Journal of Animal Physiology and Animal Nutrition

DOI: 10.1111/jpn.12384

ORIGINAL ARTICLE

Effect of treating alfalfa silage with pistachio by-products extract on Saanen dairy goats performance and microbial nitrogen synthesis

A. Mokhtarpour[1,2], A. A. Naserian[2], F. Pourmollae[3] and M. H. Ghaffari[2]

1 Research Center of Special Domestic Animals, University of Zabol, Zabol, Iran
2 Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran, and
3 Agricultural Jihad Organization of Khorasan-Razavi, Mashhad, Iran

Summary

A lactation experiment was conducted to determine the influence of addition of pistachio by-products extract (PBE) to alfalfa silage (AS) on performance, rumen fermentation, milk yield and composition, and microbial nitrogen synthesis. Eight multiparous dairy goats (1.8 ± 0.25 kg of milk yield) were used in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments to compare two types of AS (supplemented with or without PBE) with two levels of dietary crude protein (14% vs. 16% CP). Dietary treatments were (i) AS with 14% CP of DM diet without PBE (14%CP−PBE), (ii) AS with 14% CP of DM diet with PBE (14% CP + PBE), (iii) AS with 16% CP of DM diet without PBE (16%CP−PBE) and (iv) AS with 16% CP of DM diet with PBE (16%CP + PBE). PBE was sprayed on fresh alfalfa at a ratio of 500 ml/kg alfalfa DM to get the final concentration of 1% tannin as tannic acid equivalent on DM basis. Intake of CP was greater ($p < 0.01$) in goats fed 16% CP diets than those fed 14% CP diets, regardless of PBE supplementation. Supplementation of PBE tended to decrease ($p = 0.09$) rumen NH_3-N concentration regardless of the level of CP in the diet. Supplementation of PBE tended ($p = 0.09$) to decrease total purine derivatives regardless of the level of CP in the diet with no significant change in microbial nitrogen supply. Efficiency of microbial nitrogen synthesis (EMNS) had a tendency ($p = 0.07$) to decrease in PBE supplemented diets. There was also a tendency ($p = 0.10$) for more EMNS in 14% CP fed goats than those fed 16% CP diets. Therefore, AS supplemented with PBE may lead to less concentration of ruminal NH_3-N because of decreased degradation of CP by rumen micro-organisms in response to pistachio by-products tannins.

Keywords pistachio by-products extract, purine derivatives, dairy goats

Correspondence A. Mokhtarpour, Research Center of Special Domestic Animals, University of Zabol, Zabol, Iran. Tel: +98-915-5052007; Fax: +98-54-32226765; E-mail: Am.Mokhtarpour@uoz.ac.ir

Received: 2 January 2015; accepted: 10 July 2015

Introduction

Ensiling is a common method of forage preservation on farms (Mohammadzadeh et al., 2012). Alfalfa silage (AS) is high in protein that is rapidly degraded in the rumen (Krizsan et al., 2007). It has been demonstrated that the nutritive value of AS is limited by rapid protein degradation to non-protein nitrogen (NPN) compounds during wilting and ensiling (Muck et al., 2003), which ranges from 43% to 87% of total crude protein (CP) (Muck, 1987; Nagel and Broderick, 1992). Non-protein nitrogen in AS typically is promptly hydrolysed to NH_3-N in the rumen (Givens and Rulquin, 2004), and the majority enters the blood rapidly across the rumen walls (Min et al., 2003). The ammonia in the blood is converted to urea in the liver and excreted in the urine (Min et al., 2003). Thus, excessive CP fermentation in the rumen of goats adapted to CP-poor plants can exceed the capacity of rumen bacteria to use it and can cause adverse effects ranging from depressed feed intake and animal performance to death from ammonia toxicity (urea poisoning) (Huntington et al., 2006).

There are several ways to decrease protein degradation in the rumen such as heat treating of feed (to alter protein conformations) or the addition of antimicrobial compounds such as ionophores to the diet (Flythe and Kagan, \2010). However, these methods may have some disadvantages; for example, ionophores in animal feeds have been banned because of

the appearance of their residues in animal products (Calsamiglia et al., 2007). Another way to decrease the rate and extent of protein degradation during ensiling and in the rumen is to decrease CP degradation, potentially through using tannins (McNabb et al., 1996), which are polyphenols that can bind proteins and prevent bacterial proteolysis (Tabacco et al., 2006).

Pistachio by-products (PB; sun-dried or as silage) have recently come to be used in diets for dairy cows (15% PB of DM) (Mokhtarpour et al., 2012), dairy goats (30–32% PB of DM) (Ghaffari et al., 2014b; Sedighi-Vesagh et al., 2015) and sheep (30% PB of DM) (Norouzian et al., 2011; Ghaffari et al., 2014a). Pistachio by-products contain 9.06–14.57% of DM total phenolics (TP) and 4.97–8.67% of DM total tannins (TT) depending on the extraction process, particle size of samples, extraction time and temperature (Mokhtarpour et al., 2014). Commonly, extracted plant secondary metabolites can be carried requires large volumes of costly solvents such as methanol, ethanol or acetone (Makkar, 2003). However, water has also been used as extraction solvent to decrease cost and make pistachio by-products extract (PBE) to be technically and economically feasible treatment for animal diets (Jolazadeh et al., 2015). Recently, Rajaei-Sharifabadi and Naserian (2014) reported that lambs fed a diet supplemented with PB extract (PBE; 0.91% tannin) had higher average daily gain than lambs fed a diet without PBE supplementation.

While PB is as a good source of plant polyphenols and its effect on digestibility, rumen fermentation and performance have been identified (Ghaffari et al., 2014a; Sedighi-Vesagh et al., 2015), there is limited information on protein degradation and dairy goat's performance of AS + PBE. This study, therefore, was conducted to evaluate the effects of PBE addition to AS on chemical composition, intake, digestibility, milk production and composition, and microbial nitrogen synthesis in dairy goats.

Materials and methods

Pistachio by-products extract

Sun-dried PB, which contained soft external hull, twinges, leaves and bony shells, were collected from pistachio de-hulling factory in Feizabad (Khorasan-e-Razavi Province, Iran). Pistachio by-products were ground to pass a 2-mm screen and then were soaked in water with a ratio of 1:5 (w/v) at room temperature for 12 h. Extraction was performed according to Mokhtarpour et al. (2014) with modifications. The contents were filtered through cheesecloth, and then, filtrated extract was immediately used for treating fresh alfalfa.

Alfalfa silage preparation

A third regrowth of alfalfa, cultivar (*Medicago sativa* L.), was harvested at early bloom (at approximately 250 g/kg DM), chopped to a theoretical length of 3 cm using a pool type chopper (Model 965; Claas, Omaha, NE, USA), and mixed before treating with PBE at the Research Farm of Ferdowsi University of Mashhad (Mashhad, Iran). The PBE in solution was prepared on the day of ensiling and applied at solution with a ratio of 500 ml/kg alfalfa DM to obtain the final concentration of 1% tannin as tannic acid equivalent on DM basis. The same amount of water was applied to another silo when no PBE were used. Pistachio by-products extract was sprayed in a fine mist on chopped forage uniformly and under constant of mechanical mixture before packing into silos and wilted for 20 h to approximately 27% DM. Two bunker silos were filled simultaneously; one with AS + PBE and the other with AS−PBE. Approximately 5000 kg of alfalfa were put in each silo; silo filling took 1 day. Silos were covered with plastic sheet and mud immediately after filling. Silos were 2.5 m wide, 5 m long and 1 m high. Drying conditions were good, and no rainfall was recorded during harvest.

Animals, experimental diets and management

This experiment was conducted at the Research Farm of the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. Guidelines for the care and use of animals were approved by the Animal Care Committee of the university. This experiment was conducted between October 2012 and January 2013. Eight multiparous Saanen dairy goats (45.2 ± 2.14 kg BW, 63 ± 4.7 days in milk) were used in a replicated 4×4 Latin square design with a 2×2 factorial arrangement of treatments to compare two types of AS (supplemented with or without PBE) with two levels of dietary CP (14% vs. 16% CP). Dietary treatments were (i) AS with 14% CP diet without PBE (14%CP−PBE), (ii) AS with 14% CP diet with PBE (14%CP + PBE), (iii) AS with 16% CP diet without PBE (16%CP−PBE) and (iv) AS with 16% CP diet with PBE (16%CP + PBE). Each experimental period lasted 21 day with the first 14 day used for diet adaptation and 7 day for sample collection.

Goats were housed in individual metabolism cages to facilitate separate collection of faeces and urine.

They were kept in a yard protected from rain and wind and had free access to water. Experimental diets were formulated based on the AFRC (1993) recommendations to meet the nutrient requirements of lactating goats using the Small Ruminant Nutrition System (SRNS; version 1.9.4468; Tedeschi et al., 2010). Diets were offered twice daily *ad libitum* at 8:00 and 16:00 hour. Chemical composition, phenolic compounds and CP fractions of AS and the ingredients and chemical composition of the diets are presented in Tables 1 and 2 respectively.

Sampling and data collection

Alfalfa silage was sampled weekly to determine DM content, and diets were adjusted for changes in DM concentration. Feed refusal of individual goat was collected before the morning feeding and weighed daily during the measurement period within each 5-day collection periods. Nutrient apparent digestibility was estimated by complete faecal collections within each 5-day collection periods.

Goats were milked twice daily at 7:00 and 15:00 hour, and individual milk yields were recorded at each milking. Milk was sampled on consecutive morning and afternoon milkings during the collection period, and fresh subsamples were analysed daily for chemical composition.

Rumen fluid samples were taken from animals by stomach tube with a vacuum pump 2 h after the morning feeding on days 18 and 19. Ruminal pH was measured immediately with a portable digital pH-meter (WinLab, Data Line pH-meter; Windaus Labortechnik, Clausthal-Zellerfeld, Germany). Ruminal content was strained through four layers of cheesecloth. A subsample of 5 ml was combined with 5 ml of 0.2 N HCl for NH_3-N analysis. Ruminal subsamples were frozen at −20 °C until analyses.

Table 1 Chemical composition, phenolic compounds and CP fractions of AS

Item	AS−PBE	AS + PBE	SEM	p-value
Chemical composition				
Dry matter (g/kg)	277	283	2.31	0.25
CP (g/kg DM)	178	175	1.18	0.14
NDF (g/kg DM)*	428	437	4.75	0.45
ADF (g/kg DM)*	315	293	7.74	0.21
Ash (g/kg DM)	99	100	3.19	0.64
Total phenols (g/kg DM)†	10.8	17.4	1.52	0.01
Total tannins (g/kg DM)†	4.8	12.4	1.74	0.01
Condensed tannins (g/kg DM)	nd	0.5	0.99	0.01
pH	5.44	4.68		
NH_3-N (mg/dl)	14.67	6.52	1.85	0.01
Protein fractions (g/kg CP)				
Soluble protein (A + B1)	496	448	14.20	0.02
A	467	423	10.82	0.07
B1	30	25	4.70	0.52
B2	372	425	13.58	0.03
B3	82	82	5.84	0.96
C	49	45	2.60	0.56

AS−PBE, alfalfa silage without pistachio by-products extract; AS + PBE, alfalfa silage with pistachio by-products extract, A; non-protein nitrogen; B1, B2 and B3, true protein based on decreasing solubility, C; acid detergent insoluble nitrogen; ADF, acid detergent fibre; CP, crude protein; NDF, neutral detergent fibre.

*NDF and ADF were determined without sodium sulphite and amylase treatment, and expressed inclusive of residual ash (Van Soest et al., 1991).

†Expressed as grams of tannic acid equivalent per kg of dry matter.

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

	Treatments*			
	14%CP		16%CP	
Item	−PBE	+PBE	−PBE	+PBE
Diet ingredients (g/kg of DM)				
AS	500	500	500	500
Barley grain	260	260	260	260
Cotton seed meal	–	–	85	85
Wheat bran	230	230	145	145
Limestone	3.5	3.5	3.5	3.5
Salt	1.5	1.5	1.5	1.5
Vitamin–mineral mix†	5.0	5.0	5.0	5.0
Chemical composition				
Dry matter (%)	41.8	42.8	41.2	42.5
Organic matter (g/kg of DM)	918	913	914	916
CP (g/kg of DM)	142	142	162	161
ADF (g/kg of DM)‡	240	243	229	224
NDF (g/kg of DM)‡	413	409	394	393
Non-fibre carbohydrate (g/kg of DM)§	332	330	327	332
Ether extract (g/kg of DM)	32	32	31	31
Total phenols (g/kg of DM)¶	6.8	13.7	6.9	13.9
Total tannins (g/kg of DM)	2.9	6.7	2.4	6.3

ADF, acid detergent fibre; AS, alfalfa silage; CP, crude protein; NDF, neutral detergent fibre; PBE, pistachio by-products extract.

*Treatments: 14%CP−PBE = AS with 14% CP diet without pistachio by-products extract; 14%CP + PBE = AS with 14% CP diet with pistachio by-products extract; 16%CP−PBE = AS with 16% CP diet without pistachio by-products extract; 16%CP + PBE = AS with 16% CP diet with pistachio by-products extract.

†Contained (g/kg premix; DM 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, 45 mg I.

‡NDF and ADF were determined without sodium sulphite and amylase treatment, and expressed inclusive of residual ash (Van Soest et al., 1991).

§Non-fibre carbohydrates calculated as 100 − (NDF + CP + ether extract + ash) (NRC, 2001).

¶Expressed as grams of tannic acid equivalent per 1000 g of dry matter.

Urine from each goat was collected daily in plastic vessels containing 100 ml 4 N H_2SO_4 to maintain the pH level below three to prevent bacterial destruction of total purine derivatives (TPD) (Chen and Gomes, 1995). Urine was weighed once a day and mixed well, and a 0.1-daily aliquot was pooled over the 5-day collection period per animal and stored at −20 °C for later TPD (i.e. allantoin, uric acid, xanthine hypoxanthine) analyses and N content.

Chemical analyses

Daily samples of total mixed ration, feed refusals, faeces and silages were mixed thoroughly and ground to pass a 1-mm screen in a Wiley mill (standard model 4; Arthur H. Thomas, Philadelphia, PA, USA) before chemical analyses. Dry matter concentration of samples was determined by oven drying at 100 °C to a constant weight (official method 934.01; AOAC, 2005). Organic matter content was determined by ashing (2 h at 600 °C, official method 942.05; AOAC, 2005). Crude protein (Kjeldahl N × 6.25) was determined by the block digestion method using copper catalyst and steam distillation into boric acid (method 2001.11) on behr S5 distillation unit (Behr Labor-Technik GmbH, Düsseldorf, Germany) according to AOAC (2005). Concentrations of acid detergent fibre (ADF) (official method 973.18; AOAC, 2005) and neutral detergent fibre (NDF) inclusive of residual ash were determined sequentially without the use of sodium sulphite and α-amylase (Van Soest et al., 1991). The NDF and ADF components were further processed for their nitrogen content (neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) Licitra et al., 1996).

The fractionation of AS protein was performed according to Cornell Net Carbohydrates and Protein System (CNCPS) (Licitra et al., 1996), where the protein was subdivided into five fractions (A, B_1, B_2, B_3 and C) according to their solubility (Sniffen et al., 1992). The soluble protein (NPN + B_1) was obtained by the difference between CP and residual protein insoluble in borate phosphate buffer (Sniffen et al., 1992). Fraction A is equal to NPN × 6.25; fraction B_1 was obtained by the difference between the soluble protein and A; fraction B_2 was determined by the difference (CP-A-B_1-B_3-C); fraction B_3 resulted from the difference between the neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) (Sniffen et al., 1992). Fraction C was determined as ADICP (Van Soest et al., 1991). The NDICP and ADICP were obtained by multiplying NIDN and ADIN by 6.25.

To minimize changes in tannin content and activity, samples of diet, feed refusals and silages were dried in an oven at 40 °C to constant weight (Makkar, 2000). For quantification of tannins in PBE, the crude extract was first freeze-dried. Dried samples were ground to pass a 2-mm sieve and then 0.5-mm sieve. For TP measurement, 200 mg of sample was extracted in 10 ml of 70% aqueous acetone (v/v) with four replicates using an ultrasonic bath for 20 min. After centrifugation (3000 *g*, 4 °C, 10 min) the supernatant was collected and kept in refrigerator (4 °C). Non-tannin phenolic (NTP) was determined following absorption of tannins in TP extract to insoluble polyvinylpyrrolidone. Total phenolics and NTP were determined by Folin–Ciocalteu reagent using tannic acid (Merck GmbH, Darmstadt, Germany) as a standard and results were expressed as tannic acid equivalent. Total tannins were calculated as the difference between TP and NTP (Makkar, 2000). Condensed tannins (CT) concentration was measured on supernatant using the butanol-HCl reagent (Makkar, 2000). The values of CT were expressed as leucocyanidin equivalent (Makkar, 2000).

Rumen NH_3-N concentration was determined using the distillation using behr S5 Steam distillation unit (Model: D-40599; Behr Labor-Technik GmbH).

Milk samples were analysed for fat, CP, lactose, solids-non-fat and total solid content by Milk-O-Scan 605 analyser (Foss Electric, Hillerød, Denmark, AOAC International, 2005).

Microbial nitrogen supply was estimated on urinary excretion of PD excretion based on the relationship derived by Chen and Gomes (1995). Urinary PD were estimated by spectrophotometric method (Chen and Gomes, 1995). Briefly, allantoin was measured in urine by a colorimetric method at 522 nm after conversion of allantoin to phenyl hydrazone. The uric acid was measured from the reduction in optical density at 293 nm following conversion of uric acid to allantoin with uricase (Product No. U-9375; Sigma, Deisenhofen, Germany). The sum of xanthine and hypoxanthine were assayed by their conversion to uric acid with xanthine oxidase (Catalog No. X-1875, 5 Units; Sigma) with subsequent optical density at 293 nm. The urinary PD excreted in a day was used in the iteration process to calculate the microbial nitrogen supply as described (Chen and Gomes, 1995). Efficiency of microbial nitrogen synthesis (EMNS) was calculated as: EMNS = MN (g/day)/DOMR; where: DOMR = apparently digested OM in the rumen (assuming that rumen digestion was 650 g/kg OM of digestion in total tract, DOMR = DOMI × 0.65; DOMI = digestible organic matter intake according to ARC, 1984).

Statistical analysis

All results were analysed using the MIXED procedure of SAS (SAS Institute, 2003). The model was for a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments as a following:

$$Y_{ijklm} = \mu + A_i + B_j + P_k + S_l + C_m(S_l) + AB_{ij} + E_{ijklm}$$

where Y_{ijklm} = observation; μ = overall mean; A_i = fixed effect of silage type; B_j = fixed effect of CP level; P_k = fixed effect of period; S_l = random effect of square; C_m = random effect of animal in square; AB_{ij} = effect of silage type × CP level interaction; E_{ijklm} = residual error with mean 0 and variance σ^2. When a significant *F*-test was detected for means and interactions, comparisons were made using least square means (LSMEANS) for the probability. Significance was declared at $p \leq 0.05$ and trends were considered when $0.05 < p < 0.10$. All results are reported as least squares means.

Results and discussion

Chemical composition of AS

Alfalfa silage was used as the main forage in the diets. Concentrations of CP, NDF, ADF and ash were similar ($p > 0.05$) between the AS−PBE and the AS + PBE diet. The concentrations of TP (10.8 vs. 17.4 g/kg DM on average) and TT (4.8 vs. 12.4 g/kg DM on average) were higher ($p < 0.01$) in the AS + PBE diet compared with the AS−PBE diet, whereas CT concentration was not detected in the AS−PBE diet (Table 1). Supplementing AS with PBE decreased ($p < 0.01$) the pH values and NH_3-N concentration.

Reduction in pH value of AS + PBE may be due to the production of organic acids through the fermentation of water-soluble carbohydrate in PBE (McDonald et al., 2002). The content of NPN either in AS + PBE or AS−PBE was lower than those reported in the previous study (Albrecht and Muck, 1991; Tabacco et al., 2006; Guo et al., 2008), which might be due to chop length and moisture (Kung, 2010), rapid silo filling, good packing and sealing (Muck et al., 2003). The reduction in soluble protein and NH_3-N concentration and also a decreasing trend for NPN content in AS + PBE can be due to the action of tannin to bind proteins or free amino acids and preventing them from enzyme hydrolysis and plant proteolytic enzymes (Guo et al., 2007). Similarly, Guo et al. (2008) reported that treating alfalfa silage with tannic acid significantly decreased NPN and NH_3-N concentrations. Treatment with silage additives such as tannin can effectively reduce NPN formation during ensiling (Salawu et al., 1999), and a strong negative correlation between tannin concentration and soluble N content in legume silage was reported by Albrecht and Muck (1991).

Pistachio by-product extract supplementation decreased soluble crude protein (SCP), but increased ($p < 0.05$) fraction B2 and tended ($p = 0.07$) to decrease fraction A compared to AS−PBE. No significant changes ($p > 0.05$) were observed in fractions B1 and B3 due to supplementing of PBE (Table 1). We expected that the insoluble C fraction would increase due to tannins binding proteins to plant cell walls; however, no notable change ($p > 0.05$) occurred for fraction C. The overall absence of a relationship with fraction C suggests that concentration of TT (12.4 g/kg of DM) in AS + PBE is not likely to increase concentrations of indigestible protein. In the study Dehghan-Banadaky et al. (2013), PBE supplementation (containing 11% Tannin in DM of extract), decreased SCP of canola meal without significant effect on fraction B2 and increased B3 fraction compared with control.

Feed intake and apparent digestibility

Dry matter intake and total apparent digestibility of nutrients were not affected ($p > 0.05$) by treatments; however, supplementation of PBE at 14% CP level increased DMI by 10% numerically (Table 3). Most previous studies reported no effect on DMI by feeding PB (Norouzian et al., 2011; Mokhtarpour et al., 2012; Shakeri et al., 2013; Sedighi-Vesagh et al., 2015). Similar findings were noted by Gholizadeh et al. (2010) and Mokhtarpour et al. (2012), where the inclusion of PB had no significant effects on DMI and nutrient digestion in dairy cows. Carulla et al. (2005) reported that supplementing sheep diet with CT at 25 g/kg DM from *Acacia mearnsii* extract (black wattle tree) increased DMI. Deaville et al. (2010) reported that DMI in sheep fed grass silage treated with chestnut tannin (74.3 g/kg DM; hydrolysable tannin) was higher than control silage. However, Ghaffari et al. (2014b) reported that inclusion of PB at 300 g/kg of DM or 22.9 g TT/day (1.81 g of TT expressed as gram of tannic acid equivalent/kg of DM) in the diet of dairy goats decreased DMI. 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 (Waghorn et al., 1994; Aerts et al., 1999). In agreement with our findings, a lack of effect of supplementing PB at 100 g/kg DM (TP; 9.6 g tannic acid equivalent/100 g of DM) in

Table 3 Nutrient intake and total apparent digestibility of lactating goats fed AS with 14% CP or 16% CP diets without or with PBE supplementation

	Treatments*							
	14%CP		16%CP			Significance of effect		
Item	−PBE	+PBE	−PBE	+PBE	SEM	ST	CPL	ST × CPL
Intake (kg/day)								
Dry matter	1.78	1.96	1.91	1.98	0.07	0.12	0.30	0.47
Organic matter	1.64	1.78	1.74	1.82	0.06	0.25	0.30	0.59
CP	0.25	0.27	0.31	0.32	0.02	0.28	0.01	0.34
Apparent digestibility (g/kg of DM)								
Dry matter	695	683	696	706	19.2	0.77	0.42	0.44
Organic matter	712	697	713	723	20.2	0.83	0.48	0.53
CP	701	708	720	726	14.1	0.55	0.24	0.72
Neutral detergent fibre	635	614	644	633	12.2	0.19	0.21	0.47

ST, silage type effect; CPL, crude protein level; ST × CPL, interaction between silage type and crude protein level; AS, alfalfa silage; PBE, pistachio by-products extract; CP, crude protein.
*Treatments: 14%CP−PBE = AS with 14% CP diet without pistachio by-products extract; 14% CP + PBE = AS with 14% CP diet with pistachio by-products extract; 16%CP−PBE = AS with 16% CP diet without pistachio by-products extract; 16%CP + PBE = AS with 16% CP diet with pistachio by-products extract.

the diets of dairy cows on digestibility of NDF was reported by Gholizadeh et al. (2010). Likewise, Dschaak et al. (2011) observed no effects on digestibility of DM, OM, CP and ADF with the addition of quebracho condensed tannin extract (30 g/kg of DM) in the diets of dairy cows. The result in our study is inconsistent with those of Ghasemi et al. (2012) who observed that supplementing PB at 500 g/kg DM (TP; 4.25 g tannic acid equivalent/100 g of DM) in the diets of sheep increased CP digestibility. In the current experiment, similar DMI and apparent digestibility of nutrients among treatments could be due to the low tannin concentration in diets. Discrepancies between studies could partly be due to differences in the amount, chemical structure or reactivity of tannins present in diets and with the source of the plant used.

Milk yield and composition

Milk production was not affected ($p > 0.05$) by PBE supplementation in the diets (Table 4) which is consistent with results obtained by Liu et al. (2013) who reported that addition of 10 g/kg of DM chestnut tannin to the transition dairy cow's diet had no effect on milk production. Similarly, Dschaak et al. (2011) observed no difference in the milk yield of lactating dairy cows due to diet supplementation with 30 g/kg of DM of quebracho tannins extract. In the current study, however, PBE inclusion had no adverse effects on intake and nutrient digestibility, as also reflected in milk production. Regardless of the level of CP in the diet, supplementation of PBE increased ($p < 0.05$) the yield and concentrations of milk fat, and total solids concentrations (Table 4). Moreover, PBE supplementation in dairy goats increased ($p < 0.05$) the yield of milk protein and tended ($p = 0.10$) to increase milk protein concentration, regardless of the level of CP in the diet (Table 4).

Concentrations of milk lactose and solid not fat were not affected by treatments ($p > 0.05$; Table 4). Increased milk protein concentration together with the increase in milk fat concentration resulted in more milk solids in goats fed AS + PBE diets compared with those fed AS−PBE diets (11.43 vs. 12.10; Table 4).

The greater milk protein concentration and yield in goats supplemented with PBE in the current study may partly be associated with the action of PB tannins (6.5 g/kg of DM) in by-passing proteins and an increase in the flow of protein (Table 5) to the intestine, benefiting the goats by increasing the amount of amino acids available for absorption (Min et al., 2003). Conversely, Sedighi-Vesagh et al. (2015) reported that inclusion of 320 g of PB/kg of dietary DM in the diet of dairy goats altered neither milk production nor milk composition. Aguerre et al. (2010) reported that milk protein concentration in dairy cows increased with supplementation of tannin extract (4.5 g/kg of DM), whereas supplementing tannin extract (9 g/kg of DM) had no effect on milk protein content, but at 18 g/kg of DM decreased milk protein concentration. In the current study, addition of PBE to AS resulted in higher milk fat yield and concentration compared with goats fed AS−PBE, which is inconsistent with previous studies (Ghaffari et al., 2014b; Sedighi-Vesagh et al., 2015). However, the specific effects of tannins from PBE on milk fat remain

Table 4 Milk yield and composition, and ruminal fermentation characteristics of lactating goats fed AS with 14% CP or 16% CP diets without or with PBE supplementation

	Treatments*					Significance of effect		
	14%CP		16%CP					
Item	−PBE	+PBE	−PBE	+PBE	SEM	ST	CPL	ST × CPL
Milk production (kg/day)	1.64	1.63	1.69	1.68	0.05	0.72	0.11	0.99
Milk composition (%)								
Fat	3.56	3.86	3.37	3.84	0.29	0.01	0.24	0.34
Protein	3.10	3.28	3.01	3.26	0.13	0.10	0.47	0.68
Lactose	4.25	4.10	4.16	4.17	0.09	0.43	0.93	0.38
Solids-non-fat	8.07	8.19	7.92	8.24	0.16	0.11	0.54	0.29
Total solids	11.60	12.09	11.26	12.11	0.45	0.01	0.17	0.11
Milk yield (g/day)								
Fat	57.80	63.50	56.61	64.82	4.49	0.01	0.96	0.38
Protein	50.14	53.81	50.33	55.14	2.27	0.04	0.49	0.60
Lactose	69.53	66.91	70.28	70.29	1.95	0.53	0.33	0.52
Ruminal fermentation characteristics								
pH	6.38	6.43	6.61	6.57	0.10	0.93	0.09	0.48
NH_3-N (mg/dl)	32.4	27.8	32.2	27.3	1.97	0.09	0.88	0.95

ST, silage type effect; CPL, crude protein level; ST × CPL, interaction between silage type and crude protein level; AS, alfalfa silage; CP, crude protein; PBE, pistachio by-products extract.
*Treatments: 14%CP−PBE = AS with 14% CP diet without pistachio by-products extract, 14% CP + PBE = AS with 14% CP diet with pistachio by-products extract, 16%CP−PBE = AS with 16% CP diet without pistachio by-products extract; 16%CP + PBE = AS with 16% CP diet with pistachio by-products extract.

Table 5 TPD and microbial nitrogen synthesis of lactating goats fed AS with 14% CP or 16% CP diets without or with PBE supplementation

	Treatments*					Significance of effect		
	14%CP		16%CP					
Item	−PBE	+PBE	−PBE	+PBE	SEM	ST	CPL	ST × CPL
TPD (mmol/day)	38.70	37.97	38.46	35.65	0.55	0.09	0.15	0.20
Microbial nitrogen supply (g/day)	28.14	27.61	27.96	25.92	0.51	0.13	0.21	0.28
EMNS (g N/kg DOMR)†	37.07	33.93	34.67	30.30	0.98	0.07	0.10	0.62

ST, silage type effect; CPL, crude protein level; ST × CPL, interaction between silage type and crude protein level; AS, alfalfa silage; CP, crude protein; PBE, pistachio by-products extract; TPD, total purine derivatives.
*Treatments: 14%CP−PBE = AS with 14% CP diet without pistachio by-products extract; 14% CP + PBE, AS with 14% CP diet with pistachio by-products extract; 16%CP−PBE, AS with 16% CP diet without pistachio by-products extract; 16%CP + PBE, AS with 16% CP diet with pistachio by-products extract.
†EMNS, efficiency of microbial nitrogen synthesis; DOMR, apparently digested organic matter in the rumen (65% of apparently digested organic matter in total tract) according to ARC (1984).

unclear. A tendency to produce more milk fat concentration in cows fed PB silage (7.7 g tannin/kg of DM) compared with those on corn silage was reported by Mokhtarpour et al. (2012).

In agreement with our findings, Sahlu et al. (1993) reported that milk production and composition of dairy goats were not affected by dietary CP level (13% or 17% CP of DM). Crude protein intake reflected dietary protein percentage and goats fed 16% CP diets had higher CP intake than those fed 14% CP diets. The lack of response to the higher CP concentration indicates that 14% CP diet completely provide the protein requirements for this level of milk production.

Ruminal fermentation parameters

Ruminal pH was not affected ($p > 0.05$) by PBE supplementation, but it tended ($p = 0.09$) to be lower pH in 14% CP of DM diet compared with 16% CP of DM diet (Table 4). This is in line with previous findings; using PB in the diets had no effect on ruminal pH (Gholizadeh et al., 2010; Ghaffari et al., 2014a). Sahlu

et al. (1993) reported that ruminal pH was similar between goats fed diets with different CP levels (13% vs. 17% CP of DM). A trend ($p = 0.09$) for lower ruminal NH_3-N in PBE supplemented goats may be attributed to the lower concentration of NH_3-N in AS−PBE and lower degradation of diet protein in the rumen through tannin–protein complexes. Aguerre et al. (2010) observed that addition of tannin extract at 18 g/kg of DM significantly decreased ruminal NH_3-N in dairy cows. Chestnut and mimosa tannin supplementation at 14.9 g/kg of DM had no effect on ruminal pH and NH_3-N concentration of steers fed high grain diets (Krueger et al., 2010). They stated that the lack of responses can be due to the time (prior to morning feeding) and method (stomach tube) of rumen fluid sampling.

Flythe and Kagan (2010) demonstrated that soluble phenolic compounds such as isoflavones from red clover have antimicrobial activity against hyper-ammonia-producing bacteria which is responsible for deamination of amino acids in the rumen. As PB is rich in phenolic compounds such as isoflavones (Tomaino et al., 2010), lower ruminal NH_3-N in goats fed AS + PBE may be due to the effect of these plant secondary metabolites along with the effect of tannins.

Microbial nitrogen synthesis

Pistachio by-product extract supplementation tended to decrease ($p = 0.09$) TPD, regardless of the level of CP in the diet (Table 5). No significant change ($p > 0.05$) was observed in microbial N supply by treatments (Table 5). Efficiency of microbial nitrogen (g/kg DOMR) had a tendency ($p = 0.07$) to decrease in PBE supplemented diets (Table 5). There was also a tendency ($p = 0.10$) for more EMNS in 14% CP fed goats than those fed 16% CP diets (Table 5). Ghasemi et al. (2012) reported that substitution of PB at 250 g/kg of DM (19.2 g dietary tannin/kg DM) with alfalfa hay in sheep ration decreased PD excretion, but had no effect on microbial nitrogen supply and efficiency of microbial protein. However, they observed a significant decrease in PD excretion, microbial nitrogen supply and efficiency of microbial protein at 500 g/kg of DM PB (30.7 g dietary tannin/kg of DM). Zimmer and Cordesse (1996) found no effect on microbial nitrogen supply by addition of chestnut tannin at a rate of 100 g/kg of DM in sheep and goats. Yanez-Ruiz et al. (2004) also, found no difference in urinary excretion of PD in a comparative study with sheep and goats fed a diet containing two-stage olive cake (condensed tannin).

The reduction in soluble CP and NH_3-N in AS + PBE and also reduction in ruminal NH_3-N due to already formed tannin–protein complexes in AS + PBE may explain the decrease of PD excretion. However, other factors such as free phenolic compounds or free tannins may affect rumen microbial activity or interfere with their enzyme secretion or both (McSweeney et al., 2001). On the other hand, different methods for tannins quantification, nature of tannins and the ability of ruminal micro-organisms to perform efficiently in the presence of tannins may explain these effects (Yanez-Ruiz et al., 2004).

Conclusion

The results of current study revealed that PB extract decreased soluble CP and NH_3-N concentration during ensiling besides decreasing ruminal NH_3-N, dry matter, OM and CP intake and their digestibilities were not affected by dietary treatments. Inclusion of AS + PBE increased milk fat and protein concentrations without any effect on milk production. However, due to a decreasing trend in PD and EMNS, the inclusion of PB in ruminant ration should be used with caution. Further research is required to determine the optimal levels of PB tannin for properly formulating rations for dairy goat to evaluate methods of applying PBE prior to ensiling in the field.

Acknowledgements

The authors would like to express their appreciation to Ferdowsi University of Mashhad (Mashhad, Iran) for financial support of this study. We would like to thank Paul T. D. Peake, for his insightful comments on this manuscript.

References

Aerts, R. J.; McNabb, W. C.; Molan, A.; Brand, A.; Barry, T. N.; Peters, J. S., 1999: Condensed tannins from *Lotus corniculatus* and *Lotus pedunculatusexert* different effects on the *in vitro* rumen degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) protein. *Journal of the Science of Food and Agriculture* **79**, 79–85.

Agricultural and Food Research Council (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.

Agricultural Research Council (ARC), 1984: *The Nutrient Requirement of Ruminant Livestock (No.1)*. Commonw. Agric. Bureaux, Slough, UK.

Aguerre, M. J.; Wattiaux, M. A.; Capozzolo, M. C.; Lencioni, P.; Cabral, C., 2010: Effect of quebracho-chestnut tannin extracts at two dietary crude protein levels on performance and rumen fermentation of dairy cows. *Journal of Dairy Science* **93**(Suppl. 1), 445.

Albrecht, K. A.; Muck, R. E., 1991: Proteolysis in ensiled forage legumes that vary in tannin concentration. *Crop Science* **31**, 464–469.

Association of Official Analytical Chemists (AOAC), 2005: *Official Methods of Analysis*, 18th edn. Published by AOAC international, Gaithersburg, MA, USA.

Calsamiglia, S.; Busquet, M.; Cardozo, P. W.; Castillejos, L.; Ferret, A., 2007: Essential oils as modifiers of rumen microbial fermentation. *Journal of Dairy Science* **90**, 2580–2595.

Carulla, J. E.; Kreuzer, M.; Machmuller, 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.

Chen, X. B.; Gomes, M. J., 1995: *Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives-An Overview of the Technical Details*. International Feed Resources Unit, Rowett Research Institute, Bucksburn, Aberdeen, UK.

Deaville, E. R.; Givens, D. I.; Mueller-Harvey, I., 2010: Chestnut and mimosa tannin silages: effects in sheep differ for apparent digestibility, nitrogen utilisation and losses. *Animal Feed Science and Technology* **157**, 129–138.

Dehghan-Banadaky, M.; Jolazadeh, A. R.; Vahdani, N., 2013: Effects of canola meal treatment with different levels of tannins extracted from pistachio hulls on the N fractions by the Cornell Net Carbohydrate and Protein System (CNCPS). *Journal of Animal Science* **91**(E-Suppl. 2)/Journal of Dairy Science 96, E-Suppl. 1. 526.

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.

Flythe, M.; Kagan, I., 2010: Antimicrobial effect of red clover (*Trifolium pratense*) phenolic extract on the ruminal hyper ammonia-producing bacterium, *Clostridium sticklandii*. *Current Microbiology* **61**, 125–131.

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., 2012: 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.

Givens, D. I.; Rulquin, H., 2004: Utilisation by ruminants of nitrogen compounds in silage-based diets. *Animal Feed Science and Technology* **114**, 1–18.

Guo, X.; Zhou, H.; Yu, Z.; Zhang, Y., 2007: Changes in the distribution of nitrogen and plant enzymatic activity during ensilage of lucerne treated with different additives. *Grass and Forage Science* **62**, 35–43.

Guo, X. S.; Ding, W. R.; Han, J. G.; Zhou, H., 2008: Characterization of protein fractions and amino acids in ensiled alfalfa treated with different chemical additives. *Animal Feed Science and Technology* **142**, 89–98.

Huntington, G. B.; Harmon, D. L.; Kristensen, N. B.; Hanson, K. C.; Spears, J. W., 2006: Effects of a slow-release urea source on absorption of ammonia and endogenous production of urea by cattle. *Animal Feed Science and Technology* **130**, 225–241.

Jolazadeh, A. R.; Dehghan-banadaky, M.; Rezayazdi, K., 2015: Effects of soybean meal treated with tannins extracted from pistachio hulls on performance, ruminal fermentation, blood metabolites and nutrient digestion of Holstein bulls. *Animal Feed Science and Technology* **203**, 33–40.

Krizsan, S. J.; Broderick, G. A.; Muck, R. E.; Promkot, C.; Colombini, S.; Randby, A. T., 2007: Effect of alfalfa silage storage structure and roasting corn on production and ruminal metabolism of lactating dairy cows. *Journal of Dairy Science* **90**, 4793–4804.

Krueger, W. K.; Gutierrez-Banuelos, H.; Carstens, G. E.; Min, B. R.; Pinchak, W. E.; Gomez, R. R.; Anderson, R. C.; Krueger, N. A.; Forbes, T. D. A., 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.

Kung, L. Jr, 2010: *A Review on Silage Additives and Enzymes*. Department of Animal and Food Sciences University of Delaware, Newark, DE.

Licitra, G.; Hernandez, T. M.; Van Soest, P. J., 1996: Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* **57**, 347–358.

Liu, H. W.; Zhou, D. W.; Li, K., 2013: Effects of chestnut tannins on performance and antioxidative status of transition dairy cows. *Journal of Dairy Science* **96**, 5901–5907.

Makkar H. P. S. (Ed.), 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 tanninrich feeds. *Small Ruminant Research* **49**, 241–256.

McDonald, P.; Edwards, R. A.; Greenhalgh, J. F. D.; Morgan, C. A., 2002: *Animal Nutrition*, 6th edn. Longman, London, UK, pp. 451–464.

McNabb, W. C.; Waghorn, G. C.; Peters, J. S.; Barry, T. N., 1996: The effect of condensed tannins in *Lotus pedunculatuson* the solubilization and degradation of ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39; Rubisco) protein in the rumen and the sites of Rubisco digestion. *British Journal of Nutrition* **76**, 535–549.

McSweeney, C. S.; Palmer, B.; McNeill, D. M.; Krause, D. O., 2001: Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology* **91**, 83–93.

Min, B. R.; Barry, T. N.; Attwood, G. T.; McNabb, W. C., 2003: The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages. *Animal Feed Science and Technology* **106**, 3–19.

Mohammadzadeha, H.; Khorvasha, M.; Ghorbania, G. R.; Yang, W. Z., 2012: Frosted corn silage with or without bacterial inoculants in dairy cattle ration. *Livestock Science* **145**, 153–159.

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.

Mokhtarpour, A.; Naserian, A. A.; Valizadeh, R.; Danesh-Mesgaran, M.; Pourmollae, F., 2014: Extraction of phenolic compounds and tannins from pistachio by-products. *Annual Research & Review in Biology* **4**, 1330–1338.

Muck, R. E., 1987: Dry matter levels on alfalfa silage quality. I. Nitrogen transformation. *Transactions American Society of Agricultural Engineer* **30**, 7–14.

Muck, R. E.; Moser, L. E.; Pitt, R. E., 2003: *Postharvest Factors Affecting Ensiling*. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison, WI, pp. 251–304.

Nagel, S. A.; Broderick, G. A., 1992: Effect of formic acid or formaldehyde treatment of alfalfa silage on nutrient utilization by dairy cows. *Journal of Dairy Science* **75**, 140–154.

National Research Council (NRC), 2001: *Nutrient Requirements of Dairy Cattle*, 7th rev. ed. National Academic Press, Washington, DC.

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.

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.

Rajaei-Sharifabadi, H.; Naserian, A. A., 2014: Growth performance and nitrogen retention in lambs fed diets containing two different levels of crude protein supplemented with pistachio by-product extract as a source of tannins. *Research Opinions in Animal & Veterinary Sciences* **4**, 273–280.

Sahlu, T.; Fernandez, J. M.; Jia, Z. H.; Akinsoyinu, A. O.; Hart, S. P.; Teh, T. H., 1993: Effect of source and amount of protein on milk production in dairy goats. *Journal of Dairy Science* **76**, 2701–2710.

Salawu, M. B.; Acamovic, T.; Stewart, C. S.; Hvelplund, T.; Weisbjerg, M. R., 1999: The use of tannins as silage additives: effects on silage composition and mobile bag disappearance of dry matter and protein. *Animal Feed Science and Technology* **82**, 243–259.

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

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* **99**, 777–787.

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.

Sniffen, C. J.; O'Connor, J. D.; Van Soest, P. J.; Fox, D. G.; Russell, J. B., 1992: A net carbohydrate and protein system for evaluating cattle diets. II: carbohydrate and protein availability. *Journal of Animal Science* **70**, 3562–3577.

Tabacco, E.; Borreani, G.; Crovetto, G. M.; Galassi, G.; Colombo, D.; Cavallarin, L., 2006: Effect of chestnut tannin on fermentation quality, proteolysis, and protein rumen degradability of alfalfa silage. *Journal of Dairy Science* **89**, 4736–4746.

Tedeschi, L. O.; Cannas, A.; Fox, D. G., 2010: A nutrition mathematical model to account for dietary supply and requirements of energy and other nutrients for domesticated small ruminants: the development and evaluation of the small ruminant nutrition system. *Small Ruminant Research* **89**, 174–184.

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.

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 pedunculatus on its nutritive value for sheep. 1. Non-nitrogenous aspects. *Journal of Agricultural Science* **123**, 99–107.

Yanez-Ruiz, D. R.; Moumen, A.; Martın Garcıa, A. I.; Molina Alcaide, E., 2004: Ruminal fermentation and degradation patterns, protozoa population, and urinary purine derivatives excretion in goats and wethers fed diets based on two-stage olive cake: effect of PEG supply. *Journal of Animal Science* **82**, 2023–2032.

Zimmer, N.; Cordesse, R., 1996: Digestibility and ruminal digestion of non-nitrogenous compounds in adult sheep and goats: effects of chestnut tannins. *Animal Feed Science and Technology* **61**, 259–273.