruminants as a result of the specific metabolism of the ruminal production, in particular, *c*9, *t*11 CLA as the predominant isomer. However, recent studies have suggested that the endogenous synthesis of CLA by the action of Δ 9-desaturase on *trans* 18:1 fatty acids is probably more important than the ruminal production (Griinari *et al*., 2000; Santora *et al*., 2000).

When dairy cows receive diets containing fat into the rumen, there will be three processes which occur in the rumen. Firstly, lipid is hydrolyzed to be fatty acids and glycerol by extracellular enzymes produced by ruminal bacteria. Fatty acids (linoleic acid, *cis*-9 *cis*-12) are then isomerized from cis form to *trans* form at *cis*-12 position to be *trans*-11 or CLA (*cis*-9 *trans*-11). Some fatty acids are hydrogenated at *cis*-9 position to be single bond in the forms of *trans*-11 (vaccenic acid) and are further hydrogenated to be stearic acid. All the form of fatty acids can transfer to the small intestine and be absorbed to lymph vessels. Linoleic acid are then synthesized again at tissues to be CLA by Δ^9 -desaturase by adding double bond at the 9^{th} position to be in the form of *cis*-9 *trans*-11. Supplementation of high linoleic acid plant oil in the diet can probably increase the CLA content in dairy cows milk.

The objective of the present experiment was aimed at studying the increasing of CLA in milk and their performance of dairy cows through supplementation of high linoleic acid plant oils in dairy cattle feeds.

Materials and Methods

Dairy Cattle and Feeding Managements

Soybean and sunflower oils were randomly sampled from the markets. They were then analyzed for free fatty acids and especially linoleic acid. These plant oils were used in this experiment. Twenty four crossbred Holstein Friesian lactating dairy cows, averaging 22.9 ± 4.6 kg milk/d, 97 ± 41 days in milk and 451 ± 45 kg body weight, were blocked into 3 groups of 8 cows each according to a Randomized Complete Block Design (RCBD). The first group was fed the control diet, the second and the third groups were fed the control diet together with 200 g of soybean and sunflower oils per day respectively.

The experiment lasted 40 days including 10 days of adjustment period followed by six five-day periods of measurements.

Feed Intake and Milk Production

Feed offered and orts were weighed on two consecutive days of each period. Feed samples were then taken for proximate analysis (AOAC, 1990), detergent analysis (Goering and Van Soest, 1970), free fatty acids and linoleic acid (Kelly *et al*., 1998b). All cows were weighed at the start and at the end of the experiment. Milk yield was recorded daily while milk samples (evening + morning) were taken on two consecutive days in each period and being analyzed for milk compositions (Milko Scan S50, Tecator, Denmark). On days 0, 10, 20 and 30 of the experiment, milk samples were taken for free fatty acids and CLA analyses (Gas chromatography; Hewlett Packard GCD system HP 6890).

Fatty Acid Analysis

Fatty acid analysis was carried out as previous described (Hara and Radin (1978). In brief, milk fat was extracted from milk using hexane and isopropanol (3:2, vol/vol)/g of fat cake, modified from Kelly *et al*. (1998b). Heptadecanoic acid (17:0) was added as an internal standard. The fatty acid methyl esters (FAME) were analyzed by GC (HP 6890, Hewlett Packard, USA) using a CP-Sil88 column for FAME (100 m x 250 μm) (Chrompack, The Netherlands). The GC conditions were as follows: injected temperature, 240°C; detector temperature, 260°C; carrier gas, He; split ratio, 1/30; temperature program, 70°C for 4 min, followed by an increase of 13°C/min to 175°C, then 4°C/min to 215°C. Peaks were identified by comparison of retention times with those of the corresponding standards (Supelco™ 37 component FAME Mix, Sigma-Aldrich Co., USA). Identification of the peak included fatty acids between 14:0 and 22:6 and CLA isomers, i.e. *c*9, *t*11; *t*10, *c*12.

Data were subjected to analysis of variance as in the mathematical model: $X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$. The differences between means were subjected to orthogonal comparison using the Statistical Analysis System (SAS, 1988).

Results

Mean values for the chemical composition of the control diet, the diet supplemented with 200 g/d sunflower oil, the diet supplemented with 200 g/d soybean oil and corn silage are presented in Table 1. as follows: dry matter (DM) = 93.23, 92.11, 92.46 and 27.61%; crude protein (CP) = 21.52, 20.61, 20.21 and 7.57%; crude fiber (CF) = 10.36, 10.12, 9.98 and 32.37%; neutral detergent fiber (NDF) = 47.87, 44.68, 43.87 and 62.13%; acid detergent fiber (ADF) = 18.31, 17.99, 17.78 and 38.24%; and acid detergent lignin (ADL) = 5.16, 4.60, and 4.59 and 5.29% respectively. The evaluation of energy concentration in these diets were total digestible nutrients (TDN_{1x}) = 64.82, 69.48, 69.96 and 47.26%; digestible energy at production level of intake (DE_p) = 3.18, 3.49, 3.51 and 2.25 Mcal/kgDM; metabolizable energy at production level of intake (ME_p) = 2.76, 3.10, 3.11 and 1.82 Mcal/kgDM; and net energy at production level of intake (NE_{Lp}) = 1.75, 1.98, 1.99 and 1.09 Mcal/kgDM, respectively.

The fatty acid compositions of the sunflower and soybean oils are given in Table 2. Both plant oils contain high linoleic acid which will be converted to CLA in the rumen. Thus, the two oils were used in this study.

Dry matter, crude protein and net energy for lactation intakes are presented in Table 3. There were no significant differences in concentrate, grass silage and total dry matter and crude protein intakes of the experimental cows. Total DM intakes of the control, sunflower oil and soybean oil cows were 15.04, 14.19 and 14.48

Table 1. Ingredient and calculated nutrient composition of basal diets (as-fed basis)

Item	Concentrate			Corn silage
	Control	Sunflower oil	Soybean oil	
Chemical composition	---------- % of DM ----------			
Dry matter	93.23	92.11	92.46	27.61
Crude protein	21.52	20.61	20.21	7.57
Ether extract	3.80	5.79	5.81	1.37
Ash	7.51	7.36	7.21	15.37
Crude fiber	10.36	10.12	9.98	32.37
Neutral detergent fiber	47.87	44.68	43.87	62.13
Acid detergent fiber	18.31	17.99	17.78	38.24
Acid detergent lignin	5.16	4.60	4.59	5.29
Neutral detergent insoluble N	1.28	1.24	1.28	0.56
Acid detergent insoluble N	0.85	0.74	0.74	0.47
TDN_{1x} (%)	64.82	69.48	69.96	47.26
DE_P (Mcal/kgDM)	3.18	3.49	3.51	2.25
ME_P (Mcal/kgDM)	2.76	3.10	3.11	1.82
NE_{LP} (Mcal/kgDM)	1.75	1.98	1.99	1.09

[1] TDN_{1X} (%) = tdNFC + tdCP + (tdFA × 2.25) + tdNDF -7

[2] DE_{1X} (Mcal/kg) = [(tdNFC/100) × 4.2] + [(tdNDF/100) × 4.2] + [(tdCP/100) × 5.6] + [(FA/100) × 9.4]-0.3

Discount = [(TDN_{1X} - [(0.18 × TDN_{1X}) - 10.3]) × Intake)]/TDN_{1X}

DE_p (Mcal/kgDM) = DE_{1X} × Discount

[3] ME_p = [1.01 × (DE_p) - 0.45] + [0.0046 x (EE - 3)]

[4] NE_{Lp} = ([0.703 × ME_p (Mcal/kg)] - 0.19) + ([(0.097 × ME_p + 0.19)/97] × [EE - 3])

Source: NRC, (2001)

kg/d while those of grass silage were 5.55, 4.74 and 5.29 kg/d, respectively. However, cows on the plant oils group significantly consumed more ($p < 0.05$) net energy than cows on the control group.

Milk yield and milk composition of the 3 groups are given in Table 4. Milk yield and 3.5% fat corrected milk as well as the concentration and the yield of fat, protein, lactose, solid not fat and total solid concentration from the 3 group of cows were similar ($P > 0.05$).

As for the body weight at the end of the experiment and live weight change were not significantly different among the 3 groups of cows ($P > 0.05$).

Fatty acid compositions in milk fat of the 3 groups of cows are shown in Table 5. Fatty acids $C_{6:0}$, $C_{8:0}$ and $C_{16:0}$ were significantly reduced ($p < 0.05$) while $C_{18:0}$, $C_{18:1n9t}$, $C_{18:1n9c}$, and $C_{18:2n6}$ fatty acids were significantly increased ($p < 0.05$) by plant oils supplementation compared to the control diet. However $C_{18:2n6c}$ fatty acids were similar ($p > 0.05$) in all treatment diets. CLA (*cis*-9, *trans*-11 octadecadienoic) were significantly increased ($p < 0.05$) by plant oils supplementation, being 4.09, 5.50 and

Table 2. Fatty acid composition of feed and plant oils

Item	Concentrate	Corn silage	Sunflower oil	Soybean oil
	----------------------- g/100g of total fatty acid	--------------------------		
$C_{14:0}$	7.74	1.36	0.06	0.06
$C_{16:0}$	1.57	36.85	10.88	8.23
$C_{18:0}$	2.81	6.69	4.19	3.75
$C_{18:1n9c}$	24.99	11.83	21.28	30.01
$C_{18:2n6c}$	20.16	24.39	55.45	53.03
$C_{20:0}$	0.40	1.68	0.33	0.29
$C_{18:3n6}$	0.05	0.00	0.62	0.33
$C_{20:1}$	0.23	0.00	6.74	3.60
$C_{22:0}$	0.23	2.14	0.36	0.53
$C_{24:0}$	-	0.43	0.09	0.16
Others	41.82	14.63	-	-

Table 3. Dry matter, crude protein and net energy for lactation intakes of the experimental cows

Item	Control (1)	Sunflower oil (2)	Soybean oil (3)	SEM	Contrast 1 vs 2 &3	Contrast 2 vs 3
DM intake (kgDM)						
Concentrate	9.46	9.46	9.46	-	-	-
Roughage	5.55	4.74	5.29	0.81	0.4155	0.4698
Total	15.04	14.19	14.48	0.74	0.2857	0.7025
CP intake (g/d)						
Concentrate	2,036	2,036	2,036	-	-	-
Roughage	460	518	471	43.51	0.3700	0.2956
Total	2,496	2,554	2,508	43.49	0.3697	0.2956
NE_{Lp} intake (Mcal)						
Concentrate	16.60	18.80	18.80	-	-	-
Roughage	6.03	5.16	5.74	0.80	0.4250	0.4887
Total	22.63	23.99	24.57	0.87	0.0305	0.4827

6.12 mg/g fat for the control, sunflower oil and soybean oil diet, respectively. There were no significant differences in fatty acid composition between the two plant oils. Supplementation of either sunflower oil or soybean oil significantly reduced short and medium chain fatty acids while significantly increased long chain fatty acids and unsaturated fatty acids. Saturated fatty acids were not affected by either of the plant oils supplementation.

Discussion

Both concentrates supplemented with the two plant oils showed slightly lower chemical compositions than the control concentrate a except for fat and energy. This is due to the fact that plant oil contains higher energy concentration and has 86% true digestibility (NRC, 2001). Estimates of TDN_{1x}, DE_p and NE_{Lp} in the oil supplemented concentrates therefore were relatively high.

Analyses of the types and contents of fatty acids in this experiment were close to those reported by Chow (2000) and Dhiman *et al*. (1999). They reported that plant oils containing linoleic acid contents in descending order were safflower, sunflower, corn, soybean, cottonseed, sesame, rice bran, peanut and palm oil. Supplementation of sunflower and soybean oils to dairy cow's rations should increase the linoleic acid content in the diet and thus should increase the CLA content in milk.

All the forms of fatty acids can transfer to the small intestine and be absorbed to lymph vessels. These fatty acids are then synthesized again at tissues to be CLA by Δ^9- desaturase by adding double bond at the 9th position to be in the form of *cis*-9 *trans*-11. (Griinari *et al*., 1999; Baer *et al*., 2000; Abu-Ghazaleh *et al*., 2001; 2002; Whitlock *et al*. 2002). Corl *et al*. (2000) reported that 65% of CLA synthesis depended on Δ^9-desaturase.

There were no significant differences in DM and CP consumption in the present study. Two hundred grams/cow/day of plant oils did not affect feed intake. Khorasani and Kennelly (1998) reported that a supplement of more than

Table 4. Milk yield and milk composition and Body weight and BWC of the experimental cows

Item	Control (1)	Sunflower oil (2)	Soybean oil (3)	SEM	Contrast	
					1 vs 2 &3	2 vs 3
Milk yield (kg/d)	17.8	18.5	18.6	0.96	0.5907	0.9858
3.5%FCM (kg/d)	18.7	17.7	18.3	1.09	0.5959	0.7532
Fat (%)	3.79	3.29	3.48	0.22	0.1620	0.5694
Protein (%)	2.68	2.82	2.99	0.09	0.0617	0.2330
Lactose (%)	4.52	4.60	4.66	0.08	0.3416	0.7045
SNF (%)	8.14	8.34	8.61	0.14	0.0582	0.3443
Total solid (%)	11.92	11.68	12.10	0.31	0.9189	0.3880
Fat yield (g/d)	682	599	636	54.36	0.3381	0.6817
Protein (g/d)	478	514	548	23.52	0.0875	0.3669
Lactose (g/d)	807	848	860	46.04	0.4503	0.9389
SNF (g/d)	1,451	1,540	1,586	76.05	0.2681	0.7500
Total solid (g/d)	2,132	2,139	2,222	119.3	0.7828	0.6965
Final weight (kg)	450	453	447	16.39	0.9792	0.8080
BWC (g/d)	-70.84	79.17	19.05	130.4	0.4320	0.8348

SEM = standard error of the mean

BWC = body weight change

2% fish oil reduced DM intake. Similarly, Donovan *et al*. (2000) suggested that DM intake and milk yield were decreased by 2% and 3% fish oil supplementation when compared to 1% supplementation. The reason for reducing feed intake when there was high oil supplementation was probably due to palatability and feed degradation in the rumen. When oil is supplemented at a high level, oil may coat fiber particles in the rumen and thus decrease the rate of fiber degradation and reduced feed intake (Murphy *et al*., 1987; Khorasani and Kennelly, 1998). Mohammed *et al*. (1988) also reported similar results when 4% of oil was supplemented

Table 5. Fatty acid composition of milk fat of the experimental cows

Item	Control	Sunflower oil	Soybean oil	SEM	Contrast	
	(1)	(2)	(3)		1 vs 2 &3	2 vs 3
	---------------- mg/g fat ----------					
$C_{4:0}$	17.30	15.71	15.87	0.79	0.1222	0.9996
$C_{6:0}$	13.09	11.19	10.57	0.66	0.0130	0.4967
$C_{8:0}$	7.62	6.23	5.53	0.49	0.0106	0.3394
$C_{10:0}$	15.97	15.65	11.70	1.61	0.2490	0.0978
$C_{11:0}$	1.93	1.35	1.72	0.24	0.1981	0.3238
$C_{12:0}$	50.49	45.96	44.81	2.23	0.0830	0.7466
$C_{13:0}$	1.54	1.44	1.32	0.18	0.4616	0.6209
$C_{14:0}$	101.13	95.28	91.90	4.86	0.2348	0.6711
$C_{14:1}$	9.74	8.04	10.08	0.95	0.5974	0.1427
$C_{15:0}$	6.03	5.18	5.42	0.33	0.0906	0.6404
$C_{16:0}$	262.35	223.63	236.12	10.10	0.0193	0.3782
$C_{16:1}$	18.65	14.87	19.13	0.85	0.1504	0.0024
$C_{18:0}$	76.51	111.48	97.91	5.22	0.0004	0.0648
$C_{18:1n9t}$	16.39	26.49	30.29	3.05	0.0051	0.4136
$C_{18:1n9c}$	181.84	228.12	232.64	13.03	0.0079	0.8612
$C_{18:2n6t}$	0.430	1.06	1.49	0.10	0.0001	0.0072
$C_{18:2n6c}$	12.13	12.71	14.55	1.82	0.5299	0.5277
$C_{20:0}$	1.31	1.46	2.73	0.51	0.2290	0.1071
$C_{18:3n6}$	0.99	1.06	1.58	0.11	0.0304	0.0045
CLA[1]	4.09	5.50	6.12	0.57	0.0246	0.4572
$C_{22:0}$	>0.01	0.08	>0.01	0.03	0.3317	0.0781
$C_{20:3n3}$	0.04	0.16	0.09	0.06	0.2795	0.5052
$C_{22:1n9}$	0.84	0.44	0.39	0.11	0.0051	0.7709
Short[2]	107.95	97.55	91.52	5.96	0.0177	0.3174
Medium[3]	397.90	346.99	362.66	21.07	0.0334	0.4446
Long[4]	294.56	388.62	387.86	26.59	0.0008	0.9002
Saturated	553.97	533.11	522.87	27.11	0.2830	0.7046
Unsaturated	246.46	300.05	319.16	22.29	0.0047	0.4452

[1] CLA = *cis*-9, *trans*-11 *octadecadienoic acid*
[2] Short chain FA: ($C_{4:0}$ - $C_{13:0}$)
[3] Medium chain FA: ($C_{14:0}$ - $C_{17:0}$)
[4] Long chain FA: (≥ $C_{18:0}$)
SEM = standard error of the mean

in the diet. However, cows on plant oil supplements had a higher energy intake than those cows in the control group. As increase in energy intake reflected the energy concentration in plant oils.

Milk yield was not affected by plant oil supplementation. This agreed with research done by Dhiman *et al*. (2000) who supplemented 1, 2, 3 and 4% of soybean oil to dairy cattle diets and found that milk yield was similar in all treatment groups. However, previous work of Dhiman *et al*. (1999) found increases in milk yield when the diet was supplemented with extruded soybeans or extruded cotton seeds. This can be attributed to the fact that extruded oil seeds contained bypass protein and thus higher amino acids were absorption at the intestines (Solomon *et al*., 2000; Madron *et al*., 2002). Cant *et al*. (1997) suggested that milk yield would have been affected by oil when the rate of addition was 500 g/day onwards. Cows received less than 500 g/d of oil showed no effects on milk yield, fat and protein content.

Milk fat is generally synthesized from dietary fat and fat from the body reserved in the adipose tissues. However, if cows received adequate fat from feeds, mobilization of fat from body reserves is reduced (Holmes and Wilson, 1984).

Although there were no significant differences in milk composition among treatment groups, fat yield and fat content tended to be reduced in the supplemented cows. This may be due to a reduction of fiber digestion in the rumen. End products of fiber digestion were predominantly acetate and butyrate which were the precursors for milk fat synthesis in mammary glands. Reduced fiber digestion affected reduction of these volatile fatty acids and thus reduced milk fat synthesis (Khorasani and Kennelly, 1998).

Both sunflower and soybean oils contained higher long chain fatty acids ($C_{18:0}$ - $C_{22:6}$) and the fatty acid profile in milk fat reflected fatty acids in feeds, thus long chain fatty acids increased, while short ($C_{4:0}$ - $C_{13:0}$) particularly $C_{6:0}$ and $C_{8:0}$ and medium chain ($C_{14:0}$ - $C_{17:0}$) fatty acids particularly $C_{16:0}$ decreased in the present study. Donovan *et al*. (2000) and Dhiman *et al*. (1999, 2000) also found similar results. Short and medium chain fatty acids were synthesized de novo in mammary glands for which acetate was believed to be a precursor for short chain fatty acids in tissues. Oil supplementation reduced fiber digestion and thus short chain fatty acids were decreased (Banks *et al*., 1984; Grummer, 1991; Palmquist *et al*., 1993). However, Ney (1991) suggested that reduction in medium chain fatty acids reduced the risk of accumulation of cholesterol in the blood stream. Long chain fatty acids ($C_{18:0}$, $C_{18:1n9t}$, $C_{18:1n9c}$, $C_{18:2n6t}$) increased with oil supplementation in this study, which was similar to the finding of Donovan *et al*. (2000) and Dhiman *et al*. (1999, 2000).

Cows in the plant oils supplementation groups showed significantly higher CLA (*cis*-9, *trans*-11 octadecadienoic) content in milk than cows on the control group. Similarly, Dhiman *et al*. (2000) found significant increases in CLA content in milk (237% and 314%) when 3 and 4% of soybean oil were supplemented respectively. Kelly *et al*. (1998a) also found that an increase of 500% CLA in milk was supplemented by 5.3% of oil to dairy cattle diets. However, milk fat content was reduced from 3.38% to 2.25%. This is because oil containing polyunsaturated fatty acids inhibits ruminal microbial growth and fiber digestion. The inhibitory effects of oil on ruminal digestion may decrease ruminal acetate to propionate ratios. The increased mammary uptake of fatty acids of dietary origin compensated for the decrease in de novo synthesis of milk fat from acetic acid. (Khorasani *et al*., 1991)

Acknowledgement

The author would like to thank University Dairy Farm for providing the experimental cows and shed, and the Center for Scientific and Technological Equipment for providing laboratory facilities. Financial support was provided by the Research and Development Institute, Suranaree University of Technology.

References

Abu-Ghazaleh, A.A., Schingoethe, D.J., and Hippen, A.R. (2001). Conjugated linoleic acid and other beneficial fatty acid in milk from cows fed soybean meal, fish meal, or both. J. Dairy Sci., 84:1,845-1,850.

Abu-Ghazaleh, A.A., Schingoethe, A.R., Hippen, D.J., and Whitlock, L.A. (2002). Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) concentration of milk. J. Dairy Sci., 85:624-631.

Association of Official Analytical Chemists. (1990). Official Method of Analysis. 15th ed. Washington DC., 1,298 p.

Baer, R.J., Ryail, J., Schingoethe, D.J., Kasperson, K.M., Donovan, D.C., Hippen A.R. and Franklin, S.T. (2000). Composition and properties of milk and butter from cows fed fish oil. J. Dairy Sci., 84:345-353.

Banks, W., Clapperton, J.L., Girdler, A.K., and Steele, W. (1984). Effect of inclusion of different forms of dietary fatty acid on the yield and composition of cow's milk. J. Dairy Res., 51:387-395.

Cant, J.P., Fredeen, A.H., MacIntyre, T., Gunn, J., and Crowe, N. (1997). Effect of fish oil and monensin on milk fat composition in dairy cows. Can. J. Anim Sci., 77:125-131.

Chin, S.F., Liu, W., Storkson, J.M., Ha, Y.L., and Pariza, M. W. (1992). Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Comp. Anal., 5:185-197.

Chouinard, P.Y., Corneau, L., Barbano, D.M., Metzger, L.E., and Bauman, D.E., (1999). Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr., 129: 1,579-1,584.

Chow, C.K. (2000). Fatty Acid in Foods and Their Health Implications. 2nd ed. Marcel Dekker Inc., NY, USA, 1,045 p.

Corl, B.A., Baumgard, L.H., Dwyer, D.A., Griinari, J.M., Phillips, B.S., and Bauman., D.E. (2000). The role of Δ^9-desaturase in the production of *cis*-9, *trans*-11 CLA and other Δ^9-desaturated fatty acid in milk fat. J. Dairy Sci., 83(Suppl. 1):154. (Abstr.).

Dhiman, T.R., Helmink, E.D., Mcmahon, D.J., Fife, R.L., and Pariza, M.W. (1999). Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci., 82:412-419.

Dhiman, T.R., Satter, L.D., Pariza, M.W., Galli, M.P., Albright, K., and Tolosa, M.X. (2000). Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci., 83:1,016-1,027.

Donovan, D.C., Schingoethe, D.J., Baer, R.J., Ryali, J., Hippen, A.R., and Franklin, S.T. (2000). Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci., 83:2,620-2,628.

Goering, H.K., and Van Soest, P.J. (1970). Forage Fibre Analysis. Agric. Handbook No. 379, ARS./USDA, Washington. DC., p. 378-598.

Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V., and Bauman, D.E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J. Nutr., 130:2,285-2,291.

Griinari, J.M., Nurmela, K., Dwyer, D.A., Barbano, D.M., and Bauman, D.E. (1999). Variation of milk fat concentration of conjugated linoleic acid and milk fat percentage is associated with a change in ruminal biohydrogenation. J. Anim. Sci., 77(Suppl.1):117-118. (Abstr.).

Grummer, R.R. (1991). Effect of feed on the composition of milk fat. J. Dairy Sci., 74:3,244-3,247.

Ha, Y. L., Storkson, J., and Pariza, M. W. (1989). Newly recognized anticacinogenic fatty acids: indentification and quantification in natural and processed cheeses. J. Agric. Food Chem., 37:75-81.

Hara, A., and Radin, N.S. (1978). Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem., 90:420-426.

Holmes, C.W., and Wilson, G.F. (1984). Milk

Production from Pasture. Butterworths Agricultural Books., Wellington., New Zealand. 451 p.

Ip, C., Chin, S.F., Scimeca, J.A., and Pariza, M.W. (1991). Mammary cancer prevention by conjugated dienoic derivatives of linoleic acid. Cancer Res., 51:6,118-6,124.

Kelly, M.L., Berry, J.R., Dwyer, D.A., Griinari, J.M., Chouinard, P.Y., Van Amburgh, M.E., and Bauman, D.E. (1998a). Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr., 128:881-885.

Kelly, M.L., Kolver, E.S., Bauman, D.E., Van Amburgh, M.E., and Muller, D.E. (1998b). Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. J. Dairy. Sci., 81:1,630-1,636.

Khorasani, G.R., and Kennelly, J.J. (1998). Effect of added dietary fat on performance, rumen characteristics, and plasma metabolites of midlactation dairy cows. J. Dairy Sci., 81:2,459-2,468.

Khorasani, G.R., Robinson, P.H., De Boer, G., and Kennelly, J.J. (1991). Influence of canola fat on yield, fat percentage, fatty acid profile, and nitrogen fractions in Holstein milk.J.Dairy Sci., 74:1,904-1,911.

Lee, K.N., Kritehevsky, D., and Pariza, M.W. (1994). Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis., 108:19-25.

Madron, M.S., Peterson, D.G., Dwyer, D.A., Corl, B.A., Baumgart, L.H., Beerman, D.H., and Bauman, D.E. (2002). Effect of extruded full fat soybeans on conjugated linoleic acid content of intramuscular, intramuscular and subcutaneous fat in beef steers. J. Anim. Sci., 80:1,135-1,143.

Mohamed, O.E., Satter, L.D., Grummer, R.R., and Ehle, F.R. (1988). Influence of dietary cottonseed and soybean on milk production and composition. J. Dairy Sci., 71:2,677-2,688.

Murphy, M., Uden, P., Palmquist, D.L., and Wiktorson, H. (1987). Rumen and total diet digestibilities in lactating cows fed diets containing full-fat rapeseed. J. Dairy Sci., 70:1,572-1,582.

Ney, D.M. (1991). Potential for enhancing the nutritional properties of milk fat. J. Dairy Sci., 74:4,002-4,012.

Nicolosi, R.J., Rogers. E.J., Kritchevsky, D., Scimeca, J.A., and Huth P.J. (1997). Conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemia hamsters. Artery., 22:266-277.

NRC, (2001). Nutrient Requirements of Dairy Cattle, 7th ed. (revised). National Academic Science, Washington, DC. 408 p.

Palmquist, D.L., Beaulieu, A.D., and Barbano, D.M. (1993). Feed and animal factors influencing milk fat composition. J. Dairy. Sci., 76:1,753-1,771.

Pariza, M.W., Ha, Y.L., and Grim, N.K. (1987). Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid. Carcinogenesis., 8:1,881.

Santora, J.E., Palmquist, D.L., and Roehrig, K.L. (2000). Trans-vaccenic acid is desaturated to conjugated linoleic acid in mice. J. Nutr., 130:208-215.

SAS. (1988). User'Guide: Statistics. SAS Institute Inc., North Carolina. 231 p.

Solomon, R., Chase, L.E., Ben-Ghedalia, D., and Bauman, D.E. (2000). The effect of nonstructural carbohydrate and addition of full fat extruded soybeans on the concentration of conjugated linoleic acid in the milk fat of dairy cows. J. Dairy Sci., 83:322-329.

Whitlock, D.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., Baer, K.J., Ramaswamy, N., and Kasperson, K.M., (2002). Fish oil and extruded soybeans fed in combination increase conjugated linoleic acids in milk of dairy cows more than when fed separately. J. Dairy Sci., 85:234-243.