Short communication: Effects of supplementation with pomegranate seed pulp on concentrations of conjugated linoleic acid and puniic acid in goat milk

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ABSTRACT

The effects of feeding pomegranate seed pulp (PSP) on milk yield, milk composition, fatty acid profiles of milk fat, and blood metabolites were examined in this study. During a pretrial period, 27 multiparous southern Khorasan (Iran) cross-bred goats were fed a similar diet and dry matter intake, milk yield, and milk composition were recorded. After adaptation and based on pretrial records, the goats were randomly assigned to 1 of 3 experimental diets and were housed in individual stalls. Experimental diets included 0, 6, or 12% of PSP (dry matter basis) and were fed as total mixed rations ad libitum for a 45-d period. Diets were formulated to be isonitrogenous and isocaloric. Supplementation of PSP did not affect dry matter intake or average daily gain of goats. Milk yield was not affected by inclusion of PSP in the diet. Milk fat concentration of goats fed diets with 6 and 12% PSP increased, but milk fat yield, milk protein concentration, and milk solids-not-fat concentration of goats were not affected by diets. Feeding PSP did not affect blood glucose, cholesterol, urea N, triglyceride, or lipoproteins. Feeding goats a diet containing 12% PSP modified the milk fatty acid profile, including conjugated linoleic, puniic, and vacenic acids.

Key words: conjugated linoleic acid, goat milk, pomegranate seed pulp, puniic acid

Short Communication

Fatty acid (FA) composition is an important component of the nutritional quality of milk. Different FA are involved as positive or negative predisposing factors for human health (Chilliard et al., 2003; Luna et al., 2008). The term “conjugated FA” is a generic name for po-

sitional and geometric isomers of polyunsaturated FA with conjugated double bonds (Bauman et al., 2001). The *cis*-9,*trans*-11 conjugated linoleic acid (CLA) is the main CLA isomer present in milk and has been reported to have anticarcinogenic, antiatherogenic, and antidiabetic (type II) properties (Lee et al., 2005; Moon et al., 2008). Fat supplementation of dairy goat diets has potential for changing milk yield, fat content, and FA profile (Chilliard et al., 2003).

Pomegranate (*Punica granatum* L.) belongs to the Punicaceae family and it is one of the oldest known edible fruits (Seeram et al., 2006). The edible part of the pomegranate (aril) is about 55 to 60% of total fruit weight and consists of about 75 to 85% juice and 15 to 25% seeds. Pomegranate seed pulp (PSP) is a by-product of the industrial decoction of pomegranate and contains large amounts of oil, with some Iranian varieties having total lipid contents ranging from 6.6 to 19.3% (DM basis; Abbasi et al., 2008). Pomegranate seed oil consists of 65 to 80% conjugated FA, the most important of which is *cis*-9,*trans*-11,*cis*-13 octadecatrienoic acid, also called puniic acid (Abbasi et al., 2008). Puniic acid is metabolized to *cis*-9,*trans*-11 CLA via a saturation mechanism (Tsuzuki et al., 2006). Because of high puniic acid content, PSP may be used as dietary supplement to increase CLA and puniic acid concentrations of milk fat. To our knowledge, no published studies have evaluated pomegranate oil or pomegranate by-products on milk FA profile of goats. The hypothesis is that because PSP contains a considerable amount of oil, the milk FA profile may be altered. Therefore, the objective of this study was to investigate the effect of PSP supplementation on performance and specifically on milk FA profile including conjugated polyunsaturated FA, CLA isomers, and puniic acid content in southern Khorasan cross-bred goats.

All procedures for this trial were conducted according to the guidelines of the Iranian Council of Animal Care (1995). Twenty-seven multiparous southern Khorasan cross-bred goats in mid lactation (average DIM of 71

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Table 1. Ingredients, nutrient composition, and fatty acid profile of the experimental diets

Item	Diet ¹		
	Control	LPSP	HPSP
Ingredients, % of DM			
Alfalfa hay	40.0	40.0	40.0
Barley grain, ground	12.0	9.0	6.0
Corn grain, ground	32.7	29.7	26.7
Soybean meal	12.6	12.6	12.6
Pomegranate seed pulp ²	0	6.0	12.0
Sodium bicarbonate	0.9	0.9	0.9
Vitamin and mineral mixture ³	0.6	0.6	0.6
Dicalcium phosphate	0.6	0.6	0.6
Calcium carbonate	0.3	0.3	0.3
Sodium chloride	0.3	0.3	0.3
Nutrients ⁴			
DM, %	94.3 (94.5)	94.3 (94.6)	94.2 (94.5)
ME, Mcal/kg of DM	2.6 (2.4)	2.6 (2.5)	2.5 (2.3)
CP, % of DM	15.3 (14.5)	15.1 (14.0)	15.3 (13.9)
RUP, % of DM	2.9 (2.5)	3.3 (2.6)	3.2 (2.5)
RDP, % of DM	12.3 (12.0)	11.8 (11.4)	12.1 (11.3)
Ether extract, % of DM	2.2 (2.0)	2.5 (2.1)	3.2 (3.1)
NDF, % of DM	26.9 (27.7)	28.2 (28.9)	29.9 (30.5)
ADF, % of DM	19.9 (20.9)	21.0 (21.7)	22.0 (23.1)
NFC, % of DM	45.8 (44.9)	44.8 (44.6)	41.7 (41.5)
Ash, % of DM	9.8 (10.9)	9.4 (10.4)	9.9 (11.0)
Ca, % of DM	0.83 (0.86)	0.89 (0.92)	0.91 (0.92)
P, % of DM	0.53 (0.49)	0.52 (0.50)	0.50 (0.46)
Fatty acids, % of total fatty acids			
C12:0	0.02	0.02	0.02
C14:0	0	0	0
C16:0	0.51	0.53	0.55
C18:0	0.26	0.28	0.29
C18:2	1.13	1.11	1.10
C18:3 ⁵	0.21	0.21	0.21
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -13 18:3 (punicic acid)	0	0.56	1.12
C20:0	0.01	0.01	0.02

¹Control, LPSP, and HPSP diets contained 0, 6, 12% (DM basis) pomegranate seed pulp, respectively.

²Chemical composition (DM basis): gross energy = 3.96 Mcal/kg, CP = 8.85% (RDP = 77%, RUP = 23%), ether extract = 12.5%, ADF = 31.1%, NDF = 43.5%, NFC = 26.9%, ash = 9.2%, Ca = 1.73%, P = 0.22%.

³One kilogram of vitamin and mineral mixture contained 140 g of Ca; 70 g of Na; 70 g of P; 20 g of Mg; 2.4 g of Fe; 2.4 g of Zn; 2.6 g of Mn; 0.24 g of Cu; 0.0001 g of Se; 0.1 g of Co; 0.1 g of I; 400,000 IU of vitamin A; 100,000 IU of vitamin D₃; and 100 IU of vitamin E, and 3 g of beta-hydroxytoluene.

⁴The values in parentheses are the nutrient composition of feed residues.

⁵Except punicic acid.

± 12.5 , average daily milk yield of 1.09 ± 0.13 kg, and average BW of 28 ± 2.5 kg) were housed in individual stall barns for 10 d and fed a similar diet for barn adjustment and collection of pretrial records. At the onset of the 45-d trial, goats were grouped according to pretrial milk yield and then randomly assigned to 1 of the 3 experimental diets. Goats in each group were fed individually a TMR ad libitum. All diets consisted of 40% alfalfa hay and 60% concentrate (DM basis). Dietary treatments were supplemented with PSP (DM basis) at 0% (control), 6% (low PSP, **LPSP**), or 12% (high PSP, **HPSP**) levels. The ingredients, chemical composition, and FA composition of the experimental diets are given in Table 1. Diets were formulated to be isonitrogenous and isoenergetic and met NRC (2007)

recommendations. Clean water and vitamin-mineral blocks were freely available for all animals.

Goats were fed 3 times a day at 0700, 1200, and 1830 h. Samples of diets were collected weekly and voluntary feed intake was registered daily. Goats were milked twice daily at 0530 and 1730 h, and milk yield was recorded daily. Milk samples (60 mL) were collected at each milk yield recording and analyzed for fat, protein, lactose, and SNF. In wk 2, 4, and 6 of the trial period, additional milk samples were taken and frozen at -20°C for later analysis of FA profile. At the start of the experiment, goats were free from clinical mastitis. Blood samples were collected on d 1, 15, 30, and 45 of the experimental period at 0830 h. Samples from the jugular vein were collected into evacuated blood

collection tubes (Improve, Guangzhou Improve Medical Instruments Co. Ltd., Guangzhou, Guangdong, China), which contained heparin as an anticoagulant. After collecting a 10-mL blood sample from each goat, the sample was centrifuged for 15 min at $3,000 \times g$ to separate the plasma, which was then frozen at -25°C until it was analyzed for metabolites.

Feed samples were ground in a mill (1-mm screen; Type 3100, Perten, Helsinki, Finland) and the DM content was determined by oven drying (Behdad oven, Behdad Co., Tehran, Iran) at 105°C for 24 h. Crude protein content was determined according to the Kjeldahl method (AOAC, 1990) by using a Gerhardt analyzer (model VAP 50/OT, Gerhardt, Königswinter, Germany). The NDF and ADF contents were determined according to Van Soest et al. (1991) using α -amylase and sodium sulfite. Ash content was determined according to AOAC (1990) by using the electronic oven (model EF 11/88, Lenton, Sheffield, UK) and fat content was determined by using a soxhlet Gerhardt (model SE 416, Gerhardt). Analysis of the FA content of the feeds was performed according to ISIRI (1997). Milk fat, protein, lactose, and SNF were measured with a MilkoScan Foss Electric S-50 (Foss Electric, Hillerød, Denmark). For analysis of milk FA, milk fat was extracted following the Bouattour et al. (2008) method. Based on this method, milk was immediately cooled after milking and the fat fraction was separated by centrifugation for 30 min at $6,000 \times g$ and 4°C , and then stored at -20°C . Milk FA were analyzed after extraction of milk fat samples and methylation (ISIRI, 1997). Briefly, 0.1 g of the extracted milk fat was mixed with 2 mL of 1 M KOH and 5 mL of 14% boron trifluoride in methanol. The sample was methylated by incubation at 100°C for 60 min and, after cooling to room temperature, extracted with 5 mL of hexane. The FA methyl esters in the hexane layer were analyzed by a gas chromatograph (3400 Varian Star; Varian Inc., Palo Alto, CA). In this method, CP-SIL-88 capillary columns were used and helium was the carrier gas. Column temperature was initially 50°C for 1 min, increasing by $10^{\circ}\text{C}/\text{min}$ to 190°C , and was then held at 190°C for 130 min. The temperature of the injector was 270°C , and that of the detector was 300°C . Peaks were identified by comparing their retention times with those of an authentic standard FA methyl ester mixture (Sigma-Aldrich Co., St. Louis, MO). A pomegranate seed oil reference standard was used as a routine quality control and as a check for recoveries and correction factors for punicic acid (ISIRI, 1997). Blood samples were centrifuged ($3,000 \times g$ for 15 min), and plasma frozen at -20°C for later analysis. Plasma levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, urea N, cholesterol, and glucose were determined using Parsazmun Liquid Test kits

(Parsazmun Co., Karaj, Iran) and a spectrophotometer (Secomam XS2, Secomam Co., Ales, France).

Data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC) for repeated measurements. The statistical model was $y_{ijkl} = \mu + T_i + A_{j(i)} + W_k + T_i \times W_k + e_{ijkl}$, where μ is the overall mean, T_i is the fixed effect of treatments, $A_{j(i)}$ is the random effect of j th goat within i th diet, W_k is the fixed effect of repeated measurements, $T_i \times W_k$ is the interaction between T_i and W_k , and e_{ijkl} is the residual error. Significant differences were declared at $P < 0.05$ and results are presented as treatment means with SEM.

Total lipid contents of LPSP and HPSP diets were higher compared with that of control. Dry matter intake and milk yield of goats were not affected by diets (Table 2). Furthermore, feeding PSP did not affect FCM yield, ADG, or production efficiency. Although dietary fat supplementation to mid-lactation dairy cows often increases milk yield, this is not the case in goats and ewes (Mir et al., 1999), in agreement with our observations. Feeding PSP increased milk fat concentration ($P < 0.05$) but milk fat yield was not affected. Milk protein concentration, milk protein yield, milk SNF concentration, and milk SNF yield of goats were not affected by treatment. Although fat supplementation in dairy cows and sheep diets often decreases milk protein content, Chilliard et al. (2003) and Nudda et al. (2006) reported no effect in goats, which was in agreement with our results. Milk lactose concentration increased with PSP supplementation. Indirect comparisons with studies in cows show that plant oils increase milk lactose concentrations in goats but not in cows, and highlight the possibility of differences in the regulation of mammary lactose synthesis between ruminant species (Bernard et al., 2009).

Diets containing PSP altered milk fat composition. Goat milk fatty acid profile is shown in Table 3. The inclusion of PSP in the diets did not substantially change the concentration of FA from C4:0 to C10:0. Chilliard et al. (2003) and Chilliard and Ferlay (2004) also reported no changes in C4:0 to C8:0 milk fat contents after lipid supplementation with several types of basal diets enriched with polyunsaturated FA. The lack of change could be partly because short-chain FA are synthesized by metabolic pathways that are independent of acetyl-CoA carboxylase (Chilliard and Ferlay, 2004). Treatments did not influence the concentration of milk saturated FA from C10:0 to C16:0, possibly because of specific characteristics of FA metabolism in the goat mammary gland. In contrast to findings in dairy cows, enzymes involved in pathways of de novo lipogenesis in goat mammary gland seem less affected by lipid supplementation with polyunsaturated FA (Luna et al., 2008). The inclusion of 12% PSP in the

Table 2. Daily intake, milk yield, milk composition, and ADG of goats as affected by diet

Item	Diet ¹			SEM	P-value
	Control	LPSP	HPSP		
DMI, g/d	1,724	1,736	1,722	14.3	NS
Yield, g/d					
Milk	1,057	988	984	22.6	NS
Milk, 4% FCM ²	1,071	1,099	1,109	26.1	NS
Milk fat	43.2	46.9	47.7	1.77	NS
Milk protein	39.5	36.2	36.2	1.21	NS
Milk lactose	42.7	41.3	45.4	1.81	NS
Milk SNF	103	93.0	95.0	3.40	NS
Concentration, % of milk					
Milk fat	4.1 ^b	4.7 ^a	4.8 ^a	0.12	**
Milk protein	3.8	3.7	3.6	0.04	NS
Milk lactose	4.1 ^b	4.2 ^{ab}	4.3 ^a	0.03	**
Milk SNF	9.7	9.6	9.6	0.11	NS
Total ADG, g/d	106	74	96	13.6	NS
Production efficiency ³	0.62	0.63	0.64	0.17	NS

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Control, LPSP, and HPSP diets contained 0, 6, and 12% (DM basis) pomegranate seed pulp, respectively.

²4% FCM was calculated as $[(0.4 \times \text{kg of milk}) + (15 \times \text{kg of milk fat})]$.

³Production efficiency = average daily 4% FCM (g/d)/average daily DMI (g/d).

** $P < 0.01$.

diet reduced the concentration of C16:1 in goat milk fat ($P < 0.05$) compared with control diet. The content of C16:1 in milk originates both from de novo synthesis in the mammary gland and from arterial blood uptake (Nudda et al., 2006). In the HPSP diet, the reduction of C16:1 was probably due to decreased synthesis in the mammary gland, because the index of Δ^9 -desaturase activity was higher in the control group. A similar trend has been observed with other oil sources (Chilliard et al., 2003). The HPSP diet increased the concentrations of *cis*-12 C18:1, but feeding PSP decreased *cis*-11 C18:1 concentration and did not influence *cis*-9 C18:1 or *cis*-13 C18:1 contents of milk fat (Table 3). The concentration of *trans*-11 C18:1 (vaccenic acid) in milk fat was increased by feeding the HPSP diet. Although the predominant biohydrogenation end-product of unsaturated FA in the rumen is stearic acid, several monounsaturated intermediates such as vaccenic acid are often generated (Palmquist et al., 2005) and subsequently found in milk. Both α -linolenic and linoleic acids are precursors of vaccenic acid in the rumen (Nudda et al., 2006). Increases in vaccenic acid in milk fat caused increased endogenous synthesis of total CLA and *cis*-9,*trans*-11 CLA in the mammary gland; therefore, supplementation with 12% PSP was also an effective means of increasing the concentration of *cis*-9,*trans*-11 CLA in milk fat of goats. This result is in agreement with other studies that supplemented diets with extruded linseed cake and whole linseed (sources rich in linolenic acid), and reported an increased concentration of total CLA and *cis*-9,*trans*-11 CLA in goat milk fat

(Nudda et al., 2006). Feeding PSP did not influence the *trans*-10,*cis*-12 CLA and *trans*-8,*cis*-10 CLA contents of milk fat. According to Bouattour et al. (2008), *trans*-10,*cis*-12 CLA remains at trace levels in goat milk. The milk content of C18:3 n-3, which contributes to prevention of atherosclerosis in humans (Kris-Etherton and Yu, 1997), increased ($P < 0.05$) in goats fed the HPSP diet compared with the control. According to Chilliard et al. (2003), linseed oil (rich in C18:3) had a positive effect on the proportion of milk linolenic acid; in our study, PSP increased ($P < 0.05$) the proportion of *cis*-9,*trans*-12 C18:2 and *trans*-9,*cis*-12 C18:2. Although the LPSP diet did not increase the concentration of punicic acid in milk fat, supplementation with 12% PSP was an effective means of increasing the concentration ($P < 0.001$) of this important FA. This could be attributed to partial escape of punicic acid from the rumen before biohydrogenation to stearic acid, and subsequent secretion in milk fat. Supplementation with 12% PSP was also an effective means of increasing C18:3 n-3 in milk fat ($P < 0.001$), mainly because the PSP used in the study contained 75% punicic acid. The desaturase indexes were not modified for C14:0 and C18:0, but were decreased ($P < 0.05$) for C16:0 when PSP was fed. Substantial reductions in desaturase index for C16:0 have been associated with a decrease in mammary palmitoyl-CoA desaturase mRNA abundance and activity (Bernard et al., 2009).

Diet had no effect on plasma glucose, triglyceride, high-density lipoprotein concentrations, or total cholesterol. Yamasaki et al. (2006) also found no effect

Table 3. Milk fatty acid (FA) profile (% of total FA methyl esters) of goats as affected by diets

Fatty acid	Diet ¹			SEM	P-value
	Control	LPSP	HPSP		
4:0	1.28	1.43	1.48	0.058	NS
5:0	—	0.1	0.15	0.050	NS
6:0	1.81	2.07	2.16	0.124	NS
8:0	2.52	2.87	2.87	0.156	NS
8:1	0.13	0.13	0.14	0.034	NS
10:0	10.08	10.57	10.78	0.303	NS
10:1	0.44	0.45	0.42	0.029	NS
12:0	5.33	5.00	4.94	0.192	NS
<i>trans</i> -12:1	0.15	0.14	0.18	0.255	NS
<i>cis</i> -12:1	0.30	0.21	0.30	0.248	NS
14:0	10.22	10.31	9.85	0.305	NS
<i>trans</i> -14:1	0.19	0.17	0.17	0.018	NS
<i>cis</i> -14:1	0.39	0.32	0.32	0.038	NS
15:0	1.36	1.26	1.12	0.017	NS
15:1	0.29	0.24	0.27	0.026	NS
16:0	29.65	31.91	29.20	1.001	NS
<i>trans</i> -16:1	0.54 ^a	0.45 ^{ab}	0.39 ^b	0.040	*
<i>cis</i> -16:1	1.06 ^a	0.98 ^a	0.70 ^b	0.070	**
17:0	1.01	0.92	0.98	0.094	NS
17:1	0.45	0.33	0.37	0.062	NS
17:2	0.28 ^a	0.21 ^b	0.18 ^b	0.015	***
18:0	6.87	6.88	7.85	0.513	NS
<i>cis</i> -9 18:1	17.09	15.95	16.39	0.508	NS
<i>cis</i> -11 18:1	0.44 ^a	0.32 ^b	0.34 ^b	0.020	**
<i>cis</i> -12 18:1	0.22 ^b	0.23 ^b	0.37 ^a	0.020	***
<i>cis</i> -13 18:1	0.20	0.18	0.23	0.018	NS
<i>cis</i> -15 18:1	0.10 ^a	0.06 ^b	0.09 ^{ab}	0.0009	*
<i>trans</i> -11 18:1	1.05 ^b	1.15 ^b	1.89 ^a	0.159	**
<i>cis</i> -14 18:1 + <i>trans</i> -16 18:1	0.09	0.06	0.08	0.009	NS
(<i>cis</i> -9, <i>trans</i> -13 + <i>trans</i> -8, <i>cis</i> -12) 18:2	0.05	0.03	0.05	0.014	NS
<i>cis</i> -9, <i>trans</i> -12 18:2	0.19 ^b	0.20 ^{ab}	0.24 ^a	0.014	*
<i>trans</i> -9, <i>cis</i> -12 18:2	0.18 ^b	0.18 ^b	0.25 ^a	0.013	**
<i>cis</i> -9, <i>cis</i> -15 18:2	0.05	0.05	0.04	0.013	NS
<i>cis</i> -9, <i>trans</i> -11 18:2	0.11 ^b	0.13 ^b	0.29 ^a	0.014	**
<i>trans</i> -10, <i>cis</i> -12 18:2	0.001	0.003	0.003	0.0010	NS
<i>trans</i> -8, <i>cis</i> -10 18:2	0.003	0.004	0.007	0.0014	NS
Total conjugated linoleic acids	0.14 ^b	0.16 ^b	0.37 ^a	0.058	*
18:2 n-6	2.61	2.36	2.42	0.114	NS
<i>cis</i> -9, <i>cis</i> -12, <i>trans</i> -15 18:3	0.09	0.08	0.04	0.023	NS
<i>trans</i> -9, <i>cis</i> -12, <i>cis</i> -15 18:3	0.07	0.05	0.06	0.008	NS
18:3 n-3	0.74 ^b	0.75 ^b	1.30 ^a	0.082	***
<i>cis</i> -9, <i>trans</i> -11, <i>cis</i> -13 18:3	0.15 ^b	0.46 ^b	1.19 ^a	0.12	***
20:0	0.16	0.16	0.22	0.022	NS
20:1	0.27	0.23	0.23	0.013	NS
22:0	—	0.052	0.079	0.033	NS
22:1	0.04 ^b	0.07 ^b	0.22 ^a	0.031	***
22:6	0.25 ^a	0.15 ^b	0.16 ^{ab}	0.029	*
Desaturase index ²					
C14	0.05	0.04	0.04	0.004	NS
C16	0.05 ^a	0.04 ^b	0.04 ^b	0.009	**
C18	0.73	0.72	0.70	0.014	NS

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Control, LPSP, and HPSP diets contained 0, 6, and 12% (DM basis) pomegranate seed pulp, respectively.

²Calculated for each fatty acid according to Bouattour et al. (2008) as (product of Δ^9 -desaturase)/(product of Δ^9 -desaturase + substrate of Δ^9 -desaturase); that is, for C14: C14:1/(C14:1 + C14:0), for C16: C16:1/(C16:1 + C16:0), and for C18: C18:1/(C18:1 + C18:0).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

on total cholesterol in mice fed pomegranate seed oil. Bernard et al. (2009), however, reported higher total plasma cholesterol in goats fed diets supplemented with sunflower seed oil or linseed oils. These differences could be attributed to the type of fat and FA composition in

PSP and other fat sources. In conclusion, PSP could be used successfully as a replacement for cereal grains and other energy-rich dietary supplements because of its relatively high fat content. Supplementation of PSP, which is rich in punicic acid, altered milk FA profile in

goats. Dietary PSP supplementation at a level of 12% was an effective means of increasing concentrations of punicic acid, *cis*-9,*trans*-11 CLA, C18:3 n-3, and total CLA in goat milk fat. Despite the adverse effect of some CLA on milk fat content (e.g., *trans*-10,*cis*-12 CLA), feeding PSP did not decrease milk fat concentration or yield.

REFERENCES

- Abbasi, H., K. Rezaei, and L. Rashidi. 2008. Extraction of essential oils from the seeds of pomegranate using organic solvents and supercritical CO₂. *J. Am. Oil Chem. Soc.* 85:83–89.
- AOAC. 1990. Official Methods of Analysis. 15th ed. Vol. 1. Assoc. Offic. Anal. Chem., Arlington, VA.
- Bauman, D. E., B. A. Corl, L. H. Baumgard, and J. M. Griinari. 2001. Conjugated linoleic acid (CLA) and the dairy cow. Pages 221–250 in *Recent Advances in Animal Nutrition*. P. C. Garnsworthy and J. Wiseman, ed. Nottingham University Press, Nottingham, UK.
- Bernard, L., M. Bonnet, C. Leroux, K. J. Shingfield, and Y. Chilliard. 2009. Effect of sunflower-seed oil and linseed oil on tissue lipid metabolism, gene expression, and milk fatty acid secretion in alpine goats fed maize silage-based diets. *J. Dairy Sci.* 92:6083–6094.
- Bouattour, M. A., R. Casals, E. Albanell, X. Such, and G. Caja. 2008. Feeding soybean oil to dairy goats increases conjugated linoleic acid in milk. *J. Dairy Sci.* 91:2399–2407.
- Chilliard, Y., and A. Ferlay. 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reprod. Nutr. Dev.* 44:467–492.
- Chilliard, Y., A. Ferlay, J. Rouel, and G. Lamberet. 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *J. Dairy Sci.* 86:1751–1770.
- Institute of Standard and Industrial Research of Iran (ISIRI). 1997. Animal and vegetable fats and oil (methods 4090 and 4091). ISIRI, Karaj, Iran.
- Iranian Council of Animal Care. 1995. Guide to the Care and Use of Experimental Animals. Vol. 1. Isfahan University of Technology, Isfahan, Iran.
- Kris-Etherton, P. M., and S. Yu. 1997. Individual fatty acid effects on plasma lipids and lipoproteins: Human studies. *Am. J. Clin. Nutr.* 65:1628S–1644S.
- Lee, K. W., H. J. Lee, H. Y. Cho, and Y. J. Kim. 2005. Role of conjugated linoleic acid in the prevention of cancer. *Crit. Rev. Food Sci. Nutr.* 45:135–144.
- Luna, P., A. Bach, M. Juarez, and M. A. De La Fuente. 2008. Effect of a diet enriched in whole linseed and sunflower oil on goat milk fatty acid composition and conjugated linoleic acid isomer profile. *J. Dairy Sci.* 91:20–28.
- Mir, Z., L. A. Goonewardene, E. Okine, S. Jaegear, and H. D. Scheer. 1999. Effect of feeding canola oil on constituents, conjugated linoleic acid and long chain fatty acids in goats milk. *Small Rumin. Res.* 33:137–143.
- Moon, H. S., H. G. Lee, C. S. Chung, Y. J. Choi, and C. S. Cho. 2008. Physico-chemical modifications of conjugated linoleic acid for ruminal protection and oxidative stability. *Nutr. Metab. (Lond.)* 5:16–24.
- National Research Council. 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. Natl. Acad. Press, Washington, DC.
- Nudda, A., G. Battaccone, M. G. Usai, S. Fancellu, and G. Pulina. 2006. Supplementation with extruded linseed cake affects concentrations of conjugated linoleic acid and vaccenic acid in goat milk. *J. Dairy Sci.* 89:277–282.
- Palmquist, D. L., A. L. Lock, K. J. Shingfield, and D. E. Bauman. 2005. Biosynthesis of conjugated linoleic acid in ruminants and humans. *Adv. Food Nutr. Res.* 50:179–217.
- Seeram, N. P., Y. Zhang, J. D. Reed, C. G. Krueger, and J. Vaya. 2006. Pomegranate phytochemicals. Pages 3–29 in *Pomegranates: Ancient Roots to Modern Medicine*. N. P. Seeram, R. N. Schulman, and D. Heber, ed. CRC Press, Taylor & Francis Group, Boca Raton, FL.
- Tsuzuki, T., Y. Kawakami, R. Abe, K. Nakagawa, K. Koba, J. Imamura, T. Iwata, I. Ikeda, and T. Miyazawa. 2006. Conjugated linolenic acid is slowly absorbed in rat intestine, but quickly converted to conjugated linoleic acid. *J. Nutr.* 136:2153–2159.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Yamasaki, M., T. Kitagawa, N. Koyanagi, H. Chujo, H. Maeda, J. Kohno-Murase, J. Imamura, H. Tachibana, and K. Yamada. 2006. Dietary effect of pomegranate seed oil on immune function and lipid metabolism in mice. *Nutrition* 22:54–59.