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Sunflower Oil Seed (Raw-or Heat-Treated) in Lactating Dairy Cow's Diets: Effects on Milk Fatty Acids Profile and Milk Production

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Abstract: The objective of this study was to investigate the effects of dietary supplementation with sunflower oil seed (Raw-or Heat-treated) in two levels of 7.5 or 15% on unsaturated fatty acids in milk fat and performances of high-yielding lactating cows. Twenty early lactating Holstein cows were used in a complete randomized design. Treatments included: CON, control (without sunflower oil seed). LS-UT, 7.5% raw sunflower oil seed. LS-HT, 7.5% heat-treated sunflower oil seed. HS-UT, 15% raw sunflower oil seed. HS-HT, 15% heat-treated sunflower oil seed. Experimental period lasted for 4 weeks with first 2 weeks used for adaptation to the diets. Supplementation with 7.5% raw sunflower seed (LS-UT) tended to decrease milk yield with 28.37 kg day^{-1} compared with the control (34.75 kg day^{-1}). Milk fat percentage was increased with the HS-UT treatment that obtained 3.71% compared with CON that was 3.39% and without significant different. Milk protein percent was decreased by high level sunflower oil seed treatments (15%) with 3.18% whereas CON treatment is caused 3.40% protein. The cows fed added Low Sunflower Heat-Treated (LS-HT) produced milk with the highest content of total unsaturated fatty acid with 32.59 g/100 g of milk fat compared with the HS-UT with 23.59 g/100 g of milk fat. Content of C_{18} unsaturated fatty acids in milk fat increased from 21.68 g/100 g of fat in the HS-UT to 22.50, 23.98, 27.39 and 30.30 g/100 g of fat from the cow fed HS-HT, CON, LS-UT and LS-HT treatments, respectively. $C_{18:2}$ isomers of fatty acid in milk were greater by LS-HT supplementation with significant effect ($p<0.05$). Total of C_{18} unsaturated fatty acids content was significantly higher in milk of animal fed added low heat-treated sunflower (7.5%) than those fed with high sunflower. In all, results of this study showed that diet cow's supplementation with sunflower oil seed tended to reduce milk production of lactating cows but can improve C_{18} unsaturated fatty acid content in milk fat. About 7.5% level of sunflower oil seed that heated seemed to be the optimal source to increase UFA production.

Key words: Sunflower seed, milk production, fatty acid profile, unsaturated fatty acid, supplementation, Iran

INTRODUCTION

Milk fat is an important determinant of milk nutritional quality. The saturated fatty acids (FA; mainly 12:0, 14:0, 16:0 and 18:0) are considered to produce negative effects when consumed in excess whereas others (18:1, 18:2 isomers and 18:3 n-3) have well-known or potential positive effects on human health (Parodi, 2005). In addition, cis-9, trans-11 18:2, the major isomer of conjugated linoleic acids in ruminant milk is anticarcinogenic and antiatherogenic in experimental animal models (Huth *et al*., 2006). Also, ruminant milk fat content and composition can be extensively modified by nutritional factors in particular fat supplementation of the diet (Shingfield *et al*., 2008). Dietary lipids modify the composition of bovine milk fat. The simplest way of altering milk fatty acids composition is to supplement the diets to cows with unsaturated lipids. The main sources of unsaturated lipids are oilseed lipids among which linseed, rapeseed, soybeans, canola and sunflower oil seed are used both on farms and for experimental research (Glasser *et al*., 2008).

Supplementing the diet of cows with plant lipids decreased the $C_{16:0}$ and medium chain fatty acids ($C_{10:0}$, $C_{12:0}$ and $C_{14:0}$) and increased the $C_{18:0}$ and C_{18} unsaturated fatty acids content of milk fat (Palmquist *et al*., 1993). There is growing interest in feeding sunflower oil seed to dairy cows because of its FA profile; oleic and linolenic acid contributes to dietary n-3 FA and promotes increased linoleic acid isomers content while decreasing the saturated FA content of ruminant milk (Chilliard *et al*., 2007). The effects of sunflower oil seed supplementation on milk yield and composition have often been studied (Glasser *et al*.,

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2008). Many studies have used whole, rolled, crushed or ground crude sunflower seed (Casper *et al*., 1988; Rafalowski and Park, 1982; Beauchemin *et al*., 2009), sunflower oil (AbuGhazaleh and Holmes, 2007; Luna *et al*., 2008) and either extruded, micronized or formaldehyde treated sunflower seed (Petit, 2003; Drackley and Schingoethe, 1986).

The addition of plant oils in the form of intact oily seeds is less effective than free oil to increase milk VA and RA content (Dhiman *et al*., 2000). But the use of free oil in the diet is not recommended in ruminants (Garnsworthy, 1997). Because, it might inhibit rumen microbial activity and affect milk production and composition (Jenkins, 1998).

However, only a few studies have directly compared different physical forms of sunflower seed whole versus rolled crude sunflower seed (Kennelly, 1996) or ground crude versus extruded sunflower seed (Beauchemin *et al*., 2009) and several researches have reported simultaneous changes in milk FA composition after lipid supplementation of dairy cow diet (Johnson *et al*., 2002; Odongo *et al*., 2007) but no research has evaluated due to using accompany difference levels and treating methods of sunflower seed on milk FA composition in dairy cows diets.

Furthermore, feeding fats high in polyunsaturated FA can alter the FA composition of milk in a manner beneficial to human health in cluding increased proportions of monounsaturated FA and polyunsaturated FA and increased concentrations of the conjugated linoleic acid isomer cis-9, trans-11 (Hu and Willet, 2002). Also feeding heat-treated sunflower oil seed as a lipid protected from ruminal hydrogenation increased the unsaturated fatty acid composition of milk lipids (Schingoethe *et al*., 1996). Hence, the objective of this study was to investigate the effect of supplementing a dairy cow diet with sources of longchain FA such as sunflower oil seed varying in their level and treating including 2 level and raw or heat-treated sunflower seeds on milk fatty acid and milk performance in lactating dairy cows.

MATERIALS AND METHODS

Animals and diets: Twenty early lactating multiparous Holstein cows were used in a complete randomized design to evaluate responses to supplementary raw or heat-treated sunflower oil seed in two levels. The sunflower oil seed were acquired from a sunflower farm in Arak, Iran. Experimental period lasted 4 weeks and was preceded by a 2 weeks period of adaptation to the diet. Diets were formulated to meet energy and protein requirements (National Research Council, 2001) of lactating cows averaging 635 kg of BW producing 32 kg day^{-1} milk with 3.8% fat. Diets are showed in Table 1. For treating of oil seed sunflower, parts of sunflower seeds were heated in 90°C within 10 min (Pellet mill equipment made 1983 Denmark®, Animal feed factory Co, Daneh Matbu-Saveh, Iran). Cows within groups were assigned randomly to one of 5 treatments and 4 replicates. Cows were fed individually and milked three daily at 6.0, 14.00 and 20.00 h. Milk production was recorded at every milking. Cows within groups were assigned randomly to one of five treatments.

The five dietary treatments (Table 1) consisted of supplements based on either raw whole sunflower oil seed (UT) and heated whole sunflower oil seed (HT) in two levels of 7.5 and 15% total diets which would lead to about 5.3 and 5.8% fat in LS and HS diets, respectively.

Thus, the five diets were designed to yield similar CP and difference in ether extract concentrations and fatty acids as well as energy. Chemical compositions of experimental diets are shown in Table 2. Diets were fed twice daily at 8.00 and 16.00 h for 10% orts. Feed consumption was recorded initial of each week. Total

Table 1: Ingredients composition of consumed experimental diets (DM basis)

Items	CON	LS-UT	LS-HT	HS-UT	HS-HT	Diet[1] SEM
Ingredients (diet%)						
Corn silage	39.670	37.580	37.580	37.340	37.340	0.614
Alfalfa hay[2]	06.650	06.450	06.450	06.030	06.030	0.125
Barley grain	12.820	11.520	11.520	08.300	08.300	0.886
Corn grain	07.920	06.920	06.920	06.120	06.120	0.307
Canola meal[3]	02.060	02.060	02.060	02.920	02.920	0.226
Cottonseed[4]	08.580	07.580	07.580	05.260	05.260	0.611
Soybean meal[5]	11.960	11.050	11.050	08.420	08.420	0.654
Wheat bran	08.520	07.520	07.520	06.100	06.100	0.455
Beet sugar pulp	01.680	01.680	01.680	02.380	02.380	0.368
Sunflower oil seed[6]-UT	00.000	07.500	00.000	15.000	00.000	2.500
Sunflower oil seed-HT	00.000	00.000	07.500	00.000	15.000	2.500
Dicalcium phosphate	00.060	00.060	00.060	00.060	00.060	0.000
Salt, vitamin and mineral permix	00.075	00.075	00.075	00.075	00.075	0.000

[1]C = Diet of Control; LS-UT = Diet of including 7.5% untreated sunflower oil seed; LS-HT = Diet of including 7.5% heat-treated sunflower oil seed; HS-UT = Diet of including 15% untreated sunflower oil seed; HS-HT = Diet of including 15% heat-treated sunflower oil seed. [2]Alfalfa forage of third cutter from a dairy farm in Markazi province, Iran. [3]Canola meal, mech. Extract (37% CP). [4]Cottonseed, Whole with lint (23.50% CP). [5]Soybean meal, solvent (44% CP). [6]Sunflower oil seed of Blazer variety provided from a farm sunflower in Markazi province, Iran

Table 2: Chemical composition of consumed experimental diets[1] (DM basis)

Item[2]	CON	LS-UT	LS-HT	HS-UT	HS-HT	Diet SEM
Chemical composition						
DM (Percentage of diet)	62.84	61.50	61.25	61.67	61.45	0.271
OM (Percentage of DM)	95.48	94.56	94.59	94.51	94.47	0.229
NE_L[3] (Mcal kg^{-1} DM)	1.48	01.59	01.59	01.62	01.62	0.033
CP (Percentage of DM)	17.25	17.30	17.25	17.15	17.25	0.054
Ether extract (Percentage of DM)	3.70	05.38	05.24	05.86	05.80	0.480
NDF (Percentage of DM)	34.60	33.40	33.35	34.25	34.40	0.267
ADF (Percentage of DM)	19.70	20.10	21.35	22.60	22.15	0.479
NFC[4] (Percentage of DM)	34.10	36.25	36.20	35.80	35.90	0.388
Ash (Percentage of DM)	4.52	05.44	05.41	05.49	05.53	0.220

[1]Analysis performed on 2 period samples. [2]DM = Dry matter; OM = Organic matter; CP = Crude protein. [3]The NEl (Mcal kg^{-1}) was determined using the National Research Council (2001) software, Version 1.0 (December 2000). [4]NFC = 100 − (CP%+NDF%+ether extract%+ash%)

mixed diets, silage, seed and protein supplement were sampled weekly, frozen and composited on a 4 weak basis. Composited samples were mixed thoroughly and sub sampled for chemical analysis. About 500 mL milk samples were obtained on 1 and 28 day from each cow. Three consecutive milking was done to determine fat, protein, lactose and total solid compositions and fatty acid profiles. About 100 mL milk subsample was frozen in -30°C until analyses to fatty acid profile.

Chemical analysis: Dried feed samples were further ground in a Cyclotec mill (1 mm screen, Toosshekan Co®, Iran). Dry matter of TMR was determined by drying at 100°C for 5 h in oven (AOAC, 2000, ID 930.15). CP determination was done by the kjeldahl method (AOAC, 2000, ID 945.01). Both ADF and NDF were measured according to the non sequential procedures of Van Soest *et al*. (1991). Fat, protein and lactose in milk were determined by Milkoscan spectroscopy (Infrared Spectroscopy Milkoscan FT 120 Foss analytical A/S Hillerod®, Denmark).

Fatty acid analysis: The fatty acid profiles of milk, sunflower seed and experimental diets were determined by gas chromatography. Feed and frozen milks samples were shipped to Urmia University (Laboratory of Chemical and Feed Analysis) for analysis using the following procedures. Milk fat was separated by centrifugation (8000× g; 45 min) and whey was removed by vacuum aspiration leaving the fat layer. Lipids were extracted with chloroform: methanol (2:1 vol/vol). Methyl esters of fatty acids from feed and milk were prepared by the transestrification procedure of Park and Goins (1994). The methyl esters of fatty acid were injected by auto sampler into an Agilent 6890 N gas chromatograph fitted with a flame-ionization detector (Agilent Technologies, Palo Alto®, CA). A 100 m ×0.25 mm ×0.2 μm film thickness fused silica column (cp-Sil88; varian in c. Palo Alto®, CA) was used to separate fatty acid methyl esters. Gas chromatography conditions were as follows: the injection volume was 0.5 μL, a split injection was used (70:1 vol/vol); ultrapure 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) in creased by 5°C min^{-1} to 100°C (held for 3 min) in creased by 10°C min^{-1} to 175°C (held for 40 min) and then increased by 5°C min^{-1} to 220°C (held for 19 min) for a total run time of 86.5 min. Data integration and quantification were accomplished with Agilent 3365 chemstation (Agilent Technologies) software.

Statistical analysis: All results were subjected to least squares ANOVA for a complete randomized design. Data were analyzed by the general line models procedure of (SAS Institute, 1997) for a Complete Randomized Design (CRD) and a CRD factorial method using the following model:

$$Yijk = \mu+Ti+Lj+Hk+Eijk$$

Where:

Yijk = Observation
μ = Mean
Ti = Treatment
i = 1-4
Lj = Level of seed
j = 1-4
Hk = Treating of seed
k = 1-5
Eijk = Residual error

Least square means were separated by Duncan's multiple range tests with significance declared at $p \leq 0.05$. Effects of treatment were tested using the random effects of cow as the error term. The means were compared by Duncan procedure. Also, data were analyzed using a 2×2 factorial arrangement (treating and levels) of treatment (without control treatment effect) using the general linear models procedure of SAS. Data were analyzed as an interaction between raw or heat-treated sunflower and 7.5 or 15% of levels and interaction between them.

RESULTS AND DISCUSSION

Complete diets (Table 1) were formulated for Holstein cows averaging 32 kg of milk/day with 17% CP (of diet DM). The respective CON, LS-UT, LS-HT, HS-UT and HS-HT TMR analyses averaged 17.25, 17.30, 17.25, 17.15 and 17.25% CP and were estimated at 1.48, 1.59, 1.59, 1.62 and 1.62 Mcal kg^{-1} NE_L using National Research Council (2001) equations. The resulting diets containing sunflower oil seed was slightly lower in NDF but higher in ADF. The CON diet contained 3.7% ether extract of diet DM whereas the LS-UT, LS-HT, HS-UT and HS-HT contained 5.38, 5.24, 5.86 and 5.80% of diets DM, respectively. Consequently, the LS diets had 0.11 Mcal kg^{-1} and HS diets had 0.14 Mcal kg^{-1} more NE_L than CON ration. In this investigation, ether extract amount in difference diets were 5.24-5.80% without CON that was 3.70%. Nonetheless, variation normally depends on dietary factors that alter the rumen environment (e.g., forage to concentrate ratio and DM intake) (Table 3).

Intake of DM, expressed in kilogram per day was significantly greater for cows fed CON diet compared with those fed sunflower seeds. Milk yield and composition is shown in Table 4. Milk yield and 4% Fat Corrected Milk (FCM) were recorded at 1-28 day of experimental period, daily. About 4% FCM milk, milk efficiency 4% FCM, fat percentage and yield, protein percentage and yield, lactose percentage and yield, SNF percentage and yield and TS percentage were not different.

Milk actual yield and TS yield were lower from sunflower treatments fed cows ($p<0.05$), yet total yield of these milk components were not different. Fat percentage was higher in milk from HS-UT cows (3.71%) and lower in milk from CON cows (3.39%) ($p = 0.75$); as well as when corrected for total yield of milk fat, the difference was negligible. Fat yield in CON and LS-HT was more than other treatments.

In this study, significant different between milk fatty acids profiles were for $C_{14:0}$, $C_{18:1\text{-}n9}$, $C_{18:2\text{-}n6C}$, $C_{18:3\text{-}n3}$, $C_{22:0}$, total UFA, total n_6, n_3+n_6, other UFA and C_{18} UFA. Lipid supplementation induces a general increase in C_{18}% at the expense of the short and medium chain FA with the exception of $C_{18:1\text{-}n9}$, $C_{18:2\text{-}n6C}$ and $C_{18:3\text{-}n6}$ which LS-HT treatment tended to increase of those fatty acids. Other treatments had limited significant effect on milk fatty acid composition.

These fatty acids were increased due of 7.5% level sunflower added to diets and by heat-treated sunflower oil seed. This would suggest that high level (15%) and raw sunflower seed was not very effective in increase of mono or polyunsaturated fatty acids in milk. The total results of milk fatty acid profiles are shown in Table 5.

Dietary composition: Because all treatments met or exceeded energy and protein requirements, little difference was expected in milk yield or composition. The dietary protein level of CON was adjusted using cottonseed and soybean meal to reduce inherent differences in the AA profile when using sunflower oil seed in the other diets. It should be noted that the sunflower oil seed consumed as raw or heat-treated, allowing disparity in protein degradability and contributing to potential differences between diets in milk production. Furthermore, the treating of sunflower seed during heating process can alter protein degradability in a different relative proportion of RDP to RUP in the diet.

Sunflower oil seed is a excellent source of oleic and linoleic acid resulting the CON diet had low level monoenoic and dienoic fatty acids whereas LS and HS diets were higher in oleic and linoleic acid ($C_{18:1}$ and $C_{18:2}$) than the CON diet. Oleic acid was more concentrate in the HS diets than in the LS and CON diets. The HS contained more linoleic acid, the dienoic fatty acid

Table 3: Milk yield and composition of milk from lactating dairy cows at 28th day of the experiment[1]

	Diet[2]								Level[3]			Treatment[4]			
Variables	CON	LS-UT	LS-HT	HS-UT	HS-HT	SEM	F	p<	L	H	p<	U	H	p<	L×T[5] (p<)
Milk yield (kg day^{-1})	34.75^{a}	28.37^{b}	33.72^{ab}	30.22^{ab}	32.75^{ab}	2.10	1.94	0.155	32.40	32.95	NS	32.15	33.20	0.071	NS
FCM 4%	31.56	26.25	31.15	28.81	29.78	2.05	1.05	0.426	30.24	30.07	NS	30.11	30.21	NS	NS
Composition															
Fat	3.39	3.50	3.51	3.71	3.41	0.19	0.16	0.756	3.56	3.49	NS	03.59	03.46	NS	NS
Protein	3.40	3.21	3.24	3.18	3.18	0.15	0.52	0.828	3.23	3.25	NS	03.21	03.27	NS	NS
Lactose	4.92	4.78	4.92	4.94	4.78	0.10	0.67	0.710	4.81	4.88	NS	04.87	04.83	NS	NS
TS	12.42	11.80	11.96	11.88	11.63	0.25	1.25	0.309	11.86	11.97	NS	11.84	12.00	NS	NS
SNF	10.14	9.83	10.00	9.97	9.84	0.15	0.55	0.809	9.90	9.97	NS	9.92	9.95	NS	NS
Yield (kg day^{-1})															
Fat	1.17	0.99	1.17	1.11	1.11	0.09	0.78	0.625	1.15	1.14	NS	1.15	1.14	NS	NS
Protein	1.19	0.90	1.09	0.96	1.04	0.07	1.54	0.191	1.03	1.07	NS	1.03	1.08	NS	NS
Lactose	1.70	1.35	1.65	1.49	1.56	0.11	1.19	0.339	1.55	1.60	NS	1.56	1.59	NS	NS
TS	4.31^{a}	3.33^{b}	4.02^{ab}	3.59^{ab}	3.81^{ab}	0.26	1.47	0.215	3.83	3.94	NS	3.80	3.98	NS	NS
SNF	3.51	2.78	3.36	3.01	3.22	0.21	1.32	0.274	3.20	3.28	NS	3.19	3.29	NS	NS

$^{a-c}$Within a row, means without a common superscript differ ($p<0.05$). [1]FCM = 4% Fat-Corrected Milk; TS = Total Solid; SNF = Solids-Not-Fat. [2]C = Diet of Control; LS-UT = Diet of including 7.5% untreated sunflower oil seed; LS-HT = Diet of including 7.5% heat-treated sunflower oil seed; HS-UT = Diet of including 15% untreated sunflower oil seed; HS-HT = Diet of including 15% heat-treated sunflower oil seed. [3]L = 7.5%, H = 15%. [4]U = Untreated, H = Heat-treated. [5]Interaction effects between levels vs treatments. [6]NS: Non Significant; $p>0.05$

Table 4: The milk fatty acids (g/100 g of total fatty acids) of 28th day from the different diets fed to cows in experiment

	Diet[1]								Level[3]			Treatment[4]			
Fatty acid[2]	CON	LS-UT	LS-HT	HS-UT	HS-HT	SEM	F	p<	L	H	p<	U	H	p<	L×T[5] (p<)
$C_{14:0}$	14.470^{ab}	14.710^{ab}	14.120^{ab}	12.050^{b}	15.130^{a}	3.22	1.47	0.215	15.940	17.270	NS[6]	16.050	17.160	NS	NS
$C_{14:1-n5}$	0.970	0.600	0.770	0.450	0.960	0.28	0.60	0.768	0.841	0.847	NS	0.764	0.924	NS	NS
$C_{16:0}$	32.680	33.100	32.910	34.730	33.660	3.09	0.46	0.873	33.970	36.050	NS	35.640	34.380	NS	NS
$C_{16:1-n7}$	1.730	2.930	1.340	1.100	3.460	0.85	1.27	0.294	1.720	2.310	NS	2.230	1.790	NS	NS
$C_{18:0}$	23.980	27.390	30.300	21.680	22.500	3.01	1.34	0.267	27.040	21.600	0.017	24.250	24.390	NS	NS
$C_{18:1-n7}$	2.190	2.350	1.620	1.260	1.830	0.35	1.09	0.402	1.740	1.480	NS	1.650	1.580	NS	NS
$C_{18:1-n9}$	18.660^{b}	21.670^{ab}	24.540^{a}	17.980^{b}	18.060^{b}	2.42	1.35	0.261	21.630	17.690	0.036	19.530	19.790	NS	NS
$C_{18:2-n6c}$	2.480^{ab}	2.720^{ab}	3.530^{a}	1.820^{b}	2.030^{ab}	0.58	1.18	0.349	3.060	1.910	0.008	2.470	2.500	NS	NS
$C_{18:3-n3}$	0.180^{a}	0.157^{ab}	0.211^{a}	0.081^{b}	0.167^{a}	0.02	2.05	0.078	0.188	0.140	0.005	0.151	0.178	0.097	NS
$C_{18:3-n6}$	0.115	0.089	0.157	0.160	0.184	0.04	1.18	0.344	0.117	0.117	NS	0.104	0.130	NS	NS
$C_{18:4-n3}$	0.348	0.400	0.233	0.370	0.227	0.09	0.87	0.549	0.303	0.248	NS	0.341	0.210	NS	NS
$C_{20:0}$	0.146	0.203	0.246	0.135	0.237	0.08	0.49	0.853	0.237	0.168	NS	0.202	0.203	NS	NS
$C_{20:4-n6}$	0.082	0.193	0.109	0.200	0.325	0.12	0.78	0.622	0.093	0.144	NS	0.109	0.128	NS	NS
$C_{20:5-n3}$	0.047	0.004	0.018	0.004	0.018	0.01	0.82	0.593	0.011	0.009	NS	0.007	0.014	NS	NS
$C_{22:0}$	0.147^{a}	0.090^{ab}	0.067^{ab}	0.043^{ab}	0.015^{b}	0.03	1.50	0.202	0.065	0.027	0.028	0.050	0.043	NS	NS
$C_{22:5-n6}$	0.076	0.031	0.050	0.079	0.005	0.03	0.75	0.649	0.060	0.038	NS	0.059	0.038	NS	NS
$C_{22:6-n3}$	0.057	0.000	0.000	0.060	0.067	0.03	0.73	0.667	0.008	0.033	NS	0.016	0.025	NS	NS
Identified	83.930	91.290	92.530	83.530	85.880	4.43	0.71	0.682	91.550	88.020	NS	90.010	89.560	NS	NS
Unidentified	16.070	8.710	7.470	16.470	14.120	4.43	0.71	0.682	8.440	11.970	NS	9.980	10.430	NS	NS
Total sat	56.980	60.130	59.930	59.940	59.430	4.13	0.95	0.492	61.760	63.260	NS	62.560	62.460	NS	NS
Total UFA	26.950^{ab}	31.150^{a}	32.590^{a}	23.590^{b}	26.950^{ab}	2.84	1.64	0.159	29.780	24.880	0.021	27.450	27.220	NS	NS
Total n_3	0.633	0.563	0.464	0.454	0.480	0.11	0.77	0.633	0.511	0.415	NS	0.498	0.428	NS	NS
Total n_6	2.760^{ab}	3.030^{ab}	3.840^{a}	2.260^{b}	2.540^{b}	0.61	1.10	0.392	3.330	2.210	0.006	2.740	2.800	NS	NS
n_3+n_6	3.390^{ab}	3.600^{ab}	4.310^{a}	2.710^{b}	3.020^{ab}	0.65	1.07	0.410	3.840	2.630	0.007	3.240	3.230	NS	NS
Other UFA	22.550^{ab}	27.550^{a}	28.280^{a}	20.870^{b}	25.640^{ab}	2.38	1.79	0.123	25.940	22.680	0.017	24.200	24.420	NS	NS
C_{18} UFA	23.980^{b}	27.390^{ab}	30.300^{a}	21.680^{b}	22.500^{b}	3.01	1.34	0.267	27.040	21.600	0.017	24.250	24.390	NS	NS

[a-c]Within a row, means without a common superscript differ (p<0.05). [1]C = Diet of Control; LS-UT = Diet of including 7.5% untreated sunflower oil seed; LS-HT = Diet of including 7.5% heat-treated sunflower oil seed; HS-UT = Diet of including 15% untreated sunflower oil seed; HS-HT = Diet of including 15% heat-treated sunflower oil seed. [2]n = Unsaturated bond numbers; c = cis; Total sat = Total of saturated fatty acids; Total UFA = Total of unsaturated fatty acids; Total n3 = Total of n3 fatty acids; Total n6 = Total of n6 fatty acids; Other UFA = The sum of unsaturated fatty acids without n3 and n6; C18 UFA = The sum of unsaturated fatty acids with 18 carbons. [3]L = 7.5%, H = 15%. [4]U = Untreated, H = Heat-treated. [5]Interaction effects between levels vs treatments. [6]NS: Non Significant; p>0.05

Table 5: BW, BCS, DMI, EI, NDF and EE intake in of cows received experimental diets[1]

	Diet[2]								Level[3]			Treatment[4]			
Variable	CON	LS-UT	LS-HT	HS-UT	HS-HT	SEM	F	p<	L	H	p<	U	H	p<	L×T[5] (p<)
Intake (kg day^{-1})															
DMI	23.57^{a}	22.42^{ab}	21.90^{b}	21.70^{b}	21.85^{b}	0.40	3.63	0.029	22.16	21.77	NS[6]	22.06	21.87	NS	NS
Energy	0.34	0.35	0.34	0.35	0.35	0.05	0.29	0.882	0.35	0.36	NS	0.35	0.35	NS	NS
NDF	8.15^{a}	7.48^{b}	7.30^{b}	7.42^{b}	7.51^{b}	0.12	5.81	0.005	7.39	7.46	NS	7.45	7.40	NS	NS
EE	0.87^{c}	1.18^{b}	1.14^{b}	1.26^{a}	1.26^{a}	0.06	37.29	0.001	1.16	1.26	0.001	1.22	1.20	NS	NS
BW, kg	648.00	636.00	661.00	628.00	664.00	15.86	1.05	0.427	639.00	640.00	NS	633.00	647.00	NS	NS
BCS	02.93	02.87	03.05	02.88	03.00	0.85	0.87	0.550	02.92	02.92	NS	02.88	02.97	NS	NS

[a-c]Within a row, means without a common superscript differ (p<0.05). [1]BW = Body Weight; BCS = Body Condition Score; DMI = Dry Matter Intake; EI = Energy Intake; NDF = Natural Detergent Fiber; EE = Ether Extract. [2]C = Diet of Control; LS-UT = Diet of including 7.5% untreated sunflower oil seed; LS-HT = Diet of including 7.5% heat-treated sunflower oil seed; HS-UT = Diet of including 15% untreated sunflower oil seed; HS-HT = Diet of including 15% heat-treated sunflower oil seed. [3]L = 7.5%, H = 15%. [4]U = Untreated, H = Heat-treated. [5]Interaction effects between levels vs treatments. [6]NS: Non Significant; p>0.05

precursor of linoleic acid isomers with demonstrated biological value for ruminal biohydrogenation via the isomerization of $C_{18:2}$ isomers. Also $C_{18:1}$ might be VA (vaccenic acid) in the rumen that was prefabricator of $C_{18:2}$ isomers (CLA).

DMI, BW and BCS: Fat, especially from sources high in unsaturated fatty acids can reduce fiber digestibility, alter the ratio of ruminal acetate to propionate and lower intake when total dietary level exceed 6-7% DM (National Research Council, 2001). About 7.5% untreated sunflower seed (LS-UT) is readily accepted by dairy cows and has no negative effect on DMI (Petit, 2003).

Moreover, feeding up to 30% of sunflower seed in the DM has no effect on DMI (Rafulowski and Park, 1982). Differences in DMI between diets containing of sunflower seed and without sunflower seed can be related to size of sunflower seed or ether extract access in those diets.

Because lack of sunflower seed in CON diet which could result in faster release from the rumen and less breakdown of the seed due to rumination. Feeding CON diet compared with sunflower seed diets could then results in less oil being released in the rumen which would limit the negative effect of oil on fiber digestion (Schauff and Clark, 1992) and thus on DMI.

We expected that higher dietary fat intake repartum could prevent excessive lipid mobilization in adipose tissue and thereby ameliorate DMI in the subsequent lactation (Duske *et al*., 2009). This would be corroborated by the fact that feeding 7.5% sunflower seed untreated in the DM has no effect on ruminal fermentation. DMI was similar for cows fed treated and untreated sunflower seed, nonetheless heat-treated sunflower seeds were caused little great DMI than raw sunflower seed. In most cases in which protection of lipid supplements against ruminal biohydrogenation improved feed intake, there was an increased fiber digestion in the rumen. Initial, final and average BW was similar among treatments. Change in BW was not affected by the diet (at least 628 in HS-UT and maximum 664 in HS-HT; $p = 0.42$). These results obtained for BCS, too (Table 3).

Milk yield and milk composition: Significant difference in milk yield was resulted of treating effects as heat-treated sunflower seed that produced 33.20 kg day^{-1} vs. raw sunflower that produced 32.15 kg day^{-1}. As obtaining results is observed milk yield with LS-UT was 28.37 and with CON was 34.75 kg day^{-1}, yet LS-HT, HS-UT and HS-HT produced 33.72, 30.22 and 32.75 kg day^{-1} milk yield without significant different between sunflower seed diets.

These results are same of obtained data by Beachemin *et al.* (2009) and Petit (2003). CON treatment increased milk production by an average of 2.07 kg day^{-1} which would mainly result of greater DMI. On the other hand, supplementation with sunflower (untreated or heat-treated and 7.5 or 15%) had no significant effect on increasing of milk yield of cows fed sunflower seeds. Greater milk production in CON could be a result of smaller ADF intake and dietary AA available for absorption by the animal (Kempton *et al*., 1979) which would contribute in improving animal production. Supplementing dairy cow diets with high amounts of plant oils often cause a drop in feed intake and therefore milk yield (Flowers *et al*., 2008; Chilliard *et al*., 2007; Rego *et al*., 2005) possibly as a result of their negative affects on feed digestibility and rumen fermentation (Jenkins, 1998).

Milk 4% FCM was no significant difference but LS-HT was caused 31.15 kg day^{-1} 4% FCM followed CON with 31.56 kg day^{-1} 4% FCM. An average of FCM produced by CON and LS-UT was 1.40 and 0.99 kg day^{-1} and milk efficiency 4% FCM was 1.30 in CON and 1.28 in LS-HT (Table 4). Petit (2003) reported that feeding lactating dairy cow diets supplemented with untreated sunflower (15.2% of DM) increased milk fat percentage.

We used 7.5 and 15% sunflower seed in this research that consumed as raw or heated. Adding sunflower seed to dairy cows diets as raw or treated and low or high level increased fat milk percentage with most effect due of low level and untreated form. In this investigation, protein percentage and yield (kg day^{-1}) was greater for cows fed CON diet compared with those fed sunflower seed. CON diet is without sunflower seed and smaller in size than sunflower diets and that might have increased its rate of passage from the rumen and increased its supply of AA for milk protein synthesis. By compared with raw or treating sunflower is resulted heating of 15% sunflower can be caused more effects for protein synthesis.

The lack of effect of treated oil seeds on milk protein concentration has been previously reported by Tymchuk *et al.* (1998) and Ashes *et al.* (1995) resulting of greater bypass of protein due to the heat treatment which would increase AA availability at the intestine level. AbuGhazaleh and Holmes (2007) reported milk protein percentages were not affected by diets containing sunflower oil but protein yields were lower for the without oil plants supplement. In the present study, concentrations of lactose, TS and SNF percentage were similar among treatments. Treating seed with heat increased production of milk protein, fat and lactose but there was no difference between cows fed 7.5 and 15% sunflower seed. Generally, oils that were effectively protected against ruminal biohydrogenation increase milk fat yield (Ashes *et al*., 1995). On the other hand, in effective protection (Petit *et al*., 2002) or low level of added fat (Tymchuk *et al*., 1998) had no effect on milk fat yield.

Milk fatty acids profile: Feeding oilseeds to lactating dairy cows is one method to change the proportion of unsaturated fatty acids in milk fat with increases as high as 40% (Kim *et al*., 1993). The response of milk FA composition integrates both rumen metabolism (hydrolysis, isomerization and biohydrogenation of dietary FA, determining duodenal FA flow and composition) and cow metabolism (lipid mobilization, mammary uptake of plasma FA, mammary de novo synthesis of FA; Chilliard *et al*., 2007). Increase in C18 percentage is resulting from an increase in mammary uptake of long-chain FA absorbed in the intestine and a decrease in mammary de novo synthesis (Glasser *et al*., 2008; Palmquist *et al*., 1993). Fatty acids in bovine milk are considered either produced de novo in the mammary gland or derived from plasma lipids. Generally, 4:0-14:0 and some 16:0 are thought to be produced de novo in the mammary gland (Moate *et al*., 2007).

Increase of $C_{18:1-n9}$, $C_{18:2-n6C}$ and $C_{18:3-n6}$ whit LS-HT treatment is in agreement with the results of Petit (2003), who reported that treating of oil seeds significantly increased $C_{18:2}$ and $C_{18:3}$ concentrations in milk. In this study, greatest effect being observed for animals fed LS-HT. Cows fed LS-HT had higher $C_{18:1n-9}$ in milk compared to the cows fed the CON, HS-UT and HS-HT diets. Oleic acid ($C_{18:1}$) was identified as either cis or trans and the total $C_{18:1}$ was determined by totaling the cis and trans isomers. There was no significant increase in $C_{18:1n-7}$ in milk fat from cows fed the sunflower seed treatments compared to the control.

Total $C_{18:1}$ in milk for the low oil seed treatments (7.5%) was higher than in milk from the control and high level sunflower seed groups. The increased concentration of $C_{18:1}$ may be partially attributed to the unsaturated fatty acids escaping rumen hydrogenation; however the desaturase enzyme in the mammary gland can also convert C_{18} to $C_{18:1}$ (Fig. 1). Inclusion of oil seed in the diet resulted in an intensification in the concentration of $C_{18:2-n6c}$ with the greatest gain observed for cows fed LS-HT and LS-UT.

Compared to the control, milk from cows fed LS-UT and LS-HT had 10.9 and 14.2% more $C_{18:2-n6C}$, respectively. Although, added dietary fat increased the linoleic acid ($C_{18:2}$) content of milk fat.

When total 18:2 was considered, treating of lipids greatly improved the milk 18:2 content whereas seed and oil supplements had only moderate effects or none at all. This confirms the high rumen BH of dietary 18:2 observed for oils and seeds (Glasser *et al*., 2008). Similar results were observed for linolenic acid ($C_{18:3}$). Linolenic acid ($C_{18:3}$) in milk originates almost entirely from the diet, however $C_{18:2}$ can also be found in body stores. Addition of LS-HT resulted in increases in $C_{18:2}$ and $C_{18:3}$ of 142 and 124%, respectively. For Ω3, linolenic acids was no significant difference among dietary treatments. The concentration of $C_{18:3-n3}$ in milk from cows fed LS-HT was higher than from cows fed the HS-UT diet. These results are similar to those previously reported by Ashes *et al*. (1995). The fatty acid composition of the TMR was not determined. Based on the assumption of 69% digestibility of fatty acids, oil seed in the diet resulted in the $C_{18:2}$ and $C_{18:3}$ being converted in the rumen to either $C_{18:0}$ or $C_{18:1}$ since there was no transfer of these fatty acids to milk fat. Low level and raw treatments of sunflower seed (7.5% and untreated) did not result in a large transfer of $C_{18:2}$ and $C_{18:3}$ into milk fat, <1 and 2%, respectively, also suggesting that these fatty acids were saturated to either $C_{18:0}$ or $C_{18:1}$. In experiments that compared different lipid sources without a control diet (which were thus not included in the models), some researchers have confirmed this observation (Kelly and Bauman, 1996; Petit, 2003; Loor *et al*., 2004) but others do not report any significant difference between 18:2 and 18:3 rich lipids on milk 18:0% (Chouinard *et al*., 1998; Petit *et al*., 2002; Ward *et al*., 2002; Brzoska, 2005).

The concentration of $C_{22:0}$ decline with the inclusion of oil seed in the diets (Table 5). Significant differences were observed for total UFA in milk among the dietary treatments. Cows fed HS-UT had the lowest level of UFA in milk compared to the other lipid treatments. UFA content of milk was affected by level of oil seed. An increase in UFA was obtained by low-level oil seed (29.78 vs. 24.88).

No significant differences were between treatments for change of total n_3 fatty acids. The concentration of total n_6 in milk fat was decreased by high sunflower seed (15%) in the diet compared to the control diet and low sunflower seed (7.5%) in diet.

Milk from cows fed LS-HT and HS-UT had highest and lowest n3+n6 fatty acid, respectively. C_{18} unsaturated and other unsaturated fatty acids in milk were obtained greater by LS-HT and smaller with HS-UT (Table 5). A decrease in total UFA, n3, n6, n3+n6, other UFA and C_{18} UFA in milk fat with the inclusion of HS-UT or HS-HT is in agreement with others (Atwal *et al*., 1991; Khorasani and Kennely, 1998) when fat was supplemented at 2% or more in the diets. Palmquist *et al*. (2005) reported that

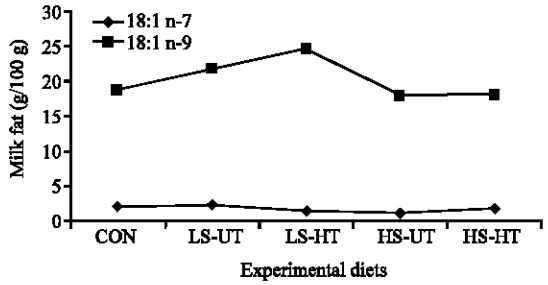

Fig. 1: $C_{18:1}$ fatty acids in milk fat of cows fed experimental diets

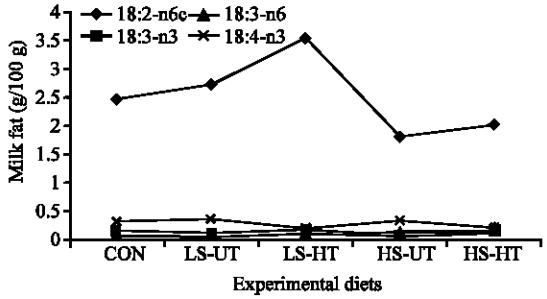

Fig. 2: $C_{18:2}$, $C_{18:3}$ and $C_{18:4}$ fatty acids in milk fat of cows fed experimental diets

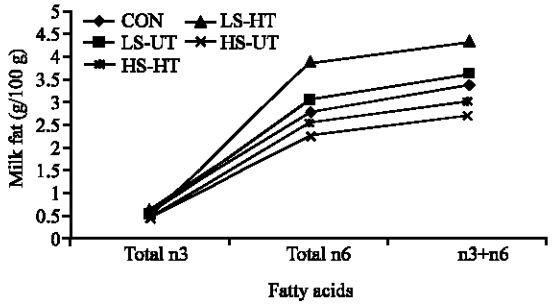

Fig. 3: n3, n6 and n3+n6 fatty acids in milk of cows fed experimental diets

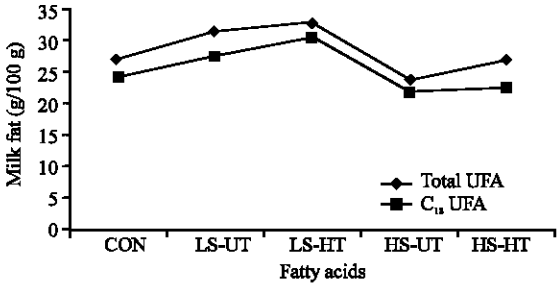

Fig. 4: Total UFA and C_{18} UFA in milk of cows fed experimental diets

reductions in mentioned fatty acids by high level oil seed supplementation may be due to lower production of acetate and beta-hydroxy-butyrate in the rumen or as a result of increased uptake of dietary long-chain fatty acids inhibiting de novo synthesis of upper mentioned fatty acids (Fig. 2-4). Moreover if cow genetics have a great effect on yields, their milk FA composition is not greatly affected (Bobe *et al*., 2009).

SUGGESTIONS

In general, heating of 7.5% sunflower seed compared with raw sunflower or 15% in diets was caused greater unsaturated fatty acids in milk suggesting that heat-treating can protect polyunsaturated fatty acids against ruminal biohydrogenation. Feeding sunflower seed would improve Ω6 and Ω6+Ω3 resulting improve nutritive value of milk from a human health point of view. Totally, using heat-treated sunflower oil seed in low level can be evince the best results for milk fatty acid quality and milk performances in early lactating dairy cows nutrition.

CONCLUSION

This study showed feeding sunflower oil seed had different results compared normal dairy cow diets. We obtained that DMI was increased by diets without oil seed. Intake of DM, expressed as a kg day^{-1} was increased by normal diets. Milk production was significant decrease only for cows fed LS-UT and increase for CON treatment and other treatments by sunflower seed. Suggesting, 7.5% sunflower seed in diet which heated can be useful for milk production results. Fat concentration was greater by all sunflower oil seed diets compared whit CON diet. Protein concentration in milk was greater for cows fed CON diet than for those fed sunflower seed.

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REFERENCES

AOAC, 2000. Official Methods of Analysis. 15th Edn., Association of Official Analysis Chemists, Arlington, VA USA.

AbuGhazaleh, A.A. and L.D. Holmes, 2007. Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. J. Dairy Sci., 90: 2897-2904.

Ashes, J.R., P.S. VincentWelch, S.K. Gulati, T.W. Scott and G.H. Brown, 1995. Manipulation of the fatty acid composition of milk by feeding protected canola seeds. J. Dairy Sci., 75: 1090-1096.

Atwal, A.S., M. Hidiroglou and J.K.G. Kramer, 1991. Effects of feeding protect and alpha-tocopherol on fatty acid composition and oxidative stability of cows milk. J. Dairy Sci., 74: 140-145.

Beauchemin, K.A., S.M. McGinn, C. Benchaar and L. Holtshausen, 2009. Crushed sunflower, flax or canola seeds in lactating dairy cow diets: Effects on methane production, rumen fermentation and milk production. J. Dairy Sci., 92: 2118-2127.

Bobe, G., G.L. Lindberg, L.F. Reutzel and M.D. Hanigan, 2009. Effects of lipid supplementation on the yield and composition of milk from cows with different β-lactoglobulin phenotypes. J. Dairy Sci., 92: 197-203.

Brzoska, F., 2005. Effect of dietary vegetable oils on milk yield, composition and CLA isomer profile in milk from dairy cows. J. Anim. Feed Sci. Technol., 14: 445-459.

Casper, D.P., D.J. Schingoethe, R.P. Middaugh and R.J. Baer, 1988. Lactational responses of dairy cows to diets containing regular and high oleic acid sunflower seeds. J. Dairy Sci., 71: 1267-1274.

Chilliard, Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel and M. Doreau, 2007. Diet, rumen biohydrogenation, cow and goat milk fat nutritional quality: A review. Eur. J. Lipid Sci. Technol., 109: 828-855.

Chouinard, P.Y., V. Girard and G.J. Brisson, 1998. Fatty acid profile and physical properties of milk fat from cows fed calcium salts of fatty acids with various unsaturation. J. Dairy Sci., 81: 471-481.

Dhiman, T.R., L.D. Satter, M.W. Pariza, M.P. Galli, K. Albright and M.X. Tolosa, 2000. Conjugated Linoleic Acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci., 83: 1016-1027.

Drackley, J.K. and D.J. Schingoethe, 1986. Extruded blend of soybean meal and sunflower seeds for dairy cattle in early lactation. J. Dairy Sci., 69: 371-384.

Duske, K., H.M. Hammon, A.K. Langhof, O. Bellmann and B. Losand *et al*., 2009. Metabolism and lactation performance in dairy cows fed a diet containing rumen-protected fat during the last twelve weeks of gestation. J. Dairy Sci., 92: 1670-1684.

Flowers, G., S.A. Ibrahim and A.A. AbuGhazaleh, 2008. Milk fatty acid composition of grazing dairy cows when supplemented with linseed oil. J. Dairy Sci., 91: 722-730.

Garnsworthy, P.C., 1997. Fats in Dairy Cow Diets. In: Recent Advances in Animal Nutrition, Garnsworthy, P.C. and D.J.A. Cole (Eds.). University of Nottingham, Nottingham, pp: 87-103.

Glasser, F., A. Ferlay and Y. Chilliard, 2008. Oilseed lipid supplements and fatty acid composition of cow milk: A meta-analysis. J. Dairy Sci., 91: 4687-4703.

Hu, F.B. and W.C. Willet, 2002. Optimal diets for prevention of coronary heart disease. JAMA, 288: 2569-2578.

Huth, P.J., D.B. Di Rienzo and G.D. Miller, 2006. Major scientific advances with dairy foods in nutrition and health. J. Dairy Sci., 89: 1207-1221.

Jenkins, T.C., 1998. Fatty acid composition of milk from Holstein cows fed oleamide or canola oil. J. Dairy Sci., 81: 794-800.

Johnson, K.A., R.L. Kincaid, H.H. Westberg, C.T. Gaskins, B.K. Lamb and J.D. Cronrath, 2002. The effect of oilseeds in diets of lactating cows on milk production and methane emissions. J. Dairy Sci., 85: 1509-1515.

Kelly, L.M. and D.E. Bauman, 1996. Conjugated linoleic acid: A potent anticarcinogen found in milk fat. Proceedings of the 58th Cornell Nutriton Conference, (CNC'96), Cornell University, Ithaca, NY USA., pp: 68-74.

Kempton, T.J., J.V. Nolan and R.A. Leng, 1979. Protein nutrition of growing lambs: Effect of nitrogen digestion of supplementing a low-protein-cellulosic diet with urea, casein, or formaldehyde-treated casein. Br. J. Nutr., 42: 303-315.

Kennelly, J.J., 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. Anim. Feed Sci. Technol., 60: 137-152.

Khorasani, G.R. and J.J. Kennely, 1998. Effect of added dietary fat on performance, rumen characteristics and plasma hormone and metabolites in midlactating dairy cows. J. Dairy Sci., 81: 2459-2468.

Kim, Y.K., D.J. Schingoethe, D.P. Casper and F.C. Ludens, 1993. Supplemental dietary fat from extruded soybeans and calcium soaps of fatty acids for lactating dairy cows. J. Dairy Sci., 76: 197-204.

Loor, J.J., K. Ueda, A. Ferlay, Y. Chilliard and M. Doreau, 2004. Short communication: Diurnal profiles of conjugated linoleic acids and trans fatty acids in ruminal fluid from cows fed a high concentrate diet supplemented with fish oil, linseed oil, or sunflower oil. J. Dairy Sci., 87: 2468-2471.

Luna, P., A. Bach, M. Juarez and M.A. de La Fuente, 2008. Effect of a diet enriched in whole linseed and sunflower oil on goat milk fatty acid composition and conjugated linoleic acid isomer profile. J. Dairy Sci., 91: 20-28.

Moate, P.J., W. Chalupa, R.C. Boston and I.J. Lean, 2007. Milk fatty acids I: Variation in the concentration of individual fatty acids in bovine milk. J. Dairy Sci., 90: 4730-4739.

National Research Council, 2001. Nutrient Requirements of Dairy Cattle. 7th Rev. Edn., National Academy of Sciences, Washington, DC.

Odongo, N.E., M.M. Or-Rashid, E. Kebreab, J. France and B.W. McBride, 2007. Effect of supplementing myristic acid in dairy cow rations on ruminal methanogenesis and fatty acid profile in milk. J. Dairy Sci., 90: 1851-1858.

Palmquist, D.L., A.D. Beaulieu and D.M. Barbano, 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci., 76: 1753-1771.

Palmquist, D.L., A.L. Lock, K.J. Shingfield and D.E. Bauman, 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. Adv. Food Nutr. Res., 50: 179-217.

Park, P.W. and R.E. Goins, 1994. *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in food. J. Food Sci., 59: 1262-1266.

Parodi, P.W., 2005. Dairy product consumption and the risk of breast cancer. J. Am. College Nutr., 24: 556S-568S.

Petit, H.V., 2003. Digestion, milk production, milk composition and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. J. Dairy Sci., 86: 2637-2646.

Petit, H.V., R.J. Dewhurst, N.D. Scollan, J.G. Proulx, W. Haresign, H. Twagiramungu and G.E. Mann, 2002. Milk production and composition, ovarian function, and prostaglandin secretion of dairy cows fed omega-3 fats. J. Dairy Sci., 85: 889-899.

Rafalowski, W. and C.S. Park, 1982. Whole sunflower seed as a fat supplement for lactating cows. J. Dairy Sci., 65: 1484-1492.

Rego, O.A., H.J.D. Rosa, P. Portugal, T. Franco, C.M. Vouzela, A.E.S. Borba and R.J.B. Bessa, 2005. The effects of supplementation with sunflower and soybean oils on the fatty acid profile of milk fat from grazing dairy cows. Anim. Res., 54: 17-24.

SAS Institute, 1997. SAS/STAT Users Guide: Statistics. Version 6.12, SAS Institute Inc., Cary, North Carolina.

Schauff, D.J. and J.H. Clark, 1992. Effects of feeding diets containing calcium salts of long-chain fatty acids to lactating dairy cows. J. Dairy Sci., 75: 2990-3002.

Schingoethe, D.J., M.J. Brouk, K.D. Lightfield and R.J. Baer, 1996. Lactational responses of dairy cows fed unsaturated fat from extruded soybeans or sunflower seeds. J. Dairy Sci., 79: 1244-1249.

Shingfield, K.J., Y. Chilliard, V. Toivonen, P. Kairenius and D.I. Givens, 2008. Trans fatty acids and bioactive lipids in ruminant milk. Adv. Exp. Med. Biol., 606: 3-65.

Tymchuk, S.M., G.R. Khorasani and J.J. Kennelly, 1998. Effect of feeding formaldehyde- and heat-treated oil seed on milk yield and milk composition. Can. J. Anim. Sci., 78: 693-700.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Ward, A.T., K.M. Wittenberg and R. Przybylski, 2002. Bovine milk fatty acid profiles produced by feeding diets containing solin, flax and canola. J. Dairy Sci., 85: 1191-1196.