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ORIGINAL ARTICLE

Effects of replacing wheat bran by pistachio skins on feed intake, nutrient digestibility, milk yield, milk composition and blood metabolites of dairy Saanen goats

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Summary

The objective of this study was to investigate the effect of pistachio skins (PiS) as a replacement of wheat bran on feed intake, nutrient digestibility, milk yield, milk composition and blood metabolites of dairy Saanen goats. Eight multiparous lactating Saanen goats (55 \pm 7.2 days post-partum, 45 \pm 2 kg body weight) were randomly assigned to one of the four dietary treatments arranged in a replicated 4×4 Latin square design. The dietary treatments were 1) 0 g/kg PiS and 210 g/kg wheat bran in the TMR (0PiS), 2) 70 g/kg PiS and 140 g/kg wheat bran in the TMR (7PiS), 3) 140 g/kg PiS and 70 g/kg wheat bran in the TMR (14PiS) and 4) 210 g/kg PiS and 0 g/kg wheat bran in the TMR (21PiS). The trial consisted of four 21-day periods, each composed of 14 days adaptation and 7 days data collection. Dry matter intake (p < 0.05) and crude protein digestibility (p < 0.01) increased linearly with increasing PiS proportions in the diet. Increasing the proportion of PiS in the diet caused a quadratic increase in apparent digestibility of dry matter (p < 0.05), and tended (p = 0.05) to increase quadratically organic matter, and ether extract digestibility. Replacing wheat bran with PiS in the diet had no effects on milk yield, whereas milk fat concentration increased linearly (p < 0.01) with increasing inclusion of PiS in the diet. As the dietary proportion of PiS increased, ruminal pH tended (p = 0.07) to increase linearly, whereas ammonia-N concentration declined in the rumen. Plasma concentrations of glucose and BUN remained unaffected, whereas triglycerides (p \leq 0.05) and cholesterol (p \leq 0.01) concentrations increased linearly with increasing inclusion of PiS in the diet. It was concluded that PiS based on local ingredients can successfully replace wheat bran in diets of dairy goats without detrimental effects on feed intake, nutrient digestibility and milk production.

Keywords by-products, pistachio skins, goats

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Introduction

Pistachio farming is a main agricultural enterprise in central Iran. Iran is the largest producer of pistachios worldwide with an annual production of approximately 500 000 tonnes of fresh pistachio by-products (PBP) (Shakeri et al., 2013). Pistachio by-products (PBP) are produced during the de-hulling of pistachio nuts after harvesting. The PBP consist of 645 g/kg skins, 250 g/kg twigs, 100 g/kg leaves and 5 g/kg kernel and bony shells (Vahmani and Naserian, 2006; Bagheripour et al., 2008). In recent years, several studies reported beneficial effects by ruminants consuming PBP likely due to their high concentrations of

crude protein (CP; 158 g/kg of DM) and ether extract (EE; 69.5 g/kg of DM) (Ghasemi et al., 2012a; Ghaffari et al., 2014a,b; Sedighi-Vesagh et al., 2015). Pistachio by-products (sun-dried or as silage) have been used successfully in diets of dairy cows (150 g/kg of DM; Mokhtarpour et al., 2012), dairy goats (300–320 g/kg of DM; Ghaffari et al., 2014b; Sedighi-Vesagh et al., 2015) and sheep (300 g/kg of DM; Norouzian et al., 2011; Ghaffari et al., 2014a). The total phenolic (TP) compounds and tannins (TT) of sun-dried PBP have been reported to be 76–156 g/kg of DM and 34–102 g/kg of DM respectively (Bagheripour et al., 2008; Ghaffari et al., 2014a,b; Sedighi-Vesagh et al., 2015). Tannins can modify microbial

populations, consequently altering variables such as nutrient digestibility, ruminal ammonia-N concentrations and VFA profiles with potential effects on animal metabolism (Jones et al., 1994; Al-Dobaib, 2009; Krueger et al., 2010). Recently, Sedighi-Vesagh et al. (2015) reported that feeding PBP at 320 g/kg of DM (31.8 g of TP/kg of DM) did not affect DMI and apparent digestibilities of DM, OM and ADF. Pistachio skins (PiS) are inexpensive by-product of the pistachio blanching industry. Pistachio skins are industrially removed from the nut and constitute about 645 g/kg of the PBP and are produced upon processing of pistachio in large amounts. Pistachio skins and wheat bran are generally comparable in terms of chemical compositions. Using PiS in the diets of ruminants can reduce dietary costs and the risk of environmental pollution. The PiS are good sources of CP (213 g/kg DM basis) and fat (196 g/kg DM basis) and provide moderate amounts of ADF (376 g/kg DM basis) and NDF (221 g/kg DM basis) (Naserian and Vahmani, 2005), thus making them a potentially good feed for lactating ruminants. In addition, PiS have a wide range and high concentration of total phenolic (TP) compounds (i.e. gallic acid, catechin, eriodictyol-7-O-glucoside, naringenin-7-O-neohesperidoside, quercetin-3-Orutinoside, eriodictyol, genistein-7-O-glucoside, genistein, daidzein and apigenin) that are all potent antioxidants and are found in greater concentrations in skins than in seeds (Tomaino et al., 2010). Few studies have examined the nutritive value of PiS for ruminant animals, and it would be interesting to find out to what level PiS can replace wheat bran in the ration of dairy goats. We hypothesized that PiS potentially have nutritive value that could be used as feedstuff in the diet of dairy goats without interfering with nutrient digestibility and milk yield. Therefore, the main objectives of this study were to investigate the effects of partial replacement of wheat bran with PiS on feed intake, nutrient digestibility, milk yield, milk composition and blood metabolites of lactating Saanen dairy goats.

Materials and methods

The experiment was conducted at the Research Farm of the Faculty of Agriculture, Ferdowsi University of Mashhad (Iran), according to the guidelines of the Iranian Council of Animal Care (1995).

Pistachio by-product preparation

Weekly samples of PiS were collected between June and August 2013 from pistachio de-hulling factories

in Feizabad (Khorasan Razavi Province, Iran). Sundried PBP that contained skins, twigs, leaves and bony shells were collected from Bardaskan Town (Babakhan, Khorasan-e-Razavi Province, Iran). Samples were composited into a single sample and chemically analysed (Table 1).

Goats, management and treatments

Eight multiparous lactating Saanen goats $(55 \pm 7.2 \text{ days post-partum}; 45 \pm 2 \text{ kg BW})$ were assigned randomly to four dietary treatments arranged in a replicated 4 × 4 Latin square design with four 21-day periods. Each experimental period consisted of 14 day of adaptation to diets and 7 days for daily data collection. The dietary treatments (Table 2) were 1) 0 g/kg PiS and 210 g/kg wheat bran in the TMR (0PiS), 2) 70 g/kg PiS and 140 g/kg wheat bran in the TMR (7PiS), 3) 140 g/kg PiS and 70 g/kg wheat bran in the TMR (14PiS) and 4) 210 g/kg PiS and 0 g/kg wheat bran in the TMR (21PiS). Goats were housed individually in metabolic cages (1.05 m \times 0.55 m)

Table 1 Chemical composition of wheat bran, pistachio skins (PiS) and pistachio by-products (PBP)

Item (g/kg dry matter)	Wheat bran	Pistachio skins	Pistachio by-products
Dry matter (g/kg)	926.1	952.0	910.0
Crude protein (g/kg DM)	170.9	217.7	114.2
Ether extract (g/kg DM)	44.6	196.0	64.5
Acid detergent fibre (g/kg DM)	116.7	155.6	205.8
Neutral detergent fibre (g/kg DM)	414.8	376.0	332.6
Lignin (g/kg DM)	17.2	26.1	97.2
Total phenols (g/kg DM)	ND†	91.0	99.5
Total tannins (g/kg DM)	ND†	44.1	66.8
Minerals			
Calcium (g/kg DM)	2.8	2.6	8.8
Phosphorus (g/kg DM)	10.5	5.0	2.1
Magnesium (g/kg DM)	4.8	1.9	3.1
Potassium (g/kg DM)	13.4	15.7	44.2
Sodium (g/kg DM)	0.4	0.3	_
Iron (mg/kg DM)	_	160.0	_
Zinc (mg/kg DM)	7.7	29.0	27.5
Copper (mg/kg DM)	_	15.0	16.2
Manganese (mg/kg DM)	15.0	16.0	_
Fatty acids (g/100 g of total fatty	y acids)		
C14:0	0	0.2	1.5
C16:0	7.9	9.7	12.3
C18:0	0.4	1.3	2.2
C18:1 cis-9	0.7	45.1	47.8
C18:2 cis-9, cis-12	2.6	42.8	26.9
C18:3 cis-9, cis-12, cis-15	0.2	0.9	4.7

†Non-detect.

Table 2 Ingredients and chemical composition of experimental diets (DM basis)

Item	Treatme	Treatments†				
	0PiS	7PiS	14PiS	21PiS		
Diet ingredients (g/kg DM)						
Alfalfa hay	350	350	350	350		
Barley grain, ground	300	300	300	300		
Beet pulp	80	80	80	80		
Cottonseed meal	50	50	50	50		
Wheat bran	210	140	70	-		
Pistachio skins	-	70	140	210		
Limestone	5	5	5	5		
Vitamin/mineral premix§	3	3	3	3		
Salt	2	2	2	2		
Chemical composition (g/kg DI	M)					
Dry matter	898	896	897	895		
Crude protein	154	157	160	164		
Neutral detergent fibre	361	360	354	352		
Acid detergent fibre	194	193	194	192		
Ether extract	26	37	48	59		
Lignin	44.8	45.4	46.0	46.6		
Calcium	7.2	7.1	7.3	7.4		
Phosphorus	5.8	5.2	4.7	4.3		
Total phenols	2.5	8.9	15.3	21.6		
Total tannins	1.6	4.5	7.8	10.9		

 \dagger 0PiS, 0 g/kg PiS, 210 g/kg wheat bran; 7PiS, 70 g/kg PiS, 140 g/kg wheat bran; 14PiS, 140 g/kg PiS, 70 g/kg wheat bran; 21PiS, 210 g/kg PiS, 0 g/kg wheat bran.

§Each kg of the vitamin–mineral premix contained (DM basis) vitamin A (50,000 IU), vitamin D3 (10,000 IU), vitamin E (1000 IU), 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 (0.1 g), I (0.1 g) and Se (0.001 g).

with unlimited access to water. Diets were formulated based upon the AFRC (1993) recommendations to meet the nutrient requirements of lactating goats that average 45 kg of BW and produce 2.7 kg/day of milk with 3.6% fat. Individual feed ingredients were mixed and fed as a totally mixed ration (TMR) once daily at 0800 h in *ad libitum* amounts (allowing 5–10% orts, as-fed basis).

Measurements and sampling procedures

Feed offered and refused and milk yield were measured daily for each goat during the last week of each period. Samples of TMR and feed refusals were collected daily from days 15 to 21 of each period, frozen and composited by goat and period. During the faecal collection periods (days 15 to 21), all faecal pellets were collected twice a day, weighed and 10% retained. Faeces and urine were separated by a metallic screen placed under the metabolic cages. Samples of TMR offered and refused and faeces were dried at

60 °C for 72 h. Dried samples were ground to pass a 1-mm screen (Retsch Cutting Mill, Retschmule, Germany) prior to chemical analyses. Apparent digestibility of nutrients was estimated by the marker ratio technique using acid-insoluble ash as an internal marker (Van Keulen and Young, 1977).

Goats were milked twice daily at 0800 and 1700 by a milking machine. Milk samples were obtained from each goat during the last 7 days of each experimental period from two consecutive milkings. Milk samples were composited according to the corresponding milk yield and kept at room temperature (*i.e.* 23 °C) with the preservative potassium dichromate.

On day 20 of each period, ruminal fluid was collected from each goat at 2 and 4 h after the morning feeding using a stomach tube and visually assessed to contain minimal amounts of saliva. After straining through four layers of cheesecloth, pH was determined using a glass electrode pH-meter (691 Metrohm, Herisau, Switzerland). Samples were frozen until analysed.

On the last day of each experimental period, blood was collected from the jugular vein into heparinized Vacutainers tubes (Vacutest[®], Arzergrande, Italy) 3 h after the morning feeding and immediately placed on ice. Tubes were centrifuged at 3000 g (4 °C) for 15 min within 2 h of sampling. The plasma was separated and stored at -20 °C for subsequent analysis.

Chemical analyses

The TMR offered and refused were mixed thoroughly across collection days and ground to pass a 1-mm screen in a Wiley mill (Ogaw Seiki, Tokyo, Japan) before chemical analyses. Feed, refusals, faecal samples and PiS were analysed for DM (method 934.01; AOAC, 1990), ether extract (EE; method 920.39; AOAC, 1990), crude protein (CP; method 988.05; AOAC, 1990), ash (AOAC, 1990; method 942.05) and lignin by the sulphuric acid method (method 973.18; AOAC, 1990) after a sequential NDF extraction (Van soest et al., 1991). Concentrations of ADF inclusive of residual ash (method 973.18c; AOAC, 1990) and NDF inclusive of residual ash were determined sequentially without the use of sodium sulphite and with the inclusion of a-amylase (Van soest et al., 1991).

Samples of PiS and diets were dried at 40 °C to constant weight to minimize changes in tannin content and activity. Dried samples were ground through a 0.5-mm screen before analysis (Makkar, 2000). Phenolic compounds were extracted using 200 mg of dried samples of TMR and PiS. The extraction process involved the addition of 10 ml of acetone–water

(70:30, v/v) left at 4 °C overnight. After centrifuging at 3000 *g* at 4 °C for 15 min, the supernatant was used to determine TP using the Folin–Ciocalteu procedure (Singleton and Rossi, 1965) and the regression equation of tannic acid standard. Total tannins were estimated indirectly after being absorbed to insoluble polyvinylpolypyrrolidone (PVPP). Concentration of TT was calculated by subtracting the TP remaining after the PVPP treatment in the assay mixture (Makkar, 2000).

Milk samples were analysed for fat, crude protein, lactose and total solids content by Milk-O-Scan 4000 infrared analyser (Foss Electric, Hillerød, Denmark).

Fatty acids in dried PiS samples were converted to methyl esters in 0.5 m sodium methoxide in methanol followed by a second methylation in acetyl chloride: methanol (1:10, vol/vol) based on a procedure described by Kramer et al. (1997) to prevent epimerization and isomerization of conjugated acids. Fatty acid methyl esters were determined using a Varian CP-3800 gas chromatograph (Varian, Palo Alto, CA, USA) equipped with auto-sampler (Varian CP-8400), flame ionization detector and a Varian capillary column (CP-sil $100 \text{ m} \times 0.25 \text{ mm} \times 0.2 \mu\text{m}$). The carrier gas was He, split ratio was 10:1, and the injector and detector temperatures were maintained at 230 °C and 250 °C respectively. One microlitre of sample was injected through the auto-sampler into the column. The oven temperature was initially set at 120 °C for 1 min, increased by 5 °C/min up to 190 °C, held at 190 °C for 30 min, increased 2 °C/min up to 220 °C and held at 220 °C for 40 min. The peak was identified and calculated based on the retention time and peak area of known standards.

Total ammonia-N concentration of the ruminal fluid was determined by micro-Kjeldahl (AOAC, 1990) using a digestion apparatus (Kjeldatherm System KT 40; Gerhardt Laboratory Instruments, Bonn, Germany).

Blood metabolite concentrations were determined by an automated biochemical analyser (Biotecnica, Targa 3000, Rome, Italy) using commercially available kits [Pars Azmoon Company, Catalogue Numbers: glucose (1-500-017), triglyceride (1-500-032), cholesterol (1-500-010) and urea nitrogen (BUN; 1-400-029), Tehran, Iran] according to the manufacturer's instructions.

Analyses for Ca, P, Mg, K, Na, Fe, Zn, Cu and Mn concentrations in PiS were performed by commercial laboratory (Northeast DHIA Forage Testing Laboratory, Ithaca, NY, USA).

Statistical analyses

Data were analysed by least squares ANOVA using the general linear models procedure of SAS (2003). The mathematical model used to analyse the data was as follows:

$$Y_{ijk1} = \mu + \alpha_i + \beta_{ij} + \gamma_k + \lambda_1 + (\alpha \lambda)_{i1} + (\alpha \gamma)_{ik} + \epsilon_{ijk1}$$

where Y_{iik1} = response variable in square i in period k in treatment 1 for cow j, μ = overall mean, α_i = effect of square i, β_{ij} = effect of cow j within square i, γ = effect of period k, λ = effect of treatment 1, $(\alpha\lambda)_{i1}$ = effect of interaction of square i with treatment 1, $(\alpha \gamma)_{ik}$ = effect of interaction of square i with period k and ε_{ijk1} = residual effect of i, j, k and 1. Linear and quadratic effects were tested using two contrast statements. The error term used to test main effect of square was cow within square. The residual error term was used to make all other tests. Least squares means were determined using the LSMEANS/PDIFF option, and statistical differences between treatments were determined following the Tukey adjustment. Significance was declared at $p \le 0.05$, and trends were considered when 0.05 .

Results and discussion

The present work reports the differences in chemical composition and phenolic content between PiS and PBP (Table 1). In the current study, PiS exhibit relatively higher CP, EE, NDF but lower ADF and lignin contents compared to PBP. More importantly, the concentrations of TP compounds and TT (condensed and hydrosable tannins) of PiS showed 91.0 and 44.1 g/kg DM comparable to those of PBP within the optimal range of 7.6% to 15.6% DM and 3.4% to 10.15% DM (Bagheripour et al., 2008; Ghasemi et al., 2012a; Ghaffari et al., 2014a,b; Sedighi-Vesagh et al., 2015). The chemical composition of PiS showed its good potentials as a feed resource in ruminant nutrition.

Feed intake and nutrient digestibility

Increasing the proportion of PiS in the diet caused a linear increase (p < 0.05) in DMI (Table 3). Several studies (Norouzian et al., 2011; Mokhtarpour et al., 2012; Shakeri et al., 2013; Sedighi-Vesagh et al., 2015) observed no effect on DMI when PiS were incorporated into the diet. However, Ghaffari et al. (2014b) replaced PBP with alfalfa hay in the diet and

Table 3 Dry matter intake and apparent nutrient digestibility of dairy goats fed increasing amounts of pistachio skins (PiS)

ltem	Treatments†				P-value contrast§	for	
	0PiS	7PiS	14PiS	21PiS	SEM	L	Q
Dry matter intake (kg/day)	1.94 ^b	2.07 ^a	2.08 ^a	2.09 ^a	0.40	*	NS
Total tract apparent digestibility	y (g/kg)						
Dry matter	717 ^c	721 ^{bc}	748 ^a	71.7 ^c	0.75	NS	*
Organic matter	725 ^a	730 ^a	757 ^a	72.6 ^b	0.88	NS	0.06
Crude protein	718 ^c	728 ^b	739 ^b	759 ^a	1.38	**	NS
Ether extract	725 ^b	729 ^b	756ª	725 ^b	0.89	NS	0.06
Neutral detergent fibre	554	524	530	525	1.10	NS	NS

†0PiS, 0 g/kg PiS, 210 g/kg wheat bran; 7PiS, 70 g/kg PiS, 140 g/kg wheat bran; 14PiS, 140 g/kg PiS, 70 g/kg wheat bran; 21PiS, 210 g/kg PiS, 0 g/kg wheat bran.

reported that inclusion of PBP at 300 g/kg of DM or 22.9 g TT/day (18.1 g of TT expressed as g of tannic acid equivalent/kg of DM) in the diet of dairy goats decreased DMI. Pistachio by-products are rich in tannins (Ghasemi et al., 2012a; Ghaffari et al., 2014a,b; Shakeri et al., 2014) which, when fed to ruminants, were shown previously to have both adverse and no effects. Greater tannin concentrations (> 50 g/kg of DM) in diets may adversely affect feed intake and nutrient utilization (Patra and Saxena, 2011), whereas lower concentrations of tannins had no influence on intake by ruminants (Barry and Duncan, 1984; Waghorn et al., 1994; Aerts et al., 1999). In the current experiment, goats fed the PiS diets consumed 22.8 g/ day of TT at the greatest tannin intake, which may have been too low to interfere with DM intake.

A quadratic response of apparent digestibility of DM (p < 0.05), OM (p = 0.06) and EE (p = 0.06) to increasing dietary PiS proportion was observed, with the highest digestibility observed for goats fed the 140 g/kg PiS diet than those fed the other diets (Table 3). Increased DMI and digestibility of DM, OM and EE may be attributed to positive impacts of PiS on ruminal fermentation. However, apparent OM and EE digestibilities were similar by goats fed the 0 and 210 g/kg PiS diets. Crude protein digestibility linearly increased (p < 0.01) with increasing intake of PiS which is consistent with Ghasemi et al. (2012b) who reported that feeding PBP at 500 g/kg of dietary DM (TP; 42.5 g of tannic acid equivalent/kg of DM) increased CP digestibility when PBP replaced lucerne hay in the diet of sheep. In contrast, Sedighi-Vesagh et al. (2015) observed that apparent total tract digestibility of CP decreased with the inclusion of 320 g of PBP/kg of dietary DM (TP; 30.2 g of tannic acid equivalent/kg of DM) when PBP replaced alfalfa hay in the diet of dairy goats. In the present study, substitution of wheat bran with PiS increased intake of EE because of greater EE content of PiS compared with wheat bran. Greater EE digestibility by dairy goats fed PiS may have resulted from greater EE intake (Palmquist, 1991). A lack of effect of supplementing PBP at 100 g/kg of DM (TP; 96 g of tannic acid equivalent/kg of DM) on digestibility of NDF was reported when PBP replaced corn silage in the diets of dairy cows (Gholizadeh et al., 2010). Discrepancies between studies could partly be due to differences in the amount, chemical structure or reactivity of tannins present in diets.

Milk yield and composition

Milk yield was not affected when PiS replaced wheat barn in the diet (Table 4). A linear increase (p < 0.01) in milk fat content occurred when the PiS dietary proportion increased, whereas milk protein, lactose, solids-non-fat and total solids concentrations were not affected by experimental treatments (Table 4). In the current study, the higher milk fat content in goats fed PiS compared with those fed wheat bran may partly be associated with an increased intake of EE due to greater levels of PiS in the diet. Likewise, Sedighi-Vesagh et al. (2015) replaced PBP with alfalfa hay in the diet and reported that inclusion of 320 g of PBP/kg of dietary DM in the diet of dairy goats altered neither milk production nor milk composition. In agreement with our findings, Dschaak et al. (2011) observed no difference in the yield and composition of milk by dairy cows due to diet supplementation with 300 g/kg of DM of extract of quebracho tannins.

 $[\]S L = \text{linear}; Q = \text{quadratic}; NS = \text{non-significant}; ** = p < 0.01; * = p < 0.05.$

a-cValues in the same row without a common superscript letter are significantly different (p < 0.05).

Table 4 Milk yield and composition of dairy goats fed increasing amounts of pistachio skins (PiS)

Item	Treatments†					P-value for contrast§	
	0PiS	7PiS	14PiS	21PiS	SEM	L	Q
Milk yield (kg/day)	2.80	2.85	2.96	2.72	0.10	NS	NS
Milk composition, %							
Fat	3.10 ^c	3.62 ^b	3.96 ^a	3.79 ^b	0.14	**	*
Protein	2.81	2.70	2.84	2.74	0.05	NS	NS
Lactose	4.94	4.97	4.90	5.02	0.05	NS	NS
Solids-not-fat	8.34	8.24	8.34	8.34	0.05	NS	NS
Total solids	11.46	11.51	11.93	11.52	0.15	NS	NS

†0PiS, 0 g/kg PiS, 210 g/kg wheat bran; 7PiS, 70 g/kg PiS, 140 g/kg wheat bran; 14PiS, 140 g/kg PiS, 70 g/kg wheat bran; 21PiS, 210 g/kg PiS, 0 g/kg wheat bran.

Ruminal fermentation and blood metabolites

Replacing wheat bran with PiS in the diet of dairy goats tended (p = 0.07) to cause a linear increase in ruminal pH (Table 5). This agrees with Ghasemi et al. (2012b) who observed that supplementing PBP by up to 500 g/kg of DM (TP; 42.5 g of tannic acid equivalent/kg of DM) in the diets increased ruminal pH from 6.03 to 6.33 of Baluchi lambs when PBP replaced alfalfa hay. Rumen ammonia-N concentration decreased linearly (p < 0.05) with increasing inclusion levels of PiS in the diet (Table 5) probably as a result of the presence of tannins in PiS binding to proteins and decreasing proteolysis of feed protein (Frutos et al., 2004; Getachew et al., 2008) and thereby ammonia-N concentration (Min et al., 2001). However, the lowest ruminal ammonia-N concentrations reported herein were still above the minimum level (≥5 mg/dl) suggested by Satter and Roffler (1975) to support rumen microbial growth. These findings are consistent with those of Ghasemi et al. (2012a) who reported lower ruminal ammonia-N concentrations in sheep fed 400 g of PBP/kg of DM (TP; 78.5 g of tannic acid equivalent/kg of DM) than those fed the control diet when PBP replaced alfalfa hay. Similarly, Shakeri et al. (2014) reported a decrease in ruminal ammonia-N concentration with increasing dietary concentration (0 to 180 g/kg) of PBP silage (TP; 145 g of tannic acid equivalent/kg of DM) when PBP silage replaced corn silage in the diets of Holstein male calves. Ghaffari et al. (2014a) also reported that the concentration of ruminal ammonia-N decreased with increasing PBP of up to 360 g/kg of dietary DM (TP; 35.2 g of tannic acid equivalent/kg of DM) when PBP replaced alfalfa hay in the diets of sheep.

Concentrations of blood triglycerides (p < 0.05) and cholesterol (p < 0.01) were linearly increased when PiS replaced wheat bran in the diet (Table 5). These responses may possibly be attributed to greater EE in the diets of goats supplemented with PiS. Some

Table 5 Ruminal pH, NH3-N concentration and blood metabolites of dairy goats fed increasing amounts of pistachio skins (PiS)

ltem	Treatments†					P-value contrast§	for
	0PiS	7PiS	14PiS	21PiS	SEM	L	Q
Ruminal pH	6.0 ^{bc}	6.2ª	6.1 ^{ab}	6.2ª	0.04	0.07	**
Ruminal NH ₃ -N, mg/dl	19.9 ^a	19.2 ^b	19.1 ^c	18.2 ^d	0.44	*	NS
Blood metabolites (mg/dl)							
Glucose	62.0	63.0	64.0	62.0	2.30	NS	NS
BUN	19.0	20.0	19.0	18.0	0.23	NS	NS
Triglycerides	10.9 ^c	12.6 ^b	12.3 ^b	15.6 ^a	1.12	*	NS
Cholesterol	95 ^d	103 ^c	114 ^b	133ª	3.52	**	NS

†0PiS, 0 g/kg PiS, 210 g/kg wheat bran; 7PiS, 70 g/kg PiS, 140 g/kg wheat bran; 14PiS, 140 g/kg PiS, 70 g/kg wheat bran; 21PiS, 210 g/kg PiS, 0 g/kg wheat bran.

 $[\]xi L = \text{linear}; Q = \text{quadratic}; NS = \text{non-significant}; ** = p < 0.01; * = p < 0.05.$

a-cValues in the same row without a common superscript letter are significantly different (p < 0.05).

L = linear; Q = quadratic; Q = non-significant; Q = quadratic; Q

a-dValues in the same row without a common superscript letter are significantly different (p < 0.05).

previous studies showed that feeding PBP at high levels decreased (Gholizadeh et al., 2010) or had no effects (Shakeri et al., 2013; Ghaffari et al., 2014a,b) on blood cholesterol concentration in ruminants.

In contrast to our expectations, glucose concentration in plasma was not affected by increasing dietary proportion of PiS. Similar results of unchanged blood glucose concentration have been reported for dairy goats (Ghaffari et al., 2014b), sheep (Ghasemi et al., 2012b) and calves (Shakeri et al., 2013) fed PBP. Sedighi-Vesagh et al. (2015) replaced PBP with alfalfa hay in the diet and reported that supplementing PBP at 320 g/kg of dietary DM (31.8 g of TP/kg of DM) to dairy goats did not affect blood glucose and cholesterol concentrations. Plant containing tannins have been shown to reduce blood glucose concentration due to the inhibitory effect on carbohydrate digestion in the rumen (Barry and Manley, 1986) and to the inhibition of the growth and activities of cellulolytic bacterial populations (Ghasemi et al., 2012a), leading to lower production of glucogenic volatile fatty acids (Makkar, 2003) and lower blood glucose concentration (Sedighi-Vesagh et al., 2015).

In contrast to our expectations, concentration of BUN was not affected (p < 0.05) by treatments. This is inconsistent with the finding by Shakeri et al. (2013) that BUN content was lower for growing male calves fed 180 g of PBP silage/kg of dietary DM (2.1 g condensed tannin expressed as g of tannic acid equivalent/kg of DM) compared with those fed corn silage as control diet (0 g/kg PBP). Sedighi-Vesagh et al. (2015) reported decreases in BUN concentration for dairy goats that were fed a diet containing 320 g of

PBP/kg of dietary DM (TP; 31.8 g of tannic acid equivalent/kg of DM) when PBP replaced alfalfa hay. Tannins have been shown to reduce ruminal degradation of feed protein due to the formation of tannin–protein complexes in the rumen (Min et al., 2001) and to the inhibition of the growth and activities of proteolytic bacterial populations (Patra and Saxena, 2011), leading to decreased BUN (Fernández et al., 2012).

Conclusion

The present study revealed that 210 g/kg PiS could be used in the diet of dairy goats without interfering with milk yield. Increasing replacement of wheat bran (up to 210 g/kg of dietary DM) with PiS improved DMI, apparent total tract digestibility of CP, milk fat concentration and pH of ruminal fluid but did not affect milk production of lactating dairy goats in mid-lactation. The TT content of PiS was not high enough to depress apparent CP digestibility or milk protein concentration when PiS were fed at 210 g/kg of dietary DM. In addition, apparent total tract digestibility of DM, OM and EE increased when PiS made up 140 g/kg of dietary DM; however, when PiS made up 210 g/kg of dietary DM, apparent digestibility of these feed components returned to baseline values. Pistachio skins can successfully replace wheat bran in diets for lactating dairy goats.

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References

Aerts, R. J.; Barry, T. N.; McNabb, W. C., 1999: Polyphenols and agriculture: beneficial effects of proanthocyanidins in forages. *Agriculture, Ecosystems and Envi*ronment 75, 1–12.

AFRC, 1993: Energy and Protein Requirements of Ruminants. An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallingford, UK.

Al-Dobaib, S. N., 2009: Effect of different levels of quebracho tannin on nitrogen utilization and growth performance of Najdi sheep fed alfalfa (*Medicago sativa*) hay as a sole diet. *Journal of Animal Science* **80**, 532–541.

Association of Official Analytical Chemists, International AOAC, 1990: Official

Methods of Analysis, 15th edn. AOAC, Arlington, VA.

Bagheripour, E.; Rouzbehan, Y.; Alipour, D., 2008: Effects of ensiling, air-drying and addition of polyethylene glycol on *in vitro* gas production of pistachio byproducts. *Animal Feed Science and Technology* **146**, 327–336.

Barry, T. N.; Duncan, S. J., 1984: The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep.
1. Voluntary intake. *British Journal of Nutrition* 51, 484–491.

Barry, T. N.; Manley, T. R., 1986: Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in *lotus sp.* and their possible consequences in ruminant nutrition. *Journal of the Science of Food and Agriculture* **37**, 248–254.

Dschaak, C. M.; Williams, C. M.; Holt, M. S.; Eun, J. S.; Young, A. J.; Min, B. R., 2011: Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. *Journal of Dairy Science* **94**, 2508–2519.

Fernández, H. T.; Catanese, F.; Puthod, G.; Distel, R. A.; Villalba, J. J., 2012: Depression of rumen ammonia and blood urea by quebracho tannin-containing supplements fed after highnitrogen diets with no evidence of self-regulation of tannin intake by sheep. *Small Ruminant Research* **105**, 126–134.

Frutos, P.; Hervas, G.; Giraldez, F. J.; Mantecon, A. R., 2004: Review: Tannins and ruminant nutrition. *Spanish Journal* of Agricultural Research **2**, 191–202.

- Getachew, G.; Pittroff, W.; Putnam, D. H.; Dandekar, A.; Goyal, S.; DePeters, E. J., 2008: The influence of addition of Gallic acid, Tannic acid, or Quebracho tannins to alfalfa hay on *in vitro* rumen fermentation and microbial protein synthesis. *Animal Feed Science and Technology* **140**, 444–461.
- Ghaffari, M. H.; Tahmasbi, A. M.; Khorvash, M.; Naserian, A. A.; Ghaffari, A. H.; Valizade, H., 2014a: Effects of pistachio by-products in replacement of alfalfa hay on populations of rumen bacteria involved in biohydrogenation and fermentative parameters in the rumen of sheep. *Journal of Animal Physiology and Animal Nutrition* 98, 578–586.
- Ghaffari, M. H.; Tahmasbi, A. M.; Khorvash, M.; Naserian, A. A.; Vakili, A. R., 2014b: Effects of pistachio by-products in replacement of alfalfa hay on ruminal fermentation, blood metabolites, and milk fatty acid composition in Saanen dairy goats fed a diet containing fish oil. *Journal of Applied Animal Research* 42, 186–193
- Ghasemi, S.; Naserian, A. A.; Valizadeh, R.; Tahmasebi, A. M.; Vakili, A. R.; Behgar, M.; Ghovvati, S., 2012a: Using pistachio hulls as a replacement for alfalfa hay in the diet of sheep causes a shift in the rumen cellulolytic bacterial population. *Small Ruminant Research* **104**, 94–98.
- Ghasemi, S.; Naserian, A. A.; Valizadeh, R.; Tahmasebi, A. M.; Vakili, A. R.; Behgar, M., 2012b: Effects of pistachio byproduct in replacement of lucerne hay on microbial protein synthesis and fermentative parameters in the rumen of sheep. *Animal Production Science* 52, 1052–1057.
- Gholizadeh, H.; Naserian, A. A.; Valizadeh, R.; Tahmasebi, A., 2010: Effect of feeding pistachio byproduct on performance and blood metabolites in Holstein dairy cows. *International Journal of Agricultural and Biological* 12, 867–870.
- Iranian Council of Animal Care, 1995: Guide to the Care and Use of Experimental Animals, Vol. 1. Isfahan University of Technology, Isfahan, Iran.
- Jones, G. A.; McAllister, T. A.; Muir, A. D.; Cheng, K. J., 1994: Growth and proteolysis by four strains of ruminal bacteria. *Applied and Environmental Microbiology* 60, 1374–1378.
- Kramer, J. G.; Fellner, V.; Dugan, M. E. R.; Sauer, F. D.; Mossoba, M. M.; Yurawecz,

- M. P., 1997: Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipid* **32**, 1219–1228.
- Krueger, W. K.; Gutierrez Bañuelos, H.; Carstens, G. E.; Min, B. R.; Pinchak, W. E.; Gomez, R. R., 2010: Effects of dietary tannin source on performance, feed efficiency, ruminal fermentation, and carcass and non-carcass traits in steers fed a high-grain diet. Animal Feed Science and Technology 159, 1–9.
- Makkar, H. P. S., 2000: Quantification of Tannins in Tree Foliage: A Laboratory Manual. FAO/IAEA Working document, Vienna, Austria.
- Makkar, H. P. S., 2003: Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin rich feeds. *Small Ruminant Research* **49**, 241–256.
- Min, B. R.; Fernandez, J. M.; Barry, T. N.; McNabb, W. C.; Kemp, P. D., 2001: The effect of condensed tannins in *Lotus corniculatus* upon reproductive efficiency and wool production in ewes during autumn. *Animal Feed Science and Technology* **92**, 185–202.
- Mokhtarpour, A.; Naserian, A. A.; Tahmasbi, A. M.; Valizadeh, R., 2012: Effect of feeding pistachio by-products silage supplemented with polyethylene glycol and urea on Holstein dairy cows performance in early lactation. *Livestock Science* **148**, 208–213.
- Naserian, A. A.; Vahmani, P., 2005: Effect of feeding pistachio skins on feed intake, milk yield and milk composition in lactating Saanen goats. Journal of Animal Science 83, Suppl. 1/Journal of Dairy Science 88, Suppl. 1 (Abstract).
- Norouzian, M. A.; Valizadeh, R.; Vahmani, P., 2011: Rumen development and growth of Balouchi lambs offered alfalfa hay pre- and post-weaning. *Tropical Animal Health and Production* **43**, 1169–1174.
- Palmquist, D. L., 1991: Influence of source and amount of dietary fat on digestibility in lactating cows. *Journal of Dairy Science* **74**, 1354–1360.
- Patra, A. K.; Saxena, J., 2011: Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Journal of the Science of Food and Agriculture 91, 24–37.

- SAS, 2003: SAS User's Guide Statistics. Version 9.1 Edition. SAS Inst., Cary, NC.
- Satter, L. D.; Roffler, R. E., 1975: Nitrogen requirement and utilization in dairy cattle. *Journal of Dairy Science* **58**, 1219–1237
- Sedighi-Vesagh, R.; Naserian, A. A.; Ghaffari, M. H.; Petit, H. V., 2015: Effects of pistachio by-products on digestibility, milk production, milk fatty acid profile and blood metabolites in Saanen dairy goats. *Journal of Animal Physiology and Animal Nutrition* doi: 10.1111/jpn.12233. [Epub ahead of print].
- Shakeri, P.; Riasi, A.; Alikhani, M.; Fazaeli, H.; Ghorbani, G. R., 2013: Effects of feeding pistachio by-products silage on growth performance, serum metabolites and urine characteristics in Holstein male calves. *Journal of Animal Physiology* and Animal Nutrition 97, 1022–1029.
- Shakeri, P.; Riasi, A.; Alikhani, M., 2014: Effects of long period feeding pistachio by-product silage on chewing activity, nutrient digestibility and ruminal fermentation parameters of Holstein male calves. *Animal* 8, 1826–1831.
- Singleton, V. L.; Rossi, J. A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16, 144–158.
- Tomaino, A.; Martorana, M.; Arcoraci, T.; Monteleone, D.; Giovinazzo, C.; Saija, A. 2010: Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie* **92**, 1115–1122.
- Vahmani, P.; Naserian, A. A., 2006: Pistachio hulls as a feed ingredients for lactating dairy goats. In: Proceedings of the British Society of Animal Science. (BSAS, Scarborough, UK), pp. 145.
- Van Keulen, J.; Young, B. A., 1977: Evaluation of acid-insoluble ash as natural marker in ruminant digestibility. *Journal of Animal Science* **44**, 282–287.
- Van soest, P. J.; Robertson, J. B.; Lewis, B. A., 1991: Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.
- Waghorn, G. C.; Shelton, I. D.; McNabb, W. C., 1994: Effects of condensed tannins in *Lotus pedunculatuson* its nutritive value for sheep. 1. Non-nitrogenous aspects. *Journal of Agricultural Science* 123, 99–107.