Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/jpn.12233

ORIGINAL ARTICLE

Effects of pistachio by-products on digestibility, milk production, milk fatty acid profile and blood metabolites in Saanen dairy goats

R. Sedighi-Vesagh[1], A. A. Naserian[1], M. H. Ghaffari[1] and H. V. Petit[2]

1 Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran, and
2 Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada

Summary

The objective of this study was to investigate the effects of pistachio by-products (PBP) on nutrient digestibility, blood metabolites and milk fatty acid (FA) profile in Saanen dairy goats. Nine multiparous lactating Saanen goats (on day 90 post-partum, 45 ± 2/kg BW) were randomly assigned to a 3 × 3 Latin square design with three treatment diets: 1) control diet (alfalfa hay based), 2) 32% PBP and 3) 32% PBP + polyethylene glycol (PEG-4000; 1 g/kg dry matter). Each period lasted 21 days, including 14 day for treatment adaptation and 7 day for data collection. Pistachio by-products significantly decreased ($p < 0.01$) crude protein (CP) digestibility compared with the control diet (64.4% vs. 58.7%), but PEG addition did not differ for CP digestibility of goats fed 32% PBP + PEG and those fed the two other diets. The digestibility of NDF tended ($p = 0.06$) to decrease for goats fed PBP compared with those fed the control diet. Yields of milk and 4% fat-corrected milk were not affected by dietary treatments. Compared with the control diet, PBP supplementation appreciably changed the proportions of almost all the milk FA measured; the main effects were decreases ($p < 0.01$) in FA from 8:0 to 16:0 and increases ($p < 0.01$) proportions of *cis*-9, *trans*-11 18:2 and *trans*-11 18:1, monounsaturated FA, polyunsaturated FA and long-chain FA. The saturated FA, short-chain FA and medium-chain FA proportions were lower ($p < 0.01$) in goats fed the two PBP supplemented diet than in those fed the control diet and PEG addition led to intermediate proportions of saturated FA, unsaturated and monounsaturated FA. Inclusion of PBP in the diet decreased ($p < 0.01$) plasma concentrations of glucose and urea nitrogen compared with the control diet. It was concluded that PBP can be used as forage in the diet of dairy goats without interfering with milk yield. Inclusion of 32% PBP in the diet of dairy goats had beneficial effects on milk FA profile but PEG addition to PBP did not contribute to enhance further milk FA profile.

Keywords pistachio by-products, polyethylene glycol, milk fatty acid, digestibility, goats

Correspondence M. H. Ghaffari, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad 91779-48978, Iran. Tel.: +98 935 781 4911; Fax: +98 511 8796845; E-mail: morteza.h.g@gmail.com

Received: 5 May 2013; accepted: 7 July 2014

Introduction

Iran is considered to be the largest producer of pistachio worldwide with an annual production of approximately 500 000 tonnes of fresh pistachio by-products (PBP) (Shakeri et al., 2012). Pistachio by-products consist of 64.5% soft external hulls, 25% twigs, 10% leaves, and 0.5% kernel and bony shells (Vahmani et al., 2006; Bagheripour et al., 2008). Using PBP as feedstuffs in ruminant nutrition reduces feeding cost and potential environmental pollution (Vahmani et al., 2006; Gholizadeh et al., 2010). In recent years, several studies have reported that PBP (sun-dried or as silage) could be integrated into livestock diets (Mokhtarpour et al., 2012; Shakeri et al., 2012; Ghasemi et al., 2012b). Pistachio by-products are high in protein (158.2 g/kg dry matter (DM)) and ether extract (EE) (69.5 g/kg DM) and, hence, a valuable feed for ruminants (Behgar et al., 2009). However, their nutritive value is subject to variation due to dehulling process, pistachio cultivar and growing conditions (Bagheripour et al., 2008). Concentrations of total phenolic compounds and total tannins (condensed and hydrosable tannins) of sun-dried PBP range from 7.6% to 15.6% DM and 3.4% to 10.15% DM respectively (Shakeri and Fazaeli, 2007; Bagheripour et al., 2008; Bohluli et al., 2009). High concentrations of phenolic compounds and total tannins may limit animal

productivity in some cases. For example, decreases in intake and digestibility of goats fed condensed tannins (CT) extracted from *Sericea lespedeza* as the standard rich herbaceous species have been reported previously (Puchala et al., 2005). Conversely, Shakeri et al. (2012) included PBP silage (2.1 g CT expressed as g tannic acid equivalent/kg of DM) as up to 18% of the diet for Holstein male calves and reported no adverse effects on dry matter intake (DMI), growth performance or blood metabolites after a long-term feeding programme. One way to improve utilization of tannin-rich forages is to form a stable complex by binding of tannins with an inert and unabsorbed molecule such as polyethylene glycol (PEG), thus preventing the binding between tannins and proteins (Badran and Jones, 1965). Indeed, PEG has shown capacity to neutralize the condensed tannins as indicated by enhanced milk production and crude protein digestibility in goats fed tannin-rich forages (Decandia et al., 2000).

Phenolic compounds have been defined as secondary metabolites of PBP (Bohluli et al., 2009; Ghasemi et al., 2012a). Quebracho tannins (*Schinopsis lorentzii*; 456 g of equivalent tannic acid/kg of DM) reduce ruminal biohydrogenation (BH) due to their ability to inhibit the activity of some ruminal micro-organisms (Vasta et al., 2009a). Conjugated linoleic acid (CLA) is a component of milk fat with many potential positive human health benefits (Parodi, 2004). Conjugated linoleic acid is produced in the rumen as a result of incomplete ruminal biohydrogenation of unsaturated fatty acid (FA) by ruminal micro-organisms (Vasta et al., 2009b). Previous studies had shown that tannins could modify the milk FA profile (Cabiddu et al., 2009; Khiaosa-Ard et al., 2009). It is thought that tannins interfere with ruminal biohydrogenation, specifically by inhibiting the last step that converts *trans*-11 18:1 to stearic acid and thereby alters milk FA profile (Khiaosa-Ard et al., 2009; Vasta et al., 2010; Toral et al., 2011). Regulation of the last step of the biohydrogenation is of major importance because *trans*-11 18:1 is the major precursor of *cis*-9, *trans*-11 CLA synthesis in the mammary gland via the action of stearoyl CoA desaturase (Griinari et al., 2000; Palmquist et al., 2005; Vasta et al., 2009b). To the best of the authors' knowledge, no published reports are available on possible effects of PBP supplementation on milk FA profile in goats. The objective of the present investigation was to determine the effect of PBP on feed intake, nutrient digestibility and blood metabolites. As its second objective, the study aims to determine whether dietary PBP affect the milk FA profile in Sannen dairy goats and whether PEG contributes to enhance utilization of PBP.

Material and methods

Animals, experimental diets and management

The experiment was conducted at the Research Farm of the Agriculture Faculty of Ferdowsi University of Mashhad (Iran) in 2011. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran. Nine multiparous lactating Saanen goats (90d post-partum, 45 ± 2 kg) were randomly assigned to three treatments in a triplicated 3 × 3 Latin square design with three diets and three 21-day periods. Each experimental period consisted of 14 day of adaptation to diets and 7 day for daily data collection. The dietary treatments (Table 1) were: (i) control (alfalfa hay based); (ii) 32% PBP (alfalfa hay in control diet replaced by PBP); and (iii) 32% PBP + 1 g/kg DM PEG (PEG-4000; Sigma-Aldrich, St Louis, MO, USA).

Table 1 Ingredients and chemical composition of experimental diets (DM basis)

Item	Treatment		
	Control	PBP	PBP+PEG†
Ingredients, %			
Alfalfa hay	32	0	0
Pistachio hulls	0	32	32
Corn silage	21	21.5	21.5
Canola meal	13	14.5	14.5
Wheat straw	15	15	15
Wheat bran	10	8	8
Canola seed	7	7	7
Vitamin–mineral mix[a]	1.2	1.2	1.2
Calcium carbonate	0.5	0.5	0.5
Salt	0.3	0.3	0.3
Chemical composition			
Metabolizable energy (Mcal/kg)	3.70	3.75	3.71
Crude protein (% of dry matter)	14.9	14.9	14.9
Ether extract (% of dry matter)	6.0	7.4	7.4
Neutral detergent fibre (% of dry matter)	32.0	31.3	31.3
Acid detergent fibre (% of dry matter)	21.1	19.4	19.4
Non-fibre carbohydrate (% of dry matter)[b]	41.6	41.2	41.2
Total phenols (g/100 g dry matter)[c]	0.72	3.18	3.02
Total tannins (g/100 g dry matter)[c]	0.39	2.12	2.04

PBP, pistachio by-product; PBP+PEG, pistachio by-product + polyethylene glycol.

[a]Contained (g/kg premix; dry matter basis): 330 000 IU of vitamin A, 60 000 IU of vitamin D, 1000 IU of vitamin E, 160 g Ca, 85 g P, 63 g Na, 45 g Mg, 2100 mg Zn, 1500 mg Mn, 535 mg Cu, 12 mg Se and 45 mg I.

[b]Non-fibre carbohydrates calculated as 100 − (crude protein + ash + neutral detergent fibre + ether extract).

[c]Expressed as grams of tannic acid equivalent per 100 g of dry matter.

†The PEG group received the PBP diet with 1 g/kg DM of polyethylene glycol-4000 added to each kg of diet.

Diets were formulated based on the AFRC (1993) recommendations to meet the nutrient requirements of lactating goats that average 45 kg of BW and produce 1.5 kg/day of milk with 3.5% of fat. Diets were fed once daily at 08:00 hours for ad libitum intake (allowing 10% refusals on as-fed basis). The goats were housed in individual metabolic cages (1.05 m × 0.55 m) and had free access to water. Sun-dried PBP, which contained soft external hull, twigs, leaves, bony shells, were collected from Bardaskan Town (Babakhan, Khorasan-e-Razavi Province, Iran). Profile of the PBP used is presented in Table 2.

Table 2 Chemical composition and fatty acid composition of pistachio by-products

Item	Pistachio by-product
Chemical composition	
DM (%)	94.0
CP (% of dry matter)	11.3
EE (% of dry matter)	6.50
ADF (% of dry matter)	30.2
NDF (% of dry matter)	22.4
NFC (% of dry matter)	42.8
Ash (% of dry matter)	9.20
TP (% of dry matter)	9.95
TT (% of dry matter)	6.68
ME (Mcal/kg)[a]	2.00
Fatty acid (g/100 g of total fatty acids)	
C12:0	0.03
C14:0	1.47
C16:0	12.34
C16:1 cis-9	0.94
C17:0	0.18
C17:1 cis-9	0.09
C18:0	2.22
C18:1 cis-9	47.80
C18:2 cis-9, cis-12	26.94
C18:3 cis-9, cis-12, cis-15	4.72
C20:0	0.15
C20:1 cis-9	0.64
C22:0	0.61
C24:0	0.61
Total identified unsaturated FA	81.13
Total identified saturated FA	17.61
Others	1.26

DM, dry matte; CP, crude protein; EE, ether extract; ADF, acid detergent fibre; NDF, neutral detergent fibre; NFC, non-fibre carbohydrates; TT, total tannins; TP, total phenols.

[a]ME, metabolizable energy, estimated by equation: ME (Mcal/ kg) = (0.027 + 0.0427 DDM) × 0.821 (Gonzalez and Everitt, 1982).

Measurements and sampling procedures

Nutrient intake and digestibility

Feed intake and milk yield were measured daily on the last week of the experiment, and data were averaged over the 7 days of each period and subjected to analysis of variance. Samples of diets and refusals were collected daily from days 15 to 21, frozen and pooled by goat and period. Composite samples of diets and refusals were mixed and subsampled by period and goat. Total faeces collection was carried out from days 15 to 21 of each period, and faecal pellets were collected twice a day. Faeces and urine were separated by a metallic screen placed under the metabolic cages at an angle of approximately 45° to avoid contamination between faeces and urine. Faecal samples were collected daily and composited (0.1 output) by animal over the 7-day collection period. Samples of diets, refusals and faeces were dried at 60 °C for 72 h, and dried samples were ground to pass a 1-mm screen (Retsch Cutting Mill, Retschmule, Germany) prior to chemical analyses. Urine from each goat was collected daily in plastic vessels containing 100 ml sulphuric acid solution (0.1, v/v) to maintain the pH level below three. Urine was weighed once a day and mixed well, and a 0.1 daily aliquot was pooled over the 7-day collection period per animal. Urine samples were stored at −20 °C until chemical analysis. Apparent digestibility of nutrients was estimated by the marker ratio technique using acid-insoluble ash as an internal marker (Van Keulen and Young, 1977).

Milk and blood sampling

The goats were milked once daily at 09:00 from days 15 to 21, and individual yields were recorded at each milking. Milk samples were obtained from each goat for the seven consecutive milkings and pooled within goat and period relative to production at each milking to obtain one composite milk sample/goat/period for chemical analysis. One sample was stored at 4 °C with a preservative (bronopol-B2) and sent to a laboratory (Food and Chemical Analysis Research Laboratory, Jahad-e-Daneshgahi, Mashhad, Iran) for determination of fat, protein and lactose. Another sample without preservative was stored at −20 °C for analysis of milk FA profile by gas chromatography. Milk yield was converted to a fat-corrected basis to evaluate the energy output in milk. Yield of 4% fat-corrected milk (FCM) was calculated as: [(0.4 × kg milk) + 15 (kg milk × milk fat/100)].

On the last day of each experimental period, blood samples were collected from the jugular vein (10 ml into sterile tubes containing EDTA solution) 4 h after the morning feeding. The samples were immediately placed on ice for processing in the laboratory. The blood samples were then centrifuged at 3000 *g* for 15 min. The plasma was frozen and stored at −20 °C until further analysis.

Chemical analysis

Samples of feed, orts and faeces were analysed for DM (method 934.01; AOAC, 1990), organic matter (method 920.39; Association of Official Analytical Chemists, 1990), ether extract (method 920.39; Association of Official Analytical Chemists, 1990) and CP (method 988.05; Association of Official Analytical Chemists, 1990) standard procedures. Concentrations of acid detergent fibre (ADF) inclusive of residual ash (method 973.18c; Association of Official Analytical Chemists, 1990) and neutral detergent fibre (NDF) inclusive of residual ash were determined sequentially without the use of sodium sulphite and with the inclusion of α-amylase (Van soest et al., 1991).

For tannin assay, samples of diets were dried at 40 °C to constant weight to minimize changes in tannin content and activity, and dried samples were ground through a 0.5-mm screen before analysis (Makkar, 2000). Phenolic compounds were extracted using 200 mg of dried samples. The extraction process involved the sample being made up to 10 ml with aqueous acetone water (700:300, v/v), and the extraction was left at 4 °C overnight. The extracts were centrifuged at 3000 *g* at 4 °C for 15 min, and the supernatant was obtained and used in the following assay. The concentration of total phenolic compounds (TP) was determined using the Folin–Ciocalteu procedure (Singleton and Rossi, 1965) and the regression equation of tannic acid (Merck GmbH, Darmstadt, Germany) standard. Total tannins (TT) 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). Plasma concentrations of glucose, urea nitrogen (BUN), cholesterol, albumin and total protein, and liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST)] in the plasma samples were determined using commercial kits (Pars Azmoon Company, Tehran, Iran) and an auto-analyser (Biotecnica, Targa 3000, Rome, Italy).

Determination of fat, protein and lactose proportions in milk was carried out using a Milk-O-Scan 4000 infrared analyser (Foss Electric, Hillerød, Denmark). Milk lipids were extracted using 2:1 (v/v) chloroform/methanol (Clark et al., 1982), and 100 μl of heneicosanoic acid C21:0 (Nuchek Prep, Elysian, MN, USA) was added as internal standard. Fatty acid methyl esters (FAME) were prepared according to the method described by Wijngaarden (1967). Fatty acid profile was determined by gas chromatography. A fused silica capillary column (WCOT Fused Silica Capillary, DANI, Model 1000, Rome, Italy) with 120 m length, 0.32 mm internal diameter and 0.2 μM film thickness on an HP 6890 GC equipped with flame ionization detector was used to qualify and quantify FAMEs. The initial column temperature was set at 180 °C for 20 min, which increased to 225 °C by increments of 5 °C/min, then to 250 °C by 10 °C/min and held for 12 min. Hydrogen was used as carrier gas with a flow of 1.7 ml/min for the first 10 min. Then, the flow was decreased to 1.3 ml/min which was kept until the end of the analysis. The detector temperature was set at 300 °C. Identification of FA was performed by comparison with the retention times of FAMEs standards (Sigma–Aldrich, Catalog #18919). The *trans*-18:1 isomers were identified by order of elution as described by Precht et al. (2001). Separations of all FA were obtained with a single chromatographic run.

Statistical analysis

All data were analysed using a triplicated 3 × 3 Latin square design using the PROC MIXED procedure of SAS 9.1.3 (SAS 2003; SAS Institute, Cary, NC, USA). The statistical model included goat as random effect, and period and treatment as fixed effects. Data were analysed according to the following model:

$$Y_{ijk} = \mu + P_i + T_j + C_k + e_{ijk}$$

where Y_{ijk} is the dependent variable, μ is overall mean, P_i is fixed effect of period (I = 1, 2 and 3), T_j is fixed effect of treatment (j = 1, 2 and 3), C_k is the random effect of goat and e_{ijk} is the residual error. Comparisons of means of the different treatments were made using Tukey's adjustment for the probability. Effects of dietary treatments are declared significant at $p < 0.05$, and trends are declared at $p < 0.10$. Results are reported as least squares means.

Results

DMI and digestibility

Intake of DM and apparent digestibilities of DM, OM and ADF did not differ significantly ($p > 0.05$) among treatments (Table 3). The digestibility of crude protein (CP) was lower ($p = 0.01$) in goats fed PBP than in those fed the control diet, and NDF digestibility tended ($p = 0.06$) to decrease for goats fed PBP compared with those fed the control diet.

Table 3 Dry matter intake and apparent nutrient digestibility of dairy goats fed diets including alfalfa hay (control) and/or pistachio by-products plus/minus PEG

	Treatments				
Parameter	Control	PBP	PBP+PEG	SEM	p-value
Dry matter intake (kg/day)	1.86	1.82	1.96	0.03	0.11
Total tract apparent digestibility (%)					
DM	69.15	69.71	68.10	0.39	0.26
OM	68.35	68.48	68.80	0.46	0.91
CP	64.37[a]	58.67[b]	61.19[ab]	0.62	0.01
NDF	63.01	60.41	62.62	0.43	0.06
ADF	46.61	42.75	43.29	0.76	0.11

PBP, pistachio by-product; PBP+PEG, pistachio by-product + PEG, DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM, standard error of the difference of least square means.
Values 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 diets including alfalfa hay (control) and/or pistachio by-products plus/minus PEG

	Treatments				
Parameter	Control	PBP	PBP+PEG	SEM	p-value
Milk production (kg/d)	1.60	1.55	1.56	0.03	0.69
4% FCM (kg/day)	1.45	1.44	1.43	0.03	0.93
Milk Composition (%)					
Fat	3.42	3.60	3.52	0.06	0.39
Protein	2.72	2.77	2.81	0.03	0.40
Lactose	4.44	4.54	4.40	0.04	0.37
Total Solid	11.30	11.65	11.47	0.09	0.27
Solids-non-fat	7.88	8.05	8.95	0.05	0.34
Milk yield (g/d)					
Fat	53.90	54.81	53.79	1.13	0.34
Protein	43.49	42.98	43.63	0.77	0.28
Lactose	71.65	70.41	68.50	1.12	0.56
Solids-non-fat	126.59	124.97	123.57	1.99	0.59

PBP, pistachio by-product; PBP+PEG, pistachio by-product + PEG; 4% FCM, fat-corrected milk for 4%; DMI, dry matter intake; SEM, standard error of the difference of least square means.
Values in the same row without a common superscript letter are significantly different ($p < 0.05$).

Yield, composition and fatty acid profile of milk

The mean of milk yield and composition are presented in Table 4. Yields of milk and 4% fat-corrected milk (FCM) averaged 1.57 and 1.44 kg/day, respectively, and they were not affected by dietary treatments. Proportions of milk fat, protein, lactose and total solids and yield of milk components averaged 3.51%, 2.77%, 4.46%, and 11.5%, respectively, and they did not differ among dietary treatments. Compared with the control diet, PBP appreciably changed ($p < 0.01$) proportions of FA in milk fat. Proportions of 8:0, 10:0, 14:0, 15:0, 16:0, 18:0 and *cis*-9 20:1 in milk fat of goats receiving PBP were lower ($p < 0.01$) than those of goats fed the control diet, whereas the inverse was observed ($p < 0.01$) for proportions of 11:0, *cis*-9 18:1, *trans*-11 18:1, *cis*-9,*cis*-12 18:2, *cis*-9,*trans*-11 18:2, *trans*-9,*trans*-12 18:2, 20:0 and 24:0 (Table 5). Feeding the PBP+PEG diet compared with the control diet decreased milk fat proportions of 4:0, 6:0, 8:0, 10:0, 12:0, 13:0, 15:0, *cis*-9 20:1 and *cis*-9 22:1 and increased those of 16:0, 18:0, *cis*-9 18:1, *cis*-9, *cis*-12, *cis*-15 18:3 and *cis*-9 24:1. Feeding the PBP diet compared with the PBP+PEG diet decreased proportions of 4:0 to 13:0, *trans*-11 18:1, 20:0 and 24:0 and increased those of 14:0, 16:0, 18:0, *trans*-9,*trans*-12 18:2, *cis*-9, *cis*-12, *cis*-15 18:3 and *cis*-9 24:1. The highest and lowest proportions of saturated FA were observed in milk fat of goats fed, the control diet and the PBP diet, respectively, and the inverse was obtained for proportions of unsaturated and monounsaturated FA. Goats fed the PBP+PEG diet had intermediate values of saturated, unsaturated and monounsaturated FA proportions. Proportions of polyunsaturated FA did not differ for goats fed the PBP+PEG and PBP diets and higher than for those fed the control diet. Proportions of short-chain FA (C4:0 to C10:0) were higher for goats fed the control diet than for those fed the PBP diet, and they were higher for goats fed the PBP diet than for those fed the PBP+PEG diet. Medium-chain FA proportions (C12:0 to C16:1) were higher for goats fed the control diet than for those fed the PBP and the PBP+PEG diets, and there was no difference between goats fed PBP and PBP+PEG. The highest and lowest proportions of long-chain FA (≥C18:0) were obtained, respectively, with the PBP+PEG diet and the control diet, and goats fed the PBP diet had intermediate values.

Plasma metabolites

Plasma concentrations of cholesterol, albumin and total protein were not affected ($p > 0.05$) by the treatments, but the PBP diet decreased ($p < 0.01$) the concentrations of glucose and BUN compared with the control diet (Table 6). Plasma concentrations of glucose and BUN did not differ for goats fed the PBP+PEG diet than those fed the other two diets. Plasma alanine aminotransferase (ALT) concentration was lower ($p < 0.01$) in goats fed the control diet than in those fed the PBP and PBP+PEG diets, but there was no difference between PBP and PBP+PEG. There were no

Table 5 Milk fatty acid (FA) profile of dairy goats fed diets including alfalfa hay (control) and/or pistachio by-products plus/minus PEG

	Treatments				
Parameter	Control	PBP	PBP+PEG	SEM	p-value
Fatty acids (g/100 g of total FA)					
4:0	2.86[a]	2.20[a]	1.67[b]	0.08	<0.001
6:0	2.62[a]	2.26[a]	1.53[b]	0.10	<0.001
8:0	2.47[a]	2.00[b]	1.61[c]	0.12	<0.001
10:0	7.39[a]	6.13[b]	5.14[c]	0.23	<0.001
11:0	0.21[b]	0.41[a]	0.14[b]	0.02	<0.001
12:0	3.51[a]	3.12[a]	2.68[b]	0.05	<0.001
13:0	0.12[a]	0.11[a]	0.09[b]	0.02	0.01
14:0	9.07[a]	7.71[b]	9.03[a]	0.18	0.01
cis-9 14:1	0.50	0.48	0.41	0.02	0.29
15:0	1.16[a]	0.87[b]	0.83[b]	0.11	0.01
cis-9 15:1	0.30	0.38	0.28	0.06	0.27
16:0	17.32[b]	16.22[c]	19.20[a]	0.61	0.01
cis-9 16:1	0.33	0.35	0.42	0.10	0.36
18:0	11.15[b]	10.37[c]	12.75[a]	0.14	<0.001
trans-11 18:1	1.16[b]	2.02[a]	1.40[b]	0.08	<0.001
cis-9 18:1	24.55[b]	25.19[a]	26.37[a]	0.26	<0.001
trans-9,*trans-12*18:2	1.98[c]	2.45[b]	3.44[a]	0.10	<0.001
cis-9,*cis*-12 18:2	3.05[b]	3.82[a]	3.52[a]	0.03	<0.001
cis-9,*trans*-11 18:2	0.75[b]	0.89[a]	0.80[a]	0.03	<0.001
cis-9,*cis*-12,*cis*-15 18:3	0.57[b]	0.56[b]	0.64[a]	0.07	<0.001
20:0	0.41[b]	1.10[a]	0.42[b]	0.01	<0.001
cis-9 20:1	1.88[a]	0.89[b]	0.78[b]	0.09	<0.001
22:0	0.13	0.10	0.07	0.01	0.09
cis -9 22:1	0.25[a]	0.12[b]	0.17[b]	0.04	<0.001
24:0	0.13[b]	0.68[a]	0.13[b]	0.01	<0.001
cis-9 24:1	0.12[b]	0.30[b]	0.42[a]	0.05	<0.001
Saturated FA*	59.09[a]	53.60[c]	55.80[b]	1.15	<0.001
Unsaturated FA†	40.84[c]	47.70[a]	45.39[b]	0.82	<0.001
Monounsaturated FA	34.50[c]	39.94[a]	36.99[b]	0.14	<0.001
Polyunsaturated FA‡	6.34[b]	7.73[a]	8.40[a]	0.14	<0.001
Short-chain FA§	5.48[a]	4.46[b]	3.21[c]	0.11	<0.001
Medium-chain FA¶	23.28[a]	19.95[b]	19.11[b]	0.22	<0.001
Long-chain FA**	71.17[c]	76.89[b]	78.88[a]	1.23	<0.001

PBP, pistachio by-product; PBP+PEG, pistachio by-product + PEG; SEM, standard error of means.

*Saturated FA = $C_{4:0} + C_{6:0} + C_{8:0} + C_{10:0} + C_{11:0} + C_{12:0} + C_{14:0} + C_{15:0} + C_{16:0} + C_{17:0} + C_{18:0} + C_{20:0}\ C_{22:0} + C_{24:0}$.

†Monounsaturated FA = $C_{14:1} + C_{15:1} + C_{16:1} + C_{17:1} + C_{18:1\ cis} + C_{18:1\ trnas} + C_{20:1} + C_{22:1} + C_{24:1}$.

‡Polyunsaturated FA = $C_{18:2\ cis} + C_{18:2\ trnas} + C_{18:3}$.

§Short-chain FA = C4:0 to C10:0.

¶Medium-chain FA = C12:0 to C16:1.

**Long-chain FA = ≥C18:0.

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

significant differences ($p > 0.05$) among the treatments for aspartate aminotransferase (AST), packed cell volume (PCV), haemoglobin concentration (Hb) and total white blood cells counts (WBC).

Discussion

Supplementation with PBP at 320 g/kg DM had no effect on DM intake as previously reported for lambs fed a diet containing 250 g/kg PBP (3.5 g CT expressed as g tannic acid equivalent/kg of DM) (Ghasemi et al., 2012b). In the present experiment, goats fed the PBP diet consumed 3.86 g/day TT, which may have been too low to interfere with DM intake and explain the lack of a beneficial effect of PEG supplementation on DM intake. Indeed, PEG supplementation has shown a more important effect on DM intakes of goats that are fed leaves (*Quercus calliprinos,*

Table 6 Blood metabolites of dairy goats fed diets including alfalfa hay (control) and/or pistachio by-products plus/minus PEG

	Treatment				
Parameter	Control	PBP	PBP+PEG	SEM	p-value
Glucose (mg/dl)	50.00[a]	40.33[b]	47.22[ab]	1.65	0.01
Cholesterol (mg/dl)	140.33	145.00	136.89	2.41	0.39
Albumin (g/l)	29.67	28.11	30.11	0.64	0.42
Total protein (g/l)	43.00	43.00	42.32	0.55	0.84
BUN (mg/dl)	32.22[a]	25.44[b]	29.11[ab]	0.69	0.01
ALT (units/l)	30.44[b]	37.66[a]	36.00[a]	0.54	0.01
AST (units/l)	67.78	67.11	67.33	0.98	0.95
PCV (%)	33.00	32.67	32.33	0.40	0.79
Hb (g/dl)	9.44	9.29	9.30	0.11	0.78
Total WBC (1000 per cubic mm)	8.33	9.17	9.33	0.51	0.69

PBP, pistachio by-product; PBP+PEG, pistachio by-product + PEG; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PCV, packed cell volume; WBC, total white blood cell count; Hb, haemoglobin; SEM, standard error of means.
Values in the same row without a common superscript letter are significantly different ($p < 0.05$).

Pistacia lentiscus, Caratonia siliqua) with higher concentrations of CT (5–7.1 g tannic acid equivalent/100 g of DM) (Silanikove et al., 1996). The lack of response to PEG supplementation could also be due to the amount of PEG supplied (1 g/kg DM) that was lower in the present experiment than in the experiments of Silanikove et al. (1996; 5–40 g/day) and Decandia et al. (2000; 25–50 g/day). In the present experiment, the antinutritional effect of tannins likely was exerted through the reduction of food protein availability as indicated by decrease CP digestibility in goats fed the PBP diet. However, PEG addition to the PBP diet did not differ CP digestibility for goats fed the PBP+PEG diet and those fed the two other diets, thus suggesting some potential for PEG to improve CP digestibility. This agrees with the enhanced CP digestibility with PEG supplementation reported for goats fed tannin-containing leaves (Silanikove et al., 1996) or woody species (Decandia et al., 2000). This finding then may suggest that PEG partly prevented the binding between tannins and proteins of the PBP diet as previously reported by Badran and Jones (1965) for tannin-rich forages.

The effects of PBP and PBP+PEG supplementation on digestibility of NDF tended to be similar to those observed for digestibility of CP. According to Barry and Manley (1986; 90 g CT/kg DM extracted from *Lotus* sp), and Barry et al. (1986; 46–106 g CT/kg DM extracted from *Lotus pedunculatus*) tannins reduce cell wall digestibility by binding bacterial enzymes and/or forming indigestible complexes with cell wall carbohydrates. However, the effect of PBP was greater on NDF than ADF digestibility, which could be related to the greater susceptibility of hemicellulolytic enzymes to tannins (Waghorn and Shelton, 1995). The effects of PEG on fibre digestibility were not as large as reported previously (Ben Salem et al., 1999; Saarisalo et al., 1999; Decandia et al., 2000), which may be due to the presence of artefacts in the fibre fractions. Indeed, lower true digestibility of NDF has been shown to result from the formation of PEG–tannin complexes that come together with the NDF as 'artefact NDF' (Makkar et al., 1995), which overestimates NDF content in faeces.

The lack of a treatment effect on digestibility of DM, OM and ADF is consistent with the results of Gholizadeh et al. (2010) who observed that supplementing PBP at 100 g/kg DM (TP; 9.6 g tannic acid equivalent/100 g of DM) in the diets of dairy cows had no effect on apparent digestibilities of DM, NDF and ADF. Conversely, Carulla et al. (2005) reported that supplementing sheep diet with CT at 25 g/kg DM from *Acacia mearnsii* extract (black wattle tree) decreased digestibilities of OM, CP, NDF and ADF. Discrepancies between experiments are likely due to differences in the amount, chemical structure or reactivity of tannins present in diets.

Goats fed the PBP diet had lower BUN than those fed control diet, which is in agreement with the results of Shakeri et al. (2012) who reported lower BUN concentrations for growing male calves fed 180 g/kg DM PBP (2.1 g CT expressed as g tannic acid equivalent/kg of DM) silage than for those fed control diet. Similar decreases in BUN concentration have been observed for sheep that were fed diets containing 11 g/kg DM quebracho tannins (Fernández et al., 2012). Tannins mainly change protein degradability in the rumen as a result of tannin–protein complexes formation (Min et al., 2001), which may decrease BUN (Fernández et al., 2012). The lower proportion of alfalfa hay in the diet of goats fed PBP could also have contributed to lower BUN concentration as ruminal degradability of protein from alfalfa might have been higher than that of PBP. Conversely, supplementation of the PBP diet with PEG resulted in BUN concentrations did not differ for goats fed the PBP+PEG diet and those fed the two other diets. Linear increases in ammonia N concentration in the rumen have been reported in parallel with increased levels of PEG supplementation (Silanikove et al., 1996), which again may corroborate that PEG partly prevented

the binding between tannins and proteins of the PBP diet. However, overprotection of protein by tannins may have limited further absorption of protein in the small intestine. Indeed, milk yield and milk composition were not affected by the treatments. Similar results have been reported for dairy goats supplemented with 140 g/kg PBP in the diet (Vahmani and Naserian, 2006) and for goats fed various proportions of PBP (Forough-Ameri and Shakeri, 2008; Rezaeenia et al., 2012). Furthermore, Cabiddu et al. (2005) reported that the administration of a PEG solution had no effects on milk yield and milk composition of sheep grazing *Sulla* (CT; 27.4 g tannic acid equivalent/kg DM).

Haematological data were used to assess the health status of goats. Alanine aminotransferase is a liver specific hepatocellular enzyme released by hepatocellular damage that increases in serum when liver cells are damaged (Mahgoub et al., 2008). Activity of ALT was increased in goats on both diets supplemented with PBP, which may indicate that PBP may lead to some liver damage as previously shown for the liver of goats fed an antinutritional factor such as total phenolics, tannins (Tabosa et al., 2000). Similar increases in ALT serum concentration have been reported for sheep fed quebracho CT at 1.5 g/kg body weight although there was no effect at lower levels (Hervas et al., 2003). Lower blood glucose concentration in goats fed PBP may be attributed to the inhibitory effect of the tannins on the digestion of carbohydrates by rumen microbes (Barry and Manley, 1986) or to lower production of glucogenic volatile fatty acids (Makkar, 2003).

The increase in milk fat proportion of *trans*-11 18:1 on the PBP diet agrees with the fact that dietary tannins modify ruminal biohydrogenation of PUFA, resulting in greater concentration of *trans*-11 18:1 in the rumen (Khiaosa-Ard et al., 2009; Vasta et al., 2009a). Similar increases in milk fat proportions of total *trans* 18:1 FA have been reported for dairy cows supplemented with quebracho CT extract (30 g/kg DM) in the diet (Dschaak et al. (2011). A higher proportions of *cis*-9, *trans*-11 CLA on the two PBP supplemented diets was expected because *trans*-11 18:1 was increased in milk fat when goats received the PBP diet and the majority of *cis*-9, *trans*-11 CLA in milk fat is synthesized endogenously from *trans*-11 18:1 as reviewed by Bauman and Lock (2006). Moreover, Toral et al. (2013) reported that dietary supplementation with quebracho tannin extract (20 g/kg DM) increased proportions of several 18:1 and 18:2 isomers in milk fat of dairy ewes, while significant differences between treatments in concentration of *trans*-11 18:1 and cis-9, *trans*-11 CLA were only observed on day 3 of the 27-day collection period and had no long-term effect on proportions of beneficial FA in milk fat. Various responses to condensed tannin supplements on the proportion of *cis*-9, *trans*-11 18:2 in milk fat of lactating ruminants have been reported, such as a decrease (Cabiddu et al., 2009), increase (Abbeddou et al., 2011) or no effect (Benchaar and Chouinard, 2009), which may be attributed to differences in dose and type of tannins and basal diet composition (Vasta et al., 2009b).

In the present experiment, the lower *trans*-11 18:1 concentration in milk fat of goats fed PBP+PEG relative to that of goats fed PBP likely resulted from higher biohydrogenation by the rumen microbes when combining PEG and PBP following partial inactivation of the PBP tannins by PEG addition. Similarly, supplementing the diet of dairy sheep grazing *Sulla* (20–40 g of CT/kg DM) with PEG increased milk fat proportions of *trans*-11 18:1 and *cis*-9, *trans*-11 CLA (Cabiddu et al., 2009).

Adding PBP to diets decreased concentrations of short-chain and saturated FA, which may be attributed to the lower de novo synthesis of short- and medium-chain FA in the mammary gland with diets rich in polyunsaturated FA (Palmquist and Beaulieu, 1993; Chilliard and Ferlay, 2004; Walker et al., 2004). Indeed, PBP used in the present experiment was rich in polyunsaturated FA (Table 2). Moreover, the increased proportions of polyunsaturated FA in milk fat of goats fed the two PBP supplemented diets likely resulted of the inhibitory effect of tannins on ruminal biohydrogenation of polyunsaturated FA. In contrast with some studies where dairy cows (Benchaar and Chouinard, 2009) and ewes were fed (Toral et al., 2011) CT, our results may suggest that PBP supplementation modify ruminal biohydrogenation, which was reflected in increased LCFA proportions in milk fat.

Conclusion

The present study revealed that PBP can be used as forage in the diet of dairy goats without interfering with milk yield or milk composition. Although PBP replacement for alfalfa hay decreased the apparent digestibility of CP, there were no effects on apparent digestibility of DM, OM and ADF. Inclusion of 32% PBP in the diet of dairy goats increased the concentration of *cis*-9, *trans*-11 CLA and *trans*-11 18:1 in milk fat but PEG addition to PBP did not contribute to enhance further milk FA profile.

Acknowledgements

The authors would like to acknowledge from Department of Animal Science of Ferdowsi University of Mashhad, (Iran) for the cooperation. We would like to thank Paul T. D. Peake for help with the manuscript and for his assistance with this project.

References

Abbeddou, S.; Rischkowsky, B.; Richter, E. K.; Hess, H. D.; Kreuzer, M., 2011: Modification of milk fatty acid composition by feeding forages and agro-industrial by-products from dry areas to Awassi sheep. *Journal of Dairy Science* **94**, 4657–4668.

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.

Association of Official Analytical Chemists, 1990: *Official Methods of Analysis*, 15th edn. AOAC Int., Arlington, VA, USA.

Badran, A. M.; Jones, D. E., 1965: Polyethylene glycols-tannin interaction in extracting enzyme. *Nature* **206**, 622–623.

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

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.

Barry, T. N.; Manley, T. R.; Duncun, S. J., 1986: The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. *British Journal of Nutrition* **55**, 123–137.

Bauman, D. E.; Lock, A. L., 2006: Conjugated linoleic acid: biosynthesis and nutritional significance. *Advanced Dairy Chemistry* **2**, 93–135.

Behgar, M.; Valizadeh, R.; Mirzaee, M.; Naserian, A. A.; Nasiri, M. R., 2009: Correlation between the physical and chemical properties of some forages and non forage fiber source. *Journal of Animal and Veterinary Advances* **8**, 2280–2285.

Ben Salem, H.; Nefzaoui, A.; Ben Salem, L.; Tisserand, L., 1999: Different means of administering polyethylene glycol to sheep: effect on the nutritive value of *Acacia cyanophylla* Lindl. foliage. *Animal Science* **68**, 809–818.

Benchaar, C.; Chouinard, P. Y., 2009: Assessment of the potential of cinnamaldehyde, condensed tannins, and saponins to modify milk fatty acid composition of dairy cows. *Journal of Dairy Science* **92**, 3392–3396.

Bohluli, A.; Naserian, A. A.; Valizadeh, R.; Eftekhar-Shahrodi, F., 2009: The effects of pistachio by-product on apparent digestibility, chewing activity and performance of early lactation Holstein cows diets. *Journal of Science and Technology and Agriculture Resource* **13**, 155–165.

Cabiddu, A.; Decandia, M.; Addis, M.; Spada, S.; Fiori, M.; Piredda, G.; Sitzia, M.; Fois, N.; Molle, G.,2005:Effect of the administration of polyethylene glycol (PEG) on the milk fatty acid composition of sheep grazing *Sulla* (*Hedysarum coronarium*). *The FAO-CIHEAM Sub-Network on Sheep and Goat Nutrition, Catania (Italy)*. 74, 11.

Cabiddu, A.; Molle, G.; Decandia, M.; Spada, S.; Fiori, M.; Piredda, G.; Addis, M., 2009: Responses to condensed tannins of flowering *Sulla* (*Hedysarum coronarium L.*) grazed by dairy sheep. Part 2: effects on milk fatty acid profile. *Livestock Science* **123**, 230–240.

Carulla, J. E.; Kreuzer, M.; Machmüller, A.; Hess, H. D., 2005: Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. *Australian Journal of Agricultural Research* **56**, 961–970.

Chilliard, Y.; Ferlay, A., 2004: Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reproduction Nutrition Development* **44**, 467–492.

Clark, R. W.; Ferris, A. M.; Fey, M.; Brown, P. B.; Hundricser, K. E.; Jensen, R. G., 1982: Changesin the lipids of human mil, from 2–16 weeks postpartum. *Journal of Pediatric Gastroenterology and Nutrition* **1**, 311–315.

Decandia, M.; Sitzia, M.; Cabiddu, A.; Kababya, D.; Molle, G., 2000: The use of polyethylene glycol to reduce the anti-nutritional effects of tannins in goats fed woody species. *Small Ruminant Research* **38**, 157–164.

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.; Villalb, J. J., 2012: Depression of rumen ammonia and blood urea by quebracho tannin-containing supplements fed after high-nitrogen diets with no evidence of self-regulation of tannin intake by sheep. *Small Ruminant Research* **105**, 126–134.

Forough-Ameri, N.; Shakeri, P., 2008: Pistachio residues as feedstuff, challenges and opportunities for development. *Proceeding of 3rd National Congress of Recycling and Reuse of Renewable Organic Resources in Agriculture*. IAU, Khorasgan, Isfahan, Iran. Waste and Waste water Research Center, 13–15 May.

Ghasemi, S.; Naserian, A. A.; Valizadeh, R.; Tahmasebi, A. M.; Vakili, A. R.; Behgar, M.; Ghovvati, S., 2012a: Using of 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 by-product 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.; Tahmasbi, A. M., 2010: Effect of feeding pistachio by-product on performance and blood metabolites in Holstein dairy cows. *International Journal of Agriculture and Biology* **12**, 867–870.

Gonzalez, C. L.; Everitt, J. H., 1982: Nutrient contents of major food plants eaten by cattle in the south Texas plains. *Journal of Range Management* **35**, 733–736.

Griinari, J. M.; Corl, B. A.; Lacy, S. H.; Chouinard, P. Y.; Nurmela, K. V. V.; Bauman, D. E., 2000: Conjugated lino-

leic acid is synthesized endogenously in lactating dairy cows by Δ^9- desaturase. *Journal of Nutrition* **130**, 2285–2291.

Hervas, G.; Frutos, P.; Javier Giraldez, F.; Mantecon, A. R.; Alvarez Del Pino, M. C., 2003: Effect of different doses of quebracho tannins extract on rumen fermentation in ewes. *Animal Feed Science and Technology* **19**, 65–78.

Khiaosa-Ard, R.; Bryner, S. F.; Scheeder, M. R. L.; Wettstein, H. R.; Leiber, F.; Kreuzer, M.; Soliva, C. R., 2009: Evidence for the inhibition of the terminal step of ruminal alpha-linolenic acid biohydrogenation by condensed tannins. *Journal of Dairy Science* **92**, 177–188.

Mahgoub, O.; Kadim, I. T.; Tageldin, M. H.; Al-Marzooqi, W.; Khalaf, S. Q.; AmbuAli, A., 2008: Pathological features in sheep fed rations containing phenols and condensed tannins. *Journal of Animal and Veterinary Advance* **7**, 1105–1109.

Makkar, H. P. S., 2000: Quantification of tannins in tree foliage. *In a laboratory manual for the FAO/IAEA coordinated research project on use of nuclear and related technique to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage*. Joint FAO/IAEA, FAO/IAEA of Nuclear Techniques in Food and Agriculture. Animal Production and Health Sub-program, FAO/IAEA Working Document IAEA, 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.

Makkar, H. P. S.; Blummel, M.; Becker, K., 1995: Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. *British Journal of Nutrition* **73**, 897–913.

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.

Palmquist, D. L.; Beaulieu, A. D., 1993: Feed and animal factors influencing milk fat composition. *Journal of Dairy Science* **76**, 1753–1771.

Palmquist, D. L.; Lock, A. L.; Shingfield, K. J.; Bauman, D. E., 2005: Biosynthesis of conjugated linoleic acid in ruminants and humans. *Advances in Food and Nutrition Research* **50**, 179–217.

Parodi, P. W., 2004: Milk fat in human nutrition. *Australian Journal of Dairy Technology* **59**, 3–59.

Precht, D.; Molkentin, J.; Destaillats, F.; Wolff, R. L., 2001: Comparative studies on individual isomeric 18:1 acids in cow, goat, and ewe milk fats by low-temperature high-resolution capillary gas-liquid chromatography. *Lipids* **36**, 827–832.

Puchala, R.; Min, B. R.; Goetsch, A. L.; Sahlu, T., 2005: The effect of a condensed tannin-containing forage on methane emission by goats. *Journal of Animal Science* **83**, 182–186.

Rezaeenia, A.; Naserian, A. A.; Valizadeh, R.; Tahmasbi, A., 2012: Effect of using different levels of pistachio by-products silage on composition and blood parameters of Holstein dairy cows. *African Journal of Biotechnology* **11**, 6192–6196.

Saarisalo, E. M.; Odenyo, A. A.; Osuji, P. O., 1999: Inoculation with adapted microbes versus addition of polyethylene glycol as methods to alleviate toxicity of *Acacia angustissima* leaves in sheep. *Journal of Agricultural Science* **133**, 445–454.

SAS, 2003: *SAS User's Guide Statistics*. Version 9.1 Edition. SAS Inst., Cary, NC.

Shakeri, P.; Fazaeli, H., 2007: Study on the use of different levels of pistachio by-product in diets of fattening lambs. *Iran Journal of Agriculture Science* **38**, 529–534.

Shakeri, P.; Riasi, A.; Alikhani, M.; Fazaeli, H.; Ghorbani, G. R., 2012: 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* **95**, 1–9.

Silanikove, N.; Gilboa, N.; Nir, I.; Perevolotsky, A.; Nitsan, Z., 1996: Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Quercus calliprinos, Pistacia lentiscus, caratonia siliqua*) by sheep. *Journal of Agricultural and Food Chemistry* **44**, 199–205.

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.

Tabosa, I. M.; Souza, J. C.; Graca, D. L.; Barbosa-Filho, J. M.; Almeida, R. N.; Rie-Correa, F., 2000: Neuronal vacuolation of the trigeminal nuclei in goats caused by ingestion of *Prosopis juliflora* pods (mesquite beans). *Veterinary and Human Toxicology* **42**, 155–158.

Toral, P. G.; Hervás, G.; Bichi, E.; Belenguer, A.; Frutos, P., 2011: Tannins as feed additives to modulate ruminal bio-hydrogenation: effects on animal performance, milk fatty acid composition and ruminal fermentation in dairy ewes fed a diet containing sunflower oil. *Animal Feed Science and Technology* **164**, 199–206.

Toral, P. G.; Hervás, G.; Belenguer, A.; Bichi, E.; Frutos, P., 2013: Effect of the inclusion of quebracho tannins in a diet rich in linoleic acid on milk fatty acid composition in dairy ewes. *Journal Dairy Science* **96**, 431–439.

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.

Vahmani, P.; Naserian, A. A.; Valizadea, R.; Nasiri Moghadam, H., 2006: Nutritive value of pistachio by-products and their effects on Holstein cows in mid-lactation. *Journal of Agricultural Science Technology* **20**, 201–210.

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.

Vasta, V.; Mele, M.; Serra, A.; Scerra, M.; Luciano, G.; Lanza, M.; Priolo, A., 2009a: Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. *Journal of Animal Science* **87**, 2674–2684.

Vasta, V.; Makkar, H. P. S.; Mele, M.; Priolo, A., 2009b: Ruminal biohydrogena-

tion as affected by tannins in vitro. *British Journal of Nutrition* **102**, 82–92.

Vasta, V.; Yanez-Ruiz, D. R.; Mele, M.; Serra, A.; Luciano, G.; Lanza, M.; Biondi, L.; Priolo, A., 2010: Bacterial and protozoal communities and fatty acid profile in the rumen of sheep fed a diet containing added tannins. *Applied and Environmental Microbiology* **76**, 2549–2555.

Waghorn, G. C.; Shelton, T. C., 1995: Effect of condensed tannin in Lotus pedunculatus on the nutritive value of ryegrass (*Lolium perenne*) fed to sheep. *Journal of Agricultural Science* **125**, 291–297.

Walker, G. P.; Dunshea, F. R.; Doyle, P. T., 2004: Effects of nutrition and management on the production and composition of milk fat and protein: a review. *Australian Journal of Agricultural Research* **55**, 1009–1028.

Wijngaarden, D., 1967: Modified rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical Chemistry* **39**, 848–849.