Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.


This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Small Ruminant Research 104 (2012) 94–98

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Short communication

# Inclusion of pistachio hulls as a replacement for alfalfa hay in the diet of sheep causes a shift in the rumen cellulolytic bacterial population

S. Ghasemi[a,*], A.A. Naserian[a], R. Valizadeh[a], A.M. Tahmasebi[a], A.R. Vakili[a], M. Behgar[b], S. Ghovvati[a]

[a] Faculty of Agriculture, Excellence Center in Animal Science, Ferdowsi University of Mashhad, P.O. Box 91775-1163, Mashhad, Iran
[b] Agricultural, Medical and Industrial Research School, P.O. Box 31485-498, Karaj, Iran

ARTICLE INFO

*Article history:*
Received 16 March 2011
Received in revised form 6 September 2011
Accepted 29 September 2011
Available online 21 October 2011

*Keywords:*
Real-time PCR
Cellulolytic bacteria
Sheep
Pistachio hull
Alfalfa hay

ABSTRACT

The objective of this study was to evaluate the effect of using pistachio (*Pistachio vera*) hulls (PH) as a replacement for alfalfa hay (AH) in the diet of Baloochi sheep on their rumen microbial and rumen fermentation characteristics. Six Baloochi sheep with a body weight of 40.1 ± 1.77 kg (mean ± SEM) fitted with rumen cannulae were assigned at random to three diets in a double 3 × 3 Latin square design. The dietary treatments were control (basal diet), low PH (LPH) diet (0.50 of AH in basal diet replaced by PH), and high PH (HPH) diet (all of the AH in the basal diet replaced by PH). The daily basal diet was 400 g AH dry matter (DM), 200 g wheat straw DM, 168 g barley grain DM, 24 g cotton seed meal DM, 6.4 g vitamin–mineral supplement DM and 1.6 g salt DM. Bacterial populations were assessed by DNA extraction of samples of rumen liquor followed by real-time polymerase chain reaction analysis. Rumen samples were evaluated for pH, volatile fatty acid (VFA) and ammonia nitrogen (AN) concentrations. The populations of total bacteria (ng per μl extracted DNA – control 564 versus LPH 407 versus HPH 315), *Fibrobacter succinogenes* and *Ruminococcus albus* decreased significantly as the level of PH in the diet increased ($P < 0.05$). The population of *Ruminococcus flavefaciens* in the rumen liquor was not affected by the LPH diet but was reduced to approximately half the level of the control in the HPH diet (NS). The replacement of AH partly or wholly in the diet caused an increase of rumen pH from 6.03 for control to 6.35 for LPH and 6.33 for HPH ($P < 0.01$). Inclusion of PH in the diet reduced total VFA concentration (control 95.0, LPH 76.3 and HPH 76.5 mmol/L; $P < 0.01$), individual VFA concentration (e.g. acetate control 47.6, LPH 35.1 and HPH 31.7 mmol/L; $P < 0.01$), and AN (control 193, LPH 173 and HPH 84.3 mg/L; $P < 0.01$) in the rumen liquor. It was concluded that the tannins in PH reduced the number of cellulolytic and total bacteria. Inclusion of PH in the diet of sheep also caused an increase in the pH and reduced the concentrations of AN and VFA of rumen liquor.

© 2011 Elsevier B.V. All rights reserved.

## 1. Introduction

Pistachio (*Pistachio vera*) hull (PH) is pistachio processing by-product produced during de-hulling of pistachio nuts after harvesting. According to the Food and Agriculture Organization (FAO, 2005) Iran is the largest pistachio producer in the world. Over the last five years total pistachio by-product production in Iran has been increased at an average rate of about 310,000 metric tons per year and becoming an environmental problem. PH is produced during de-hulling of pistachio nuts soon after harvesting. Pistachio by-product mainly consists of PH, and then peduncles, leaves and a little amount of mesocarp and kernels (Bohluli et al., 2007). PH contains 158.2 g/kg

* Corresponding author. Tel.: +98 915 5255587.
*E-mail address:* samaneh_gh_59@yahoo.com (S. Ghasemi).

0921-4488/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.smallrumres.2011.09.052

Author's personal copy



crude protein (CP), 69.5 g/kg ether extract (EE), 250 g/kg neutral detergent fiber (NDF) and 207.5 g/kg acid detergent fiber (ADF) (Behgar et al., 2009). In recent years, the results of some experiments shown that PH can be used as a feedstuff in ruminant nutrition (Vahmani and Naserian, 2006; Gholizadeh et al., 2009, 2010). However, PH contains high level of phenolic compounds and tannins, which can affect their utilization as feedstuffs by animals (McSweeney et al., 2001). Phenolic contents of PH include 75–95 g/kg total phenol and 35–45 g/kg tannin (Bohluli et al., 2007). The chemical composition and tannin content of PH are varied largely depending on variations in pistachio cultivars (Bohluli et al., 2007), harvesting time (Hashami et al., 2008), drying and de-hulling processes. This variation toward increasing in tannin content may inhibit microbial activity and digestion process in rumen and intestinal tract. Moderate amounts of tannins have been also reported to exert beneficial effects on protein metabolism in ruminants, decreasing rumen degradation of dietary protein and increasing absorption of amino acids in the small intestine and negative effects on fermentation and VFA concentrations (Frutos et al., 2004). Also, tannins and phenolic monomers are known for their antimicrobial activity (Goel et al., 2005; Guimaraes-Beelen1 et al., 2006) either on cellulolytic or proteolytic bacteria. Previous studies showed that tannin decreased cellulolytic bacteria population in *in vivo* (Newbold et al., 1997) and *in vitro* conditions (Min et al., 2005; Bhatta et al., 2009). Also, Nelson et al. (1997) showed that *Fibrobacter succinogenes* is one of the bacteria most inhibited by tannins. In this study although *Ruminococcus albus* was inhibited by high levels of tannins it was less sensitive than *F. succinogenes*.

Despite many efforts to show the effects of tannin rich plants on population of rumen microorganism in *in vitro* studies, very few reports can be found on the effect of these sources on the rumen cellulolytic bacteria population in *in vivo* condition. Authors could not found any information about the effects of PH tannin on rumen bacterial population. Because the key role of ruminal predominant cellulolytic bacteria (i.e. *F. succinogenes*, *R. albus* and *Ruminococcus flavefaciens*) in degradation of plant cell wall, recently the dynamics of cellulolytic bacterial populations in response to dietary changes have been studied using targeting 16S rRNA gene by real-time PCR in the sheep (Mosoni et al., 2007) and cattle (Wanapat and Cherdthong, 2009). The objective of the present study was to investigate the effect of PH replacement for AH on total and cellulolytic bacteria population shift by real-time PCR technique and to determine the effect of this replacement on the rumen fermentation parameters in Baloochi sheep.

## 2. Materials and methods

### 2.1. Animals and diet

Six Baloochi sheep (BW 40.1 ± 1.77 kg), fitted with ruminal cannulae were housed indoors in individual metabolism cages in a temperature controlled building (approximately 22 °C) with constant lighting. All isoenergetic and isoproteic diets were supplied as TMR, and offered at maintenance level once daily at 08:00. Clean water was freely available 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), LPH diet (0.5 of AH in basal diet replaced by PH) and HPH diet (whole of AH in basal diet replaced by PH). The diets were formulated for maintenance requirements according to AFRC (1993). Basal diet consisted of 400 g AH + 200 g wheat straw + 200 g barley base concentrate. The ingredients and chemical composition of the experimental diets are shown in Table 1. PH was the current year's annual growth, hand-harvested near Bardaskan (Iran) during summer 2009. Harvested PH was sun cured before use. Chemical composition of PH is shown in Table 1.

**Table 1**
Chemical composition of pistachio hulls (PH) used in the diets of sheep, ingredients and chemical composition of diets used to evaluate the effect of including pistachio hulls in the diet of sheep (LPH, low level of pistachio hulls; HPH, high level of pistachio hulls).

| | Diets | | |
|---|---|---|---|
| | Control | LPH | HPH |
| Ingredients (DM/day, g) | | | |
| Alfalfa hay | 400 | 200 | 0.00 |
| Wheat straw | 200 | 200 | 200 |
| Pistachio hull | 0.00 | 200 | 400 |
| Barley grain | 168 | 166 | 164 |
| Cotton seed meal | 24.0 | 25.6 | 27.2 |
| Vitamin–mineral premix | 6.40 | 6.40 | 6.40 |
| Salt | 1.60 | 1.60 | 1.60 |
| Lime | 0.00 | 0.40 | 0.80 |

| | Diets | | | |
|---|---|---|---|---|
| | Control | LPH | HPH | PH |
| Chemical composition (g/kg DM) | | | | |
| DM | 918 | 915 | 909 | 900 |
| OM | 904 | 902 | 905 | 755 |
| CP | 118 | 118 | 117 | 153 |
| EE | 14.4 | 24.8 | 35.8 | 58.0 |
| NDF | 504 | 455 | 416 | 259 |
| Phenolic compound (g/kg DM) | | | | |
| Total phenolics | 9.10 | 29.50 | 42.50 | 78.5 |
| Tannin | 4.30 | 19.2 | 30.7 | 31.6 |
| Condensed tannin | 1.00 | 3.50 | 6.50 | 8.50 |

Trial consisted of three periods and each experimental period consisted of 19 d adaptation and 1 d data collection including measurement of ruminal cellulolytic and total bacteria population and rumen fermentation parameters.

### 2.2. Rumen fluid sampling

On the last day of each period one sample per animal of ruminal fluid was collected before feeding (0800 h). Samples were squeezed loosely through cheese cloth layers and stored at −20 °C for later DNA extraction. On the same day another portion of ruminal fluid was collected at 5 h after feeding. Ruminal pH was determined immediately using a pH meter (Accumet, Fisher Scientific, USA) and samples were strained through four layers of cheesecloth, 10 ml of rumen fluid was acidified with 10 ml of 0.2 N HCl for AN (ammonia nitrogen) determination and 8 ml of rumen fluid was added to 2 ml of deproteinising solution (metaphosphoric acid 25% and 200 μl 2-ethyl butyric acid) for VFA determination. Samples were stored at −20 °C until analysis of AN and VFA.

### 2.3. Chemical analyses

Samples of feed were analyzed for DM, OM, EE and nitrogen by standard procedures (AOAC, 1995). NDF was determined according to Van Soest et al. (1991) without amylase application. Total phenols, tannins and condensed tannin (CT) (butanol procedure) in the PH and dietary treatments were determined in aqueous acetone (70:30, acetone: distilled water) extracts, as described by Makkar (2003a). AN was determined from 5 ml samples of ruminal 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).

Author's personal copy



**Table 2**
PCR primers used for amplifying target bacteria in the rumen contents of sheep.

| Target species | Forward/reverse | Primer sequence | References |
|---|---|---|---|
| Total bacteria | F | GTGSTGCAYGGYTGTCGTCA | Maeda et al. (2003) |
| | R | ACGTCRTCCMCACCTTCCTC | |
| *F. succinogenes* | F | GTTCGGAATTACTGGGCGTAAA | Zhang et al. (2008) |
| | R | CGCCTGCCCCTGAACTATC | |
| *R. flavefaciens* | F | CGAACGGAGATAATTTGAGTTTACTTAGG | Zhang et al. (2008) |
| | R | CGGTCTCTGTATGTTATGAGGTATTACC | |
| *R. albus* | F | CCCTAAAAGCAGTCTTAGTTCG | Koike and Kobayashi (2000) |
| | R | CCTCCTTGCGGTTAGAACA | |

**Table 3**
Populations of total bacteria[a] and cellulolytic bacteria[b] in the rumen contents of sheep offered a control diet without pistachio hulls (PH) or including pistachio hulls at a low (LPH) or high (HPH) level.

| | Diets | | | SE | *P* value |
|---|---|---|---|---|---|
| | Control | LPH | HPH | | |
| Total bacteria | 564a | 407ab | 315b | 54.40 | 0.02 |
| *F. succinogenes* | 1.00a | 0.80b | 0.15c | 0.06 | <0.01 |
| *R. flavefaciens* | 1.19 | 1.21 | 0.56 | 0.30 | 0.28 |
| *R. albus* | 1.02a | 0.64ab | 0.16b | 0.11 | 0.04 |

[a] ng per μl of extracted DNA.
[b] Fold change compared to control.
Within rows, means with different letters are significantly different ($P < 0.05$).

### 2.4. DNA extraction and real-time polymerase chain reaction

After thawing, rumen samples were shaken and transferred to 1.5 ml micro tubes containing glass beads and vortexed twice for 2 min with incubation on ice between shakings. This work allowed disruption of bacterial cell wall and detached bacteria from feed particles. Tubes were centrifuged at 200 × *g* for 5 min at 4 °C for the sedimentation of feeds particles. The supernatants (200 μl) were transferred to fresh 1.5 ml micro tubes and DNA extraction was performed using a genomic DNA extraction kit (AccuPrep™, Bioneer Corporation, South Korea) equipped with spin columns. Total bacterial, *F. succinogenes*, *R. flavefaciens* and *R. albus* rDNA concentrations were measured using real time PCR and the SYBR Green PCR Master Mix Kit (SYBR Green I qPCR Master Mix, Syntol, Russia) according to Valizadeh et al. (2010). The 16S rRNA gene-targeted primer sets used in the present study are described in Table 2. Amplification and detection were performed using an ABI 7300 (Applied Biosystems) sequence detection system. A bacterial rDNA standard curve was generated from DNA extracted from a mix (equal volumes) of 24 cultures of the following rumen bacterial strains grown on Hobson's medium 2 (Stewart et al., 1997): *Prevotella ruminicula 23*, *Butyrivibrio fibrisolvns SH13*, *Ruminococcus albus SY23*, *Prevotella albensis M384*, *Clostridium sticklandii 12 662*, *Peptostreptococcus anaerobius 27 337*, *Ruminococcus falvefaciens Fd1*, *Mitsuokella multiacidus D15d*, *Veillonela parvula L59*, *Prevotella bryantii B14*, *Prevotella brevis GA33*, *Lactobacillus casei LB17*, *Clostridium aminophilum 49 906*, *Streptococcus bovis ES1* and *Megasphera elsdenii J1*, all obtained from the Rowett Research Institute (Aberdeen, UK) culture collection. For total bacteria the threshold cycle of each standard dilution was determined during the exponential phase of amplification and regressed against the logarithm of known total bacterial DNA standards that had been prepared for each animal. Total bacteria population size is reported as nano gram (ng) per μl of extracted DNA.

Shift in the count of cellulolytic bacteria species of animals fed experimental diets was compared with those fed control diet in the same period using the methods of relative quantification (relative fold change in genomic DNA = $2^{-\Delta C_t}$, where $\Delta C_t = C_t$ treated $- C_t$ untreated, and $C_t$ is the cycle number at which the fluorescence generated within a reaction crosses the threshold). To achieve optimal relative expression results, all the relative comparisons were made on a constant basis of extracted DNA. Change in cellulolytic species reported as fold change in genomic DNA per μl of extracted DNA compare to control. All post-run data analyses were performed using SDS Software (Sequence Detector Software, V1.4).

### 2.5. Statistical analyses

The statistical model was:

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

where $Y_{ijkl}$ = observation *ijkl*; $\mu$ = the overall mean; $T_i$ = the effect of treatment *i*; SQ = the effect of square *j*; Period $(\mathrm{SQ})_{kj}$ = the effect of period *k* within square *j*; Sheep $(\mathrm{SQ})_{lj}$ = the effect of sheep *l* within square *j* and $\varepsilon_{ijkl}$ = random error with mean 0 and variance $\sigma^2$. Data were analyzed using the GLM procedure of SAS (V 9.0). Before statistical analyzing data were tested for normality using Proc UNIVARIATE in SAS (V 9.0).

## 3. Results

### 3.1. Population of total bacteria and cellulolytic bacteria in the rumen

The populations of total bacteria (ng per μl extracted DNA – control 564 versus LPH 407 versus HPH 315), *F. succinogenes* and *R. albus* decreased significantly as the level of PH in the diet increased ($P < 0.05$). The population of *R. flavefaciens* in the rumen liquor was not affected by the LPH diet but was reduced to approximately half the level of the control in the HPH diet (NS) (Table 3).

### 3.2. Ruminal fermentation parameters

Ruminal pH was increased (from 6.03 for control to 6.35 for LPH and 6.33 for HPH) with increasing the level of PH in the diets ($P < 0.01$) (Table 4). Inclusion of PH in the diet reduced total VFA concentration (control 95.0, LPH 76.3 and HPH 76.5 mmol/L; $P < 0.01$), individual VFA concentration (e.g. acetate control 47.6, LPH 35.1 and HPH 31.7 mmol/L; $P < 0.01$), and AN (control 193, LPH 173 and HPH 84.3 mg/L; $P < 0.01$) in the rumen liquor (Table 4).

Author's personal copy



**Table 4**
Rumen pH, and concentrations of ammonia nitrogen (AN) and volatile fatty acids (VFA) in rumen liquor of sheep fed diets including alfalfa hay (control) and/or pistachio hulls (PH) at one of two levels (low, L; high. H).

| | Diets | | | SE | *P* value |
|---|---|---|---|---|---|
| | Control | LPH | HPH | | |
| Rumen pH | 6.03b | 6.35a | 6.33a | 0.01 | <0.01 |
| Rumen AN (mg/L) | 193a | 173a | 84.3b | 1.82 | <0.01 |
| Total VFA (mmol/L) | 95.0[a] | 76.3[b] | 76.5[b] | 3.42 | <0.01 |
| *Individual VFA (mmol/L)* | | | | | |
| Acetate | 47.6a | 35.1b | 31.7b | 2.97 | <0.01 |
| Propionate + iso butyrate | 23.8a | 16.5c | 21.1b | 0.76 | <0.01 |
| Butyrate | 22.1 | 24.6 | 23.6 | 0.83 | 0.14 |
| Iso valerate | 1.40a | 0.00b | 0.00b | 0.06 | <0.01 |

Within rows, means with different letters are significantly different ($P < 0.05$).

## 4. Discussion

Population of total bacteria, *F. succinogenes*, *R. albus* and concentrations of AN, total VFA, acetic, propionic + iso butyric, and iso valeric acids in the rumen were decreased and ruminal pH was increased with increasing the level of PH in diets. These results are consistent with some previous studies (Newbold et al., 1997; Min et al., 2005; Bhatta et al., 2009). As mentioned before PH replacement for AH increased the levels of tannin in dietary treatments which may affect the population of cellulolytic and total bacteria in the rumen. Moreover, Guimaraes-Beelen1 et al. (2006) reported that tannins have negative effect on cellulolytic bacteria.

Lower ruminal AN concentration in sheep fed PH may have resulted from a greater concentration of tannins that bound to proteins and decreased proteolysis of feed protein and subsequently lowered the concentration of AN in the rumen fluid (Min et al., 2005). Some part of decrease in bacterial population might be related to this fact that tannins inhibit rumen microbial function by reducing the availability of AN for microbial use.

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 reduction in total and cellulolytic bacteria in the rumen supports these findings. The concentration of iso valeric acid was zero in PH containing diets. Iso-acids are derived from amino acid catabolism by cellulolytic bacteria in the rumen (Mackie and White, 1990). Concentrations of iso-acids were lower in the presence of PH tannin because the protein was protected from bacterial deamination. This result is consistence with the effect of PH on the production of AN in the rumen. Also, the reduction in cellulolytic bacteria population in the rumen supports these findings. Similar results were observed by Bhatta et al. (2009) and Krause et al. (2004). In contrast to our finding, Yildiz et al. (2005) reported no different in AN, VFA level and ruminal pH, in lambs receiving oak leaves. Inconsistency in these results to our finding could be related to difference in type of tannins, concentrations and dietary ingredient (Makkar, 2003b). Higher ruminal pH in sheep fed PH contained diets may have resulted from lower AN and VFA concentrations in the rumen. Ben Salem et al. (1997) reported an increase in ruminal pH with increasing amount of *Acacia cyanophylla* leaves in Barbarine sheep fed on AH.

## 5. Conclusion

The results from the present study indicate that inclusion of PH in the diet of Baloochi sheep decreased population of total and cellulolytic bacteria in the rumen. Moreover, PH replacement for AH decreased rumen AN and VFA concentrations and increased rumen pH. Future studies should focus on protozoa and proteolytic bacteria along with cellulolytic bacteria to determine the mechanism of tannin action in the rumen and interaction of these microorganisms together. Also, using tannin binding agents (e.g. PEG) and extracted tannins may help to define the overall effect of tannins on ruminal microorganisms in *in vivo* experiment.

## Acknowledgment

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.
AOAC, 1995. Official Methods of Analysis, 15th ed. Association of Official Agricultural Chemists, Washington, DC.
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. J. Anim. Vet. Adv. 8 (11), 2280–2285.
Ben Salem, H., Nefzaoui, A., Ben Salem, L., Tisserand, J.L., 1997. Effect of Acacia cyanophylla Lindl. foliage supply on intake and digestion by sheep fed lucerne hay-based diets. Anim. Feed Sci. Technol. 68, 101–113.
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. J. Dairy Sci. 9, 5512–5522.
Bohluli, A., Naserian, A.A., Valizadeh, R., Eftekarshahroodi, F., 2007. The chemical composition and in vitro digestibility of pistachio by-product. Proc. Br. Soc. Anim. Sci., 224.
FAO, Food and Agriculture Organization of the United Nations. 2005. Available from http://www.faostat.fao.org (accessed 12.12.10).
Frutos, P., Hervas, G., Giraldez, F.J., Mantecon, A.R., 2004. Review. Tannins and ruminant nutrition. Span. J. Agric. Res. 2 (2), 191–202.
Gholizadeh, H., Naserian, A.A., Valizadeh, R., Tahmasebi, A., 2009. Effects of feeding pistachio hull and interaruminal infusion of urea on feed intake, ruminal and abomasum N–$NH_3$ and blood metabolites in Iranian Baloochi sheep. Proc. Br. Soc. Anim. Sci., 163.
Gholizadeh, H., Naserian, A.A., Valizadeh, R., Tahmasebi, A., 2010. Effect of feeding pistachio byproduct on performance and blood metabolites in Holstein dairy cows. Int. J. Agric. Biol. 12, 867–870.

Author's personal copy



Goel, G., Puniya, A.K., Singh, K., 2005. Xylanolytic activity of ruminal *Streptococcus bovis* in presence of tannin acid. Annu. Microbiol. 55, 295–297.

Guimaraes-Beelen1, P.M., Berchielli, T.T., Buddington, R., Beelenl, R., 2006. Effects of condensed tannins from northeastern semi-arid shrubs on growth and cellulolytic activity of *Ruminococcus flavefaciens* FD1. Arq. Bras. Med. Vet. Zootec. 58, 910–917.

Hashami, O., Naserian, A., Tahmasbi, A.M., Valizadeh, R., Mohammadabadi, T., 2008. Degradability of pistachio by-products at different harvesting times. Proc. Br. Soc. Anim. Sci., 251.

Koike, S., Kobayashi, Y., 2000. Development and use of competitive PCR assays for the rumen cellulolytic bacteria: *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*. FEMS Microbiol. Lett. 204, 361–366.

Komolong, M.K., Barber, D.G., McNeil, D.M., 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. Anim. Feed Sci. Technol. 92, 59–72.

Krause, D.O., Smith, W.J.M., McSweeney, S., 2004. Use of community genome arrays (CGAs) to assess the effects of Acacia angustissima on rumen ecology. Microbiology 150, 2899–2909.

Mackie, R.I., White, B.A., 1990. Recent advances in rumen microbial ecology and metabolism: potential impact on nutrient out put. J. Dairy Sci. 73, 2971–2995.

Maeda, H., Fujimoto, C., Haruki, Y., Maeda, T., Kokeguchi, S., Petelin, T., Arai, H., Tanimoto, I., 2003. Quantitative real-time PCR using TaqMan and SYBR green for Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, tetQ gene and total bacteria. FEMS Immunol. Med. Microbiol. 39, 81–86.

Makkar, H.P.S., 2003a. Quantification of tannins in tree and shrub foliage. In: Makkar, H.P.S. (Ed.), A Laboratory Manual. Kluwer Academic Publishers, p. 102.

Makkar, H.P.S., 2003b. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Res. 49, 241–256.

McSweeney, C.S., Palmer, B., Bunch, R., Krause, D.O., 2001. Effect of the tropical forage Calliandra on microbial protein synthesis and ecology in the rumen. J. Appl. Microbiol. 90, 78–88.

Min, B.R., Attwood, G.T., McNabb, W.C., Molan, A.L., Barry, T.N., 2005. The effect of condensed tannins from Lotus corniculatus on the proteolytic activities and growth of rumen bacteria. Anim. Feed Sci. Technol. 121, 45–58.

Mosoni, P., Chaucheyras-Durand, F., Bera-Maillet, C., Forano, E., 2007. Quantification by real-time PCR of cellulolytic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates: effect of a yeast additive. J. Appl. Microbiol. 103, 2676–2685.

Nelson, K.E., Pell, A.N., Doane, P.H., Giner-Chavez, B.I., Schofield, P., 1997. Chemical and biological assays to evaluate bacterial inhibition by tannins. J. Chem. Ecol. 23, 1175–1194.

Newbold, C.J., Elhassan, S.M., Wang, J., Ortega, M.E., Wallace, R.J., 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. Br. J. Nutr. 78, 237–249.

SAS (Statistical Analysis System), 2001. User's Guide: Statistics, Version 9.1. SAS Inst., Carry, NC, USA.

Stewart, C.S., Flint, H.J., Bryant, M.P., 1997. The Rumen Bacteria. The Rumen Microbial Ecology. Blackie Academic, Professional, London, New York.

Vahmani, P., Naserian, A.A., 2006. Pistachio hulls as a feed ingredients for lactating dairy goats. Proc Br. Soc. Anim. Sci., 145.

Valizadeh, R., Behgar, M., Mirzaee, M., Naserian, A.A., Vakili, A.R., Ghovvati, S., 2010. The effect of physically effective fiber and soy hull on the ruminal cellulolytic bacteria population and milk production of dairy cows. Asian-Aust. J. Anim. Sci. 23, 1325–1332.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in ration to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Vanzant, E.S., Cochran, R.C., 1994. Performance and forage utilization by beef cattle receiving increasing amounts of alfalfa hay as a supplement to low-quality, tallgrass-prairie forage. J. Anim. Sci. 72, 1059–1067.

Wanapat, M., Cherdthong, A., 2009. Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in swamp buffalo. Curr. Microbiol. 58, 294–299.

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. Anim. Feed Sci. Technol. 122, 159–172.

Zhang, C., Guo, Y., Yuan, Z., Wu, Y., Wang, J., Liu, J., Zhu, W., 2008. Effect of octadeca carbon fatty acids on microbial fermentation, methanogenesis and microbial flora in vitro. Anim. Feed Sci. Technol. 146, 259–269.