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Dry Matter Intake, Milk Yield and Milk Fatty Acid Composition of Dairy Cows Fed Raw or Microwave Irradiated Safflower Seed as a Substitution to Cottonseed

Research Article

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ABSTRACT

The present study was conducted to evaluate the effects of using raw or microwave irradiated safflower seed on dry matter intake, milk production and composition, milk fatty acid profile, blood parameters, and nutrient apparent digestibility in early lactation cows. Nine multiparous Holstein cows at early lactation were randomly assigned to one of three treatments based on 3 × 3 balanced latin square design. Dietary treatments included whole linted cottonseed (control), 40 g/kg DM raw safflower seed (RSS), and 40 g/kg DM microwave irradiated safflower seed (MSS). Results showed no significant effect of dietary treatments on milk production, milk fat, protein, and lactose content, and dry matter intake. Feeding RSS and MSS diets increased milk long chain fatty acids, C18:1 trans, C18:2, and polyunsaturated fatty acid concentrations, while milk C16 fatty acid ($P<0.05$) decreased. The cis-9, trans-11 conjugated linoleic acid (CLA) concentration tended to be higher in cows fed MSS diet. It was concluded that there are no negative impacts of raw and microwave irradiated safflower seed supplementation on lactation performance, while milk quality was meliorated by increasing unsaturated fatty acid concentrations.

KEY WORDS fatty acid profile, lactating cow, microwave irradiation, milk composition, safflower seed.

INTRODUCTION

Elevated concentrations of cholesterol and low-density lipoprotein (LDL) in human blood serum due to using milk predominant long chain fatty acids (C14:0-C18:0) causes the higher risk of cardio-vascular diseases (Mansbridge *et al.* 1997). Increasing the ratio of unsaturated to saturated fatty acid along with conjugated linoleic acid (CLA) common and useful isomers (C18:2 cis-9, trans-11 and trans-10, cis-12) in dairy milk fat was well-studied (Aydin, 2005; Troegeler-Meynadier and Enjalbert, 2005). Based on previous studies, CLA had some positive effects such as reduction in body fat deposition, type II diabetes prevalence, atherosclerosis, while improving bone mineralization and immune system along with anti-carcinogenic properties (Belury, 2002). In order to improve unsaturated fatty acid (FA) content of milk fat, some oilseeds were fed to ruminants. Researches indicated high linoleic acid content of safflower seed (*Carthamus tinctorius*) which can be fed to ruminants (Alizadeh *et al.* 2010). In an experiment, adding 6% of diet dry matter (DM) safflower oil raised cis-9, trans-11 CLA content of milk (Bell *et al.* 2006). safflower seed have some phenolic glycosides (2-hydroxy-arctiin and matairesinol monoglucoside) as antinutritional factor and it can be altered dry matter intake (DMI) in ruminant (Kim *et al.* 2006) but Nagatsu (2004) reprted that its amount in safflower is reduced by breeding for improving seed yield. No negative effects were determined on milk production, DMI

and ruminal fermentation when safflower seed added up to 5% DM of diet during early lactation, but milk CLA increased when 25 g/kg of safflower seed were used in diet Alizadeh *et al.* (2012). Previous studies reported effects of oilseeds heat processing techniques on fatty acids bio hydrogenation in rumen (Samadi *et al.* 2018) and CLA content of milk fat (Troegeler-Meynadier *et al.* 2006). In this regard, it was reported that feeding diets with extruded or roasted soybean increased milk fat CLA content two to three times higher than control diet with raw grounded soybeans (Chouinard *et al.* 2001). Nowaday ussing of microwave irradiation in ruminant nutrition is common, because of its less preparing time, faster heating and saving in energy and time, acurate controled process and improving in nutritive quality of treated feed (Sumnu, 2001; Lashkari *et al.* 2015).

Accordingly, the present study was designed to evaluate the effects of dietary adding of raw safflower seed (RSS) or microwave irradiated safflower seed (MSS) on DMI; blood parameters; milk production, composition, and fatty acid profile; and nutrient apparent digestibility in early lactation dairy cows.

MATERIALS AND METHODS

Animals, diets and treatment

Nine multiparous Holstein cows averaging: 38.6 kg/d of milk yield, 33 ± 8 days in milk and 3 ± 1 lactations were randomly assigned to 3 × 3 Latin square design at the beginning of the experiment. Cows were housed in individual stalls.

The experiment included three 21 d periods in a way that in each period the first 14 d used for adaptation and the last 7 d used for sampling and data collection. All cows had free access to drinking water, were fed individually twice a day, and were milked three times a day. Safflower seeds (variety S-3110) were purchased from a commercial supplier in Hashtrood city, Eastern Azerbaijan province, Iran. For microwave treatment, safflower seeds were placed in a Pyrex pan (28×28 cm) with the height of 5 cm and were subjected to microwave irradiation (Butane microwave oven BC380W, Iran; 2450 MHz frequency) for 3 min and then stored at 4 ˚C for a maximum of 7 d. National research council recommendations were used for formulizing diets. Dietary treatments included: control group (100 g whole cottonseeds+0 g/kg DM safflower seeds), RSS group (60 g whole cottonseeds+40 g/kg DM RSS), and MSS group (60 g whole cottonseeds+40 g/kg DM MSS). Total mixed ration (TMR) were then fed to cows twice a day (Tables 1). Moreover, it should be mentioned that each kg of safflower seed (based on DM) contained 941 g DM, 168 g crude protein (CP), 435 g neutral detergent fiber (NDF), 337 g acid detergent fiber (ADF), and 261 g ether extracts (EE). Also, fatty acids composition of safflower and cottonseed were determined by gas chromatography-mass spectrometry (GC-MS) (Savage *et al.* 1997; Savage *et al.* 1999) and each kg of safflower seed oil extract contained 87 g palmitic acid, 26 g stearic acid, 113 g oleic acid, 742 g linoleic acid, and 2 g linolenic acid, while these values for each kg of cottonseed oil extract were 273, 21, 176, 493, and 1 g, respectively.

Dry matter intake, feed analyses, and nutrient digestibility

The amount of TMR which were offered to cows considered as maximum as possible to ensure having at least 10% daily refusals. TMR Samples accompanied by rectum fecal samples were obtained daily during d 14 to d 21 (sampling days) of each experimental period. Samples of feed and feces stored at −20 ˚C until chemical analysis. Afterward, samples thawed at 20-25 ˚C, oven dried (55 ˚C for 48 h) samples grounded using Wiley mill adjusted to 1 mm screen.

All feed samples were analyzed for NDF, ADF, N, ether extract, ash (AOAC, 1990). Acid detergent insoluble ash contents used to determine apparent total tract nutrient digestibility (Van Keulen and Young, 1997). The NDF and ADF fractions included residual ash.

Milking, milk composition analysis, and fatty acids analysis

Cows were milked three times a day at 02:00, 10:00, and 18:00 h. Milk production was recorded at last 5 d of each period. In order to ensure no effect of mastitis on milk yield and composition, cows were monitored for udder inflammation or milk clots in nipples before each milking. Individual milk samples were collected in pre-labeled 50 mL plastic tubes at each milking and preserved using potassium dichromate. Milk samples compositions were determined using Milk-O-Scan (Funke Gerber, LactoStar), and FA composition of milk samples were determined for individual cows. Extraction and derivation of fat prior to GC-MS detection were according to Savage *et al.* (1997) and Savage *et al.* (1999) technique. Agilent gas chromatography (2001, Palo Alto, CS, USA) with a 30 m to 0.25 mm HP-5MS capillary column coupled with a flame-ionization detector, selective quadrupole mass detector (Agilent Technologies, Palo Alto, CA) operating in EI mode at 70 eV was used for GC-MS analysis.

Injector and detector ports temperatures were set at 250 and 150 ˚C, respectively. Column initial temperature held at 60 ˚C for 3 min and then, increased at a rate of 5 ˚C per min to 220 ˚C. Finally, column temperature held at 220 ˚C for 10 min.

Table 1 Ingredients and chemical composition of the diets in g/kg of diet dry matter (DM)

Ingredients	Diets[1]		
	Control	RSS	MSS
Alfalfa hay	200	200	200
Corn silage	200	200	200
Whole linted cottonseed	100	60	60
Safflower seed	-	40	-
Microwave irradiated safflower seed	-	-	40
Ground barley grain	190	190	190
Ground corn grain	60	60	60
Soybean meal	130	130	130
Cottonseed meal	30	30	30
Wheat bran	60	60	60
Calcium carbonate	10	10	10
Minerals and vitamins supplement[2]	10	10	10
Sodium chloride	5	5	5
Sodium bicarbonate chemical	5	5	5
Chemical composition (g/kg)			
Crude protein (CP)	175	173	173
Ether extract (EE)	42	43	43
Neutral detergent fiber (NDF)	315	315	315
Acid detergent fiber (ADF)	201	200	200
NFC[3]	414	420	420
Calcium	9	9	9
Phosphorus	5	5	5
Net energy for lactation (NE_L, MJ/kg DM)	6.99	6.99	6.99

[1] Control: diet with 100 g cottonseeds and no safflower seeds; Raw safflower seed (RSS): diet with 60 g cottonseeds and 40 g raw safflower seeds and microwave irradiated safflower seed (MSS): diet with 60 g cottonseeds and 40 g microwave irradiated safflower seeds per kg of diet DM.
[2] Minerals and vitamins supplement contained: Ca: 196 g; P: 96 g; Na: 71 g; Mg: 19 g; Fe: 3 g; Cu: 0.3 g; Mn: 2 g; Zn: 3 g; Co: 100 ppm; I: 100 ppm; Se: 0.1 ppm; vitamin A: 50 × 105 IU; vitamin D: 10 × 105 IU and vitamin E: 0.1 g.
[3] Non fiber carbohydrates (NFC)= 1000 − (g NDF+g CP+g EE+g ash per kg of diet DM).

Blood sampling and serum analysis

Blood samples were collected from the coccygeal vein at d 21 of each experimental period. Blood samples were centrifuged at 4000 rpm for 10 min and serum were obtained and stored at −20 ˚C until analysis. Serum glucose, cholesterol, and blood urea nitrogen (BUN) were measured using enzymatic colorimetric method and by commercial kits (Pars Azmon Co., Iran). Also, non-esterified fatty acids (NEFA) concentrations and beta-hydroxybutyric acid (BHBA) were determined using enzymatic procedure by a commercial kit (Randox, UK).

Statistical analysis

Mixed procedure of SAS software was used to analyze data (SAS, 2001).

The experimental model included fixed effects of square, dietary treatment, day, and their interactions, while cows within square and period within square considered as random variables.

Before any analysis, data were tested for normal distribution of the residuals by PROC UNIVARIATE of SAS software. Tukey-Kramer test was applied for the comparison of group differences.

RESULTS AND DISCUSSION

Results of the present study not only showed that the DMI was not influenced by RSS and MSS supplementation treatments compared to control diet but also, these similarities indicate no negative effects of treatments on DMI (P>0.05; Table 2). Addition of RSS and MSS in the diets did not alter apparent total tract digestibility of DM, organic matter (OM), and NDF. Addition of RSS and MSS in the diets had no effect on milk production, 4% fat-corrected milk (FCM), and energy-corrected milk (ECM) (Table 3). Milk components including fat, protein yield and lactose were similar among the treatments (P>0.05). No significant differences (P>0.05; Table 3) determined in efficiency of milk production. The results showed that adding RSS and MSS in the diets had no effect (P>0.05) on milk yield and feed efficiency (milk yield/DMI) among treatments. There were no differences (P>0.05; Table 4) in the concentration of short-chain (C6-C12) and medium-chain (C14-C17) fatty acids in milk fat except for C14:1 and C16 in which C14:1 was lower in cows fed the RSS and MSS diets than those fed the control diet (P<0.05), and C16 fatty acid was decreased by MSS dietary treatment.

Table 2 Dry matter intake and total tract digestibility

Item	Treatment diets[1]			SEM	P-value
	Control	RSS	MSS		
Dry matter intake (kg/d)	27.4	26.9	27.2	0.59	0.831
Nutrient digestibility (g/kg)					
Dry matter	622.7	619.3	627.7	8.67	0.794
Organic matter	656.9	661.4	665.8	8.38	0.758
Neutral detergent fiber	494.7	523.6	506.0	24.6	0.696

[1] Control: diet with 100 g cottonseeds and no safflower seeds; Raw safflower seed (RSS): diet with 60 g cottonseeds and 40 g raw safflower seeds and microwave irradiated safflower seed (MSS): diet with 60 g cottonseeds and 40 g microwave irradiated safflower seeds per kg of diet DM.
SEM: standard error of the means.

Table 3 Milk production and composition

Item	Treatment diets[1]			SEM	P-value
	Control	RSS	MSS		
Milk production (kg/d)	39.91	39.69	38.97	2.42	0.074
4% fat-corrected milk (kg/d)[2]	34.40	33.15	32.32	1.56	0.194
Energy-corrected milk (kg/d)[3]	36.97	35.97	35.04	1.62	0.198
Efficiency (milk production/DMI)	1.47	1.50	1.42	0.09	0.832
Milk composition (g/kg)					
Fat	31.2	29.4	28.9	1.62	0.289
Protein	28.5	28.1	28.3	0.60	0.828
Lactose	47.2	47.0	47.2	0.04	0.890
Milk composition (kg/d)					
Fat	1.22	1.13	1.11	0.056	0.154
Protein	1.12	1.11	1.09	0.049	0.425
Lactose	1.89	1.88	1.84	0.105	0.356

[1] Control: diet with 100 g cottonseeds and no safflower seeds; Raw safflower seed (RSS): diet with 60 g cottonseeds and 40 g raw safflower seeds and microwave irradiated safflower seed (MSS): diet with 60 g cottonseeds and 40 g microwave irradiated safflower seeds per kg of diet DM.
[2] Fat corrected milk with 4% fat (NRC, 2001).
[3] Energy correctd milk (ECM)= [0.327 × milk yield (kg/d)) + (12.95×fat yield (kg/d)) + (7.2×protein yield (kg/d)]
SEM: standard error of the means.

Table 4 Fatty acid profile of milk fat (g/kg of total milk fatty acids)

Fatty acids[2]	Treatment diets[1]			SEM	P-value
	Control	RSS	MSS		
C6	30.1	28.7	29.3	2.65	0.932
C8	22.9	22.7	22.5	2.61	0.990
C10	46.5	43.2	42.3	3.28	0.374
C12	41.1	37.3	37.6	3.04	0.624
SCFA	140.6	131.9	131.7	8.09	0.656
C14	126.4	132.4	129.9	3.32	0.397
C14:1 trans	9.2^{a}	8.0^{b}	7.6^{b}	0.43	0.041
C15	18.2	19.2	18.9	1.09	0.811
C16	278.7^{a}	267.2^{ab}	266.3^{b}	3.67	0.039
C17	13.2	12.7	12.4	1.55	0.930
MCFA	439.6	445.9	435.4	6.37	0.340
C18	90.7^{b}	101.1^{a}	102.3^{a}	3.82	0.076
C18:1 trans	22.3^{b}	27.0^{a}	27.8^{a}	1.54	0.040
C18:1 cis	169.3	175.6	177.9	7.00	0.588
C18:2	29.5^{c}	33.2^{b}	36.9^{a}	1.54	0.001
cis 9 trans 11 CLA	3.3	3.8	4.8	0.09	0.356
LCFA	315.3^{b}	340.8^{a}	349.9^{a}	10.71	0.025
SFA	668.0	664.7	661.8	9.82	0.887
USFA	233.7	247.7	255.2	9.34	0.129
MUFA	200.8	210.6	213.4	7.67	0.396
PUFA	32.9^{c}	37.1^{b}	41.8^{a}	1.50	0.001
Others	104.5	72.4	83.0	-	-

[1] Control: diet with 100 g cottonseeds and no safflower seeds; Raw safflower seed (RSS): diet with 60 g cottonseeds and 40 g raw safflower seeds and microwave irradiated safflower seed (MSS): diet with 60 g cottonseeds and 40 g microwave irradiated safflower seeds per kg of diet DM.
[2] Fatty acid composition was expressed as g/kg of fatty acid methyl esters: SCFA: short-chain fatty acids (C6 to C12); MCFA: medium-chain fatty acids (C14 to C17); LCFA: long-chain fatty acids (C18); SFA: saturated fatty acids; USFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids and CLA: conjugated linoleic acid.
The means within the same row with at least one common letter, do not have significant difference (P>0.1).
SEM: standard error of the means.

Adding RSS and MSS in the diet increased C18:0, C18:1 trans, and C18:2 fatty acids in milk.

In general, polyunsaturated fatty acids were increased by RSS and MSS treatments (P<0.05). Based on the results, milk C18:0 fatty acid was increased by adding 40 g/kg of diet with RSS and MSS. An increase (P<0.05) in the content of C18:1 trans and a tendency to increase in C18:1 cis was observed in cows fed RSS and MSS diets. Results show that the concentration of cis-9, trans-11 CLA in milk tended to increase when RSS and MSS added to the diets. Inclusion of MSS in the diet numerically increased concentration of cis-9, trans-11 in milk compared with the cows fed RSS.

Serum concentrations of glucose, urea, NEFA and BHBA were not affected by the addition of RSS and MSS in the diets, but serum cholesterol (P>0.05) concentration was increased by RSS and MSS dietary treatments (Table 5).

It was reported that adding safflower seed up to 50 g/kg diet (Alizadeh *et al.* 2010; Dschaak *et al.* 2011) and heat treated safflower seed such as roasting (Alizadeh *et al.* 2012) had no effect on DMI. Godfrey and Dhiman (2006) reported that grounded or extruded safflower seed can be fed up to 20 g/kg of diet DM without having negative effects on DMI; on the other hand, a decrease in DMI was observed by Stegeman *et al.* (1992) when feeding safflower seed at 100 g/kg of diet. The reduction in DMI by feeding higher amount of safflower seed can be partly due to some phenolic glycosides (2-hydroxy-arctiin and matairesinol monoglucoside) in safflower seeds which produce a bitter taste and have cathartic effects (Alizadeh *et al.* 2012; Kim *et al.* 2006). It seems that along with breeding for improving seed yield, seed oil content and its quality, that caused a concomitant reduction in phenolic glycosides content of the seed. Therefore, observing no difference in DMI by adding safflower seed in the diet may be because of its considerably low phenolic glycosides content which could not induce low feed intake. In agreement with our results, it was reported that heat treatment like micronization and extrusion of oil seeds such as flaxseed had no effect on DMI (Gonthier *et al.* 2005).

Polyunsaturated fatty acids can alter the apparent digestibility of DM, OM, and particularly NDF by acting on cell membrane of gram-positive bacteria, especially cellulolytic bacteria (Martin *et al.* 2008). In this study, RSS and MSS supplementations in the diet had no effects on apparent total tract digestibility of DM, OM, and NDF which are in agreement with findings of Dschaak *et al.* (2011) and Alizadeh *et al.* (2010). In the study of Dschaak *et al.* (2010) an increase in total tract digestibilities of DM and OM was observed, which was opposite to the results of the present study, while their result for NDF was in agreement with our results. Present results indicated no changes in rumen cellulolytic activities by using safflower seed, which then resulted in no differences in fiber digestion by adding safflowe seed or NutraSaff safflower seed (Safflower Technologies International, Sidney, MT) in the diet. Observing different results among studies may be explained by the presence of different types and varieties of safflower seeds. Our findings not only agreed on milk production and composition including milk fat, lactose, and protein among treatments with other researchers, but also adding RSS and MSS in the diets had no effect on milk fat yield (Alizadeh *et al.* 2010; Alizadeh *et al.* 2012; Dschaak *et al.* 2010; Dschaak *et al.* 2011).

Dschaak *et al.* (2010) reported that increasing NSS - which had higher oil and less NDF than commercial safflower seed- supplementation in diet (up to 40 g/kg, the same amount of our research) linearly decreased milk fat yield. Mainly, differences in physiological condition of experimental animals, chemical composition of safflower seeds and diet composition can cause various responses.

Chilliard *et al.* (2007) reported that milk fat decreased when the diet included low forage, but rich in C18:2 n-6 FA whereas using oilseeds in forage based diets with lipids having higher amount of C18:2 n-6 did not affect milk fat synthesis. Heat treatment of oil seeds such as flaxseed resulted in different effects on milk fat concentration. Some studies showed that using different amounts of micronized flaxseed, 1 kg/d (Soita *et al.* 2003) or 10% of diet (Gonthier *et al.* 2005) including raw, micronized or extruded flaxseed resulted to similar milk fat and milk fat yield comparing to control diet with no flaxseed. Feed efficiency (milk yield/DMI) was similar among treatments. Similarly, feed efficiency did not differed by adding safflower seed (Dschaak *et al.* 2010; Dschaak *et al.* 2011) and crushed safflower seed (Oguz *et al.* 2014) in lactating dairy cow diets.

It was well documented in many reviews that milk fatty acid composition differed, when oil seeds like safflower seed incorporated in dairy diets. Inclusion of RSS and MSS up to 40 g/kg diet had no significant effect on concentration of short-chain fatty acids (SCFAs) in milk. In agreement with our results, Alizadeh *et al.* (2012) found that inclusion of safflower seed in the diet had no effect on concentrations of SCFAs in milk fat, whereas Dschaak *et al.* (2010) showed that milk fat concentrations of SCFAs was increased by dietary supplementation with safflower.

More than 90% of milk fatty acids are from blood circulation and de novo synthesis (Neville Picciano, 1997), in which SCFAs and medium-chain fatty acids (MCFAs) are synthesized in mammary glands using acetate and butyrate coming from ruminal fermentation and long-chain fatty acids (LCFAs) are transferred from circulating lipids in blood (Mansbridge and Blake, 1997).

Table 5 Serum metabolites

Serum metabolite	Treatment diets[1]			SEM	P-value
	Control	RSS	MSS		
Glucose (mg/dL)	58.7	56.3	56.1	1.40	0.342
Cholesterol (mg/dL)	265.6[b]	272.3[a]	276.6[a]	5.59	0.046
BUN (mg/dL)	15.6	16.1	17.2	2.31	0.163
BHBA (mg/dL)	10.23	9.81	9.71	0.352	0.201
NEFA (mmol/L)	3.27	3.34	3.40	0.284	0.284

[1] Control: diet with 100 g cottonseeds and no safflower seeds; Raw safflower seed (RSS): diet with 60 g cottonseeds and 40 g raw safflower seeds and microwave irradiated safflower seed (MSS): diet with 60 g cottonseeds and 40 g microwave irradiated safflower seeds per kg of diet DM.
BUN: blood urea nitrogen; BHBA: beta-hydroxybutyric acid and NEFA: non-esterified fatty acids.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Our finding was in agreement with those of Dhiman *et al.* (1995) they found that dietary supplementation with LCFAs increase the milk fat concentration and decrease de novo synthesis of SCFAs and MCFAs in the mammary gland.

Dschaak *et al.* (2011) reported more C16:0 in milk obtained from treatment having higher levels of C16:0 in its composition, which was similar to our control treatment. C16:0 FA levels linearly decreases when safflower level was increased up to 40 g/kg DM of diet (Dschaak *et al.* 2010). De novo synthesis of C16:0 may be inhibited more effectively by C18:2 (like in safflower seeds) than C18:3 (like in flaxseed) Glasser *et al.* (2008), therefore the level of C16:0 can decrease when diet accompanied by safflower seeds.

C18:0 fatty acids in milk increased by inclusion RSS and MSS seed up to 40 g/kg diet. Stearic acid (C18:0) concentration decreased by supplementing diet with 10 g/kg DM safflower seed, while higher levels (30 or 40 g/kg DM) had no effect compared to cottonseed supplementation (Dschaak *et al.* 2010). Higher levels of C18:0 in milk is reported by Cortes *et al.* (2010) when polyunsaturated fatty acids (PUFAs) source like safflower was used in diet. Stearic acid (C18:0), one of LCFA, is secreted to the milk either from dietary source or by bio hydrogenated C18 unsaturated FA in the rumen. The content of C18:1 trans and C18:1 cis tended to be greater (P<0.05) in cows fed RSS and MSS diet. Dhiman *et al.* (1995) reported that dietary supplying of C18:2 and C18:3 fatty acids increased C18:1 fatty acid in milk through incomplete ruminal bio hydrogenation of PUFAs. The 18:1 trans fatty acid is one of the intermediate of fatty acids bio hydrogenation in the rumen (Jenkins *et al.* 2008). In agreement with our results, Dschaak *et al.* (2011) reported similar effects on C18:1 trans and cis isomers of milk fatty acids by adding safflower seed in the diets of cows.

A numerical increase in the levels of cis-9, trans-11 CLA was indicated for RSS and MSS supplemented diets. A parallel increase occurred in cis-9, trans-11 CLA and C18:1 trans-11 FA, because C18:1 trans-11 FA is the main precursor of cis-9, trans-11 CLA in milk.

Bauman and Griinari (2001) reported that Δ^9-desaturase can convert C18:1 trans-11 FA to cis-9, trans-11 CLA with in the mammary glands or other tissues. A linear increase in the concentration of cis-9, trans-11 CLA was reported by Dschaak *et al.* (2010) when diet was supplemented with safflower. Addition of MSS in the diet numerically increased concentration of cis-9, trans-11 CLA in milk compared with the cows fed RSS.

The scenario of simultaneous heating and pressure due to rapid vaporization of internal mist inside the seed during microwave irradiation causes a rupture prior to the seed coat which enhances the release of oil and fatty acids from seeds. Therefore, more CLA biosynthesis occurs because of more C18:2 fatty acids are released.

The diet supplementation with lipid or oil seeds normally increases serum cholesterol (Cant *et al.* 1993) and in the present study addition of RSS and MSS increased serum cholesterol. This might be related to the greater ether extract content of the RSS and MSS diets compared with control diet since DMI remained similar. Similarly, Gonthier *et al.* (2005) reported serum concentration of cholesterol which was increased by adding flaxseed to the diet, but heat treatment of flaxseed (micronization and extrusion) had no effect on a cholesterol concentration in blood serum.

CONCLUSION

Our results suggest that safflower seed can be used as a suitable replacement for cottonseed as a means of fat supplementation in dairy diets. Also, results showed that feeding 40 g/kg DM diet safflower seed to lactating dairy cows has no negative effects on lactation performance and milk fat yield. Because of observing an increase in C18:1 trans and cis-9, trans-11 CLA concentration in the milk by the addition of microwave irradiation safflower seed, this processing can be used for safflower seed treatment in animal nutrition industry.

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