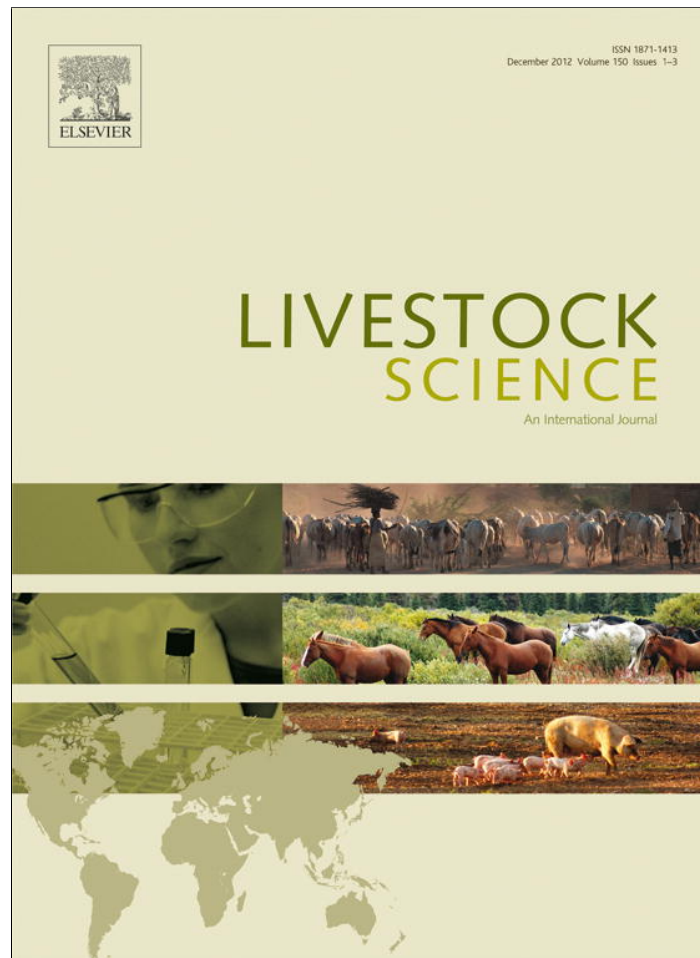
Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



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

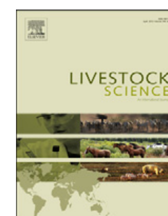
Author's personal copy

Livestock Science 150 (2012) 342–348

Contents lists available at SciVerse ScienceDirect

Livestock Science

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



Partial and total substitution of alfalfa hay by pistachio byproduct modulated the counts of selected cellulolytic ruminal bacteria attached to alfalfa hay in sheep

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

[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

ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form
27 September 2012
Accepted 28 September 2012

Keywords:
Real-time PCR
Cellulolytic bacteria
Attachment
Sheep
Pistachio hull

ABSTRACT

This study was conducted to evaluate the effect of partial and total substitution of alfalfa hay (AH) by pistachio (*Pistacia vera*) by-product (PB) on counts of selected cellulolytic ruminal bacteria attached to AH by real-time PCR technique and to determine the effect of this replacement on degradability of AH in Baloochi sheep. Six Baloochi sheep, averaging 40.1 ± 1.77 kg BW, fitted with ruminal cannula were assigned at random to three diets in a double 3 × 3 Latin square design. The dietary treatments were control (basal diet), low PB (LPB) diet (0.50 of AH in basal diet replaced by PB), and high PB (HPB) diet (all of the AH in the basal diet replaced by PB). 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. Alfalfa hay was placed in to the rumen for *in situ* degradability of DM, organic matter (OM) and microbial attachment. Bacterial populations were assessed by DNA extraction of incubated alfalfa samples followed by real-time polymerase chain reaction analysis. The population of *Fibrobacter succinogenes* and *Ruminococcus albus* that were attached to AH decreased with increasing the level of PB in diets ($P<0.05$). The effect of dietary treatments on the population of *Ruminococcus flavefaciens* and total bacteria was not significant. PB replacement for AH decreased ruminal DM and OM degradability of AH ($P<0.05$). It was concluded that tannins from PB reduced the attachment of cellulolytic bacteria as well as *in situ* degradability of AH.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Pistachio farming is one of main activity in agriculture sector of central Iran and according to the Food and Agriculture Organization (FAO, 2005) Iran is the largest pistachio producer in the world. Pistachio by product (PB) contains 158.2 g/kg 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 PB can be used as a feedstuff for ruminants (Gholizadeh et al., 2009, 2010; Shakeri and Fazaeli, 2005; Vahmani and Naserian, 2006). However, PB contains high level of phenolic compounds such as tannins, which can affect their nutrient utilization by animals (McSweeney et al., 2001). Phenolic contents of PB include 75–95 g/kg total phenol and 35–45 g/kg tannin (Bohluli et al., 2007).

Microbial adhesion to plant material is the first step in the degradation of insoluble nutrients (Cheng et al., 1990).

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

1871-1413/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.livsci.2012.09.024

Author's personal copy

The cellulolytic bacteria *Ruminococcus albus, Ruminococcus flavefaciens*, and *Fibrobacter succinogenes* adhere to or are found in close proximity to plant cell wall material undergoing degradation in the rumen (Bento et al., 2005).

Tannins may affect the adhesion of rumen microorganisms to the substrate through binding to the substrate (Artz et al., 1987; Asquith and Butler, 1986; Barry et al., 1986; Makkar et al., 1995), or to the bacterial cell surface (Bae et al., 1993; Jones et al., 1994; Molan et al., 2001). It has been suggested that phenolic monomers decrease cellulose and xylan digestion by inhibiting the attachment of ruminal cellulolytic bacteria like *F. succinogenes* to fiber particles (Akin et al., 1988; Varel and Jung, 1986). Also, filter paper digestion and endoglucanase activity by *F. succinogenes*, were inhibited in a dose dependent manner by purified condensed tannin (CT) (Bae et al., 1993).

However, it is notable that the effects of tannins on attachment of bacteria to cell wall vary depending on the tannin type and the plant material (Bento et al., 2005). Very few reports can be found on the effect of tannin sources and the concentration at which they affect the attachment of rumen cellulolytic bacteria to plant cell wall.

In previous works, the abilities of rumen cellulolytic bacteria to attach to plant fibers and the mechanisms of this attachment have been studied using pure cultures (Minato and Suto, 1978; Mosoni et al., 1997; Pegden et al., 1998). Although numerous important findings were obtained from these *in vitro* studies, it is difficult to track specific bacterial species quantitatively *in vivo* using traditional enumeration techniques such as culturing. The kinetics of fiber-attachment of ruminal cellulolytic bacteria was studied by different methods, such as PCR based methods (Koike et al., 2003) and isotope labeling methods (Bento et al., 2005). Authors could not found any information about the effects of PB tannin on the attachment of rumen cellulolytic bacteria to plant cell wall. The objective of the present study was to investigate the effect of partial and total substitution of AH by PB on counts of selected cellulolytic ruminal bacteria attached to AH by real-time PCR technique and to determine the effect of this replacement on degradability of AH in Baloochi sheep.

2. Material 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 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), LPB diet (0.5 of AH in basal diet replaced by PB) and HPB diet (whole of AH in basal diet replaced by PB). The diets were formulated for maintenance requirements according to AFRC (1993). Basal diet consisted of 400 g AH DM + 200 g wheat straw DM + 200 g barley based concentrate DM. The ingredients and chemical composition of the experimental diets are shown in Table 1. PB was the current year's annual growth, hand-harvested near Bardaskan (Iran) during summer 2009. Harvested PBHH was sun cured before use. Chemical composition of PB is shown in Table 2.

Trial consisted of three periods and each experimental period consisted of 19d adaptation and 7d data collection including *in sacco* incubation of AH and measurement of rumen cellulolytic bacteria attachment to AH.

2.2. Sample preparation and *in sacco* incubation of AH

Alfalfa hay as a source of fiber was used for nylon bag degradability of DM and OM. Samples were milled (2 mm) and then stored at 4 °C until ready for incubation. *In sacco* degradability was determined according to the method of Orskov (1984) and it was not effective because the rate of passage was not measured. During the experimental periods, about 5 g (DM basis) of AH sample was placed in a 12 cm × 8.5 cm, 50 μm pore size polyester bag.

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

	Diets		
	Control	LPB†	HPB†
Ingredients (g/kg DM)			
Alfalfa hay	400.0	200.0	0.0
Wheat straw	200.0	200.0	200.0
Pistachio by-product	0.0	200.0	400.0
Barley grain	168.0	166.0	164.0
Cotton seed meal	24.0	25.6	27.2
Vitamin–mineral premix	6.4	6.4	6.4
Salt	1.6	1.6	1.6
Lime	0.0	0.4	0.8
Chemical composition (g/kg DM)			
DM	918.1	915.6	909.5
OM	904.7	902.3	905.4
CP	118.0	118.0	117.4
EE	14.4	24.8	35.8
NDF	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

† LPB (low pistachio by product), HPB (high pistachio by product).

Table 2
Chemical composition of pistachio by-product (PB) (g/kg DM).

Item	Amount
DM	900.0
OM	755.3
EE	58.0
CP	153.1
NDF	259.4
Total phenol	78.5
Tannin	31.6
Condensed tannin	8.5

Author's personal copy

Table 3
PCR primers utilized for amplifying the target bacteria.

Target species	Forward/reverse	Primer sequence	References
Total bacteria	F	GTGSTGCAYGGYTGTCGTCA	Maeda et al. (2003)
	R	ACGTCRTCCMCACCTTCCTC	
F.succinogens	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 (2001)
	R	CCTCCTTGCGGTTAGAACA	

The incubations were performed in triplicate (three bags per time) in the rumen of each sheep immediately prior to feeding.

Bags were removed from the rumen at 2 h, 4 h, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h. For time 0, nylon bags were rinsed thoroughly under tap water until the rinsed water is cleared. After removal from the rumen, bags were thoroughly washed under running cold water until it ran clear, and dried in an air-forced oven at 65 °C for 48 h to determine DM losses. Residues were analyzed for OM by standard procedures (AOAC, 1998) to determine OM losses. The percentage disappearance of DM and OM from the bags at each incubation time was calculated from the proportion remaining after incubation in the rumen.

2.3. Attachment of rumen cellulolytic bacteria and total bacteria to AH

Attachment of total and rumen cellulolytic bacteria to AH was determined according to the method of Koike et al. (2003). Using PCR based method for measuring attachment of ruminal cellulolytic bacteria, is a new method. On last day of each period, four nylon bags containing chopped AH were placed into the rumen of each sheep immediately prior to feeding. Bags were removed from the rumen at 24 h after incubation. The bags were rinsed thoroughly in water (38 °C) until it ran clear and then squeezed by hand to remove excess water, and then stored at −20 °C until analysis for microbial attachment.

2.4. Chemical analyses

Samples of feed were analysed for DM, OM, EE and nitrogen by standard procedures (AOAC, 1998). NDF was determined according to Van Soest et al. (1991) without amylase application. Total phenols, tannins and CT (butanol procedure) in PB and dietary treatments were determined in aqueous acetone (70:30, acetone:distilled water) extracts, as described by Makkar (2003).

2.5. DNA extraction

The incubated bags were freeze dried. Then the samples (20–50 mg) were homogenized with a mortar and pestle and transferred to 1.5 ml tubes containing glass beads and 200 μl tissue lysis buffer 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 feed particles. The supernatants (200 μl) were transferred to fresh 1.5 ml tubes and DNA extraction was performed using a genomic DNA extraction kit (AccuPrepTM, Bioneer Corporation, South Korea) equipped with spin columns.

2.6. Real-time polymerase chain reaction design and assay conditions

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). The 16S rRNA gene-targeted primer sets used in the present study are described in Table 3. Templates (1 μl) were added to amplification reactions (25 μl) containing 0.6 μl of primer mixture containing 10 pmol of each primer, 11.5 μl of SYBR Green I qPCR Master Mix and 12 μl of deionized water. SYBR Green I qPCR Master Mix contained KCl, Tris–HCL (pH 8.8), 6.25 mM $MgCl_2$, dNTP, Taq DNA polymerase, Tween, and SYBR Green I. A no-template (sterile distilled water) negative control was loaded on each plate run to screen for contamination and dimmer formation and to set the background fluorescence for plate normalization. Amplification and detection were performed using an ABI 7300 (Applied Biosystems) sequence detection system under the following conditions: initial denaturation at 95 °C for 5 min was followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 61 °C for 15 s, extension at 72 °C for 30 s, and then by the melting curve program (60–95 °C with a heating rate of 0.1 °C per second and a continuous fluorescence measurement). Simultaneously, DNA extracted from each animal was subjected to real-time qPCR for all of the bacteria (total and 3 cellulolytics). 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*

Author's personal copy

Table 4
Population of attached bacteria to alfalfa hay (AH) cell wall.

	Diets				
	Control	LPB[†]	HPB[†]	SEM	*P* value
Total bacteria[d]	6373	3341	6235	1076.58	0.13
F. succinogenes[e]	1.635[a]	1.047[b]	0.955[c]	0.019	< 0.01
R. flavefaciens[e]	1.000	1.127	1.057	0.103	0.70
R. albus[2]	1.437[a]	0.860[b]	0.322[c]	0.087	< 0.01

[a] Within rows, means with different letters are significantly different ($P<0.05$).
[b] Within rows, means with different letters are significantly different ($P<0.05$).
[c] Within rows, means with different letters are significantly different ($P<0.05$).
[d] ng per μl of extracted DNA.
[e] Fold change compared to control.
[†] LPB (low pistachio by product), HPB (high pistachio by product).

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.

Count of cellulolytic bacteria species attached to AH incubated in sheep fed PB containing diets were compared with those fed control diet 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. All post-run data analyses were performed using SDS Software (Sequence Detector Software, V1.4).

2.7. Statistical analyses

Dry matter and OM disappearances were fitted to the model described by Orskov and McDonald (1979).

$P=a+b(1-e^{-ct})$, where P represents the loss from the bag after t h, a the fraction that immediately disappears from the bag (intercept), b the fraction that is potentially degraded over time and c the rate of degradation of fraction b. The equation was fitted to *in situ* degradation profiles using the NLIN procedure of the SAS (V. 9.0) package.

The statistical model was:

$Y_{ijkl}=\mu+T_i+SQ_j+\text{Period }(SQ)_{kj}+\text{Sheep }(SQ)_{lj}+\varepsilon_{ijkl}$, where Y_{ijkl}=observation $ijkl$; $\mu=$ 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 ε_{ijkl}=random error with mean 0 and variance $\sigma 2$. All 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). The tukey test was used to assess the significance of differences between treatment means where the overall treatment effect was significant ($P<0.05$).

3. Results

3.1. Attachment of total and cellulolytic bacteria to AH cell wall

Data generated from real-time PCR assays for total bacteria are expressed as ng per μl of extracted DNA, while quantity of cellulolytic bacteria (*F. succinogenes*, *R. flavefaciens* and *R. albus*) are expressed as fold changes in genomic DNA compared to control. Population of *F. succinogenes* and *R. albus* that were attached to AH cell wall decreased with increasing the level of PB in diets ($P<0.05$) (Table 4). The effect of dietary treatments on the population of *R. flavefaciens* and total bacteria was not significant.

3.2. Dry matter and organic matter degradability of AH

In situ ruminal DM and OM degradability and the rumen degradation parameters (a, b and c values) for DM and OM of AH are given in Table 5. The degradability was not effective. The results indicate that ruminal DM and OM degradability, immediately degradable fraction (a), potential degradable fraction (b) and rate of degradation(c) of AH decreased as the level of PB in the diet increased ($P<0.05$).

4. Discussion

Population of *F. succinogenes* and *R. albus* that were attached to AH decreased with increasing the level of PB in diets of sheep. PB replacement for AH increased the levels of tannin in dietary treatments (9.1 g/kg, 29.5 g/kg and 42.5 g/kg for control, LPB and HPB, respectively) which may affect the attachment of cellulolytic bacteria to AH cell wall. Similar to our finding, Bento et al. (2005) observed that the addition of mimosa tannin to cellulose reduced microbial attachment compared with cellulose alone in *in vitro* trials. Previous studies demonstrated that

Author's personal copy

Table 5
Ruminal degradation profiles of alfalfa hay (AH).

	Diets				
	Control	LPB[†]	HPB[†]	SEM	*P* value
DM					
a-value (g/g incubated)	0.4023[a]	0.40[b]	0.3787[c]	0.00031	< 0.01
b-value(g/g incubated)	0.3755[a]	0.3698[b]	0.3427[c]	0.00089	< 0.01
c (per hour)	0.094[a]	0.089[b]	0.076[c]	0.0007	< 0.01
$a+b$ (g/g incubated)	0.7778[a]	0.7699[b]	0.7214[c]	0.00098	< 0.01
OM					
a-value (g/g incubated)	0.4085[a]	0.4021[b]	0.3838[c]	0.00047	< 0.01
b-value(g/g incubated)	0.3624[a]	0.3598[a]	0.3477[b]	0.00088	< 0.01
c (per hour)	0.124[a]	0.111[b]	0.101[c]	0.0015	< 0.01
$a+b$ (g/g incubated)	0.7709[a]	0.7619[b]	0.7315[c]	0.00133	< 0.01

[a] Within rows, means with different letters are significantly different ($P < 0.05$).
[b] Within rows, means with different letters are significantly different ($P < 0.05$).
[†] LPB (low pistachio by product), HPB (high pistachio by product).

the presence of tannin in pure cultures *in vitro* resulted in the formation of tannin–protein complexes on the cell surface of *F. succinogenes*, suggesting that the tannin may interfere with the adhesion process (Bae et al., 1993). Therefore, it is likely that the reduction in microbial attachment is related to binding of tannin to bacterial cell surface (Molan et al., 2001). Moreover, Makkar et al. (1995) showed that tannin may affect the adhesion of rumen microorganisms to the substrate through binding to it.

In the present study, PB tannin may have bound to AH cell wall resulting in a reduction in the availability of binding sites on cell wall for microorganisms. The results presented here are in agreement with previous findings, suggesting that tannins may form strong complexes with substrate and reduce adhesion of microbes (Makkar et al., 1995). Moreover, tannins are known for their antimicrobial activity (Goel et al., 2005; Guimaraes-Beelen1 et al., 2006) either on cellulolytic or proteolytic bacteria and previous studies showed that tannin reduced cellulolytic bacteria population in *in vivo* (Newbold et al., 1997) and *in vitro* trials by inhibiting microbial growth (Bhatta et al., 2009; Min et al., 2005). Our previous study showed that PB tannin reduced cellulolytic bacteria population in the rumen of sheep (Ghasemi et al., 2012). So, the reduced microbial attachment caused by tannin, is supported by the reduction in microbial population in the rumen (Ghasemi et al., 2012). In the present study, *R. flavefaciens* counts were not affected by PB tannin. This different susceptibility of bacteria to tannins may have resulted from the different mechanisms of attachment to substrate (Bento et al., 2005).

In the present study partial and total substitution of AH by PB increased EE (control 14.4 g/kg DM versus HPB 35.8 g/kg) and decreased NDF (control 504.7 g/kg DM versus HPB 415.7 g/kg) content of experimental diets (Table 1). It is well known that these 2 parameters affect microbial activity and their attachment to substrate. However, the level of EE in HPB diet was below the general recommendation (60–70 g/kg DM) that affect microbial activity (Jenkins, 1993; NRC, 2001). Wiemer et al. (1999) reported that decrease dietary NDF to 22.9% of DM did not affect cellulolytic bacteria in the rumen of lactating dairy cows. They suggest that shift in ruminal microbial population is resulted from interaction between many factors that diet is one of these components. In the present study the level of NDF in HPB was 41.57% of DM that was higher than this amount.

Ruminal DM and OM degradability, immediately degradable fraction (*a*), potential degradable fraction (*b*) and rate of degradation(*c*) of AH decreased with inclusion of more PB in diets. Similar observations were obtained by Balogun et al. (1988), Hervas et al. (2000), Makkar et al. (1995), and Martinez et al. (2004) when studying different doses of tannins. The reduction in attachment of bacteria to AH cell wall supports these findings. The reduction of the extent of degradation of the AH by the treatments was basically due to a marked reduction of the immediately degradable fraction (*a*) and lower rate of degradation (*c*) which was observed in HPB treatment. Kumar and Vaithiyanathan (1990) have proposed that tannins reduced the rate of plant fiber degradation in the rumen by reducing the availability of protein nitrogen and sulphur for microbial use. As mentioned before, PB tannin may have bound to substrate cell wall and resulting in a reduction in degradability of them. Similar to our finding, Bohluli and Naserian (2007) showed that PB could limit digestibility of AH and soybean meal in *in vitro* condition. Moreover, Bae et al. (1993) reported that digestion of filter paper by *F. succinogenes* was reduced in the presence of tannin.

5. Conclusion

In conclusion, the results from the present study indicate that inclusion of PB in diet of Baloochi sheep decreased the attachment of cellulolytic bacteria to AH. Moreover, PB replacement for AH decreased ruminal DM and OM degradability, immediately degradable fraction (*a*), potential degradable fraction (*b*) and rate of degradation(*c*) of AH. Future studies should focus on using tannin binding agents (e.g., PEG) to define the overall effect

Author's personal copy

of tannins on attachment of ruminal microorganisms and degradability of different sources of feed in *in sacco* incubation.

Conflict of Interest

None of the authors of the manuscript entitled 'Partial and total substitution of alfalfa hay by pistachio byproduct modulated the counts of selected cellulolytic ruminal bacteria attached to alfalfa hay in sheep' has declared any conflict of interest within the last years which may arise from being named as an author on the manuscript.

Acknowledgments

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.

Akin, D.E., Rigsby, L.L., Theodorou, M.K., Hartley, R.D., 1988. Population changes of fibrolytic rumen bacteria in the presence of phenolic acids and plant extracts. Anim. Feed Sci. Technol. 19, 261–275.

Artz, W.E., Bishop, P.D., Dunker, A.K., Schanus, E.G., Swanson, B.G., 1987. Interaction of synthetic proanthocyanidin dimer and trimer with bovine serum albumin and purified bean globulin fraction G-1. Agric. Food. Chem. 35, 417–421.

Association of Official Analytical Chemists, 1998. Official Methods of Analysis, sixteenth ed. AOAC, Washingtown, DC.

Asquith, T.N., Butler, L.G., 1986. Interactions of condensed tannins with selected proteins. Phytochemistry 25, 1591–1593.

Bae, H.D., McAllister, T.A., Yanke, J., Cheng, K.J., Muir, A.D., 1993. Effects of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Appl. Environ. Microbiol. 59, 2132–2138.

Balogun, R.O., Jones, R.J., Holmes, J.H.G., 1988. Digestibility of some tropical browse species varying in tannin content. Anim. Feed Sci. Technol. 76, 77–88.

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

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.

Bento, M.H.L., Acamovic, T., Makkar, H.P.S., 2005. The influence of tannin, pectin and polyethylene glycol on attachment of 15N-labelled rumen microorganisms to cellulose. Anim. Feed Sci. Technol. 122, 41–57.

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., 2007. Pistachio hull tannin affected digestibility of soybean meal and alfalfa during *in vitro* digestion. J. Dairy Sci. 90 (1), 99.

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.

Cheng, K.J., McAllister, T.A., Kudo, H., Costerton, J.W., 1990. The importance of adhesion in the microbial digestion of plant materials. In: Hoshino, S. (Ed.), The Rumen Ecosystem, The Microbial Metabolism and its Regulation, pp. 129–135.

FAO, Food and Agriculture Organization of the United Nations. 2005. 〈http://www.faostat.fao.org〉 (accessed 12. 12. 2010.).

Ghasemi, S., Naserian, A.A., Valizadeh, R., Tahmasebi, A.M., Vakili, A.R., Behgar, M., Ghovvati, S., 2012. Inclusion Of Pistachio Hulls As a Replacement for Alfalfa Hay in the Diet of Sheep Causes a Shift in the Rumen Cellulolytic Bacterial Population. Small ruminant Res. 104, 94–98.

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.

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.

Hervas, G., Frutos, P., Serrano, E., Mantecon, A.R., Giraldez, F.J., 2000. Effect of tannic acid on rumen degradation and intestinal digestion of treated soya bean meals in sheep. J. Agric. Sci. 135, 305–310.

Jenkins, T.C., 1993. Lipid methabolism in the rumen. J. Dairy Sci. 76, 3851–3863.

Jones, G.A., McAllister, T.A., Muir, A.D., Cheng, K.J., 1994. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Appl. Environ. Microbiol. 60, 1374–1378.

Koike, S., Kobayashi, Y., 2001. 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.

Koike, S., Pan, J., Kobayashi, Y., Tanaka, K., 2003. Kinetics of *in sacco* fiber-attachment of representative ruminal cellulolytic bacteria monitored by competitive PCR. J. Dairy Sci. 86, 1429–1435.

Kumar, R., Vaithiyanathan, S., 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30, 21–38.

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., Blummel, M., Becker, K., 1995. *In vitro* effects of and interactions between tannins and saponins and fate of tannins in the rumen. J. Sci. Food Agric. 69, 481–493.

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

Martinez, T.F., Moyano, F.J., Diaz, M., Barroso, F.G., Alarco, F.J., 2004. Ruminal degradation of tannin-treated legume meals. J. Sci. Food Agric. 84, 1979–1987.

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.

Minato, H., Suto, T., 1978. Technique for fractionation of bacteria in rumen microbial ecosystem. II. Attachment of bacteria isolated from bovine rumen to cellulose powder in vitro and elution of bacteria attached therefrom. J. Gen. Appl. Microbiol. 24, 1–16.

Molan, A.L., Attwood, G.T., Min, B.R., McNabb, W.C., 2001. The effect of condensed tannins from *Lotus pedunculatus* and *Lotus corniculatus* on the growth of proteolytic rumen bacteria *in vitro* and their possible mode of action. Can. J. Microbiol. 47, 626–633.

Mosoni, P., Fonty, G., Gouet, P., 1997. Competition between ruminal cellulolytic bacteria for adhesion to cellulose. Curr. Microbiol. 35, 44–47.

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.

NRC, 2001. Nutrient Requirement of Dairy Cattle, seventh ed. National Academic Press, Washington, DC., USA.

Orskov, E.R., 1984. Evaluation of crop residues and agro-industrial by-products using the nylon bag method. In: Preston, T.R., Kossila, V.V., Goodwin, J., Reed, S. (Eds.), Proceedings of FAO/ILCA Expert Consultation, ILCA, Addis Ababa, Ethiopia.

Author's personal copy

Orskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J. Agric. Sci. 92, 499–503.
Pegden, R.S., Larson, M.A., Grant, R.J., Morrison, M., 1998. Adherence of the Gram-positive bacterium *Ruminococcus albus* to cellulose and identification of a novel form of cellulose-binding protein which belongs to the Pil family of proteins. J. Bacteriol. 180, 5921–5927.
SAS User's Guide: Statistics, Version 9.0 Edition. SAS Inst. Inc., Cary, NC.
Stewart, C.S., Flint, H.J., Bryant, M.P., 1997. The Rumen Bacteria. The Rumen Microbial Ecology. Blackie Academic and Professional, London and New York.
Shakeri, P., Fazaeli., H., 2005. Effect of Diets Contained Pistachio by-product on the Performance of Fattening Lambs. Iv International Symposium on Pistachio and Almond. ISHS, Tehran, Iran, Pp. 180-181.
Vahmani, P., Naserian, A.A., 2006. Pistachio hulls as a feed ingredients for lactating dairy goats. Proc. Br. Soc. Anim. Sci., p.145.
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.
Varel, V.H., Jung, H.G., 1986. Influence of forage phenolics on ruminal fibrolytic bacteria and *in vitro* fiber degradation. Appl. Environ. Microbiol. 52, 275–280.
Wiemer, P.J., Waghorn, G.C., Odt, C.L., Mertens, D.R., 1999. Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. J. Dairy Sci. 82, 122–134.
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.