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Short communication

Antioxidant status of dairy goats fed diets containing pomegranate seed oil or linseed oil

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ABSTRACT

Twenty-four *Mahabadi* goats (DIM = 45 ± 6 d, BW = 38.4 ± 3.8 kg, average daily milk yield = 1.05 ± 0.14 kg) were used in a completely randomized design experiment for 42 days to evaluate the effects of pomegranate seed oil and linseed oil on their performance, blood metabolites and antioxidant status. Experimental diets were: 1- control diet (basal diet without added oil, COND), 2- basal diet with 25 g/kg DM pomegranate seed oil (PSOD) and 3- basal diet with 25 g/kg DM linseed oil (LSOD). Dry matter intake (DMI), milk yield, milk protein, milk lactose, ADG and production efficiency in dietary treatments were similar in different groups ($P > 0.05$), but milk fat increased by addition of linseed and pomegranate seed oils to diet ($P < 0.05$). Blood metabolites (glucose, total cholesterol, triglyceride, LDH and LDL) were not affected by dietary treatments ($P > 0.05$). Relative to the COND, PSOD increased total antioxidant capacity (TAC), whereas LSOD decreased TAC of milk and blood ($P < 0.05$). In contrast, compared with the COND, milk and blood malondialdehyde (MDA) content decreased in goats fed PSOD, whereas LSOD increased MDA content of milk and plasma ($P < 0.001$). Also, milk somatic cell count decreased with PSOD compared with COND and LSOD ($P = 0.001$). The highest level of milk total polyphenols was found in goats fed PSOD ($P < 0.0001$). Results suggest that it is possible to improve antioxidant status and milk fat of dairy goats by feeding pomegranate seed oil (25 g/kg DM) as a natural antioxidants.

1. Introduction

In recent years, a lot of attention has been directed to healthy fatty acids (FA), especially n-3 polyunsaturated fatty acids (PUFAs), and conjugated fatty acids (CFAs), such as rumenic acid (*cis*-9, *trans*-11conjugated linoleic acid (CLA), RA), and conjugated linolenic acid (CLnA) content of ruminant milk and dairy products. Till now the dietary inclusion of PUFA-rich lipids has been the most commonly investigated nutritional strategy to enhance the content of these valuable FAs in ruminant products (Raes et al., 2004; Wood et al., 2008). Some specific seed oils, such as karela oil (Dhar and Bhattacharyya, 1998), tung oil (Igarashi and Miyazawa, 2000; Suzuki et al., 2001), and pomegranate oil (Suzuki et al., 2001) are rich source of CLnA. Emami et al. (2016) reported that addition of 25 g/kg DM pomegranate seed oil (PSO) or linseed oil (LSO) increased RA and n-3 PUFA in goat milk. However, this may increase the occurrence of spontaneous oxidized flavor in milk (Granelli et al., 1998). Therefore, the addition of antioxidants to animal diets has emerged as a strategy for increasing the commercial value of ruminant milk and meat. Although synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are widely used in the animal nutrition and food industries to prevent lipid oxidation, the consumer concern over their safety and toxicity have initiated search for natural sources of antioxidants, such as polyphenols (Nuala et al., 2006).

Pomegranate (*Punicagranatum*), belonging to family Punicaceae (Lansky and Newman, 2007), is native to the Iran plateau and the Himalayas in north Pakistan and Northern India. 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 fruits are widely consumed fresh or processed into juice, jams, syrup and sauce. The edible portion (aril) of fruit is about 55–60% of the total fruit weight and consists of about 75–85% juice and 15–25% seeds (Al-Maiman and Ahmad, 2002). Pomegranate seeds (even after juice extraction) are rich in polyphenolic compounds that have potent antioxidant and antimicrobial properties (Wang et al., 2004; Dahham et al., 2010). The lipid profiles of pomegranate seeds have also gained a great attention for their high content of PUFA and other bioactive compounds (Pande and Akoh, 2009). The PSO consists of approximately 80% CLnA,

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with high content of *cis*-9, *trans*-11, *cis*-13 C18:3 isomer (punicic acid; PUA), a specific FA for this oil. 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). It also be found that PSO may contain significant levels of tocopherols (Caligiani et al., 2010), which are well-known natural antioxidants.

Previous studies showed that phenolic compounds of pomegranate by products can be transferred from diet to the ruminant milk (Shabtay et al., 2012) and meat (Kotsampasi et al., 2014; Emami et al., 2015a) and increased total antioxidant capacity (TAC) of these products. Therefore, the objective of this study was to investigate the effects of addition PSO and LSO to diet on performance, blood metabolites and especially antioxidant status of *Mahabadi* dairy goats.

2. Materials and methods

2.1. Animals and diets

Twenty-four *Mahabadi* goats (DIM = 45 ± 6 d, BW = 38.4 ± 3.8 kg, average daily milk yield = 1.05 ± 0.14 kg) were assigned to three groups of 8 each, in a completely randomized design. All diets were consisted of 45% forage and 55% mixed concentrate (on DM basis; Table 1). The control group received a basal diet, without added oil (COND), the other two groups received diets containing either 25 g/kg pomegranate seed oil (PSOD) or 25 g/kg linseed oil (LSOD), on DM basis. The oil was added to diet instead of barley grain. The soybean meal of oil containing diets was slightly increased to prevent the reduction of CP level of diet. Diets were formulated to be isocaloric and isonitrogenous and to meet NRC (2007) requirements. Goats were housed in individual stalls and the experimental period lasted 42 d. The first 14 d corresponded to the adaptation to experimental diets. Goats were fed a TMR twice daily in equal amounts (0800 and 1700 h) for ad libitum consumption. Amounts fed and refused were recorded daily. The goats were weighed at 1, 21 and 42 d of the trial period before morning feeding. Animals involved in this study were cared according to the guidelines of the Iranian Council of Animal Care (1995). All standard procedures concerning animal care and management were taken throughout the entire period of the experiment.

Table 1
Ingredients and chemical composition of experimental diets.

	Diets[a]		
	COND	PSOD	LSOD
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[b]	–	2.5	–
LSO[c]	–	–	2.5
Calcium carbonate	0.99	0.99	0.99
Minerals and vitamins prermix[d]	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)[e]	43.8	41.9	41.9
Ca (% DM)	0.84	0.73	0.73
P (% DM)	0.37	0.41	0.41

[a] COND = diet without added oil; PSOD = diet contain of 25 g/kg DM of pomegranate seed oil; LSOD = diet contain of 25 g/kg DM of linseed oil.
[b] FA content (% of total FA): 3.8, C16:0; 2.6, C18:0; 6.9, C18:1; 0.5, *trans*-C18:2; 7.4, *Cis*-C18:2; 0.6, C20:0; 0.7, C20:1; 0.4, C18:3(except punicic acid); 77.2, *cis*-9, *trans*-11, *cis*-13- CLnA (punicic acid); 0.2, C22:0; Total phenolic compounds (mg GAE/g):12.64.
[c] FA content (% of total FA): 5.8, C16:0; 3.9, C18:0; 21.5, C18:1; 17.1, *Cis*-C18:2; 51.7, C18:3; Total phenolic compounds (mg GAE/g):1.63.
[d] 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).
[e] Non-fibrous carbohydrates (NFC) were estimated according to the equation: NFC = 100 − (NDF + CP + EE + Ash).

2.2. Measurements

Blood samples were collected on d 1, 21 and 42 of the experimental period before morning feeding. Samples from the jugular vein were collected into evacuated collection tubes containing heparin. After collecting blood samples were centrifuged (3000 × *g* for 15 min), and plasma was then frozen at −80 °C until it was analyzed for TAC and malondialdehyde (MDA) and some other metabolites (glucose, total cholesterol, triglyceride, LDH and LDL).

Milk production was recorded and milk samples were taken weekly from each goat. Milk composition was analyzed by MilkoScan analyzer (Foss Electric, Hillerød, Denmark) and milk somatic cell counts (SCC) were measured using an optical somatic cell counting analyser (Fossomatic 4000, Foss Electric,Hillerod, Denmark). During weeks 1, 3 and 6 of the experiment milk samples were collected and stored immediately at −80 °C and then analyzed for TAC, MDA, flavonoids and total polyphenols.

Samples of TMR were collected three times (during three consecutive days) during trial and stored at −20 °C for chemical analysis.

2.3. Laboratory analysis

Diet 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).

Plasma samples were analyzed for glucose, total cholesterol, triglyceride, LDH and LDL content by using a commercially assay kits (Pars-azmon Co., Tehran, Iran). The level of MDA in plasma and milk was determined using thiobarbituric acid method according to Placer et al. (1966). The results were obtained in terms of nmol/mL and determined using the colorimetric method. TAC in milk was determined by ferric reducing antioxidant power (FRAP) method (Benzie and Strain, 1996) with some modifications according to Smet et al. (2008). Before measuring the absorbance, a centrifugation step was introduced (1300 × *g* for 5 min), resulting in a clear medium.

Plasma TAC was measured using Randox kit (Crumlin, CountyAntrim, UK) in which 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfanate,ABTS) is incubated with a peroxidase and H_2O_2 to produce the radical cation ABTS+. This has a stable blue-green color, which is measured at 600 nm. Antioxidants in plasma samples cause suppression of this color production to a degree that is proportional to their concentration.

After methanol extraction, milk and oil total content of polyphenols were determined using the Folin–Ciocalteu procedure (Singleton and Rossi, 1965) and concentrations of polyphenol compounds were expressed as gallic acid equivalents (GAE;mg/l of milk and mg/g of oil). One hundred microliters of extract was mixed with 750 µL Folin-Ciocalteu reagent and left to stand at 22 °C for 5 min. Next, 750 µL of aqueous sodium bicarbonate solution (g/L) was added to the mixture. After 90 min at 22 °C, absorbance was measured at 725 nm.

Flavonoid contents of milk samples were measured at 425 nm by