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Heat-treated or Raw Sunflower Seeds in Lactating Dairy Cows 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 raw or heat-treated sunflower oil seed with 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: 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 wk, with first 2 wk used for adaptation to the diets. 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 treatment that obtained 3.71% compared with CON that was 3.39% and without significant different. Milk protein percent was decreased 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/100g of milk fat compared with the HS-UT with 23.59 g/100g of milk fat. Content of C_{18} unsaturated fatty acids in milk fat increased from 21.68 g/100g of fat in the HS-UT to 22.50, 23.98, 27.39 and 30.30 g/100g 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} UFA (Unsaturated Fatty Acid) content in milk fat. 7.5% level of sunflower oil seed that heated seemed to be the optimal source to increase UFA production.

Keywords**—**fatty acid profile, milk production, sunflower seed

I. INTRODUCTION

DIETARY lipid supplementation has been extensively researched and used as a management tool to increase milk and milk composition yields in high-yielding dairy cows [1]. Unsaturated milk fatty acid specially linoleic and linolenic acid has been reported to have a wide range of beneficial effects for humans [2], [3] and [4]. High-yielding dairy cows produce a lot of milk in a lactation period, but they are sensitive to metabolic disorders, such as acidosis, ketosis and fatty liver. It is better large amount of milk production be associated with nutrient high quality like unsaturated fatty acids. Lipid type and form influences responses to dietary lipid supplementation. Supplementation of diets with plant lipid is one strategy recognized to rich in unsaturated fatty acids (FA), through biohydrogenation of FA [5]. The *cis*-9, *trans*-11 linoleic acid is formed in the rumen as an intermediate in the biohydrogenation of linoleic acid or in tissues by *Δ*9-desaturase from vaccenic acid (VA, *trans*-11 C18:1). Another intermediate in the ruminal biohydrogenation of oleic, linoleic, and linolenic acids [6](Bauman, 1999). Recent studies have estimated that more than 90% of milk *cis*-9, *trans*-11 linoleic acid isomers are made by the activity of the *Δ*9-desaturase enzyme [7], [8] and [9]. Other researchers demonstrated that dietary supplementation of vegetable oil seeds high in unsaturated fatty acid gave the greatest response and there is a clear dose-dependent increase in milk fat content of unsaturated fatty acids [10] and [11]. Modification in milk fatty acids composition through nutrition may result in positive or adverse changes in milk production and nutritional properties of dairy products [12]. Supplementation with plant oils and seeds such as cottonseed, soybean and sunflower with a high linoleic acid content has been confirmed as an effective nutritional strategy to enrich ruminant milk fat in isomers of $C_{18:2}$ (rumenic acid, RA), the biologically most active Conjugated linoleic acid isomers and in *trans-11* $C_{18:1}$ (vaccenic acid, VA) its precursor in the mammary gland [13]. 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 [14]. But the use of free oil in high doses in the diet is not recommended in ruminants [15]. Because it might inhibit rumen microbial activity and affect milk production and composition [16]. Furthermore, feeding fats high in polyunsaturated FA can alter the FA composition of milk [17] in a manner beneficial to human health, including increased proportion of mono and polyunsaturated FA and increased concentration of the linoliec acid isomers [18]. Sunflower seed would be a good choice from a consumer viewpoint, as it is rich in polyunsaturated fatty acids with sunflower being a source of linoleic acid (66-70% of the total fatty acid). Sunflower seed was chosen because it is readily available to dairy producers and it range in FA profiles [19]. Sunflower increase the proportion of unsaturated fatty acids in milk compared to cows fed no supplemental fat [20].

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II. Materials and Methods

A. Animals and diets

Twenty early lactating multiparous Holstein cows were used in a complete randomized design to evaluate responses to supplementary heat-treated or raw sunflower seed in two levels. The sunflower seeds were acquired from a sunflower farm in Arak, Iran. Experimental period lasted 4 wk and was preceded by a 2 wk 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 [21]. Diets are showed in table I. For treating of oil seed sunflower, parts of sunflower seeds were heated in 90°C within 10 minutes (Pellet mill equipment made 1983 Denmark. Animal feed factory Daneh Matbu-Saveh, Iran). Cows within groups were assigned randomly to one of five treatments and 4 replicates. Cows were fed individually and milked three daily at 0060, 1400 and 2000h. Milk production was recorded at every milking. Cows within groups were assigned randomly to one of five treatments. The five dietary treatments (Table II) consisted of supplements based on either raw whole sunflower seed (UT) and heated whole sunflower 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 II. Diets were fed twice daily at 0800 and 1600h for 10% orts. Feed consumption was recorded initial of each week. Total mixed diets, silage, seed and protein supplement were sampled weekly, frozen, and composited on a 4-wk basis. Composited samples were mixed thoroughly and sub sampled for chemical analysis. 500 ml milk samples were obtained on d 1 and 28 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. Body weight and BCS of cows were determined first and end of experiment [22].

TABLE I Ingredients composition of consumed experimental diets (DM basis)

	Diet[1]					
Item	CON	LS-UT	LS-HT	HS-UT	HS-HT	SEM
Ingredients (% of Diet)						
Corn silage	39.67	37.58	37.58	37.34	37.34	0.61
Alfalfa hay[2]	6.65	6.45	6.45	6.03	6.03	0.12
Barley grain	12.82	11.52	11.52	8.30	8.30	0.88
Corn grain	7.92	6.92	6.92	6.12	6.12	0.30
Canola meal[3]	2.06	2.06	2.06	2.92	2.92	0.22
Cottonseed[4]	8.58	7.58	7.58	5.26	5.26	0.61
Soybean meal[5]	11.96	11.05	11.05	8.42	8.42	0.65
Wheat bran	8.52	7.52	7.52	6.10	6.10	0.45
Beet sugar pulp	1.68	1.68	1.68	2.38	2.38	0.36
Sunflower[6]-UT	0.0	7.50	0.0	15.00	0.0	2.50
Sunflower[6]-HT	0.0	0.0	7.50	0.0	15.00	2.50
Salt	0.02	0.02	0.02	0.02	0.02	0.0
Vitamin permix	0.025	0.025	0.025	0.025	0.025	0.0
Mineral permix	0.025	0.025	0.025	0.025	0.025	0.0

[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.

B. 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 5h in oven (Method 930.15-AOAC, 2000) [23]. CP determination was done by the kjeldahl method (Method 945.01- AOAC, 2000). Both ADF and NDF were measured according to the non sequential procedures of Van Soest methods [24]. Fat, protein and lactose in milk were determined by Milkoscan spectroscopy (Infrared spectroscopy milkoscan FT 120 Foss analytical A/S Hillerod, Denmark).

C. 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., Folch et al., 1957). Methyl esters of fatty acids from feed and milk were prepared by the transestrification procedure of Park and Goins (1994) [25]. 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, CA). 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 0.5µl, a split injection was used (70:1 vol/vol); ultrapure hydrogen was the carrier gas; and the injector and detector temperatures were 250 and 300ºC, respectively. The initial temperature was 70ºC (held for 1 min), increased by 5ºC per min to 100ºC (held for 3 min), increased by 10ºC per min to 175ºC (held for 40 min), and then increased by 5ºC per min to 220ºC (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.

D. Statistical analysis

All results were subjected to least squares ANOVA for a complete randomized design. Data were combined and analyzed using the MIXED procedures of SAS 9.1-2002 with cow as experimental unit [26]. Effects of treatment were tested

using the random effects of cow as the error term. Least square means were separated by least significant difference when 1-way ANOVA f-test were significant at $P < 0.05$. The means were compared by Duncan procedure. In addition, 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.

TABLE II CHEMICAL COMPOSITION OF CONSUMED EXPERIMENTAL DIETS[1] (DM BASIS)

	Diet					
Item[2]	CON	LS-UT	LS-HT	HS-UT	HS-HT	SEM
Chemical Composition						
DM % of Diet	62.84	61.50	61.25	61.67	61.45	0.27
OM % of DM	95.48	94.56	94.59	94.51	94.47	0.22
NE_L[3]	1.48	1.59	1.59	1.62	1.62	0.03
CP % of DM	17.25	17.30	17.25	17.15	17.25	0.05
Ether extract	3.70	5.38	5.24	5.86	5.80	0.48
NDF % of DM	34.60	33.40	33.35	34.25	34.40	0.26
ADF(% of DM)	19.70	20.10	21.35	22.60	22.15	0.47
NFC[4] %of DM	34.10	36.25	36.20	35.80	35.90	0.38
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]The NEl (Mcal/kg DM) was determined using the NRC (2001) software, Version 1.0 (December 2000).
[4]NFC = 100 – (CP% + NDF% + ether

III. RESULTS AND DISCUSSION

A. Dietary composition

Complete diets (Table I) 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 lower in NDF but higher in ADF. 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. 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 more NE_L than CON ration. 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 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).

B. 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 to 7% DM [21]. 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 (e.g., 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. 7.5% untreated sunflower seed (LS-UT) is readily accepted by dairy cows and has no negative effect on DMI [27]. Moreover, feeding up to 30% of sunflower seed in the DM has no effect on DMI [28]. 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 [29] 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 [30]. 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 III).

C. Milk Yield and Milk Composition

Milk composition is reported in Table V. Milk yield and 4% fat corrected milk (FCM) were recorded at 1d to 28d of experimental period, daily. 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. Significant difference in milk yield was resulted of

TABLE III 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															
1-day	649	637	664	626	665	15.78	1.29	0.291	640	640	NS	632	647	NS	NS
28-day	648	636	661	628	664	15.86	1.05	0.427	639	640	NS	633	647	NS	NS
Average[7]	-0.03	-0.03	-0.10	0.07	-0.03	0.03	1.12	0.33	0.0	0.0	NS	-0.03	0.0	NS	NS
BCS															
1-day	2.93[ab]	2.88[ab]	3.05[a]	2.81[ab]	3.00[a]	0.08	1.42	0.232	2.90	2.88	NS	2.93	2.85	NS	NS
28-day	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; $P > 0.05$.
[7]Average BW; g = Gain; l = Less.

treating effects as heat-treated sunflower seed that produced 33.20 kg/d vs. raw sunflower that produced 32.15 kg/d. As obtaining results is observed milk yield with LS-UT 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 [19] and [27]. 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 seed (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 [31] 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 [32], [1] and [33] possibly because of their negative affects on feed digestibility and rumen fermentation [16]. Milk 4% FCM was no significant difference. However, LS-HT was caused 31.15 kg/d 4% FCM followed CON with 31.56 kg/d 4% FCM. 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 V). 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. Petit (2003) reported that feeding lactating dairy cow diets supplemented with untreated sunflower (15.2% of DM) increased milk fat percentage [27]. We used 7.5 and 15% sunflower seed in this research which consumed 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. Protein concentration in milk had not significant different between treatments. 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 seed 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 seed 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 [34] and [35] resulting of greater bypass of protein due to the heat treatment, which would increase AA availability at the intestine level. Abughazaleh et al., (2007) reported milk protein percentages were not affected by diets containing sunflower oil, but protein yields were lower for the without oil plants supplement [36]. 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 [35]. On the other hand, ineffective protection (Petit et al., 2002), or low level of added fat [34] had no effect on milk fat yield (Figures 1 and 2).

TABLE IV. Milk yield, and composition of milk from lactating dairy cows at 1d and 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*<
Milk 1 day														
Yield kg/d	35.70	31.30	35.37	33.20	35.15	1.90	1.10	0.390	33.33	33.72	NS[6]	31.80	35.26	NS
FCM 4%	31.00	27.13	29.71	28.93	32.39	1.75	1.28	0.322	28.42	30.66	NS	28.03	31.05	NS
Milk 28 day														
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
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
Efficiency	1.30	1.06	1.28	1.22	1.22	0.09	0.78	0.626	1.19	1.24	NS	1.21	1.22	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
Protein	3.40	3.21	3.24	3.18	3.18	0.15	0.52	0.838	3.23	3.25	NS	3.21	3.27	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
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
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
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
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
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
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
SNF	3.51	2.78	3.36	3.01	3.22	0.21	1.33	0.274	3.20	3.28	NS	3.19	3.39	NS

[a-b]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, $P > 0.05$.

D. 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%, [37], [38] and [39]. Generally, 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. 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; [40]). Lipid supplementation induces a general increase in C18 percentage at the expense of the short- and medium-chain FA, resulting from an increase in mammary uptake of long-chain FA absorbed in the intestine and a decrease in mammary de novo synthesis [41] and [42]. 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 [43] and [44]. 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, other treatments had limited significant effect on milk fatty acid composition (Table IV). 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. This is in agreement with the results of [27], 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. $C_{14:0}$ concentrations in milk from cows fed HS-UT was lower than that milk from cows fed the other diets. Cows fed LS-HT had higher $C_{18:1n\text{-}9}$ 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 $C_{18:1n\text{-}7}$ 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 $C_{18:1}$ (Figure 3). Inclusion of oil seed in the diet resulted in an intensification in the concentration of $C_{18:2\text{-}n6c}$ 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 $C_{18:2\text{-}n6c,}$ 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 [41]. 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 omega 3 linolenic acids was no significant difference among dietary treatments. The concentration of $C_{18:3\text{-}n3}$ 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 [35]. 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, less than 1 and 2%, respectively, also suggesting that these fatty acids were saturated to either $C_{18:0}$ or $C_{18:1}$. In experiments which compared different lipid sources without a control diet (which

were thus not included in the models), some workers have confirmed this observation [10], [27] and [45], but others do not report any significant difference between 18:2- and 18:3-rich lipids on milk 18:0 percentage [46], [47], [48] and [49]. The concentration of $C_{22:0}$ decline with the inclusion of oil seed in the diets (Table IV). 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 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 (Table IV). Milk from cows fed LS-HT and HS-UT had highest and lowest n_3+n_6 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 IV). A decrease in total UFA, n_3, n_6, n_3+n_6, other UFA and C_{18} UFA in milk fat with the inclusion of HS-UT or HS-HT is in agreement with others [50] and [51], when fat was supplemented at 2% or more in the diets. Palmquist and Jenkins (1980) 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 [52]. Moreover, if cow genetics have a great effect on yields, their milk FA composition is not greatly affected [53]. (Figures 4, 5 and 6)

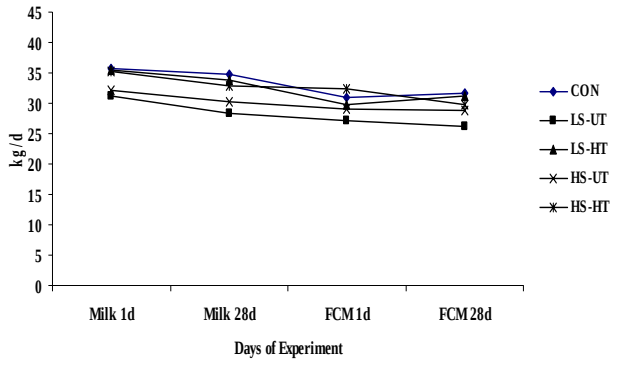

Fig. 1. Milk yield and 4%FCM at 1st and 28th day of experiment

IV. 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/d, 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 level, 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. 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 omega 6 and omega 6 plus omega 3, 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.

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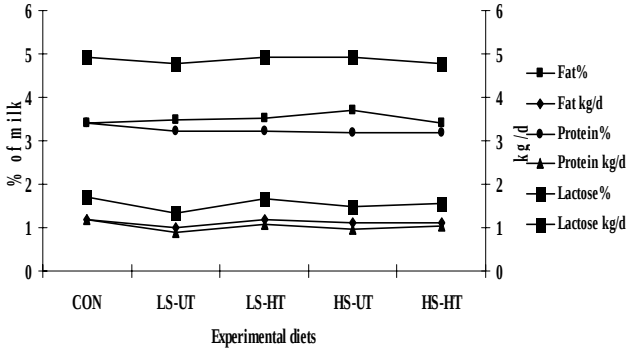

Fig. 2. Fat, protein and lactose (% of milk and kg/d) of cows fed experimental diets

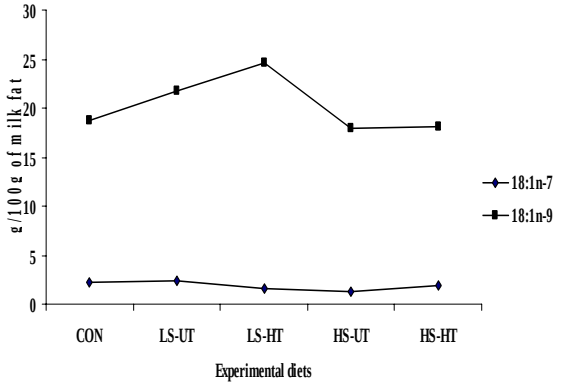

Fig. 3. $C_{18:1}$ fatty acids in milk fat of cows fed experimental diets

TABLE V. The milk fatty acids (g/100 g of total fatty acids) of 28th-day from the different diets fed to cows in experiment

Fatty acid[2]	Diet[1] CON	LS-UT	LS-HT	HS-UT	HS-HT	SEM	F	P<	Level[3] L	H	P<	Treatment[4] U	H	P<	L×T[5] P<
C4:0	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
C4:1n-5	0.97	0.60	0.77	0.45	0.96	0.28	0.60	0.768	0.841	0.847	NS	0.764	0.934	NS	NS
C6:0	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
C6:1n-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
C8:0	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
C10:1n-7	2.19	2.35	1.62	1.36	1.83	0.35	1.09	0.402	1.74	1.48	NS	1.65	1.58	NS	NS
C10:1n-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
C10:2n-6c	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
C10:3n-3	0.180[a]	0.157[ab]	0.211[a]	0.081[b]	0.167[ab]	0.02	2.05	0.078	0.188	0.140	0.005	0.151	0.178	0.09	NS
C10:3n-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
C10:4n-3	0.348	0.400	0.233	0.370	0.237	0.09	0.87	0.549	0.303	0.248	NS	0.341	0.210	NS	NS
C12:0	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
C12:1n-6t	0.082	0.193	0.109	0.200	0.325	0.12	0.78	0.622	0.093	0.144	NS	0.109	0.138	NS	NS
C12:1n-5	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
C13:0	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
C13:1n-3	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
Unidentified	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
Total Sat	56.98	60.13	59.93	59.94	59.43	4.13	0.95	0.492	61.76	63.26	NS	62.46	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 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	2.76[ab]	3.03[ab]	3.84[a]	2.26[b]	2.54[b]	0.61	1.10	0.393	3.33	2.21	0.006	2.74	2.80	NS	NS
n_3+n_6	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
C_{18} UFA	23.98[a]	27.39[a]	30.30[a]	21.68[b]	25.50[ab]	3.01	1.34	0.267	27.04	21.60	0.017	24.25	24.39	NS	NS

[a-b]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 and treatments. [6]NS: Non Significant; $P > 0.05$

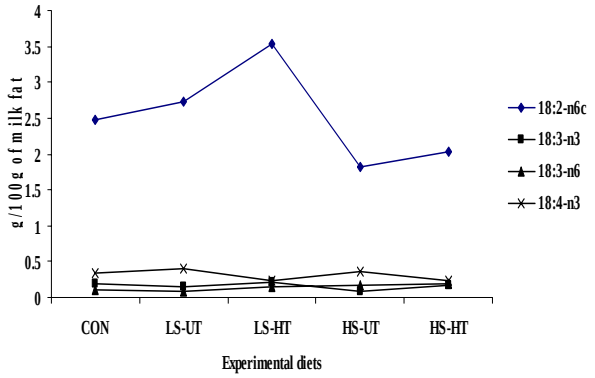

Fig. 4. $C_{18:2}$, $C_{18:3}$ and $C_{18:4}$ fatty acids in milk fat of cows fed experimental diets

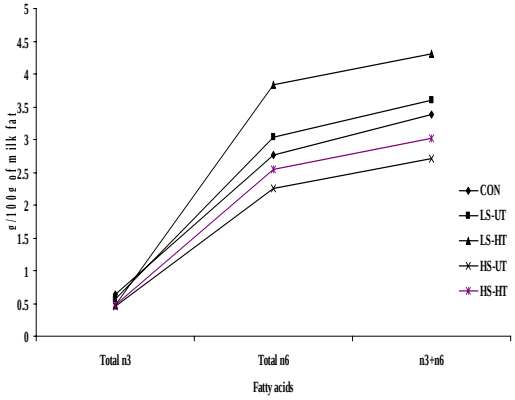

Fig. 5. n_3, n_6 and n_3+n_6 fatty acids in milk of cows fed experimental diets

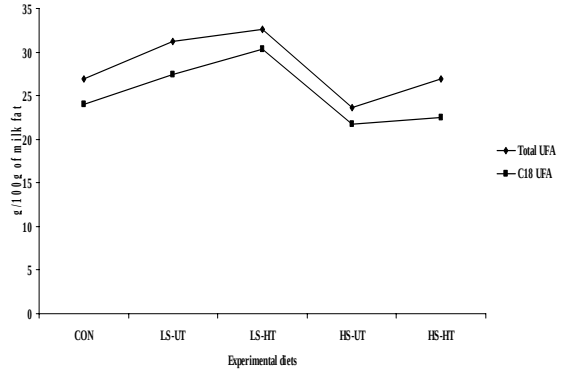

Fig. 6. Total UFA and C_{18} UFA in milk of cows fed experimental diets

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