# HEAT-TREATED OR RAW SUNFLOWER SEED IN LACTATING DAIRY COWS DIETS: EFFECTS ON MILK FATTY ACIDS PROFILE AND MILK PERFORMANCE

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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 (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 wk. 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% level sunflower treatments with 3.18% whereas CON treatment is caused 3.40% protein. 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/100g of milk fat. Content of $C_{18}$ UFA in milk fat increased from 21.68 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 $C_{18}$ 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 reduce milk production of lactating cows but can improve $C_{18}$ UFA content in milk fat. It seems that 7.5% heated sunflower oil seed can to be the optimal source to increase UFA production.*

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

## INTRODUCTION

Milk fat is an important determinant of milk quality. The saturated fatty acids are considered to produce negative effects, whereas others (18:1, 18:2 isomers, and 18:3n-3) have 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 soybeans, canola, and sunflower oil seed are used both on farms and experimental work (Glasser *et al.*, 2008). Supplementing the diet of cows with plant lipids decreased the $C_{16:0}$ and medium chain fatty acids 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 n3 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.*, 2008). Many studies have used whole, rolled, crushed, or ground crude sunflower seed (Beauchemin *et al.*, 2009, and Drackley & schingoethe, 1986). Using of free oil in diets is not recommended in ruminants

(Garnsworthy, 1997), because it might inhibit rumen microbial activity and affect milk production and composition. However, only a few studies have directly compared different physical forms of sunflower seed (Kennelly, 1996 and Beauchemin *et al.,* 2009). Several studies have reported simultaneous changes in milk FA composition after lipid supplementation of dairy cow diet, but no work has evaluated due to using accompany difference levels and treating methods of sunflower seed on milk FA. Furthermore, feeding fats high in unsaturated FA can alter the FA composition of milk in a manner beneficial to human health. Hence, the objective of this study was to investigate the effect of supplementing dairy cow diets with 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.

## MATERIAL AND METHOD

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 wk. 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. 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 Co, Daneh Matbu- Saveh, Iran). Cows within groups were assigned randomly to one of five treatments and 4 replicates. Cows were milked three daily at 0060, 1400 and 2000h. Milk production was recorded at every milking. The five dietary treatments 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. The five diets were designed to yield similar CP and difference in ether extract concentrations and fatty acids as well as energy. Diets were fed twice daily at 0800 and 1600h for 10% orts. Feed consumption was recorded initial of each week. Total mixed diets 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 28d 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. Dried feed samples were ground in a Cyclotec mill 1-mm screen. Dry matter of TMR was determined by AOAC method, 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 procedures of Van Soest. Fat, protein and lactose in milk were determined by Milkoscan spectroscopy (Infrared Spectroscopy Milkoscan FT 120 Foss A/S Hillerod®, Denmark). The fatty acid profiles of milk were determined by gas chromatography. Frozen milks samples were shipped to Urmia University for analysis by using of Agilent 6890N gas chromatograph fitted with a flame-ionization detector (Agilent Technologies, Palo Alto®, CA). All results were subjected to least squares ANOVA for a complete randomized design. Data were analyzed by the general line models procedure of SAS (SAS 9.1, 2002®).

## RESULTS

TMR diets analyses averaged 17.25 CP and were estimated at 1.60 Mcal/kg $NE_L$ using NRC (2001) equations. The CON diet contained 3.7% ether extract of diet DM, whereas the other diets contained about 5.50%. Consequently, the LS diets had 0.11 Mcal/kg and HS diets had 0.14 Mcal/kg more $NE_L$ than CON ration. In this investigation ether extract amount in difference diets. Nonetheless, variation normally depends on dietary factors that alter the rumen environment. Intake of DM, was significantly greater for cows fed CON diet compared with those fed sunflower seeds. Milk yield and composition is reported in Table 1. Milk yield and FCM were recorded at 1d to 28d of experimental period, daily. 4% FCM milk, milk efficiency, fat percentage and yield, protein percentage and yield, lactose percentage were not different. Milk actual yield was 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 $C_{14:0}$, $C_{18:1-n9}$, $C_{18:2-n6C}$, $C_{18:3-n3}$, $C_{22:0}$, total UFA, total $n_6$ and $C_{18}$ UFA. Lipid supplementation induces a general increase in C18 percentage at the expense of the short- and medium-chain FA, with the exception of $C_{18:1-n9}$, $C_{18:2-n6C}$ and $C_{18:3-n6}$ 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 unsaturated fatty acids in milk. The total results of milk fatty acid profiles are shown in Table 2.

## DISCUSSIONS

In this experiment little differences were expected in milk yield or composition. 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 $C_{18:1}$ 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, 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. 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 (NRC, 2001). 7.5% untreated sunflower seed (LS-UT) is readily accepted by dairy cows and has no negative effect on DMI (Petit, 2003). 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 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. 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 sunflower. In most cases in which protection of lipid supplements against ruminal hydrogenation improved feed intake, there was an increased fiber digestion in the rumen. Significant difference in milk yield was resulted of 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. These results are same of obtained data by Beachchemin *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. Supplementation with sunflower had no significant effect on increasing of milk yield. Greater milk production in CON could be a result of smaller ADF intake and dietary AA available for absorption by the animal 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) possibly as a result of their negative affects on feed digestibility and rumen fermentation. 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 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 2). 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 AA for milk protein synthesis. By compared with raw or treating sunflower is resulted heating of 15% sunflower can be caused more effects for synthesis of protein. 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. In the present study, concentration of lactose was 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.*, 1992). On the other hand, ineffective protection or low level of added fat (Tymchuk *et al.*, 1998) had no effect on milk fat yield. Feeding oilseeds to lactating dairy cows is one method to change the proportion of unsaturated fatty acids in milk fat (Kim *et al.*, 1993). The response of milk FA composition integrates both rumen metabolism and cow metabolism (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 and 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 16:0 are thought to be produced de novo in the mammary gland (Moate *et al.*, 2007). Increase of $C_{18:1\text{-}n9}$, $C_{18:2\text{-}n6C}$ and $C_{18:3\text{-}n6}$ 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 $C_{18:1n\text{-}9}$ in milk compared to the cows fed the CON, HS-UT and HS-HT diets. $C_{18:1}$ 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 7.5% sunflower 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 $C_{18:1}$. 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 (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. Addition of LS-HT resulted in increases in $C_{18:2}$ and $C_{18:3}$ of 142% and 124%. For omega 3 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 Ashes *et al.*, (1995). $C_{18:2}$ 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. 7.5% and untreated sunflower seed did not result in a large transfer of $C_{18:2}$ and $C_{18:3}$ into milk fat, 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 *et al.*, 1996 and Petit, 2003), but others do not report any significant difference between 18:2- and 18:3-rich lipids on milk 18:0 percentage (Brzoska, 2005). Cows fed HS-UT had the lowest level of UFA in milk compared to the other lipid treatments. 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. $C_{18}$ unsaturated and other unsaturated fatty acids in milk were obtained greater by LS-HT and smaller with HS-UT (Table 2). A decrease in total UFA, $n_3$, and $n_6$ in milk fat with the inclusion of HS-UT or HS-HT is in agreement with others (Palmquist *et al*, 2005).

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

| | Diet | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Variable | CON | LS-UT | LS-HT | HS-UT | HS-HT | SEM | F | *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 |
| FCM 4% | 31.56 | 26.25 | 31.15 | 28.81 | 29.78 | 2.05 | 1.05 | 0.426 |
| Fat % | 3.39 | 3.50 | 3.51 | 3.71 | 3.41 | 0.19 | 0.16 | 0.756 |
| Protein % | 3.40 | 3.21 | 3.24 | 3.18 | 3.18 | 0.15 | 0.52 | 0.828 |
| Lactose % | 4.92 | 4.78 | 4.92 | 4.94 | 4.78 | 0.10 | 0.67 | 0.710 |
| Fat kg/d | 1.17 | 0.99 | 1.17 | 1.11 | 1.11 | 0.09 | 0.78 | 0.625 |
| Protein kg/d | 1.19 | 0.90 | 1.09 | 0.96 | 1.04 | 0.07 | 1.54 | 0.191 |
| Lactose kg/d | 1.70 | 1.35 | 1.65 | 1.49 | 1.56 | 0.11 | 1.19 | 0.339 |

$^{a-c}$Within a row, means without a common superscript differ ($P < 0.05$).

Table 2. Milk fatty acids (g/100 g of total fatty acids) profiles of 28th-day in experiment

| | Diet | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid[1] | CON | LS-UT | LS-HT | HS-UT | HS-HT | SEM | F | *P*< |
| $C_{14:0}$ | 14.47$^{ab}$ | 14.71$^{ab}$ | 14.12$^{ab}$ | 12.05$^{b}$ | 15.13$^{a}$ | 3.22 | 1.47 | 0.215 |
| $C_{14:1-n5}$ | 0.97 | 0.60 | 0.77 | 0.45 | 0.96 | 0.28 | 0.60 | 0.768 |
| $C_{16:0}$ | 32.68 | 33.10 | 32.91 | 34.73 | 33.66 | 3.09 | 0.46 | 0.873 |
| $C_{16:1-n7}$ | 1.73 | 2.93 | 1.34 | 1.10 | 3.46 | 0.85 | 1.27 | 0.294 |
| $C_{18:0}$ | 23.98 | 27.39 | 30.30 | 21.68 | 22.50 | 3.01 | 1.34 | 0.267 |
| $C_{18:1-n7}$ | 2.19 | 2.35 | 1.62 | 1.26 | 1.83 | 0.35 | 1.09 | 0.402 |
| $C_{18:1-n9}$ | 18.66$^{b}$ | 21.67$^{ab}$ | 24.54$^{a}$ | 17.98$^{b}$ | 18.06$^{b}$ | 2.42 | 1.35 | 0.261 |
| $C_{18:2-n6c}$ | 2.48$^{ab}$ | 2.72$^{ab}$ | 3.53$^{a}$ | 1.82$^{b}$ | 2.03$^{ab}$ | 0.58 | 1.18 | 0.349 |
| $C_{18:3-n3}$ | 0.180$^{a}$ | 0.157$^{ab}$ | 0.211$^{a}$ | 0.081$^{b}$ | 0.167$^{a}$ | 0.02 | 2.05 | 0.078 |
| $C_{18:3-n6}$ | 0.115 | 0.089 | 0.157 | 0.160 | 0.184 | 0.04 | 1.18 | 0.344 |
| $C_{18:4-n3}$ | 0.348 | 0.400 | 0.233 | 0.370 | 0.227 | 0.09 | 0.87 | 0.549 |
| $C_{20:0}$ | 0.146 | 0.203 | 0.246 | 0.135 | 0.237 | 0.08 | 0.49 | 0.853 |
| $C_{20:5-n3}$ | 0.047 | 0.004 | 0.018 | 0.004 | 0.018 | 0.01 | 0.82 | 0.593 |
| $C_{22:6-n3}$ | 0.057 | 0.0 | 0.0 | 0.060 | 0.067 | 0.03 | 0.73 | 0.667 |
| Total UFA | 26.95$^{ab}$ | 31.15$^{a}$ | 32.59$^{a}$ | 23.59$^{b}$ | 26.95$^{ab}$ | 2.84 | 1.64 | 0.159 |
| Total $n_3$ | 0.633 | 0.563 | 0.464 | 0.454 | 0.480 | 0.11 | 0.77 | 0.633 |
| Total $n_6$ | 2.76$^{ab}$ | 3.03$^{ab}$ | 3.84$^{a}$ | 2.26$^{b}$ | 2.54$^{b}$ | 0.61 | 1.10 | 0.392 |
| $C_{18}$ UFA | 23.98$^{b}$ | 27.39$^{ab}$ | 30.30$^{a}$ | 21.68$^{b}$ | 22.50$^{b}$ | 3.01 | 1.34 | 0.267 |

$^{a-c}$Within a row, means without a common superscript differ ($P < 0.05$). [1]Total Sat = Total of saturated fatty acids; Total UFA = Total of unsaturated fatty acids; Total n3 = Total of n-3 fatty acids; Total n6 = Total of n-6 fatty acids; C18 UFA = The sum of unsaturated fatty acids with 18 carbons.

## CONCLUSIONS

We obtained that milk production were decreased by oil seed. Fat concentration was greater by all sunflower oil seed diets. Protein concentration in milk was greater for cows fed CON. 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, resulting improve nutritive value of milk from a human health. 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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