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Full Length Research Paper

The influence of feeding plant oils on milk production and fatty acid content

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This investigation was done to research the effects of vegetable oils on UFA content in milk fat and performances of high-yielding lactating cows. Sixteen lactating Holstein cows in early lactation were used in a complete randomized design. Experimental diets included: 1) Control (without oil, CON); 2) Diet with 2% soybean oil (SBO); 3) Diet with 2% sunflower oil (SFO); and 4) Diet with 2% canola oil (CLO). Supplementation with vegetable oils tended to decrease DMI, resulting in reduced milk production. Lowest milk production obtained by SBO treatment with 26.44 kg/day compared with CON diet (32.85 kg/day). The cows whose feed had SBO and SFO added, produced milk with the highest content of UFA, as C18:1 content was 26.82 g/100 g milk fat by SFO compared with CON (20.86). Content of C18:2 obtained 3.53 g/100 g milk fat by SFO that was highest compared to other treatments. These results were observed for C18:3 isomers, too. All observations for C18 UFA were significant (P<0.05). Despite, obtained results about CLO was unintelligible. In general, supplementation of dairy cow diets with vegetable oils tended to enrich UFA content in milk fat significantly. Sunflower oil seemed to be the optimal source to increase UFA production.

Key words: Vegetable oil, fatty acid, dairy cow.

INTRODUCTION

Public health concerns are driving research into modifying the FA profiles of cow's milk, particularly toward less SFA and more PUFA (Glasser et al., 2008). The SFA are considered to produce negative effects particularly when consumed in excess, whereas UFA has well-known or potential positive effects on human health (Parodi, 2005). In addition, milk of ruminants fat content and composition can be extensively modified by nutritional factors (Shingfield et al., 2008). The simplest way of altering milk FA composition is to supplement the diets of cows with unsaturated lipids. The main sources of unsaturated lipids are oil plants and oilseed lipids, such as soybean, canola, and sunflower (Glasser et al., 2008). Supplementing the diets of cows with plant lipids decreased the medium chain fatty acids (C10:0, C12:0, C14:0 and C:16) and increased the C18 UFA content of milk fat (Palmquist et al., 1993).

There is growing interest in feeding soybean, sunflower and canola oil to dairy cows because they have oleic and linolenic acid which contributes to dietary n3 FA and promotes increased linoleic acid isomers content while decreasing the SFA content of milk (Chilliard et al., 2009). The effects of vegetable oils supplementation on milk yield and composition have often been studied (Zheng et al., 2005; AbuGhazaleh and Holmes, 2007; and Luna et al., 2008) that reported adding kinds of vegetable oils to dairy cow diets are caused to decrease SFA and increase linoleic acid. Furthermore, feeding fats high in PUFA content can alter the FA composition of milk (Bu et al., 2007) in a manner beneficial to human health, including increased proportions of MUFA and PUFA and increased concentrations of the linoleic acid isomers (Hu and Willett, 2002). Hence, the objective of this study was to investigate the effects of supplementing

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Abbreviations: 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	Diets			
	CON	SBO	SFO	CLO
Ingredients, % of DM				
Alfalfa hay[1]	6.45	6.22	6.22	6.22
Corn silage	43.56	43.82	43.82	43.82
Corn grain	5.92	6.13	6.13	6.13
Barley	11.52	8.32	8.32	8.32
Wheat bran	8.52	8.67	8.67	8.67
Cottonseed[2]	9.08	9.60	9.60	9.60
Soybean meal[3]	8.35	8.37	8.37	8.37
Canola meal[4]	5.50	5.76	5.76	5.76
Soybean oil[5]	-	2.00	-	-
Sunflower oil	-	-	2.00	-
Canola oil	-	-	-	2.00
Salt	0.025	0.025	0.025	0.025
Sakarosh[6]	0.96	0.96	0.96	0.96
Mineral permix	0.025	0.025	0.025	0.025
Vitamin permix	0.025	0.025	0.025	0.025
Chemical composition % of DM				
DM (% of Diet)	62.50	61.67	61.50	62.34
CP (% of DM)	16.72	15.75	16.60	16.95
Ether extract (% of DM)	1.95	3.85	3.75	3.69
NDF (% of DM)	45.13	44.96	40.40	45.07
ADF (% of DM)	22.11	26.03	27.55	29.02
NFC[8] (% of DM)	31.66	30.98	34.46	30.00
Ash (% of DM)	4.54	4.46	4.79	4.33

[1]Alfalfa forage of third cutter from a dairy farm in Markazi Province, Iran. [2]Cottonseed, whole with lint (23.50% CP). [3]Soybean meal, solvent (44% CP). [4]Canola meal, mech. Extract (37% CP). [5]Soybean oil, sunflower oil and canola oil were purchased from a local market in Arak, Iran. [6]Sakarosh is a compound made by Biosaff (*Saccharomyses Cervicea*) and Sodium Bicarbonate that purchased from Kanidam Co, Tehran, Iran. [7]Analysis performed on 2 period samples. DM = Dry matter; CP = crude protein; [8]NFC = 100 – (CP% + NDF% + ether extract% + ash%).

dairy cow diet with sources of long chain FA such as soybean, sunflower and canola oil on milk fatty acid and milk performance in lactating dairy cows.

MATERIALS AND METHODS

Animals and diets

Sixteen early lactating multiparous Holstein cows averaging 635 kg of BW, producing 32 kg/day milk in four treatments were used in a complete randomized design to evaluate responses to supplementary vegetable oil in diets. The experimental period lasted 4 weeks and was preceded by a 2 week 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/day milk with 3.8% fat. Diets are shown in Table 1. Treatments included: 1) CON, without oil; 2) SBO, with 2% soybean oil; 3) SFO, with 2% sunflower oil, and 4) CLO, with 2% canola oil. Cows within groups were assigned randomly to one of four treatments and four replicates. Cows were fed individually and milked three times daily at 0060, 1400 and 2000 h.

Milk production was recorded at every milking. The four dietary treatments (Table 1) consisted of supplements based on either soybean oil, sunflower oil and canola oil by 2% level which would lead to about 3.65 to 3.85% fat in oily diets compared to 1.95% in the control diet. Thus, the four diets were designed to yield similar CP and difference in ether extract and fatty acids concentrations as well as energy. Feed consumption was recorded as initial of each week. Composited samples were mixed thoroughly for chemical analysis. 500 ml milk samples were obtained on day 28 from each cow. Three consecutive milking was done to determine fat, protein, lactose and fatty acid profiles.

Chemical analysis

Dried feed samples were further ground in a Cyclotec mill. Dry matter of TMR (total mixed ration) was determined by method of AOAC (2000), ID 930.15. CP determination was done by the Kjeldahl method described by AOAC (2000), ID 945.01. Both ADF

Table 2. DMI, Milk yield, and composition of milk from lactating dairy cows.

Variable	Diets[1]				SEM	F	P<
	CON	SBO	SFO	CLO			
DMI	22.43[a]	21.21[ab]	20.87[b]	20.63[b]	0.35	3.55	0.02
Milk yield, kg/d	32.85[a]	26.44[b]	31.35[ab]	29.25[ab]	3.20	2.05	0.05
FCM[2] 4%	30.45	25.64	30.20	27.75	2.33	1.15	0.33
Composition							
Fat	3.37	3.53	3.51	3.66	0.18	0.16	0.65
Protein	3.41	3.32	3.22	3.18	0.16	0.45	0.85
Lactose	4.87	4.65	4.66	4.79	0.10	0.67	0.87
Yield kg/day							
Fat	1.21	0.93	1.10	1.07	0.10	0.75	0.73
Protein	1.12	0.88	1.00	0.93	0.06	1.57	0.20
Lactose	1.59	1.22	1.46	1.40	0.13	1.21	0.42

[a-c]Within a row, means without a common superscript differ (P < 0.05). [1]CON = Diet of Control; SBO = diet of including 2% soybean oil; SFO = diet of 2% sunflower oil; CLO = diet of including 2% canola oil; [2]FCM = 4% fat-corrected milk.

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 and experimental diets were determined by gas chromatography. Frozen milk samples were shipped to the laboratory of chemical and feed analysis for analysis using the following procedures. Milk fat was separated by centrifugation (8000×g; 45 min), and whey was removed by vacuum aspiration leaving the fat layer. Lipids were extracted with chloroform: methanol (2:1 vol/vol). Methyl esters of fatty acids from feed and milk were prepared by transesterification. 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℃, respectively. Data in tegration and quantification were accomplished with Agilent 3365 chemstation 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 CRD (complete randomized design) using the following model:

$Yij = \mu + Ti + Eij;$

Where: Yij = observation; μ = mean; Ti = treatment, i = 1,2,3,4; j = 1,2,3,4; and Eij = residual error.

Least square means were separated by Duncan's multiple range tests with significance declared at P ≤ 0.05. Effects of treatment were tested using the random effects of cow as the error term. The means were compared by Duncan's procedure.

RESULTS

Complete diets (Table 1) were formulated for Holstein cows averaging 32 kg of milk/day with 17% CP (of diet DM). The respective CON, SBO, SFO and CLO analyses averaged 16.72, 15.75, 16.60 and 16.95% CP. The CON diet contained 1.97% ether extract of diet DM, whereas the SBO, SFO and CLO contained 3.85, 3.75 and 3.69% of diets DM, respectively. Consequently, the oil diets had near 2% ether extract more than the CON ration. Nonetheless, variation normally depends on dietary factors that alter the rumen environment (e.g., forage-to-concentrate ratio and DMI). Intake of DM, expressed in kg per day was significantly greater for cows fed CON diet compared with those fed oil diets. Milk yield and composition is reported in Table 2. Milk yield and 4% FCM (fat corrected milk) were recorded at 1 to 28 days of experimental period, daily. FCM, fat, protein and lactose were not different.

Milk actual yield were lower from oil diets and was significant between CON compare with SBO diet (P<0.05). Fat percentage was higher in milk from CLO cows (3.66%) and lower in milk from CON cows (3.39%) but without significant difference. Fat yield in CON and SFO was more than other treatments. In this study, significant difference between milk FA profiles were for C18:1n-9, C18:2n-6cis, C18:3n-3, UFA, Ω6, Ω3+Ω6, and C18 UFA. Vegetable oils supplementation induces a general increase in C18 percentage at the expense of the

Table 3. The milk fatty acids (g/100 g milk fat) from the different diets fed to cows.

Fatty acids	Diets[1]				SEM	F	P<
	CON	SBO	SFO	CLO			
C14:0	13.32	13.89	13.96	12.35	2.98	1.56	0.27
C14:1n-5	0.92	0.65	0.79	0.53	0.31	0.65	0.78
C16:0	31.63	32.90	32.85	34.65	3.09	0.46	0.87
C16:1n-7	1.65	2.96	1.54	1.20	0.85	1.27	0.32
C18:0	22.87	27.38	30.00	22.76	3.01	1.34	0.29
C18:1n-7	2.12	2.45	1.94	1.37	0.35	1.09	0.42
C18:1n-9	18.74^{b}	21.55^{ab}	24.88^{a}	17.76^{b}	2.48	2.35	0.04
C18:2n-6cis	2.48^{ab}	2.78^{ab}	3.53^{a}	1.76^{b}	0.58	3.23	0.02
C18:3n-3	0.176^{a}	0.159^{ab}	0.215^{a}	0.083^{b}	0.02	2.05	0.07
C18:3n-6	0.103	0.087	0.121	0.153	0.04	1.18	0.36
C18:4n-3	0.244	0.385	0.321	0.386	0.09	0.87	0.63
C20:4n-6	0.088	0.176	0.109	0.206	0.12	0.78	0.64
Total UFA[2]	26.95^{ab}	31.15^{a}	32.59^{a}	23.59^{b}	2.84	1.64	0.159
Total Ω3	0.633	0.563	0.464	0.454	0.11	0.77	0.633
Total Ω6	2.76^{ab}	3.03^{ab}	3.84^{a}	2.26^{b}	0.61	1.10	0.392
Ω3+ Ω6	3.39^{ab}	3.60^{ab}	4.31^{a}	2.71^{b}	0.65	1.07	0.410
C18 UFA[3]	23.98^{b}	27.39^{ab}	30.30^{a}	21.68^{b}	3.01	1.34	0.267

$^{a-c}$Within a row, means without a common superscript differ ($P < 0.05$). [1]CON = Diet of Control; SBO = diet of including 2% soybean oil; SFO = diet of 2% sunflower oil; CLO = diet of including 2% Canola oil; [2]total UFA = total of unsaturated fatty acids; [3]C18 UFA = the sum of unsaturated fatty acids with 18 carbons.

short- and medium-chain FA, with the exception of C18:1n-9, C18:2n-6cis and C18:3n-6 that SFO treatment tended to increase those fatty acids. Other treatments had a limited significant effect on milk fatty acid composition. The results of milk FA profiles are shown in Table 3.

DISCUSSION

Dietary composition

Because all treatments met or exceeded energy and protein requirements, little differences were observed in milk yield or composition. The dietary protein level of CON was adjusted using cottonseed and soybean meal. Soybean and Sunflower oils are an excellent source of linoleic acid and canola oil is a source of oleic acid. The resulting CON diet had low-level monoenoic and dienoic fatty acids. Whereas SBO, SFO and CLO diets were higher in linoleic and oleic acid (C18:2 and C18:1) than the CON diet. Oleic acid was more concentrated in the CLO diets than in the SBO, SFO and CON diets. The SBO and SFO 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.

DMI, milk yield and milk composition

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). Results showed that 2% of vegetable oils are readily accepted by dairy cows and has no negative effect on DMI (Petit, 2003). Moreover, feeding up to 30% of oil seed in the DM has no effect on DMI (Rafalowski and Park, 1982). Differences in DMI between CON diets and plant oil diets can be related to ether extract access in those diets. Because lack of oil in CON diet, could result in no negative effect on rumen fermentation (Chilliard et al., 2009). Feeding CON diet compared with plant oil diets could then result in less oil being released in the rumen, which would limit the negative effect of oil on fiber digestion (Schauff and Clark, 1992) and thus on DMI. We expected that higher dietary fat intake repartum could prevent excessive lipid mobilization in adipose tissue and thereby ameliorate DMI in the subsequent lactation (Duske et al., 2009). This would be corroborated by the fact that feeding 2% oil in the DM has no effect on ruminal fermentation. In most cases in which protection of lipid supplements against ruminal biohydrogenation improved feed intake, there was an increased fiber digestion in the rumen (Table 3).

Significant difference in milk yield resulted from CON diets compared to SBO diet that produced 32.85 vs. 26.44 kg/day. Obtained results had observed milk yield

with SBO, SFO and CLO being 26.44, 31.35 and 29.25 kg/day without significant difference respectively. These results are same with obtained data by Beauchemin et al. (2009) and Petit (2003). CON treatment increased milk production by an average of 2.07 kg/day, which would mainly result of greater DMI. Supplementing dairy cow diets with high amounts of plant oils often cause a drop in feed intake and therefore lowers milk yield (Flowers et al., 2008; Chilliard et al., 2009; Rego et al., 2008) possibly as a result of their negative effects on feed digestibility and rumen fermentation (Jenkins, 1998). FCM had no significant difference, but CON diet caused 30.45 kg/day 4% FCM; followed SFO with 30.20 kg/day. Petit (2003) reported that feeding lactating dairy cow diets supplemented with plant fats increased milk fat percentage. We used 2% oil in this study that consumed about 3.7% ether extract. Adding plant oils to dairy cow diets as soybean or sunflower or canola increased fat milk percentage with most effect due to canola oil.

In this investigation, protein percentage and yield (kg/day) was greater for cows fed CON diet compared with other diets. The lack of effect oils on milk protein concentration has been previously reported by Tymchuk et al. (1998) and Ashes et al. (1995). AbuGhazaleh et al. (2003) reported that 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 were similar among treatments. Generally, oils that were effectively protected against ruminal biohydrogenation increase milk fat yield (Ashes et al., 1995). 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 plant oils 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., 2009)]. 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 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, C4:0 to C14:0 and some C16:0 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 SFO treatment is in agreement with the results of Casper et al. (1988). In this study, the greatest effect was observed for animals fed SFO and SBO. Cows fed SFO had higher C18:1n-9 in milk compared to cows fed the CON and CLO 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 experimental diets compared to the control. Total C18:1 in milk for the SBO was higher than in milk from the control and other diets. The increased concentration of C18:1 may be 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. These results are in agreement with AbuGhazaleh and Holmes (2007) and Luna et al. (2008). Inclusion of oil in the diet resulted in an intensification in the concentration of C18:2n-6cis with the greatest gain observed for cows fed SFO. Compared to the control, milk from cows fed SFO had 70% more C18:2n-6cis. Although added dietary fat increased the linoleic acid (C18:2) content of milk fat. This confirms the high rumen biohydrogenation of dietary 18:2 observed for oils (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 SFO resulted in increases in C18:2 and C18:3 of 70 and 122%, respectively. For omega 3 linolenic acids there were no significant differences among dietary treatments. The concentration of C18:3n-3 in milk from cows fed SFO and CON was higher than from cows fed the CLO 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, oils 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 of oils did not result in a large transfer of C18:2 and C18:3 into milk fat.

Also suggesting that these fatty acids were saturated to either C18:0 or C18:1. In the experiments that have compared different 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; 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). Significant differences were observed for total UFA in milk among the dietary treatments. Cows fed CLO had the lowest level of UFA in milk compared to the other lipid treatments. UFA content of milk was affected by sunflower and soybean oil. SFO and SBO (33.44 and 31.19) obtained an increase in UFA.

No significant differences were found between treatments for change of total Ω3 fatty acids. The concentration of total Ω6 in milk fat was increased by 3.76, 3.04 and 2.67 of the SFO, SBO and CON compared

to the CLO. Milk from cows fed SFO and CLO had highest and lowest Ω3+Ω6 fatty acid, respectively. C18:0 UFA fatty acids in milk was obtained greater by SFO and smaller with CLO (Table 3). An increase in UFA, Ω6, Ω3+ Ω6 and C18:0 UFA in milk fat with the inclusion of plant oils is in agreement with others (Palmquist and Jenkins, 1980) when fat was supplemented at 2% or more in the diets. Palmquist et al. (1993) reported that reductions in mentioned fatty acids by high level oil 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 the aforementioned fatty acids. 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 that feeding diets containing vegetable oils had different results compared to normal dairy cow diets. We obtained that DMI was increased by normal diets. Milk production was significantly decreased only for cows fed SBO and increased for CON treatment and other oil treatment. Protein concentration in milk was greater for cows fed CON diet than for those fed oil. In general, supplementation of 2% sunflower or soybean oils caused greater UFA in milk and omega 6, resulting in improved nutritive value of milk from a human health point of view.

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