www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Growth performance and nitrogen retention in lambs fed diets containing two different levels of crude protein supplemented with pistachio by-product extract as a source of tannins

Rajaei Sharifabadi H.* and Naserian A.A.

Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

The effects of pistachio by-products aqua extract (PBE) on growth performance and retention of dietary nitrogen (N) were investigated in finishing lambs fed diets containing 11.3% DM (low protein; LP) or 13.9% DM (high protein; HP) crude protein. Two groups (n = 10) of Baluchi ram lambs (4-5 months old, 26.25±3.30 kg initial BW) were allotted to LP and HP treatments. In each group, one half of the lambs received experimental diets mixed with 500 ml liquid fresh PBE per kg DM of diet. Thus, four experimental diets were tested: LP diet without PBE supplementation; LP-PEB, LP diet with PBE supplementation; LP+PBE, HP diet without PBE supplementation; HP-PBE and HP diet with PBE supplementation; HP+PBE. Feed intake, average daily gain (ADG) and feed conversion ratio (FCR) were recorded for a 50 d experimental period. Plasma urea (PUN) and ruminal NH_3-N concentrations were measured at 0, 2 and 4 h after morning feeding on the 35th d of the experiment. Apparent total tract digestibility of DM, organic matter (OM) and N as well as urinary losses and retention of N were also determined for 5 days at the end of the experiment. Although there was a tendency for feed intake to be lower (P=0.102) in LP compared with HP fed lambs, the dietary CP level had no effect on ADG and FCR. In contrast, PBE supplementation improved both growth rate (P=0.021) and FCR (P=0.104) of lambs. The urinary losses of N and also PUN and ruminal NH_3-N concentrations at h 2 and 4 after feeding were decreased and the retention of N was increased by PBE supplementation of diets. However, these effects of PBE were only significant in the lambs fed HP diet. The concentrations of PUN (0 and 4 h after feeding) and ruminal NH_3-N (4 h after feeding) were affected positively by dietary CP level. Although the lambs feeding on the LP diet retained a higher percentage of N intake or N digested, no difference in daily N retained was observed between LP and HP fed lambs. The experimental treatments had no effect on DM, OM and N apparent digestibility. Our results indicated that the low concentration of PBE tannins have a protein-sparing potential depend on dietary CP density, thereby improving growth performance and N retention in finishing lambs.

Keywords: Growth performance; pistachio by-product; lamb; N retention; tannins

To cite this article: Rajei Sharifabadi HR and AA Naserian, 2014. Growth performance and nitrogen retention in lambs fed diets containing two different levels of crude protein supplemented with pistachio by-product extract as a source of tannins. Res. Opin. Anim. Vet. Sci., 4(5): 273-280.

Introduction

The ruminant animals are very inefficient for dietary nitrogen (N) utilization and there is a growing interest in improving intake-N efficiency in order to reduce feed costs as well as to abate environmental pollution. The low efficiency is mainly associated with N losses as a result of microbial degradation of dietary proteins to ammonia (Lapierre and Lobley, 2001). Hence, many feed additives have been developed to improve N efficiency by decreasing of ammonia production in the rumen, among which ionophore antibiotics have been very successful (Hutjens, 1992). However, anxiety about antibiotic residues in milk and meat has limited use of antibiotics in the animal feeds.

***Corresponding author:** Rajaei Sharifabadi H, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran; Tell: +98-9371940594

As a safe antibiotic alternative, the plant phenolic compounds, particularly tannins, have a protective potential against protein degradation in the rumen and increase rumen escape protein and supply of essential amino acids for the small intestine (Lamy et al., 2011). It is generally agreed that tannins improve N metabolism and efficiency of protein utilization in the ruminants depending on several factors such as their concentration, type and molecular weight (Patra and Saxena, 2011) as well as diet composition, animal species and physiological state of the animal (McSweeny et al., 2001). Due to the beneficial effects of tannins on protein metabolism, some commercial tannins such as quebracho have been proposed for improving N utilization in the ruminants (Frutos et al., 2000; Fernández et al., 2012). Thus a protein-sparing effect can be expected for tannins. There is evidence that protein-sparing compounds such as monensin (Perry et al., 1983; Hanson and Klopfenstein, 1979) and rumen-protected CLA (Schiavon et al., 2010; Schiavon et al., 2012) have been used successfully for reduction of dietary CP density and N losses in ruminants. Recently, Hart et al. (2011) used low tannin pea silage (LTPS) for reducing percentage of soybean meal in the ration of lambs and suggested a concentration sparing effect for LTPS. However, a little information is available on the effects of tannins on animal performance when dietary crude protein (CP) density is lower than the recommended optimum level.

Pistachio by-products (PB), a pistachio de-hulling by-products soon after harvest, contain high level of phenolic compounds, including tannins (Bagheripour et al., 2008; Mokhtaropur et al., 2012). It is has been reported that the phenolic compounds of PB are water extractable (Mokhtarpour et al., 2014). Therefore, PB and also its aqua extract can be used as an affordable source of tannins in pistachio producing countries such as Iran.

The subject of the current study was to investigate the effect of the pistachio by-products extract, as a source of tannins, on the performance and N retention of lambs fed two different levels of protein with their diets.

Materials and Methods

The animals in this study were selected from the flock at Animal Research Station of Ferdowsi University of Mashhad, Mashhad, Iran and were housed and cared for in accordance with the institutionally approved animal care and use protocol. Pistachio by-products were provided from pistachio de-hulling factories in Feizabad (Khorasan e Razavi, Iran).

Two isoenergetic diets containing 11.3% DM (Low protein; LP) or 13.9% of DM (high protein; HP) crude protein were prepared with 30% corn silage and 70% concentrate consisting of whole barley grain, soybean meal, vitamin and mineral premix, calcium carbonate and salt (Table 1). Two groups (n=12 lambs per group) of Baluchi ram lambs (4-5 months old, 26.25±3.30 kg BW) were housed in 1×1 m individual cages and fed individually with the low protein (LP) or high protein (HP) diets twice daily at 0700 and 1400. Fresh water was freely available during the experiment. In each experimental group, one half of the lambs (n = 5) received diets mixed with 500 ml of liquid fresh pistachio by-products aqua extract (PBE) per kg DM of diet. Thus, four dietary treatments were tested: LP diet without PBE supplementation; LP-PEB, LP diet with PBE supplementation; LP+PBE, HP diet without PBE supplementation; HP-PBE and PH diet with PBE supplementation; HP+PBE. The PBE was freshly prepared after 24 h soaking of pistachio by-products in the cold water (1:3 w/v) and filtering through two layers of cheesecloth. After a 14-day adaptation period, feed consumption and body weight gain of lambs were recorded and feed conversion ratio were calculated for 50-days.

On the 35th d of the experimental period, rumen fluid and blood samples were collected 0, 2 and 4 h after morning feeding from two lambs in each group. Rumen fluid samples were collected using a stomach tube (oral lavage) and filtered through two layers of cheesecloth and then 10 ml sub sample was acidified by 10 ml 0.2 N HCl. Plasma samples were separated by centrifugation (3000 g, 15 min) of five ml blood samples collected from the jugular vein in EDTA-containing tubes. Rumen fluid and plasma samples were stored at -20^{o}C until subsequent analysis. From day 45 to 50 of the experimental period, two lambs from each experimental group were moved to metabolism cages. The lambs were fed experimental diets and daily total fecal and urine output were collected. Feed, orts and feces were weighted daily and their samples were immediately dried at 60^{o}C for 48 h. The acidified urine (by 200 ml 2 M H_2SO_4 to maintain urine pH <3.0) was weighted and 100 ml samples were stored at -20^{o}C.

All chemical analysis was performed in triplicate. Dried samples of feed, orts and faeces were milled to pass through a 1 mm screen. Total ash was determined after 4 h dry-ashing at 550^{o}C and organic matter was calculated as the difference between the weight of original sample and the weight of incinerated ash. Feed, orts and faeces as well as urine were analyzed for N content by Kjeldahl method (AOAC 1997, Method 990.02). The ammonia N concentration of rumen fluid was determined by the method of Varner et al. (1953) as was described by Rust et al. (1965) with a few modifications. Briefly, acidified rumen fluid sub samples were centrifuged at 15,000 g for 15 min and a 2 ml sample of the supernatant was distilled by a distillation unit (Behr distillation unit S5, Germany)

after adjustment of the pH with a sodium borate buffer. The ammonia was collected in 3% boric acid and titrated with 0.01 N HCl using a pH titration unit (Titroline® easy, Germany). An autoanalyzer (Biosystems A 15; 08030 Barcelona, Spain) was used for determination of plasma urea concentrations. The fresh PBE was analyzed for total phenolic compounds (TP) and total tannins (TT) by Folin-Ciocalteu reagent using tannic acid as standard (Makkar, 2000). The condensed tannins content of PBE was also determined by butanol-HCl method (Porter et al., 1986) and expressed as leucocyanidin equivalent.

Statistical Analysis

The data for feed intake, body weight, growth rate and FCR as well as digestibility and N retention were analyzed using the GLM procedure of SAS using the model

$Y = \mu + CP_i + PBE_j + (CP \times PBE)_{ij} + \varepsilon_{ij}$

where μ is the overall mean, CP_i is the effect of CP level (i = LP and HP), PBE_j is the effect of PEB level (j = with PBE supplementation; +PBE and without PBE supplementation; -PBE), CP×PBE is the interaction between CP and PBE level and ε_{ij} is the associated error. For data collected over time within each sampling period (ruminal NH_3-N and PUN concentrations), a repeated-measures analysis was performed using the MIXED procedure of SAS. The means were compared using Duncan's multiple range test and differences among treatments were considered significant when $P<0.05$, whereas when $0.10 \geq P \geq 0.05$, differences were considered to indicate a trend toward a significant effect.

Results

A summary of the mean initial and final body weight as well as ADG, DMI and FCR of lambs is presented in Table 2. The effects of dietary CP density on both DMI and ADG of lambs were non-significant ($P>0.05$). There was, however, a tendency for DMI to be lower ($P=0.102$) in LP fed lambs compared with HP lambs. The efficiency of feed intake expressed as FCR was similar between LP and HP groups. In contrast, PBE supplementation improved growth rate ($P=0.021$) and also FCR ($P=0.104$) of lambs. The effect of PBE supplementation on ADG was only about 3% for lambs fed LP diet, whereas a 16% increase ($P=0.08$) in ADG was observed by adding of PBE to HP diet.

As a result of similar feed intake between LP and HP lambs, the intake of N in HP was significantly higher ($P<0.001$) than LP fed lambs (Table 3). DM, OM and N digestibility were not affected by dietary CP density or PBE supplementation. The lambs feeding on the LP diet excreted lower N in fecal ($P=0.004$) and also in urine ($P<0.001$) than HP fed lambs. Although

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

	Experimental diets[1]			
	LP-PBE	LP+PBE	HP-PBE	HP+PBE
Dietary ingredients (g/kg)				
Corn silage	300	300	300	300
Whole barely grain	660	660	560	560
Soybean meal	25	25	125	125
Vitamin and mineral premix[2]	7.5	7.5	7.5	7.5
CaCO3	5	5	5	5
Salt	2.5	2.5	2.5	2.5
Chemical composition[3]				
ME (Mcal/kg of DM)	2.6	2.6	2.7	2.7
CP (% of DM)	11.3	11.3	13.9	13.9
RDP (% of DM)	8.4	8.4	10.6	10.6
RUP (% of DM)	2.9	2.9	3.3	3.3
RDP/RUP ratio	2.9	2.9	3.2	3.2
TP (% of DM)	ND	1.9	ND	1.9
TT (% of DM)	ND	0.91	ND	0.91
CT (% of DM)	ND	0.27	ND	0.27

[1]LP-PBE: low protein diet without pistachio by-products aqua extract (PBE) supplementation; LP+PBE: low protein diet with PBE supplementation; HP-PBE: high protein diet without PBE supplementation; HP+PEG: high protein diet with PBE supplementation; [2]Vitamin and mineral premix (per kg DM): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1000 IU of vitamin E, 160 g Ca, 85 g P, 63 g Na, 45 g Mg, 2100 mg Zn, 1500 mg Mn, 535 mg Cu, 12 mg Se, 45 mg I; [3]ME: metabolisable energy; CP; crude protein; RDP: rumen degradable protein; RUP: rumen undegradable protein; TP: total phenolics; TT: total tannins; CT: condensed tannins.

daily retention of N was very similar between LP and HP (6.7 vs. 6.8 g/d, $P=0.375$), the LP lambs retained a higher percentage of N intake or N digested compared with HP. Similarly, urinary losses of N were decreased ($P=0.008$) due to PBE supplementation. However, the urinary excretion of N for lambs in LP-PBE, LP+PBE and HP+PBE was similar and lower than HP-PBE group. The N retention was also higher for lambs received PBE in the diets. This effect of PBE was depended on dietary CP level and significant only when lambs fed HP diet. The greatest daily N retention value was observed in HP+PBE, whereas the lambs in other groups retained similar amount of N.

There was no difference in ruminal NH_3-N concentration among lambs before feeding. For all experimental groups, the ruminal concentration of NH_3-N was significantly reduced 2 h after morning feeding (Fig. 1a). Nevertheless, the level of ruminal ammonia 2 h after feeding was higher in HP-PBE than other groups. The increase of NH_3-N concentration in the rumen fluid of HP fed lambs from 2 to 4 h after feeding was retarded by adding PBE to the diet. In contrast, similar levels of ruminal ammonia were observed between h 2 and 4 after feeding for LP lambs with no effect due to PBE supplementation (8.0 vs. 7.9 mg/dL).

Table 2: Effects of crude protein (CP) level, pistachio by-products aqua extract (PBE) and CP×PBE interaction on feedlot performance of lambs

	CP level		PBE		CP × PBE interaction[2]				Root MSE	P value		
Item[1]	LP (n=10)	HP (n=10)	-PBE (n=10)	+PBE (n=10)	LP-PBE (n=5)	LP+PBE (n=5)	HP-PBE (n=5)	HP+PBE (n=5)		CP	PBE	CP×PBE
IBW (kg)	26.2	27.1	27.0	26.4	26.1	26.3	27.9	26.4	3.74	0.619	0.726	0.649
FBW (kg)	36.5	37.8	36.9	37.3	36.2	35.7	37.6	37.9	3.98	0.506	0.830	0.980
DMI (g/d)	949.4	1071.0	1011.5	1008.9	990.3	908.6	1032.7	1109.3	146.50	0.102	0.972	0.288
ADG (g/d)	204.6	212.4	197.7	219.3	201.7	207.6	193.8	231.0	16.92	0.365	0.021	0.080
FCR (F:G)	4.7	5.0	5.1	4.6	4.9	4.4	5.2	4.8	0.59	0.180	0.104	0.892

[1]IBW: initial body weight; FBW: final body weight; DMI: dry mater intake; ADG: average daily gain; FCR: feed conversion ratio**;** [2]LP-PBE: low protein diet without PBE supplementation; LP+PBE: low protein diet with PBE supplementation; HP-PBE: high protein diet without PBE supplementation; HP+PEG: high protein diet with PBE supplementation.

Table 3: Effects of crude protein (CP) level, pistachio by-products aqua extract (PBE) and CP×PBE interaction on dry mater (DM), organic matter (OM) and nitrogen (N) digestibility, fecal and urine N excretion and N retention of lambs

	CP level		PBE		CP × PBE interaction[1]					P value		
Item	LP (n=4)	HP (n=4)	-PBE (n=4)	+PBE (n=4)	LP-PBE (n=2)	LP+PBE (n=2)	HP-PBE (n=2)	HP+PBE (n=2)	Root MSE	CP	PBE	CP×PBE
DMI (g/d)	1083.3	1088.3	1100.1	1071.5	1083.7	1082.8	1116.5	1060.2	56.32	0.905	0.512	0.525
OMI (g/d)	1036.7	1032.0	1044.2	1024.5	1034.4	1039.1	1054.0	1010.0	59.34	0.915	0.663	0.593
N intake (g/d)	19.9	24.2	22.1	22.0	19.8	19.9	24.4	24.1	0.21	<0.001	0.453	0.369
Digestibility												
DM (% of DM intake)	76.4	75.3	76.3	75.3	77.7	75.0	74.9	75.6	2.75	0.596	0.634	0.433
OM (% of OM intake)	78.8	78.3	79.0	78.2	78.9	78.8	79.0	77.7	2.29	0.779	0.666	0.736
N (% of N intake)	71.1	70.4	71.1	70.4	71.4	70.8	70.9	70.0	1.58	0.593	0.543	0.945
N excretion												
Faecal (g/d)	5.7	7.1	6.4	6.5	5.7	5.8	7.1	7.2	0.33	0.004	0.609	0.966
Urine (g/d)	7.4	10.3	9.3	8.4	7.5^{a}	7.3^{a}	11.1^{c}	9.5^{b}	0.26	<0.001	0.008	0.016
Urine (% of N intake)	37.4	42.3	41.7	38.1	37.9^{a}	36.9^{a}	45.5^{c}	39.2^{b}	1.25	0.005	0.015	0.040
N retained												
g/d	6.7	6.8	6.4	7.1	6.7^{a}	6.7^{a}	6.2^{a}	7.4^{b}	0.16	0.375	0.005	0.007
% of N intake	33.7	28.1	29.5	32.3	33.6^{c}	33.9^{c}	25.4^{a}	30.8^{b}	0.70	<0.001	0.004	0.007
% of N digested	47.4	39.9	41.4	45.9	47.0^{c}	47.9^{c}	35.8^{a}	44.0^{b}	0.85	<0.001	0.002	0.004

[1]Within each row, means with common superscript do not differ (P>0.05); LP-PBE: low protein diet without PBE supplementation; LP+PBE: low protein diet with PBE supplementation; HP-PBE: high protein diet without PBE supplementation; HP+PEG: high protein diet with PBE supplementation.

In comparison with HP, the lambs fed LP diet had lower (P=0.024) plasma concentration of urea before morning feeding. An increase in PUN was observed in both LP (30 %, P=0.001) and HP (13%, P=0.048) fed lambs 2 h after feeding (Fig. 1b). There was, however, no difference in PUN concentration measured 2 h after feeding between LP and HP lambs. On h 4 after feeding, the PUN concentration in LP fed lambs was dropped to the same level that was detected before the morning feeding, whereas the levels of PUN between h 2 and 4 after feeding were similar in HP lambs. Although PBE had no effect on the PUN concentration of lambs before feeding, we observed a lower level of PUN 2 and 4 h after feeding due to PBE supplementation of HP diet only.

Discussion

A trend to significant (P=0.102) lower DMI observed in lambs fed LP diet is likely due to lower dietary RDP/RUP ratio. In ruminants, it has been reported that the voluntary DMI can be affected negatively by decrease of dietary RDP/RUP ratio (Valkeners et al., 2008). In our experiment, 80% of the soybean meal in the HP diet was replaced with whole barley grain in the LP diet; consequently the LP had a lower RDP/RUP ratio than HP diet (Table 1). Feed intake may also be affected by the present of tannins in the diet as a result of decreasing palatability or negatively affecting digestion because of the astringency caused by formation of complexes between tannins and salivary glycoproteins (Landau et al., 2000). However, depression of feed intake in animals fed low tannin diets (<5.0 % DM) is uncommon (Patra and Saxena, 2011), as also observed in the present experiment.

There are some reports that indicate improvement in performance for lambs fed increasing levels of dietary CP (Haddad et al., 2001; Ebrahimi et al., 2007). In the current study, however, a 2.6% increase in dietary CP density from 11.3 to 13.9 % had no effect on

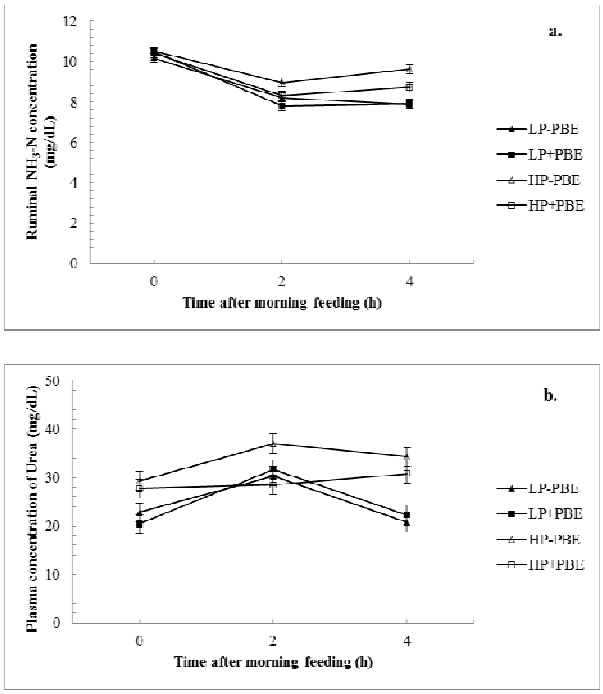

Fig. 1: Ruminal NH_3-N (a) and plasma urea (b) concentrations with time after morning feeding across periods for LP-PBE: low protein diet without pistachio by-products aqua extract (PBE) supplementation; LP+PBE: low protein diet with PBE; HP-PBE: high protein diet without PBE supplementation; HP+PEG: high protein diet with PBE supplementation treatments.

ADG and FCR. Close to these levels of dietary CP, Dayani et al. (2011) reported no difference in ADG and FCR of lambs fed diets contained 12 or 14% CP. Although a 2% increase in dietary CP level from 14 to 16% improved performance of Awassi lambs, there was no difference in ADG and also FCR between lambs fed 12 and 14% CP contained diets (Haddad et al., 2001). In contrast, the low concentration of tannins (9.1 g/kg DM, Table 1) prepared by adding PBE to the diets significantly (P=0.02) increased ADG as well as improved FCR by about 10% (P=0.104).The advantage of tannins, specially condensed tannins (CT), at a moderate concentration (20-40 g CT/kg DM) on the growth performance of ruminant animals has widely been reported (Patra and Saxena, 2011). Montossi (1995) suggested a minimum effective CT concentration of 5 g/kg DM for positive impact on ruminant performance. The beneficial effect of PBE supplementation on ADG for lambs in the LP was about 3%, whereas ADG improved by 16% in HP groups. Previously, we also noticed a significant interaction between low concentration CT (4.06 g/Kg DM) and dietary CP density on growth performance with a greater response in growing lambs feeding LP diets containing licorice leaves as a source of tannins (data unpublished). Valizadeh et al. (2010) obtained an ADG 230 g/day in Baluchi lambs fed diets containing 14.7% CP, which is very similar to those we observed in HP+PBE group (231 g/day, Table 2). These authors also reported no differences in growth rate and FCR of lambs due to feeding different levels (0, 10, 20 and 30% of diet DM) of pistachio by-products. Our results, however, hinted that there is likely to exist a relationship between dietary CP density and the protein-sparing effect of tannins, thereby the effectiveness of tannins is more obvious when CP is at a marginal level for impact on performance. It has also been demonstrated that the beneficial protein-sparing effects of monensin (Hanson and Klopfenstein, 1979) or rumen-protected CLA (Schiavon et al., 2010) on the ruminant performance were depended on dietary CP level; the greatest response in FCR occurred in animals feeding on the low CP density diets.

The overall means for total tract digestibility of DM, OM and N were about 76, 78 and 71%, respectively, with no effect due to experimental treatments, which are comparable with those reported in animals fed high concentrate diets containing optimum levels of CP (Devant et al., 2000; Haddad et al., 2001). Although there is evidence that nutrient digestibility can be affected by dietary CP level mainly associated with N supply for bacterial growth (Veira et al., 1980; Willms et al., 1991), the limitation of N for microbial activity in our study seems unlikely. Willms et al. (1991) observed similar DM, OM and also N digestibility between lambs fed 12 and 14% CP diets. Similarly, no difference in nutrient digestibility was reported in Awassi lambs fed diets containing 12, 14, 16 or 18% CP (Haddad et al., 2001). Oldham and Smith (1982) suggested that increasing dietary CP level above 10 to 12% had little effect on DM digestibility in non lactating ruminants. Likewise, the lack of differences in nutrient digestibility between -PBE and +PBE (Table 3) is likely attributed to low concentration of tannins in the diets. Generally, the high concentrations of tannins (>55 g CT/kg DM) reduce nutrients digestibility in the ruminants (Min et al., 2003).

Although N digestibility was similar between LP and HP lambs, statistically significant lesser values of absolute fecal N output (g/d) were observed in lambs fed LP diet (Table 3). Similar fecal N excretion was reported in heifers (Devant et al., 2000) and lambs (Ludden et al., 2002a) fed different levels of dietary CP. These authors also suggested that both N intake and digestibility improved by increasing levels of dietary CP. However, when there was no difference in N digestibility between lambs fed diets containing 12 and 14% CP, Willms et al. (1991) observed a lower daily fecal excretion of N in lambs with lower N intake. Additionally, urinary excretion of N was increased as a

result of higher N intake in HP lambs (Table 3). Similarly, urinary N losses were increased as dietary CP concentration increased from 10 to 15% in lambs supplemented with cottonseed meal (Cole, 1999). A linear increase in urinary N excretion was also observed in lambs fed increasing levels of dietary CP (Ludden et al., 2002a).

The reduction of urinary N losses caused by LP or PBE treatment may be a reflection of lower plasma concentrations of urea (Kohn et al., 2005). PUN concentration can be influenced by level and also degradability of dietary protein (Dabiri and Thonney, 2004). Moreover, dietary CP and RDP levels positively affect ruminal ammonia concentration (Devant et al., 2000; Ludden et al., 2002b). Therefore the lesser ruminal NH_3-N (Fig. 1a) and PUN (Fig. 1b) concentrations were expected for LP fed lambs due to intake of the diet with lower levels of both CP and RDP than HP fed lambs (Table 1). Similarly, the PBE supplementation also reduced PUN and ruminal NH_3-N concentrations after feeding likely in response to the protective effect of PBE tannins against ruminal degradation of protein (Patra and Saxena, 2011). Pistachio by-products silage (PBS) reduced ruminal NH_3-N and serum urea N concentrations in dairy cows in comparison with corn silage or polyethylene glycol (as a tannin binder) treated PBS (Mokhtarpour et al., 2012). Besides tannins, pistachio by-products contains many bioactive phenolics such as naringenin and quercetin, which are isoflavone (Tomaino et al., 2010). Flythe and Kagen (2010) demonstrated that isoflavones from red clover have antimicrobial activity against hyper ammonia-producing bacteria. Therefore, lower PUN and ruminal NH_3-N concentration in lambs fed PBE supplemented diets may also be attributed to other PBE phenolics. On h 2 after feeding, although we observed a reduction in ruminal NH_3-N concentration for all groups, which is in agreement with the findings of Devant et al. (2000), the ammonia concentrations in rumen fluid of LP-PBE, LP+PBE and HP+PBE lambs were similar and significantly lower than HP-PBE group. At the same time, the increase of PUN concentration was affected negatively by PBE supplementation of HP diet. Similar interaction effects of dietary CP level and PBE supplementation on ruminal NH_3-N and PUN concentrations were detected 4 h after feeding. In consistent to our results, Fernández et al. (2012) suggested that tannins supplements have a higher potential to reduce ruminal NH_3-N and BUN in sheep when fed HP diets. Moreover, it has been reported that protein precipitation effect of phenolic compounds is affected positively by protein concentration in the solution (Ozdal et al., 2013). Similar to the current study, however, the dietary RDP level of LP was also lower than HP diet in the experiment of Fernández et al. (2012). Therefore, the influence of dietary RDP level on the tannins potential for reduction of urinary N losses as well as PUN and ruminal NH_3-N concentration is unclear.

The similar positive N balance between LP and HP lambs (Table 3) suggests that N in the LP diet was not limiting growth. This is more likely a result of improving in N efficiency due to lower N losses in LP fed lambs. Devant et al. (2000) reported similar ADG and N retention in growing heifers fed diets containing 14 or 17% CP. This author also observed a higher efficiency of N intake in heifers fed 14% CP diet compared with 17% CP diet. The retention of N was increased by PBE supplementation of diets. Although PBE supplementation had no effect on N retained in LP fed lambs, but improved N retention in HP lambs by 16%. Thus the maximal N retention was observed in HP+PBE group. Assuming that N retention in sheep is 29 g/kg of body weight gain (Kohn et al., 2005), the predicted body weight gains correspond to N retained values are 194.3 g/d for LP fed lambs and 179.8 and 214.6 g/d for HP-PBE and HP+PBE groups respectively, comparable with observed ADG (Table 2).

Conclusion

The primary purpose of this study was to improve the efficiency of dietary N utilization in order to reduce production costs and environmental pollution. Our results showed that reduction of dietary CP density from 13.9 to 11.3% improved N retained by the decrease of N losses, without any negative effects on feed intake, growth rate and also digestibility of DM, OM and N in Baluchi ram lambs. In addition, supplementation of diets with PBE increased growth rate and retention of N depend on dietary CP levels; the greatest response occurred in animals feeding on the HP diets. Based on the results of the current study, we suggested that there is likely a relationship between dietary CP density and the protein-sparing effect of tannins, thereby the effectiveness of tannins is more obvious when CP is at a marginal level for impact on performance. Moreover, in agreement with Fernández et al. (2012), the higher potential of PBE tannins for depression of PUN and ruminal NH_3-N concentration was observed in lambs when fed HP diet. However, the influence of dietary RDP level on these effects of tannins is unclear.

Acknowledgement

We would like to thank Mr. A. Mokhtarpour and Mr. H. Karimzadeh for help in laboratory analysis. We appreciate the cooperation of the staff of the Animal Research Station during the conduct of the experiment.

References

AOAC., 1997. Official Methods of Analysis. 16th ed. Assoc. Offic. Anal. Chem., Arlington, VA, USA.

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

Cole, N.A. 1999. Nitrogen retention by lambs fed oscillating dietary protein concentrations. *Journal of Animal Science*, 77: 215-222.

Dabiri, N. and Thonney, M.L. 2004. Source and level of supplemental protein for growing lambs. *Journal of Animal Science*, 82: 3237-3244.

Dayani, O., Dadvar, P. and Afsharmanesh, M. 2011. Effect of dietary whole cottonseed and crude protein level on blood parameters and performance of fattening lambs. *Small Ruminant Research*, 97: 48-54.

Devant, M., Ferret, A., Gasa, J., Calsamiglia, S. and Casals, R. 2000. Effects of protein concentration and degradability on performance, ruminal fermentation, and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100 to 230 kg body weight. *Journal of Animal Science*, 78: 1667-1676.

Ebrahimi, R., Ahmadi, H.R., Zamiri, M.J. and Rowghani, E. 2007. Effect of energy and protein levels on feedlot performance and carcass characteristics of Mehraban ram lambs. *Pakistan Journal of Biological Sciences*, 10: 1697-16984.

Fernández, H.T., Catanese, F., Puthod, G., Distel, R.A. and Villalba, J.J. 2012. Depression of rumen ammonia and blood urea by quebracho tannin-containing supplements fed after high-nitrogen diets with no evidence of self-regulation of tannin intake by sheep. *Small Ruminant Research*, 105: 126-134.

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

Frutos, P., Hervás, G., Giráldez, F.J., Fernández, M. and Mantecón, A.R. 2000. Digestive utilization of quebracho-treated soya bean meals in sheep. *Journal of Agricultural Science*, 134: 101-108.

Haddad, S.G., Nasr, R.E. and Muwalla, M.M. 2001. Optimum dietary crude protein level for finishing Awassi lambs. *Small Ruminant Research*, 39: 41-46.

Hanson, T.L. and Klopfenstein, T. 1979. Monensin, protein source and protein levels for growing steers. *Journal of Animal Science*, 48: 474-479.

Hart, K.J., Sinclair, L.A., Wilkinson, R.G. and Huntington, J.A. 2011. Effect of whole-crop pea (*Pisum sativum* L.) silages differing in condensed tannin content as a substitute for grass silage and soybean meal on the performance, metabolism, and carcass characteristics of lambs. *Journal of Animal Science*, 89: 3663-3676.

Hutjens, M.F. 1992. Selecting feed additives. In Large Dairy Herd Management. H. H. Van Horn and C. J. Wilcox, ed. Am. Dairy Sci. Assoc., Champaign, IL.

Kohn, R.A., Dinneen, M.M. and Russek-Cohen, E. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *Journal of Animal Science*, 83: 879-889.

Lamy, E., Rawel, H., Schweigert, F.J., Silva, F.C., Ferreira, A., Costa, A.R., Antunes, C., Almeida, A.M., Coelho, A.V. and Sales-Baptista, E. 2011. The effect of tannins on Mediterranean ruminant ingestive behavior: The role of the oral cavity. *Molecules,* 16: 2766-2784.

Landau, S., Perevolotsky, A., Bonfil, D., Barkai, D. and Silanikove, N. 2000. Utilization of low quality resources by small ruminants in Mediterranean agro-pastoral systems: the case of browse and aftermath cereal stubble. *Livestock Production Science*, 64: 39-49.

Lapierre, H. and Lobley, G.E. 2001. Nitrogen recycling in the ruminant: A review. *Journal of Dairy Science*, 84(E. Suppl.): E223-E236.

Ludden, P.A., Wechter, T.L. and Hess, B.W. 2002a. Effects of oscillating dietary protein on nutrient digestibility, nitrogen metabolism, and gastrointestinal organ mass in sheep. *Journal of Animal Science*, 80: 3021-3026.

Ludden, P.A., Wechter, T.L. and Hess, B.W. 2002b. Effects of oscillating dietary protein on ruminal fermentation and site and extent of nutrient digestion in sheep. *Journal of Animal Science*, 80: 3336-3346.

Makkar, H.P.S. 2000. Quantification of Tannins in Tree Foliage. A Laboratory Manual FAO/IAEA Working Document, Vienna, Austria.

McSweeny, C.S., Palmer, B., McNeill, D.M. and Krause, D.O. 2001. Microbial interaction with tannins: Nutritional consequences for ruminants. *Animal Feed Science and Technology*, 91: 83-93.

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

Mokhtarpour, A., Naserian, A.A., Tahmasbi, A.M. and Valizadeh, R. 2012. Effect of feeding pistachio by-products silage supplemented with polyethylene glycol and urea on Holstein dairy cows performance in early lactation. *Livestock Science*, 148: 208-213.

Mokhtarpour, A., Naserian, A.A., Valizadeh, R., Danesh Mesgaran, M. and Pourmollae, F. 2014. Extraction of phenolic compounds and tannins

from pistachio by-products. *Annual Research & Review in Biology*, 4: 1330-1338.

Montossi, F. 1995. Comparative studies on the implications of condensed tannins in the evaluation of *Holcus lanatus* and *Lolium* spp. swards for sheep performance. Ph.D. Thesis. Massey University, New Zealand.

Oldham, J.D. and Smith, T. 1982. Protein-energy interrelationships for growing and for lactating cattle. Ln: E.L. Miller, I.H. Pike and A.J.H. Van Es (Ed.) Protein Contribution of Feedstuffs for Ruminants. Butterworths, London.

Ozdal, T., Capanoglu, E. and Altay, F. 2013. A review on protein-phenolic interactions and associated changes. *Food Research International*, 51: 954-970.

Patra, A.K. and Saxena, J. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture*, 91: 24-37.

Perry, T.W., Shields, D.R., Dunn, W.J. and Mohler, M.T. 1983. Protein levels and monensin for growing and finishing steers. *Journal of Animal Science*, 57: 1067-1076.

Porter, L.J., Hrstich, L.N. and Chan, B.G. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, 25: 223-230.

Rust, J.W., Jacobson, N.L., McGilliard, A.D. and Hotchkiss, D.K. 1965. Supplementation of dairy calf diets with enzymes. II. Effect on nutrient utilization and on composition of rumen fluid. *Journal of Animal Science*, 24: 156-160.

Schiavon, S., Tagliapietra, F., Dal Maso, M., Bailoni, L. and Bittante, G. 2010. Effects of low-protein diets and rumen-protected conjugated linoleic acid on production and carcass traits of growing double-muscled Piemontese bulls. *Journal of Animal Science*, 88: 3372-3383.

Schiavon, S., Tagliapietra, F., Dalla Montà, G., Cecchinato, A. and Bittante, G. 2012. Low protein diets and rumen-protected conjugated linoleic acid increase nitrogen efficiency and reduce the environmental impact of double-muscled young Piemontese bulls. *Animal Feed Science and Technology*, 174: 96-107.

Tomaino, A., Martorana, M., Arcoraci, T., Monteleone, D., Giovinazzo, C. and Saija, A. 2010. Antioxidant activity and phenolic profile of pistachio (Pistacia vera L., variety Bronte) seeds and skins. *Biochimie*, 92: 1115e1122.

Valizadeh, R., Norouzian, M.A., Salemi, M., Ghiasi, E. and Yari, M. 2010. Effects of feeding pistachio by-products on hematology and performance of Baluchi lambs. *Journal of Animal and Veterinary Advances*, 9: 1115-1119.

Valkeners, D., Théwis, A., van Laere, M. and Beckers, Y. 2008. Effect of rumen-degradable protein balance deficit on voluntary intake, microbial protein synthesis, and nitrogen metabolism in growing double-muscled Belgian Blue bulls fed corn silage-based diet. *Journal of Animal Science*, 86: 680-690.

Varner, J.E., Bulen, W.A., Vanecko, S. and Burrell, R.C. 1953. Determination of ammonium, amide, nitrite and nitrate nitrogen in plant extracts. *Analytical Chemistry*, 25: 1528.

Veira, D.M., Macleod, G.K., Burton, J.H. and Stone, J.B. 1980. Nutrition of the weaned Holstein calf. II. Effect of dietary protein level on nitrogen balance, digestibility and feed intake. *Journal of Animal Science*, 50: 945.

Willms, C.L., Berger, L.L., Merchen, N.R., Fahey Jr, G.C. and Fernando, R.L. 1991. Effects of increasing crude protein level on nitrogen retention and intestinal supply of amino acids in lambs fed diets based on alkaline hydrogen peroxide-treated wheat straw. *Journal of Animal Science*, 69: 4939-4950.