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Effect of pomegranate seed oil as a source of conjugated linolenic acid on performance and milk fatty acid profile of dairy goats

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ABSTRACT

The objective of this experiment was to investigate the effects of dietary pomegranate seed oil and linseed oil on lactational performance, ruminal fermentation parameters, nutrients digestibility and milk fatty acid (FA) content, particularly conjugated linoleic acid (CLA), conjugated linolenic acid (CLnA) and vaccenic acid (VA) of dairy goats. Twenty-four *Mahabadi* goats in mid lactation were assigned to three dietary treatments: 1- control diet, basal diet without added oil (CON), 2- diet supplemented with 25 g/kg pomegranate seed oil (PSO), and 3- diet supplemented with 25 g/kg linseed oil (LSO), on a dry matter (DM) basis. Feed intake, milk yield and 4% fat-corrected milk yield were similar for goats fed different diets. Milk fat concentration ($P < 0.01$) and fat/protein ratio ($P < 0.0001$) of goats fed PSO and LSO diets increased, while milk protein, lactose and solid not fat concentrations were not affected by diets ($P > 0.05$). Addition of vegetable oils to diet had no effect on apparent digestibility of nutrients and ruminal fermentation parameters ($P > 0.05$). The proportions of VA ($P < 0.001$) and C18:1 (*trans*-9+*trans*-10, $P < 0.01$) acids were increased in milk fat from goats fed PSO and LSO diets compared with goats fed CON diet. The concentration of *cis*-9, *trans*-11 CLA (rumenic acid) increased with oil supplements ($P < 0.0001$) and was greatest for goats fed PSO diet. Compared with CON and LSO diets, feeding PSO diet increased *cis*-9, *trans*-11, *cis*-13 C18:3 CLnA (punicic acid) in milk fat ($P < 0.0001$). The concentrations of monounsaturated fatty acids (MUFA; $P < 0.05$), polyunsaturated fatty acids (PUFA; $P < 0.0001$) and n-3PUFA ($P < 0.0001$) increased and n-6/n-3 PUFA ratio ($P < 0.0001$) decreased with oil supplemented diets. In conclusion, feeding PSO and LSO to dairy goats was a useful way to increase milk fat, CLA, and VA content of milk and to reduce the n-6/n-3 PUFA ratio without negative effects on intake, milk yield, and nutrients digestibility.

1. Introduction

Conjugated fatty acid (CFA) is the general term of positional and geometric isomers of polyunsaturated fatty acids (PUFAs) with conjugated double bonds. Beneficial effect of these FAs, mainly composed of conjugated linoleic acid (CLA) and conjugated linolenic acid (CLnA), on human health is approved (Crumb and Vattem, 2011; Tanaka et al., 2011). Ruminant's products may contain some of these FAs. The *cis*-9, *trans*-11 C18:2 (rumenic acid; RA) is the predominantly naturally occurring CLA in the meat and milk of ruminants that originates from either incomplete biohydrogenation of PUFAs in the rumen or mammary endogenous synthesis from *trans*-11 C18:1 (vaccenic acid; VA), an intermediate of rumen biohydrogenation (Grinari and Bauman, 1999). The CLnA content of foods derived from ruminants depend on their dietary consumption of alpha-linolenic acid and also biohydrogenation of the linoleic acid in rumen. Dietary inclusion of PUFAs-rich lipids has been the most commonly investigated nutritional strategy to increase the CFA content of ruminant's milk and meat (Raes et al., 2004; Wood et al., 2008). Chilliard et al. (2003) reported that dietary linseed oil (LSO) or sunflower oil increased the VA and CLA content in the milk fat of goats. The CLnA occurs abundantly in some specific seed oils, such as karela oil (Dhar and Bhattacharyya, 1998), tung oil (Igarashi and Miyazawa, 2000; Suzuki et al., 2001), and pomegranate seed oil (PSO, Suzuki et al., 2001). Pomegranate (*Punica granatum* L.) is a deciduous shrub that is native to Iran (Sarkhosh et al., 2006). Iran is one of the most important pomegranate producers and exporters in the world, and its total production in 2014 was 990,000 t (AMI, 2014). Pomegranate seed as a byproduct of pomegranate processing is about 20% (w/w) of the whole fruit (Tehranifar et al., 2010). The seed oils of 25 pomegranates varieties grown in Iran contained oil in the range of about 66.3–193 g/kg dry matter (Fadavi et al., 2006). The PSO consists of approximately 80% CLnA, with high content of *cis*-9, *trans*-11, *cis*-

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13 C18:3 isomer (punicic acid; PUA), a specific FA for this oil. Pomegranate seeds are also rich in polyphenolic compounds that have potent antioxidant and antimicrobial properties (Wang et al., 2004; Dahham et al., 2010). Cold pressing is a method of oil extraction that involves no heat or chemical treatment, and during this process, polyphenolic compounds are extracted into the cold pressed oil in significant quantities (Parry and Yu, 2004). Several studies have shown that feeding fattening and dairy goats with pomegranate seed pulp (PSP, contains about 120 g/kg oil) has increased the concentrations of CLnA and CLA in their meat and milk (Emami et al., 2015b; Modaresi et al., 2011; Razzaghi et al., 2015a). Due to the high level of annual production of pomegranate in Iran and the fact that no comprehensive research has been done about the effect of PSO feeding on FA composition of ruminant's milk or meat, the present study was designed to determine the effects of dietary PSO or LSO on lactational performance and milk FA composition of *Mahabadi* dairy goats.

2. Materials and methods

2.1. Animals, experimental diets and management

Animals were cared for according to the guidelines of the Iranian Council of Animal Care (1995) and all procedures and guidelines involving animals were approved by the Animal Experiment Committee at Tehran University, Iran. Twenty-four *Mahabadi* goats in mid lactation (DIM=45 ± 6 d, BW=38.4 ± 3.8 kg, average daily milk yield of 1.05 ± 0.14 kg) were randomly assigned to three experimental diets (n =8 per group). Goats were located in individual pens (1.8 m×1.6 m), and before the beginning of the experimental period (6 weeks), they were gradually adapted to the experimental diets for 2 weeks. Treatments consisted of a control diet (CON), without added oil, and two diets supplemented with either 25 g/kg pomegranate seed oil (PSO) or 25 g/kg linseed oil (LSO), on a dry matter (DM) basis. Diets containing of 55% concentrate and 45% forage (DM basis), were formulated to be isocaloric and isonitrogenous and to meet NRC (2007) requirements. Ingredients and chemical composition of diets and the FA profile of oil supplements, are presented in Tables 1, 2, respectively. Goats were fed ad libitum a TMR twice daily in equal portions at 0800 and 1700 h. Amounts fed and refused were recorded daily.

2.2. Sampling, measurements and analyses

2.2.1. Feed and fecal

Samples of TMR and orts were collected three times (during three consecutive days) during trial and stored at −20 °C for chemical analysis. During the last 7 d of the experiment, fecal samples were collected every morning around the feeding time. Diet and fecal samples were separately pooled and ground in a hammer mill with a 1 mm screen (Arthur Hill Thomas Co., Philadelphia, PA) and analyzed (three replicates) for DM (945.15), ash (967.05), crude protein (CP, Kjeldahl N×6.25, 990.03) and ether extract (EE, 945.16) according to AOAC (1990). The NDF content of samples were analyzed (Fibertec 1010, Tecator, Sweden) according to Van Soest et al. (1991). Acid-insoluble ash (AIA) content was used as an internal marker to determine the apparent digestibility of DM, OM, EE, CP and NDF as reported by Van Keulen and Young (1977).

2.2.2. Rumen fluid

Samples of rumen fluid were collected from goats on week 6 at 2 h after the morning feeding using a stomach tube attached to an erlenmeyer flask connected to vacuum pump. The rumen fluid was filtered through four layers of cheesecloth, and pH was determined immediately using a portable pH meter (Sentron, model A102-003) and samples were prepared for protozoa counting according to the procedure described by Veira et al. (1983). The filtrate fluid (5 ml) was preserved by adding 1 ml of 25% metaphosphoric acid solution for later determination of volatile fatty acids (VFAs), and 5 ml of rumen fluid was combined with 5 ml of HCl (0.2 N) for later measurement of ammonia nitrogen (NH3-N) concentration, and stored at −20 °C until analysis. Rumen fluid samples were thawed centrifuged at 12000g for 2 min. A 1.2 ml aliquot of supernatant was removed to a new centrifuge tube and combined with 0.2 ml of internal standard (crotonic acid). After 30 min, the sample was centrifuged at 12000×*g* for 10 min and the supernatant fluid was analyzed for VFA by gas chromatography (Hewlett-Packard, model 5890, Avondale, PA). Helium was used as the carrier gas and oven initial temperatures was 105 °C, 1 min, increasing 5 °C per minute to 195 °C, held at 195 °C for 5 min. The temperature of injector was 225 °C, and that of detector was 250 °C. Ruminal NH3-N concentration was determined according with procedure of Crooke and

Table 1
Ingredients and chemical composition of experimental diets.

	Diets[a]		
	CON	PSO	LSO
Ingredient (% of DM)			
Alfalfa hay	22.53	22.53	22.53
Corn silage	22.47	22.47	22.47
Barley grain, ground	27.94	24.39	24.39
Corn grain, ground	10.48	10.48	10.48
Canola meal	5.82	5.82	5.82
Soybean meal	5.82	6.87	6.87
Wheat bran	2.33	2.33	2.33
PSO	–	2.5	–
LSO	–	–	2.5
Calcium carbonate	0.99	0.99	0.99
Minerals and vitamins premix[b]	0.81	0.81	0.81
Sodium bicarbonate	0.58	0.58	0.58
Salt	0.23	0.23	0.23
Chemical composition			
ME, Mcal/kg of DM	2.55	2.66	2.66
DM (%)	68	68	68
CP (% DM)	15	15	15
Ether extract (% DM)	2.9	5.3	5.3
Ash (% DM)	7.2	7.2	7.2
NDF (% DM)	31.1	30.6	30.6
NFC (% DM)[c]	43.8	41.9	41.9
Ca (% DM)	0.84	0.73	0.73
P (% DM)	0.37	0.41	0.41

[a] CON=diet without added oil; PSO=diet contain of 25 g/kg DM of pomegranate seed oil; LSO=diet contain of 25 g/kg DM of linseed oil.
[b] Containing vitamin A (250,000 IU/kg), vitamin D (50,000 IU/kg) and vitamin E (1500 IU/kg), manganese (2.25 g/kg), calcium (120 g/kg), zinc (7.7 g/kg), phosphorus (20 g/kg), magnesium (20.5 g/kg), sodium (186 g/kg), iron (1.25 g/kg), sulfur (3 g/kg), copper (1.25 g/kg), cobalt (14 mg/kg), iodine (56 mg/kg) and selenium (10 mg/kg).
[c] Non-fibrous carbohydrates (NFC) were estimated according to the equation: NFC=100−(NDF+CP+EE+Ash).

Table 2
Fatty acid composition of pomegranate seed oil and Linseed oil.

Fatty acids (% of total fatty acids)	Pomegranate seed oil	Linseed oil
C16:0	3.8	5.8
C18:0	2.6	3.9
C18:1	6.9	21.5
Trans-C18:2	0.5	–
Cis-C18:2	7.4	17.1
C20:0	0.6	–
C20:1	0.7	–
C18:3[a]	0.4	51.7
Cis-9, *trans*-11, *cis*-13-CLnA (punicic acid)	77.2	–
C22:0	0.2	–

[a] Except punicic acid for pomegranate seed oil.