**CSIRO** PUBLISHING

*Animal Production Science*, 2012, **52**, 1052–1057
http://dx.doi.org/10.1071/AN11287

# Effects of pistachio by-product in replacement of lucerne hay on microbial protein synthesis and fermentative parameters in the rumen of sheep

*S. Ghasemi*[A,C], *A. A. Naserian*[A], *R. Valizadeh*[A], *A. M. Tahmasebi*[A], *A. R. Vakili*[A] *and M. Behgar*[B]

[A]Faculty of Agriculture, Excellence Center in Animal Science, Ferdowsi University of Mashhad, PO Box 91775-1163, Mashhad, Iran.
[B]Agricultural, Medical and Industrial Research School, PO Box 31485-498, Karaj, Iran.
[C]Corresponding author. Email: samaneh_gh_59@yahoo.com

**Abstract.** This study was conducted to evaluate the effect of partial and total substitution of lucerne hay with pistachio by-product on nutrient intake, apparent digestibility, rumen fermentation, digesta kinetics, blood metabolites, nitrogen retention and microbial protein synthesis in Baloochi sheep. Six sheep with a bodyweight of 40.1 ± 1.77 kg fitted with ruminal and abomasal cannulae were assigned at random to three diets in a double 3 × 3 Latin-square design. The dietary treatments included a control (basal diet), low pistachio by-product diet (50% of the lucerne hay in the basal diet was replaced by pistachio by-product), and high pistachio by-product diet (all of the lucerne hay in the basal diet was replaced by pistachio by-product). The daily basal diet was 500 g/kg DM lucerne hay, 250 g/kg DM wheat straw, 210 g/kg DM barley grain, 30 g/kg DM cotton seed meal, 8 g/kg DM vitamin–mineral supplement and 2 g/kg DM salt. Faeces and urine were collected for 8 days and used to estimate digestibility, nitrogen retention and microbial protein synthesis. Sheep were dosed ruminally with Cr-EDTA (2.27 g/L) and rumen samples were evaluated for pH, volatile fatty acids, ammonia nitrogen concentrations and digesta kinetics. The intake of neutral detergent fibre, purine derivatives excretion, volatile fatty acid concentrations in the rumen and ammonia nitrogen in the abomasum decreased ($P < 0.05$). Ether extract intake and ruminal pH was increased at 5 h after feeding ($P < 0.05$) as the level of pistachio by-product in the diet increased. Total pistachio by-product replacement for lucerne hay increased ($P < 0.05$) apparent digestibility of nitrogen, ether extract, organic matter and nitrogen retention and decreased ($P < 0.05$) microbial protein synthesis, efficiency of microbial protein synthesis and ammonia nitrogen in the rumen. The inclusion of pistachio by-product had no effect on blood metabolites and digesta kinetics. Based on these results it was concluded that pistachio by-product can be considered as a useful replacement for lucerne hay in the diet of Baloochi sheep without any negative impacts on their responses. Moreover, pistachio by-product inclusion in diet improved nitrogen metabolism in Baloochi sheep.

Received 2 August 2011, accepted 31 May 2012, published online 22 August 2012

## Introduction

According to the Food and Agriculture Organisation (FAO 2005) Iran is the largest pistachio (*Pistachio vera*) producer in the world. Excluding Iran, the top four pistachio producers in the world are United States, Syria, Turkey and China. Over the last 5 years total pistachio by-products (PB) production in Iran has been increased at an average rate of ~310 000 t per year. PB primarily consists of pistachio hull, and then peduncles, leaves and a little amount of mesocarp and kernels (Bohluli *et al*. 2007). PB contains 158.2 g/kg crude protein (CP), 69.5 g/kg ether extract (EE), 250 g/kg neutral detergent fibre (NDF) and 207.5 g/kg acid detergent fibre (ADF) (Behgar *et al*. 2009). Using PB in the diets of ruminants reduces the feed cost and environmental pollution (Valizadeh *et al*. 2009). In recent years, the results of some experiments demonstrated that PB could be integrated into livestock feeding (Vahmani and Naserian 2006; Gholizadeh *et al*. 2009, 2010). However, PB contains a high level of phenolic compounds and tannins, which can affect their utilisation by animals (Gholizadeh *et al*. 2010). Phenolic contents of PB include 75–95 g/kg total phenol and 35–45 g/kg tannin (Bohluli *et al*. 2007). The chemical composition and tannin content of PB vary with the cultivars of pistachio (Bohluli *et al*. 2007), harvesting time (Hashami *et al*. 2008), drying and de-hulling processes (Bohluli *et al*. 2007). Moderate amounts of tannins have been reported to exert beneficial effects on protein metabolism in ruminants (Frutos *et al*. 2004). The reduction of ruminal protein degradation may be the most significant and well known effect of tannins (Frutos *et al*. 2004). In general, this reduction in protein degradation is associated with a lower production of ammonia nitrogen (AN) and greater non-ammonia nitrogen (NAN) flow to the duodenum (Frutos *et al*. 2004). The mechanisms by which tannins reduce ruminal degradation of different dietary components are not entirely clear. Among the most accepted are substrate privation, enzyme inhibition and direct action on rumen

microorganisms (McSweeney *et al*. 2001*a*). Some previous studies showed that feeding PB at high levels decreased feed intake, blood glucose, cholesterol, triglyceride, ruminal AN concentration, and increased abomasum AN and blood urea nitrogen (BUN) in Baloochi sheep and dairy cows (Gholizadeh *et al*. 2009, 2010). Also, Mahdavi *et al*. (2010) suggested that high levels of PB had negative effects on lamb growth. However, there is little information about PB feeding in ruminants. The present study was conducted to evaluate the effects of PB replacement for lucerne hay (LH) on nutrient intake and digestion, rumen fermentation pattern, digesta kinetics, blood metabolites and microbial protein synthesis (MPS) in Baloochi sheep.

## Materials and methods

### *Animals and diets*

Six Baloochi sheep (BW 40.1 ± 1.77 kg), fitted with ruminal and abomasal cannula were housed indoors in individual metabolism cages suitable for separate collection of faeces and urine in a temperature-controlled building (~22°C) with constant lighting. All isoenergetic and isoproteic diets were supplied as total mixed ration, and offered at maintenance level once daily at 0800 hours. The sheep had access to clean water at all times. Sheep were randomly assigned in a double 3 × 3 Latin-square design to dietary treatments. Dietary treatments consisted of control diet (basal diets), low PB diet (50% of LH in the basal diet replaced by PB) and high PB diet (all of LH in the basal diet replaced by PB). The substitution of LH with PB was made on a DM basis. The diets were formulated for maintenance requirements according to AFRC (1993). The basal diet consisted of 500 g/kg DM LH + 250 g/kg DM wheat straw + 250 g/kg DM concentrate. The ingredients and chemical composition of the experimental diets are shown in Table 1. Pistachio by-product was the current year's annual growth, hand-harvested near Bardaskan (Iran) during summer 2009. Harvested PB was sun cured before use. Chemical composition of PB is shown in Table 2.

The trial consisted of three periods and each composed of 14-day adaptation and 8-day data collection including measurement of nutrients intake and digestion, N balance and digesta flow.

### *Nutrient intake and digestibility and N balance*

During the 8 days of the collection period, the amount of feed given and the feed left over after feeding were weighted and individual samples were collected daily and composited by animal for DM determination. Composited samples were dried (60°C) and ground to pass through a 1-mm screen (Retsch Cutting Mill, Retschmule, Germany) and then used for analysis.

Fecal samples (0.1 daily outputs) were collected and composited by animal over the 8-day collection period. The composited fecal samples were then mixed well and duplicate batches were dried to a steady mass at 60°C over 72 h, for determination of DM content, and then ground to pass through a 1-mm screen for later chemical analyses. Urine from each animal was collected daily in plastic vessels containing 100 mL sulfuric acid solution (0.1, v/v) to maintain a pH level below three, weighed, mixed well and a 0.1 daily aliquot was pooled over the 8-day collection period per animal. The bulked 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).

**Table 1. Ingredients and chemical composition of diets used to evaluate the effect of including pistachio by-product in the diet of sheep**

LPB, low level of pistachio by-product; HPB, high level of pistachio by-product

| Component | Diets | | |
|---|---|---|---|
| | Control | LPB | HPB |
| Ingredients (g/kg DM) | | | |
| Lucerne hay | 500.0 | 250.0 | 0.0 |
| Wheat straw | 250.0 | 250.0 | 250.0 |
| Pistachio by-product | 0.0 | 250.0 | 500.0 |
| Barley grain | 210.0 | 207.5 | 205.5 |
| Cotton seed meal | 30.0 | 32.0 | 34.0 |
| Vitamin–mineral premix | 8.0 | 8.0 | 8.0 |
| Salt | 2.0 | 2.0 | 2.0 |
| Lime | 0.0 | 0.5 | 1.0 |
| Chemical composition (g/kg DM) | | | |
| DM (g/kg) | 918.1 | 915.6 | 909.5 |
| Organic matter | 904.7 | 902.3 | 905.4 |
| Crude protein | 118.0 | 116.0 | 114.0 |
| Ether extract | 14.4 | 24.8 | 35.8 |
| Neutral detergent fibre | 504.7 | 454.9 | 415.7 |
| Phenolic compound (g/kg DM) | | | |
| Total phenolics | 9.1 | 29.5 | 42.5 |
| Tannin | 4.3 | 19.2 | 30.7 |
| Condensed tannin | 1.0 | 3.5 | 6.5 |

**Table 2. Chemical composition of pistachio by-product**

| Item | Content (g/kg DM) |
|---|---|
| DM | 900.0 |
| Organic matter | 755.3 |
| Ether extract | 58.0 |
| Crude protein | 153.1 |
| Neutral detergent fibre | 259.4 |
| Total phenols | 78.5 |
| Total tannins | 31.6 |
| Condensed tannins | 8.5 |

### *Blood metabolism*

Blood samples (10 mL) were taken from each animal from the jugular vein in the last day of each period just 2 h after the 0800 hours feeding. Blood was allowed to clot at room temperature for 30 min, centrifuged at 3000*g* for 15 min at 4°C and serum stored at −20°C for analysis.

### *Digesta flow measurement and rumen fermentation*

Animals were dosed with 300 mL Cr-EDTA (2.77 g/L) from Days 15 to 22 of each period. Samples of ruminal content (fluid strained through cheesecloth) were obtained at 2, 5, and 24 h after dosing on the last day of each period (Day 22). Ruminal fluid samples were centrifuged at 10 000*g* for 30 min at 4°C and clear

fluid was stored at −20°C for Cr determination. Ruminal fluid volume, dilution rate, fluid flow rate and turnover time were calculated by regressing the natural logarithm of Cr concentrations against sampling times (Warner and Stacy 1968). Additional rumen samples were collected at 0, 2 and 5 h after feeding on Day 21. Ruminal pH was measured using a pH meter (Metrohm 691, Sweden) and samples were strained through four layers of cheesecloth, 10 mL of rumen fluid were acidified with 10 mL of 0.2 *N* HCl for AN determination and 8 mL of rumen fluid (5 h after feeding) was added to 2 mL of deproteinising solution (250 mL/L of metaphosphoric acid and 200 μL 2-ethyl butyric acid) for volatile fatty acid (VFA) determination. Samples were stored at –20 C until they could be analysed. On Day 21 of each period abomasum fluid samples were obtained from abomasal cannula 5 h post feeding. Samples were stored at –20 C until analysis.

### Chemical analyses

Samples of feed, orts and faeces were analysed for DM, organic matter (OM), EE and N by standard procedures (AOAC 1995). NDF was determined according to Van Soest *et al.* (1991) without amylase and sodium sulfite application. Total phenols and tannins and condensed tannin (butanol procedure) in the PB and dietary treatments used in the experiment were determined in aqueous acetone (70 : 30, acetone : distilled water; v/v) extracts, as described by Makkar (2003*a*). Urine samples were analysed for N and purine derivatives as an index of MPS according to Chen and Gomes (1995). The amount of the four purine derivatives (uric acid, allantoin, xanthine and hypoxanthine) excreted in the urine was expressed as gram of microbial N per kilogram of digestible OM daily intake using coefficients established on sheep (Chen and Gomes 1995) after correction for metabolic weight. Glucose, BUN, potassium, iron and creatinine were determined by autoanalyzer (Selectra XL). AN was determined from 5 mL samples of ruminal and abomasal fluid in 30 mL saturated sodium tetra borate solution by steam distillation and librated ammonia was captured in 2% boric acid solution and titrated against 0.2 *N* HCl (Komolong *et al.* 2001). Ruminal VFA concentrations were determined by gas chromatography (Vanzant and Cochran 1994). Cr concentration in ruminal fluids was determined using an atomic absorption spectrometer with an air-acetylene flame.

### Statistical analyses

The statistical model is as follows:

$$Y_{ijkl} = \mu + T_i + SQ_j + \text{Period}\,(SQ)_{kj} + \text{Sheep}\,(SQ)_{lj} + \varepsilon_{ijkl}$$

where $Y_{ijkl}$ = observation ijkl; μ = the overall mean; $T_l$ = the effect of treatment i; $SQ_j$ = the effect of square j; Period $(SQ)_{kj}$ = the effect of period k within square j; Sheep $(SQ)_{lj}$ = the effect of sheep l within square j and $\varepsilon_{ijkl}$ = random error with mean 0 and variance σ2.

Data were analysed using the GLM procedure of SAS (SAS 2001). Before the statistical analysis data was tested for normality using the Proc UNIVARIATE in SAS (SAS 2001). The Tukey test was used to assess the significance of differences between treatment means where the overall treatment effect was significant ($P < 0.05$).

## Results

### Intake, apparent digestibility and N balance

Data on intake, apparent digestibility of nutrients and N balance are given in Tables 3 and 4. DM, OM and CP intakes were similar among all dietary treatments, but NDF intake decreased ($P = 0.03$) while EE intake increased ($P = 0.01$) with increasing the level of PB in the diets. Total PB replacement for LH in the diet was increased ($P < 0.05$) the total apparent digestibility of CP, EE and OM. There was also a decrease ($P < 0.01$) in the amount of N excreted in the faeces, urine and an increase in N retention.

### Blood metabolites

Serum BUN, glucose, creatinine, potassium and iron are shown in Table 5. The inclusion of PB in the diets had no effect on blood metabolites.

### Ruminal fermentation parameters

Ruminal pH at 5 h after feeding was increased ($P < 0.01$) with increasing the level of PB in the diets (Table 6). Inclusion of PB in the diet reduced ($P < 0.01$) total VFA concentration, individual VFA concentration and AN in the rumen and abomasums liquor (Table 6).

**Table 3. Nutrient intake and apparent digestibility by sheep fed dietary treatments**

Within rows, means followed by different letters are significantly different at $P = 0.05$. LPB, low level of pistachio by-product; HPB, high level of pistachio by-product

| | Diets | | | s.e. | *P*-value |
|---|---|---|---|---|---|
| | Control | LPB | HPB | | |
| Intake (g/day) | | | | | |
| DM | 734.5 | 731.9 | 648.9 | 26.6 | 0.2 |
| Organic matter | 723.7 | 723.3 | 636.6 | 28.6 | 0.2 |
| Crude protein | 94.4 | 94.5 | 79.3 | 3.7 | 0.1 |
| Neutral detergent fibre | 403.8a | 403.9a | 296.9b | 11.3 | 0.03 |
| Ether extract | 11.5b | 19.8ab | 23.3a | 1.7 | 0.01 |
| Diet digestibility (g/g) | | | | | |
| DM | 0.86 | 0.87 | 0.88 | 0.011 | 0.20 |
| Organic matter | 0.75b | 0.76b | 0.80a | 0.005 | 0.01 |
| Crude protein | 0.65b | 0.65b | 0.76a | 0.003 | 0.01 |
| Ether extract | 0.60b | 0.61b | 0.79a | 0.019 | 0.02 |
| Neutral detergent fibre | 0.70 | 0.71 | 0.73 | 0.015 | 0.36 |

**Table 4. Nitrogen (N) balance in sheep fed dietary treatments (g/day)**

Within rows, means followed by different letters are significantly different at $P = 0.05$. LPB, low level of pistachio by-product; HPB, high level of pistachio by-product

| | Diets | | | s.e. | *P*-value |
|---|---|---|---|---|---|
| | Control | LPB | HPB | | |
| N intake | 15.10 | 15.11 | 12.69 | 0.59 | 0.10 |
| Fecal N | 5.28a | 5.10a | 3.04b | 0.29 | <0.01 |
| Urinary N | 3.98a | 4.28a | 2.79b | 0.22 | <0.01 |
| N balance | 5.84b | 5.73b | 6.86a | 0.154 | <0.01 |

**Table 5. Blood urea nitrogen (BUN), glucose, potassium, iron and creatinine concentrations in sheep fed dietary treatments**
LPB, low level of pistachio by-product; HPB, high level of pistachio by-product

| | Diets | | | s.e. | *P*-value |
|---|---|---|---|---|---|
| | Control | LPB | HPB | | |
| BUN (mg/L) | 186.0 | 140.0 | 130.0 | 29.2 | 0.32 |
| Glucose (mg/L) | 753.3 | 720.00 | 723.3 | 27.9 | 0.70 |
| Creatinine (mg/L) | 10.6 | 10.0 | 11.3 | 0.3 | 0.20 |
| Potassium (mg/L) | 216.2 | 199.4 | 194.3 | 15.6 | 0.69 |
| Iron (µg/L) | 3567.0 | 1983.0 | 2497.0 | 848.4 | 0.52 |

**Table 6. Ruminal pH, ammonia nitrogen (AN), volatile fatty acids (VFA) concentrations and AN concentration of abomasum in sheep fed dietary treatments**
Within rows, means followed by different letters are significantly different at $P = 0.05$. LPB, low level of pistachio by-product; HPB, high level of pistachio by-product

| | Diets | | | s.e. | *P*-value |
|---|---|---|---|---|---|
| | Control | LPB | HPB | | |
| Rumen pH | | | | | |
| Before feeding | 7.14 | 7.09 | 7.23 | 0.151 | 0.82 |
| 2 h post feeding | 6.22 | 6.28 | 6.40 | 0.109 | 0.60 |
| 5 h post feeding | 6.03b | 6.35a | 6.33a | 0.012 | <0.01 |
| Rumen AN (mg/L) | | | | | |
| Before feeding | 106.6a | 104.10a | 63.60b | 6.26 | <0.01 |
| 2 h post feeding | 241.4a | 210.70a | 84.80b | 13.56 | <0.01 |
| 5 h post feeding | 193.6a | 173.00a | 84.30b | 18.20 | <0.01 |
| Abomasum AN (mg/L) | 328.8a | 276.80ab | 221.40b | 25.50 | <0.01 |
| Total VFA (mmol/L) | 95.00a | 76.33b | 76.50b | 3.426 | <0.01 |
| Individual VFA (mmol/L) | | | | | |
| Acetate | 47.63a | 35.13b | 31.66b | 2.971 | <0.01 |
| Propionate + isobutyrate | 23.83a | 16.53c | 21.16b | 0.765 | <0.01 |
| Butyrate | 22.13 | 24.66 | 23.66 | 0.831 | 0.14 |
| Isovalerate | 1.40a | 0.00 | 0.00 | 0.061 | <0.01 |

### *Digesta flow*

Fluid dilution rate, total turnover time, ruminal fluid volume and fluid flow rate (outflow from the rumen) are shown in Table 7. The inclusion of PB in the diets had no effect on digesta kinetics.

### *Estimation of microbial protein synthesis*

Purine derivatives excretion decreased ($P < 0.01$) with increasing the level of PB in the diets (Table 8). Total substitution of LH by PB decreased ($P = 0.01$) MPS and efficiency of MPS (Table 8).

## Discussion

### *Intake, apparent digestibility and N balance*

In the present study partial and total substitution of LH by PB increased EE (control 14.4 g/kg DM versus high PB 35.8 g/kg) and decreased NDF (control 504.7 g/kg DM versus high PB 415.7 g/kg) content of experimental diets (Table 1). It is well known that these two parameters affect digestion, metabolic responses and microbial activity. However, the level of EE in the high PB diet was below the general recommendation (60–70 g/kg DM) that affect these parameters (Jenkins 1993; NRC 2001). Weimer *et al*. (1999) reported that decreased dietary NDF to 22.9% of DM did not affect cellulolytic bacteria in the rumen of lactating dairy cows. In the present study the level of NDF in high PB was 41.57% of DM that was higher than this amount.

**Table 7. Fluid dilution rate, total turnover time, ruminal fluid volume and fluid flow rate (out flow from the rumen) in sheep fed dietary treatments**
LPB, low level of pistachio by-product; HPB, high level of pistachio by-product

| | Diets | | | s.e. | *P*-value |
|---|---|---|---|---|---|
| | Control | LPB | HPB | | |
| Fluid dilution rate (%/h) | 4.83 | 4.53 | 4.30 | 0.607 | 0.83 |
| Total turnover time (h) | 20.94 | 24.45 | 24.50 | 2.916 | 0.67 |
| Ruminal fluid volume (L) | 6.64 | 5.62 | 5.41 | 1.419 | 0.38 |
| Fluid flow rate (L/h) | 0.42 | 0.25 | 0.23 | 0.095 | 0.46 |

**Table 8. Rumen microbial protein synthesis (MPS) and urinary excretion of purine derivatives by sheep fed dietary treatments**
Within rows, means followed by different letters are significantly different at $P = 0.05$. LPB, low level of pistachio by-product; HPB, high level of pistachio by-product

| | Diets | | | s.e. | *P*-value |
|---|---|---|---|---|---|
| | Control | LPB | HPB | | |
| Purine derivatives excretion (mmol/day) | 12.49a | 11.30b | 8.37c | 0.149 | <0.01 |
| Microbial nitrogen supply (g/day) | 10.73a | 9.68a | 6.92b | 0.206 | 0.01 |
| Efficiency of MPS (g microbial nitrogen/kg DOMI) | 37.04a | 34.79a | 22.52b | 0.886 | 0.01 |

As mentioned above, PB used in this study had higher fat content (58 g/kg) compared with LH, thus the intake of EE was increased with increasing the level of PB in the diets. NDF content of PB was less than NDF content of LH (259.4 versus 500 g/kg), therefore the intake of NDF decreased with inclusion of PB in the diets. The replacement of PB for LH tended to decrease N intake, due to poor palatability of PB. Ben Salem *et al*. (2005) claimed that tannins cause an astringent sensation in the mouth of the animal, this sensation results in a negative feedback which reduces the consumption of tannin-rich feeds. DM, OM and N intake were similar across treatments. Similar results were observed by others (Vahmani and Naserian 2006; Getachew *et al*. 2008*a*; Gholizadeh *et al*. 2010). Gholizadeh *et al*. (2009) reported that replacement of PB for beet pulp decreased DM intake in Baloochi sheep. Inconsistency in these results and our findings could be related to difference in digestion potential of sugar beet pulp and PB. After 72 h rumen incubation, 96 and 89% of DM had disappeared for beet pulp and PB, respectively (Grenet and Barry 1990; Mahdavi *et al*. 2008).

Total replacement of PB for LH increased OM, CP and EE apparent digestibilities. Higher EE digestibility in sheep fed PB may have resulted from greater EE intake (Palmquist 1991).

Moreover, more CP digestibility in sheep fed PB may have resulted from lower N intake and greater N balance. DM and NDF apparent digestibilities were not affected by the experimental diets. These results are consistent with some previous studies (Getachew *et al*. 2008*a*). In contrast to our findings, Zimmer and Cordesse (1996) observed that DM, OM and NDF digestibilities were decreased by hydrolysable tannins. Moreover, Bagheripour *et al*. (2008) showed that OM degradability of pistachio hull was decreased by tannins and addition of polyethylene glycol (PEG) improved *in vitro* OM degradability. Although using PEG can help to define the overall effect of PB tannins, authors could not find any study in which diets containing PB treated with PEG.

Total replacement of PB for LH decreased N excretion in faeces and urine therefore, N retention was increased. Similar results were observed by Driedger and Hatfield (1972), Mathieu and Jouany (1993), Hervás *et al*. (2000) and Poncet and Remond (2002). Frutos *et al*. (2004) reported that, tannins decrease the rate of protein degradation and de-amination in the rumen and, therefore, lower ruminal AN and urinary N loss. Moreover, Getachew *et al*. (2008*a*) observed no effect of tannin on N retention.

### *Blood metabolites*

In the present study, blood metabolites were not affected by the experimental diets. In contrast to our findings, Gholizadeh *et al*. (2009) observed that replacement of PB for beet pulp increased serum BUN and AN in abomasums. Increase in abomasums AN caused an increased in serum BUN in this experiment. In another study, Nastis and Malechek (1981) showed that replacement of oak for lucerne raised BUN concentration.

### *Ruminal fermentation parameters*

Concentrations of AN in the rumen and abomasums, total VFA, acetic, propionic + isobutyric, and isovaleric acids in the rumen were decreased and ruminal pH at 5 h after feeding was increased with increasing level of PB in diets. Lower ruminal AN concentration in sheep fed PB may have resulted from a greater concentration of tannins that bound to proteins and decreased proteolysis resulting in a reduction of AN in rumen fluid (Singh *et al*. 2001; Frutos *et al*. 2004; Getachew *et al*. 2008*a*, 2008*b*). Depression in VFA concentrations in the present study might be related to lower microbial activity of rumen in the presence of tannins (Bhatta *et al*. 2009). The concentration of iso valeric acid was very low in PB-containing diets. Iso-acids are derived from amino acids catabolism by cellulolytic bacteria in the rumen (Mackie and White 1990). Concentrations of iso-acids were lower in the presence of PB tannin because the protein was protected from bacterial deamination. This result is consistent with the effect of PB on the production of AN in the rumen, similar results were observed by Bhatta *et al*. (2009) and Getachew *et al*. (2008*a*). Also, Silanikove *et al*. (1996) reported that VFA production during *in vivo* and *in vitro* fermentation was reduced when the substrate contained different kinds of tannin. Higher ruminal pH in sheep fed PB contained diets may have resulted from lower AN and VFA concentrations in the rumen. In contrast to our finding, Yildiz *et al*. (2005) reported no difference in AN, VFA level and ruminal pH, in lambs receiving oak leaves.

### *Digesta flow*

Fluid dilution rate can affect MPS efficiency (Van Soest 1982) and ruminal fermentation (Stell and Galyean 1985). The replacement of PB for LH had no effect on digesta kinetics. Similar results were reported by Poncet and Remond (2002) in sheep.

### *Estimation of microbial protein synthesis*

In the present study, replacement of PB for LH decreased purine derivatives excretion, MPS and efficiency of MPS. According to Zimmer and Cordesse (1996), addition of tannins had no significant effect on the flow of MP in sheep and goats. Yildiz *et al*. (2005) found that MPS increased without alteration in VFA production in rumen fluid of lambs receiving oaks. In the present study, one of the reasons for decreasing MPS might be lower AN concentration in the rumen (McSweeney *et al*. 2001*b*).

## Conclusion

The results from the present study indicated that PB can be considered as a useful replacement for LH without any negative impact on sheep responses. Moreover, PB inclusion in the diet improved N metabolism in Baloochi sheep. Further research is needed to determine the optimal level in the diets PB for fattening sheep. Using tannin-binding agents (e.g. PEG) and extracted tannins will also help to define the overall effect of tannins on animal performance.

## Acknowledgements

This study was financially supported by Animal Science Department of Ferdowsi University of Mashhad, Iran.

## References

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 (1995) ‘Official methods of analysis.’ 17th edn. (AOAC International: Gaithersburg, MD)

Bagheripour E, Rouzbehan Y, Alipour D (2008) Effect 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. doi:10.1016/j.anifeedsci.2008.01.002

Behgar M, Valizadeh R, Mirzaee M, Naserian AA, Nasiri MR (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, Makkar HPS, Hochlef H, Ben Salem I, Ben Salem L (2005) Effect of early experience and adaptation period on voluntary intake, digestion, and growth in Barbarine lambs given tannin-containing (*Acacia cyanophylla* Lindl. foliage) or tannin-free (oaten hay) diets. *Animal Feed Science and Technology* **122**, 59–77. doi:10.1016/j.anifeedsci.2005.04.014

Bhatta R, Uyeno Y, Tajima K, Takenaka A, Yabumoto Y, Nonaka I, Enishi O, Kurihara M (2009) Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. *Journal of Dairy Science* **92**, 5512–5522.

Bohluli A, Naserian AA, Valizadeh R, Shahroodi FE (2007) The chemical composition and in vitro digestibility of pistachio by-product. ‘In Proceedings of British Society of Animal Science’. p. 224. (BSAS: Scarborough, UK)

Chen XB, Gomes MJ (1995) 'Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: an overview of the technical details.' (Rowett Research Institute: Bucksburne, Aberdeen)

Driedger A, Hatfield E (1972) Influence of tannins on the nutritive value of soybean meal for ruminants. *Journal of Animal Science* **34**, 465–468.

FAO (2005) Food and Agriculture Organization of the United Nations. Available at http://www.faostat.fao.org [Verified 12 December 2010]

Frutos P, Hervas G, Giraldez FJ, Mantecon AR (2004) Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research* **2**, 191–202.

Getachew G, Pittroff W, DePeters EJ, Putnam DH, Dandekar A, Goyal S (2008*a*) Influence of tannic acid application on alfalfa hay: *in vitro* rumen fermentation, serum metabolites and nitrogen balance in sheep. *Animal* **2**, 381–390. doi:10.1017/S1751731107001486

Getachew G, Pittroff W, Putnam DH, Dandekar A, Goyal S, DePeters EJ (2008*b*) The influence of addition of Gallic acid, Tannic acid, or Quebracho tannins to alfalfa hay on in vitro rumen fermentation and microbial protein synthesis. *Animal Feed Science and Technology* **140**, 444–461. doi:10.1016/j.anifeedsci.2007.03.011

Gholizadeh H, Naserian AA, Valizadeh R, Tahmasebi A (2009) Effects of feeding pistachio hull and interaruminal infusion of urea on feed intake, ruminal and abomasum N-NH3 and blood metabolites in Iranian Balochi sheep. In 'Proceedings of British Society of Animal Science'. p.163. (BSAS: Scarborough, UK)

Gholizadeh H, Naserian AA, Valizadeh R, Tahmasebi A (2010) Effect of feeding pistachio byproduct on performance and blood metabolites in Holstein dairy cows. *International Journal of Agriculture and Biology* **12**, 867–870.

Grenet E, Barry P (1990) *In vivo* and *in sacco* digestibility and rumen microbial degradation of cell walls of soyabean and rape integuments and of dehydrated beet pulp in sheep, observed by scanning electron microscopy. *The Journal of Agricultural Science* **115**, 429–435. doi:10.1017/S0021859600075882

Hashami O, Naserian A, Tahmasbi AM, Valizadeh R, Mohammadabadi T (2008) Degradability of pistachio by-products at different harvesting times. In 'Proceedings of British Society of Animal Science'. p. 251. (BSAS: Scarborough, UK)

Hervás G, Frutos P, Serano E, Mantecon AR, Giraldez JF (2000) Effect of tannic acid on rumen degradation and intestinal digestion of treated soyabean meals in sheep. *The Journal of Agricultural Science* **135**, 305–310. doi:10.1017/S0021859699008151

Jenkins TC (1993) Lipid metabolism in the rumen. *Journal of Dairy Science* **76**, 3851–3863. doi:10.3168/jds.S0022-0302(93)77727-9

Komolong MK, Barber DG, McNeil DM (2001) Post ruminal protein supply and N retention of weaner sheep fed on a basal diet of Lucerne hay (*Medicago sativa*) with increasing levels of quebracho tannins. *Animal Feed Science and Technology* **92**, 59–72. doi:10.1016/S0377-8401(01)00246-2

Mackie RI, White BA (1990) Recent advances in rumen microbial ecology and metabolism: potential impact on nutrient out put. *Journal of Dairy Science* **73**, 2971–2995. doi:10.3168/jds.S0022-0302(90)78986-2

Mahdavi A, Zagharu M, Zahedifar M, Nikkhah A, Aghashahi AR (2008) The effect of different feeding levels of dried pistachio epicarp on fattening performance of Afshari lamb breed of Iran. *Journal of Agricultural Sciences and Natural Resources* **15**, 139–148.

Mahdavi M, Zaghari M, Zahedifar M, Nikkhah A, Alemi F, Hosseini A, Mirabdolbaghi Z, Lotfolahiyan H (2010) The effects of dried pistachio epicarp on lambs' performance. *Advances in Animal Biosciences* **1**, 236. doi:10.1017/S2040470010003791

Makkar HPS (2003*a*) Quantification of tannins in tree and shrub foliage. In 'A laboratory manual'. (Ed. HPS Makkar) p. 102. (Kluwer Academic Publishers: Vienna)

Mathieu F, Jouany JP (1993) Effect of chestnut tannin on the fermentability of soyabean meal nitrogen in the rumen. *Animal Research* **42**, 127. doi:10.1051/animres:19930210

McSweeney CS, Palmer B, Bunch R, Krause DO (2001*a*) Effect of the tropical forage calliandra on microbial protein synthesis and ecology in the rumen. *Journal of Applied Microbiology* **90**, 78–88. doi:10.1046/j.1365-2672.2001.01220.x

McSweeney CS, Palmer B, Mc Neill DM, Krause DO (2001*b*) Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology* **91**, 83–93. doi:10.1016/S0377-8401(01)00232-2

Nastis AS, Malechek JC (1981) Digestion and utilization of nutrients in oak browse by goats. *Journal of Animal Science* **53**, 283–290.

NRC (2001) 'Nutrient requirement of dairy cattle.' 7th edn. (National Academic Press: Washington, DC)

Palmquist DL (1991) Influence of source and amount of dietary fat on digestibility in lactating cows. *Journal of Dairy Science* **74**, 1354–1360. doi:10.3168/jds.S0022-0302(91)78290-8

Poncet C, Remond D (2002) Rumen digestion and intestinal nutrient flows in sheep consuming pea seeds: the effect of extrusion or chestnut tannin addition. *Animal Research* **51**, 201–216. doi:10.1051/animres:2002021

SAS (2001) 'SAS/STAT user's guide.Version 9.' (SAS Institute: Cary, NC)

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*, and *Ceratonia siliqua*) by goats. *Journal of Agricultural and Food Chemistry* **44**, 199–205. doi:10.1021/jf950189b

Singh B, Bhat TK, Sharma OP (2001) Biodegradation of tannic acid in an *in vitro* ruminal system. *Livestock Production Science* **68**, 259–262. doi:10.1016/S0301-6226(00)00227-X

Stell RE, Galyean ML (1985) Relationship of rumen fluid dilution rate to rumen fermentation and dietary characteristics of beef steers. *Journal of Animal Science* **60**, 1061.

Vahmani P, Naserian AA (2006) Pistachio hulls as a feed ingredients for lactating dairy goats. 'In Proceedings of the British Society of Animal Science'. p. 145. (BSAS: Scarborough, UK)

Valizadeh R, Naserian AA, Vahmani P (2009) Influence of drying and ensiling pistachio by-products with urea and molasses on their chemical composition, tannin content and rumen degradability parameters. *Journal of Animal and Veterinary Advances* **8**, 2363–2368.

Van Keulen J, Young BA (1977) Evaluation of acid-insoluble ash as natural marker in ruminant digestibility. *Journal of Animal Science* **44**, 282–287.

Van Soest PJ (1982) 'Nutritional ecology of the ruminant.' (O & B Books: Corvallis, OR)

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in ration to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Vanzant ES, Cochran RC (1994) Performance and forage utilization by beef cattle receiving increasing amounts of alfalfa hay as a supplement to low-quality forage. *Journal of Animal Science* **72**, 1059–1067.

Warner ACI, Stacy BD (1968) The fate of water in the rumen. I. A critical appraisal of the use of soluble markers. *The British Journal of Nutrition* **22**, 369–387. doi:10.1079/BJN19680046

Weimer PJ, Waghorn GC, Odt CL, Mertens DR (1999) Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. *Journal of Dairy Science* **82**, 122–134. doi:10.3168/jds.S0022-0302(99)75216-1

Yildiz S, Kaya I, Unal Y, Aksu Elmali D, Kaya S, Cenesiz M, Kaya M, Oncuer A (2005) Digestion and body weight change in Tuj lambs receiving oak (*Quercus hartwissiana*) leaves with and without PEG. *Animal Feed Science and Technology* **122**, 159–172. doi:10.1016/j.anifeedsci.2005.04.005

Zimmer N, Cordesse R (1996) Digestibility and ruminal digestion of nonnitrogenous compounds in adult sheep and goats: effects of chestnut tannins. *Animal Feed Science and Technology* **61**, 259–273. doi:10.1016/0377-8401(95)00940-X