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Full Length Research Paper

The changes of milk fatty acids profile and milk performances by using of whole sunflower oil seed (raw or treated) in lactating Holstein cow's diets

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The objective of this study was to investigate the effects of dietary supplementation with sunflower oil seed (raw- or heat-treated) in two levels (7.5 or 15%) on UFA in milk fat and performances of high-yielding lactating cows. Twenty early lactating Holstein cows were used in a complete randomized design. Treatments included: 1) CON, control (without sunflower oil seed). 2) LS-UT, 7.5% raw sunflower oil seed. 3) LS-HT, 7.5% heat-treated sunflower oil seed. 4) HS-UT, 15% raw sunflower oil seed. 5) HS-HT, 15% heat-treated sunflower oil seed. Experimental period lasted for 4 weeks. Supplementation with 7.5% raw sunflower seed (LS-UT) tended to decrease milk yield, with 28.37 kg/d compared with the control (34.75 kg/d). Milk fat percentage was increased with the HS-UT that obtained 3.71% compared with CON that was 3.39% (P>0.05). Milk protein percent was decreased by 15% sunflower treatments with 3.18% whereas CON treatment caused 3.40%. The cows fed added LS-HT produced milk with the highest content of total UFA with 32.59 g/100g of milk fat compared with the HS-UT with 23.59 g/100 g of milk fat. Content of C18 UFA in milk fat increased from 21.68 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 diets, respectively. C18:2 isomers of fatty acid were greater by LS-HT supplementation with significant effect (P<0.05). Total of C18 UFA content was significantly higher in milk of animal fed added 7.5% heat-treated sunflower. In all, results showed that diet cow's supplementation with sunflower oil seed tended to improve C18 UFA content in milk fat. It seems 7.5% heated sunflower oil seed can be the optimal source to increase UFA production and improve some milk performances.

Key words: Sunflower seed, milk production, fatty acid profile.

INTRODUCTION

Milk fat is an important determinant of milk nutritional quality. The SFA (mainly 12:0, 14:0, 16:0 and 18:0) are considered to produce negative effects when consumed in excess, whereas UFA (18:1, 18:2 isomers and 18:3n-3) have well-known or potential positive effects on human health (Parodi, 2005). 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 work (Glasser et al., 2008). Supplementing the diet of cows with plant lipids decreased the C16:0 and other medium chain fatty acids (C10:0, C12:0 and C14:0) and increased the C18:0, and C18 UFA content of milk fat (Palmquist et al., 1993). There is growing interest in feeding sunflower oil seed to dairy cows because of its FA profiles; oleic and linolenic acid contributes to dietary n-3 FA and promotes increased linoleic acid isomers content while decreasing the SFA content of ruminant milk

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Abbreviation: UFA, Unsaturated fatty acid; **SFA,** saturated fatty acid; **FA,** fatty acid; **MUFA,** monounsaturated fatty acid; **PUFA,** polyunsaturated fatty acid.

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

Item	Diet[1]					
	CON	HS-UT	LS-HT	LS-UT	HS-HT	SEM
	Ingredients (% of Diet)					
Corn silage	39.67	37.58	37.58	37.34	37.34	0.614
Alfalfa hay[2]	6.65	6.45	6.45	6.03	6.03	0.125
Barley grain	12.82	11.52	11.52	8.3	8.3	0.886
Corn grain	7.92	6.92	6.92	6.12	6.12	0.307
Canola meal[3]	2.06	2.06	2.06	2.92	2.92	0.226
Cottonseed[4]	8.58	7.58	7.58	5.26	5.26	0.611
Soybean meal[5]	11.96	11.05	11.05	8.42	8.42	0.654
Wheat bran	8.52	7.52	7.52	6.1	6.1	0.455
Beet sugar pulp	1.68	1.68	1.68	2.38	2.38	0.368
Sunflower oil seed[6]-UT	0	7.5	0	15	0	2.5
Sunflower oil seed-HT	0	0	7.5	0	15	2.5
Dicalcium phosphate	0.06	0.06	0.06	0.06	0.06	0
Salt, Vitamin and Mineral permix	0.075	0.075	0.075	0.075	0.075	0

[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.

(Chilliard et al., 2007). The effects of sunflower oil seed supplementation on milk yield and composition have often been studied (Glasser et al., 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 (vaccenic acid) and RA (rumenic acid) content (Dhiman et al., 2000). However, 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). Several studies 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 work 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, including increased proportions of MUFA and PUFA and increased concentrations of the conjugated linoleic acid isomer *cis*-9, *trans*-11 (Hu and Willett, 2002). Also, feeding heat-treated sunflower oil seed as a lipid protected from ruminal hydrogenation increased the unsaturated fatty acid composition of milk lipids plus milk production (Schingoethe et al., 1996). Hence, the objective of this study was to investigate the effect of supplementing a dairy cow diet with sources of long chain FA such as sunflower oil seed varying in their level and treating including two 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 in five treatments and four replicate were used in a complete randomized design to evaluate responses to supplementary sunflower oil seed in 7.5 or 15% levels and raw or heat-treated forms. 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 (NRC, 2001) of lactating cows averaging 635 kg of BW producing 32 kg/d milk with 3.8% fat. Diets are showed in Table 1. For treating of oil seed sunflower, parts of sunflower seeds were heated at least in 90°C within 10 min (Pellet mill equipment made Denmark®, Animal feed factory Co, Daneh Matbu- Saveh, Iran). Cows within groups were assigned randomly to one of five treatments and four replicates. Cows were fed individually and milked three times daily at 0060, 1400 and 2000h. Milk production was recorded at every milking. The five dietary treatments (Table 1) consisted of supplements based on either raw sunflower oil seed (UT) and heated 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

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

Item[2]	Diet					
	CON	LS-UT	LS-HT	HS-UT	HS-HT	SEM
	Chemical composition					
DM (% of Diet)	62.84	61.5	61.25	61.67	61.45	0.271
OM (% of DM)	95.48	94.56	94.59	94.51	94.47	0.229
NE_L[3] (Mcal/kg DM)	1.48	1.59	1.59	1.62	1.62	0.033
CP (% of DM)	17.25	17.3	17.25	17.15	17.25	0.054
Ether extract (% of DM)	3.7	5.38	5.24	5.86	5.8	0.48
NDF (% of DM)	34.6	33.4	33.35	34.25	34.4	0.267
ADF (% of DM)	19.7	20.1	21.35	22.6	22.15	0.479
NFC[4] (% of DM)	34.1	36.25	36.2	35.8	35.9	0.388
Ash (% of DM)	4.52	5.44	5.41	5.49	5.53	0.22

[1]Analysis performed on 2 period samples. [2]DM = dry matter; OM = organic matter; CP = crude protein. [3]NEl (Mcal/kg) was determined using the NRC 2001 software, version 1.0 (December 2000). [4]NFC = 100 – (CP% + NDF% + ether extract% + ash%).

and HS diets, respectively. Thus, the five diets were designed to yield similar protein 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 0800 and 1600 h for 10% orts. Feed consumption was recorded at an initial of each week. Total mixed diets, silage, seed and protein supplement were sampled weekly, frozen and composited on a 4 weeks basis. Composited samples were mixed thoroughly and subsampled for chemical analysis. 500 ml milk samples were obtained on 1 and 28 d from each cow. Three consecutive milking was done to determine fat, protein, lactose and total solid compositions and fatty acid profiles. 100 ml milk subsample was frozen in -30ºC 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 (total mixed ration) 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. 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 they were 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 6890N gas chromatograph fitted with a flame-ionization detector (Agilent Technologies, Palo Alto®, USA). A 100 m ×0.25 mm × 0.2 µm film thickness fused silica column (cp-Sil88; varian, Inc. 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), increased by 5°C per min to 100°C (held for 3 min), increased by 10°C per min to 175°C (held for 40 min) and then increased by 5°C per min 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 linear models procedure of SAS (SAS 9.1, 2002®) for 1) A CRD (complete randomized design); and 2) A CRD factorial method using the following model:

$Yijk = \mu + Ti + Lj + Hk + Eijk$; where: Yijk = observation; μ = mean; Ti = treatment, i = 1,2,3,4; Lj = level of seed, j = 1,2,3,4; Hk= treating of seed, k = 1,2,3,4,5; and 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

Complete diets (Table 1) were formulated for Holstein cows averaging 32 kg of milk/d 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 NE_L using NRC (2001) equations. The resulting diets containing sunflower oil seed was slightly

Table 3. BW, BCS, DMI, EI, NDF and EE intake in of cows received experimental diets[1].

	Diet[2]								Level[3]			Treatment[4]			L×T[5]
Variable	**CON**	**LS-UT**	**LS-HT**	**HS-UT**	**HS-HT**	**SEM**	**F**	***P<***	**L**	**H**	***P<***	**U**	**H**	***P<***	***P<***
							Intake, kg/d								
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	636	661	628	664	15.86	1.05	0.427	639	640	NS	633	647	NS	NS
BCS	2.93	2.87	3.05	2.88	3.00	0.85	0.87	0.55	2.92	2.92	NS	2.88	2.97	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; $P > 0.05$.

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 and HS diets had 0.14 Mcal/kg of more NE_L than CON ration. In this investigation ether extract amount in difference diets were 5.24 to 5.80% without CON that was 3.70%. Nonetheless, variation normally depends on dietary factors that alter the rumen environment (for example, forage-to-concentrate ratio and DM intake). 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 reported in Table 4. Milk yield and 4% FCM (fat corrected milk) were recorded at 1 to 28 d of experimental period, daily. FCM, milk efficiency 4% FCM, fat percentage and yield, protein percentage and yield, lactose percentage and yield, SNF (solid non fat) percentage and yield, and TS (total solid) 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 FA profiles were for C14:0, C18:1n-9, C18:2n-6cis, C18:3n-3, C22:0, UFA, n6, n3+n6 and C18 UFA. Lipid supplementation induces a general increase in C18 percentage at the expense of the short- and medium-chain FA, with the exception of C18:1n-9, C18:2n-6cis and C18:3n-6 that 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 (UT) seed was not very effective in Increase of MUFA or PUFA in milk fat. The total results of milk FA profiles are shown in Table 5.

DISCUSSION

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 amino acid profiles 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 heat process can alter protein degradability in a different relative proportion of RDP (ruminal degradable protein) to RUP (ruminal undegradable protein) in the diet. Sunflower oil

Table 4. Milk yield and composition of milk from lactating dairy cows at 28th -day of the experiment[1].

	Diet[2]								Level[3]			Treatment[4]			L×T[5]
Variable	**CON**	**LS-UT**	**LS-HT**	**HS-UT**	**HS-HT**	**SEM**	**F**	***P<***	**L**	**H**	***P<***	**U**	**H**	***P<***	***P<***
Milk															
Yield, kg/d	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	3.59	3.46	NS	NS
Protein	3.40	3.21	3.24	3.18	3.18	0.15	0.52	0.828	3.23	3.25	NS	3.21	3.27	NS	NS
Lactose	4.92	4.78	4.92	4.94	4.78	0.10	0.67	0.710	4.81	4.88	NS	4.87	4.83	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/d															
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 5. 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]			L×T[5]
Fatty acid[2]	**CON**	**LS-UT**	**LS-HT**	**HS-UT**	**HS-HT**	**SEM**	**F**	***P<***	**L**	**H**	***P<***	**U**	**H**	***P<***	***P<***
C14:0	14.47[ab]	14.71[ab]	14.12[ab]	12.05[b]	15.13[a]	3.22	1.47	0.215	15.94	17.27	NS[6]	16.05	17.16	NS	NS
C14:1n-5	0.97	0.60	0.77	0.45	0.96	0.28	0.60	0.768	0.841	0.847	NS	0.764	0.924	NS	NS
C16:0	32.68	33.10	32.91	34.73	33.66	3.09	0.46	0.873	33.97	36.05	NS	35.64	34.38	NS	NS
C16:1n-7	1.73	2.93	1.34	1.10	3.46	0.85	1.27	0.294	1.72	2.31	NS	2.23	1.79	NS	NS
C18:0	23.98	27.39	30.30	21.68	22.50	3.01	1.34	0.267	27.04	21.60	0.017	24.25	24.39	NS	NS
C18:1n-7	2.19	2.35	1.62	1.26	1.83	0.35	1.09	0.402	1.74	1.48	NS	1.65	1.58	NS	NS
C18:1n-9	18.66[b]	21.67[ab]	24.54[a]	17.98[b]	18.06[b]	2.42	1.35	0.261	21.63	17.69	0.036	19.53	19.79	NS	NS

Table 5. Contd.

C18:2n-6cis	2.48^{ab}	2.72^{ab}	3.53^{a}	1.82^{b}	2.03^{ab}	0.58	1.18	0.349	3.06	1.91	0.008	2.47	2.50	NS	NS
C18:3n-3	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
C18:3n-6	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
C18:4n-3	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
C20:0	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
C20:4n-6	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
C20:5n-3	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
C22:0	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
C22:5n-6	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
C22:6n-3	0.057	0.0	0.0	0.060	0.067	0.03	0.73	0.667	0.008	0.033	NS	0.016	0.025	NS	NS
Identified	83.93	91.29	92.53	83.53	85.88	4.43	0.71	0.682	91.55	88.02	NS	90.01	89.56	NS	NS
Unidentified	16.07	8.71	7.47	16.47	14.12	4.43	0.71	0.682	8.44	11.97	NS	9.98	10.43	NS	NS
Total Sat	56.98	60.13	59.93	59.94	59.43	4.13	0.95	0.492	61.76	63.26	NS	62.56	62.46	NS	NS
Total UFA	26.95^{ab}	31.15^{a}	32.59^{a}	23.59^{b}	26.95^{ab}	2.84	1.64	0.159	29.78	24.88	0.021	27.45	27.22	NS	NS
Total n3	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 n6	2.76^{ab}	3.03^{ab}	3.84^{a}	2.26^{b}	2.54^{b}	0.61	1.10	0.392	3.33	2.21	0.006	2.74	2.80	NS	NS
n3+n6	3.39^{ab}	3.60^{ab}	4.31^{a}	2.71^{b}	3.02^{ab}	0.65	1.07	0.410	3.84	2.63	0.007	3.24	3.23	NS	NS
Other UFA	22.55^{ab}	27.55^{a}	28.28^{a}	20.87^{b}	25.64^{ab}	2.38	1.79	0.123	25.94	22.68	0.017	24.20	24.42	NS	NS
C18 UFA	23.98^{b}	27.39^{ab}	30.30^{a}	21.68^{b}	22.50^{b}	3.01	1.34	0.267	27.04	21.60	0.017	24.25	24.39	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; 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; $P > 0.05$.

seed is an 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 (C18:1 and C18:2) than the CON diet. Oleic acid was more concentrated in the HS diets than in the LS and CON diets. The HS contained more linoleic acid, the dienoic fatty acid precursor of linoleic acid isomers with demonstrated biological value for ruminal biohydrogenation via the isomerization of C18:2 isomers. Also C18:1 might be VA (vaccenic acid) in the rumen that was prefabricator of C18:2 isomers as CLA (Conjugated linoleic acid).

DMI, BW and BCS

Fat, especially from sources high in UFA, can reduce fiber digestibility, alter the ratio of ruminal acetate to propionate and lower intake, when total dietary level exceed 6 to 7% DM (NRC, 2001). 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 was 28.37 and with CON was 34.75 kg/d, yet LS-HT, HS-UT and HS-HT produced 33.72, 30.22 and 32.75 kg/d milk yield without significant different between sunflower seed diets. These results are same of obtained data by

Beauchemin et al. (2009) and petit (2003). CON treatment increased milk production by an average of 2.07 kg/d, 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 amino acid 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/d 4% FCM followed CON with 31.56 kg/d. An average of FCM produced by CON and LS-UT was 1.40 and 0.99 kg/d 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/d) 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 amino acid for milk protein synthesis. By comparing 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 amino acids availability at the intestine level. Heat treating of oilseeds reduce protein degradation and should increase dietary protein escaping degradation in the rumen.

Ruminal degradation of whole sunflower seed was more slowly in the rumen than some other treating. Abughazaleh et al. (2007) reported milk protein percentages were not affected by diets containing sunflower oil, but protein yields were lower for those without oil plants supplement. In the present study, concentrations of lactose, TS and SNF percentage were 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., 1992). On the other hand, ineffective 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 UFA 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 C18:0 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 to 14:0 and some 16:0 are thought to be produced de novo in the mammary gland (Moate et al., 2007; Grummer, 1991). Increase of C18:1n-9, C18:2n-6cis and C18:3n-6 whit LS-HT treatment is in agreement with the results of Petit (2003) who reported that treating of oil seeds significantly increased C18:2 and C18:3 concentrations in milk. In this study, greatest effect being observed for animals fed LS-HT. Cows fed LS-HT had higher C18:1n-9 in milk compared to the cows fed the CON, HS-UT and HS-HT diets.

Oleic acid (C18:1) was identified as either *cis* or *trans* and the total C18:1 was determined by totaling the *cis* and *trans* isomers. There was no significant increase in C18:1n-7 in milk fat from cows fed the sunflower seed treatments compared to the control. Total C18:1 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 C18:1 may be similar among treatments. Treating seed with heat partially attributed to the unsaturated fatty acids escaping rumen hydrogenation; however the desaturase enzyme in the mammary gland can also convert C18:0 to C18:1 (Figure 1). Inclusion of oil seed in the diet resulted in an intensification in the concentration of C18:2n-6cis 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 C18:2n-6cis, respectively. Although added dietary fat increased the linoleic acid (C18:2) 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 biohydrogenation of dietary 18:2 observed for oils and seeds (Glasser et al., 2008). Similar results were observed for linolenic acid (C18:3). Linolenic acid (C18:3) in milk originates almost entirely from the diet, however, C18:2 can also be found in body stores. Addition of LS-

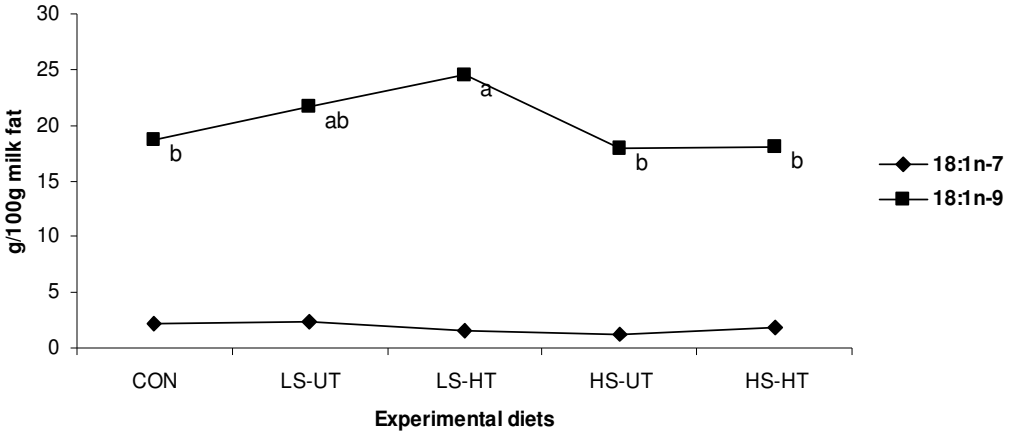

Figure 1. C18:1 fatty acids in milk fat of cows fed experimental diets.

HT resulted in increases in C18:2 and C18:3 of 142 and 124%, respectively. By heat process of sunflower oil seed, fat coupled with the fiber of the seed hull and PUFA pass to intestine. For omega 3 linolenic acids was no significant difference among dietary treatments.

The concentration of C18:3n-3 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 C18:2 and C18:3 being converted in the rumen to either C18:0 or C18:1 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 C18:2 and C18:3 into milk fat, less than 1 and 2%, respectively. Also suggesting that these fatty acids were saturated to either C18:0 or C18:1. In the experiments that have compared different in lipid sources without a control diet, which were not included in the models, some researchers have confirmed this observation (Kelly et al., 1996; Petit, 2003, 2004; Loor et al., 2004). However, others do not report any significant difference between 18:2 and 18:3 rich lipids on milk 18:0 percentage (Chouinard et al., 1998; Petit et al., 2002; Ward et al., 2002; Brzoska, 2005). The concentration of C22:0 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. Low-level oil seed (29.78 vs. 24.88) obtained an increase in UFA. No significant differences were between treatments for change of total n3 fatty acids. The concentration of total n6 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. C18:0 unsaturated and other unsaturated fatty acids in milk were obtained greater by LS-HT and smaller with HS-UT (Table 5). A decrease in UFA, n3, n6, n3 + n6 and C18:0 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 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 (Figures 2, 3, 4 and 5). Moreover, if cow genetics have a great effect on yields, their milk FA composition is not greatly affected (Bobe et al., 2009).

Conclusion

This study showed feeding diets containing 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/d, was increased by normal diets. Milk production was significantly 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 with CON diet. Protein concentration in milk was greater for cows fed CON diet than for those fed sunflower seed. In general, heating of 7.5% sunflower seed compared with raw sunflower or 15% in diets caused greater UFA in milk, suggesting that heat-treating can protect PUFA against ruminal biohydrogenation. Feeding sunflower seed would improve omega 3 and 6, resulting improve nutritive value of milk from a human health point of view.

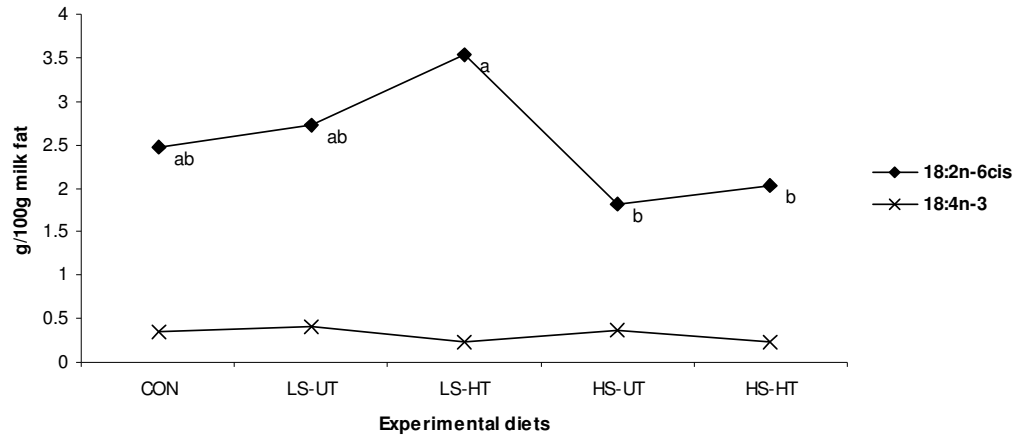

Figure 2. C18:2, and C18:4 fatty acids in milk fat of cows fed experimental diets.

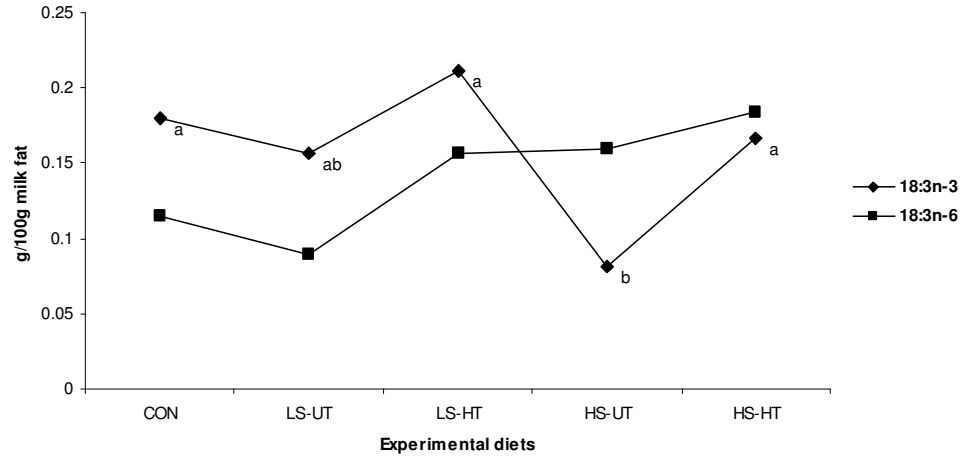

Figure 3. C18:3 fatty acids in milk fat of cows fed experimental diets.

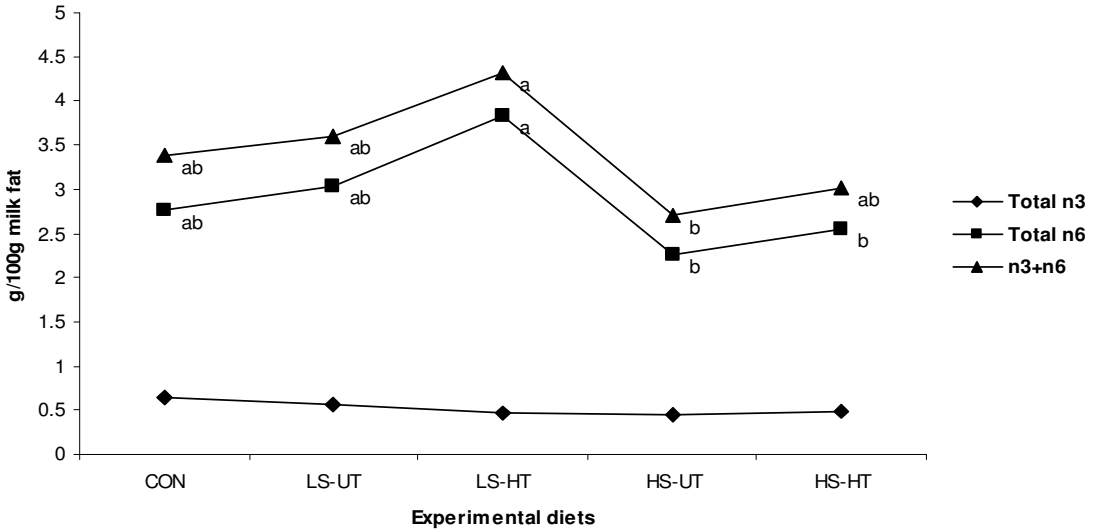

Figure 4. n3, n6 and n3+n6 fatty acids in milk of cows fed experimental diets.

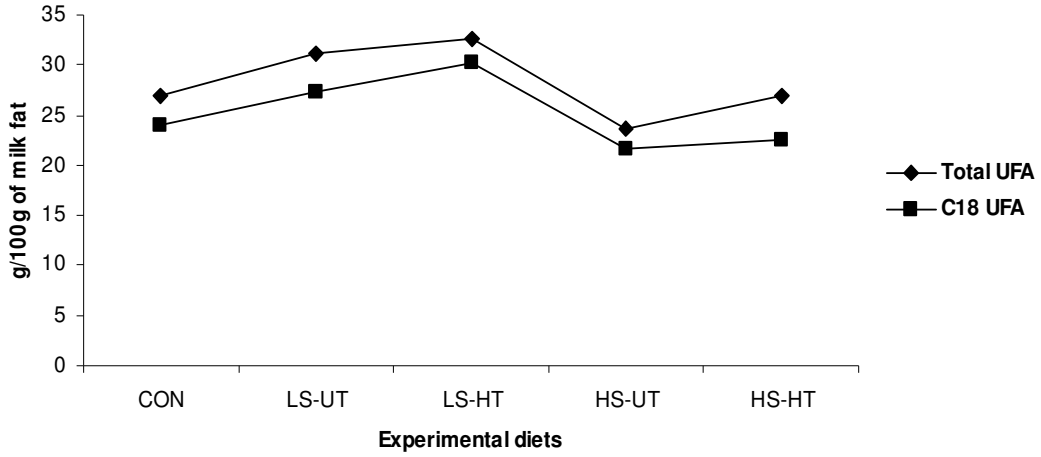

Figure 5. Total UFA and C18 UFA in milk of cows fed experimental diets.

Totally, using heat-treated sunflower oil seed in low level can be evince the best results for milk fatty acid quality and some milk performances such as fat and protein milk in early lactating dairy cows nutrition.

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