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Effect of Oak (*Quercus libani* Oliv.) Leave Tannin on Ruminal Fermentation of Sheep

M. J. Abarghuei[1], Y. Rouzbehan[1*], and D. Alipour[2]

ABSTRACT

Six rumen fistulated adult sheep were used to assess the effect of tannins (hydrolysable tannin; HTs) in oak leaves (*Quercus Libani* Oliv.) on ruminal fermentation parameters in a change-over design experiment for 28 days in 3 periods. Polyethylene glycol (PEG) was used to deactivate the tannins. The three dietary treatments were control (alfalfa hay, barley grain, wheat bran, wheat straw); OL (oak leave, barley grain, wheat bran and urea) and OL+ 80 g PEG. Animals were held in individual pens and metabolism cages. They were adapted to experimental conditions for 21 days before the commencement of the measurement periods. In each period, the digestibilities of dry matter (DMD), organic matter (OMD), NDF (NDFD), crude protein (CPD) and ruminal parameters (pH, ammonia, bacteria and protozoa population), and microbial protein synthesis were measured using urinary purine derivatives in sheep. The DMD, OMD, NDFD and CPD were decreased by oak leaves and the addition of PEG improved CPD (P<0.05). The ruminal pH values for all diets were within the normal range. Ruminal ammonia was similar among the treatments (p>0.05). Hydrolysable tannins in OL diets decreased (P<0.05) urinary allantoin in comparison to the control diet. Addition of PEG increased (P<0.05) allantoin. The uric acid, xanthine and hypoxanthine excretion in urine were not affected by the diet. Feeding OL diet decreased the microbial N in sheep, whereas addition of PEG improved it. The total protozoa count in sheep offered OL diet declined in comparison to those fed the control diet; however, addition of PEG had no effect on it. Sheep fed OL diet had significantly less cellulolytic and proteolytic bacteria than those fed the control diet (P<0.05), but improved (P< 0.05) with feeding of PEG along with OL. It was concluded that diets containing *Q. Libani* leaves had lower ruminal fermentability than diet containing alfalfa and that supplementation of PEG in OL diet improved the fermentability.

Keywords: Microflora, Oak leave, Polyethylene glycol, Rumen, Sheep, Tannin.

INTRODUCTION

Approximately 3 million ha of forest are covered by various oak species, mainly dominated by *Quercus persica*, *Quercus infectoria* and *Quercus libani*, in the west of Iran (Fatahi, 1995). In this region, oak leaves are the main source of forage for goats and sheep, since scarcity of animal feed is the major constraint to animal production in this area. However, *Quercus* species have been reported to contain high levels of hydrolysable tannins (Yousef Elahi and Rouzbehan, 2008). Reed (1995) reported that the value of these leaves (which contain 200 g HTs/kg DM) as feed for ruminants is offset by their negative effect on protein utilization and the risk of toxicity (mortality and morbidity) when its intake was high. However, when the level of HTs consumption is low *i.e.* 0.15-0.3 g

[1] Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, P. O. Box: 14115-336, Islamic Republic of Iran.
* Correspondence author, e-mail: rozbeh_y@modares.ac.ir
[2] Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamadan, Islamic Republic of Iran.

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HTs/ $kg^{0.75}$, Yildiz *et al.* (2005) noted that at least half of the hay ration in sheep can be replaced with oak leaves. Although clinical and pathological signs associated with this toxicosis have been described (Spier *et al*., 1987; Yeruham *et al*., 1998), little is known about the effect of consumption of these leaves on the ruminal fermentation (Doce *et al*., 2009). For example, Yildiz *et al*. (2005) noted that ruminal pH, ammonia, short chain fatty acid and microbial protein production levels did not differ between sheep fed 185 and 370 g oak leaves *i.e.* 0.1-0.3 g HTs /$kg^{0.75}$ and the control (hay mixture and concentrate). On the other hand, young Pyrenean oak leaves (0.3 g HTs /$kg^{0.75}$) have a negative effect on *in vitro* organic matter digestibility (OMD) and ruminal ammonia concentration (Doce *et al*., 2009).

Although the incorporation of polyethylene glycol (PEG), which binds with and inactivates tannins, is quite effective, success of its adoption depends on the cost**:** benefit ratio (Makkar, 2003)., In Iran, one of the largest oil producers in the world, PEG is produced from oil. The production capacity of PEG exceeds 7000 MT per year. Therefore, under the Iranian conditions, assessment of the economical viability of including this supplement in the OL diets is necessary.

Previously, *in vitro* work suggested that *Q. libani* was nutritionally the best among *Q.* species as ruminant feed (Yousef Elahi and Rouzbehan, 2008). In Iran, alfalfa is the main forage used in the feeding of ruminants; however, it is expensive, particularly in dry seasons. Therefore, it was decided to assess the effect of replacing alfalfa forage with *Q. libani* on the ruminal parameters (pH, ammonia concentration, microbial protein, bacterial and protozoa counts) of Ghezel sheep. We also examined the effects on the ruminal parameters of PEG treatment in deactivating tannins in *Q. libani.*

MATERIAL AND METHODS

Oak Leave and alfalfa

Oak leaves (*Quercus libani)* were obtained from Kordestan Province, in Baneh city of Iran. Leaves were harvested by hand and sun-dried during the summer (2007). The chemical composition, metabolisable energy and organic matter digestibility (g kg^{-1}DM) of oak leave and alfalfa which were used in the experimental diets are shown in Table 1.

Animal Studies

Six rumen fistulated sheep, (Ghezel breed, twelve months of age with live body weight of 61.8± 2.9 kg) were used in a 3 x 3 change-over design experiment with each period consisting of three 3-wk periods. Ingredient composition of the three experimental diets, viz., control, oak leaves based (OL) and OL plus polyethylene glycol (OL+PEG), is presented in Table 2. PEG (MW-6000) per day was offered as a mixture with concentrate supplement at the dose of 80 g d^{-1}. The ratio of PEG: HTs was 1.5:1. Animals were offered food at 1.2 of the maintenance level kg/day

Table 1. Chemical composition (g kg^{-1} DM), metabolisable energy (MJ kg^{-1} DM) and organic matter digestibility (g kg^{-1}DM) of oak leave and alfalfa.

	Chemical Composition					
Feed	Dry matter	Crude protein	Crude fat	ADFom	ME	OMD
Oak leave	959	116	27	316	6.3	400
Alfalfa	960	150	28	341	8.7	539

ADFom: acid detergent Fiber; GP: gas production; ME: metabolisable energy and OMD; organic matter digestibility. OMD was measured using *in vitro* gas production method.

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Table 2. Ingredients and nutrient composition (g kg^{-1} DM) or as stated for the experimental diets given to sheep.

Ingredients	Diets		
	Control	OL [a]	OL+PEG [b]
Alfalfa	390	-	-
Oak leave	-	709	629.25
Wheat bran	83	206.8	189.3
Barley	250	70.9	65.3
Wheat straw	277	-	-
Urea	-	13.3	11.75
PEG	-	-	104.4
Nutrient composition			
Dry matter (g kg^{-1} DM)	942	934	933
Organic matter	966	947	965
Ash	34	53	35
ERDP: FME ratio	11.1	11.1	11.1
Neutral detergent Fiber	443	472	372
Total phenolic compounds	-	57.6	57.6
Total tannin	-	50.9	50.9
Condensed tannin	-	3.8	3.8
Free gallic acid	-	36.3	36.3
Total gallic acid	-	106	106
Gallotannin	-	69.7	69.7

[a] Oak leave, [b] Polyethylene glycol

±0.2 (AFRC, 1993). Sheep were fed their respective diet twice daily, at 08:00 and 17:00 h with free access to mineral block and water. Animals were adapted to the experimental diets and metabolism crates for 21 days and 7 days for feces and urine collection, which were sub-sampled (from each sheep on each treatment), weighed and a 10% sample was stored for later analysis. The digestibility coefficient of organic matter (OMD) and NDF (NDFD) for the control, OL or OL+PEG diets were calculated by difference according to Givens *et al*. (2000). Daily feed intake for the digestibility trial was measured during collection period. Samples of feed offered, feed refusal, and feces were collected every morning. Urine from individual sheep was collected for 7 days with a buckets containing 100 ml of 10% (V/V) sulfuric acid solution (containing 10 ml of concentrated sulfuric acid in 100 ml of distilled water), to keep the final pH below 3. Urine collection buckets were placed below the urine outlets in the metabolic cages. Urine collected every morning from individual animal was measured and appropriate dilutions were made. A sub sample of 20 ml was stored at −20 °C for the estimation of purine derivatives (Chen and Gomes, 1995).

Rumen Fermentation

Approximately 100 ml of ruminal fluid was collected before the first feeding (zero h) and at 3, 6 and 8 h after feeding. Rumen liquor samples were strained through (SRL) two layers of cheesecloth. The pH value was measured immediately using a pH meter (WTW multilab 540 Ionalyzer, Weilheim, Germany). A sub sample of 5ml was combined with 1 ml of 0.2 M HCl for ammonia-N analysis. Sub sample was frozen at −20 °C until laboratory analyses.

Enumeration of Rumen Protozoa and Bacteria

Rumen ciliates were identified according to the method of Dehority (2003). Rumen fluid was collected using a stomach tube from individual animals before the first

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feeding offer (zero h). Rumen fluid was filtered through two layers of cheesecloth. A two ml of rumen fluid was pipetted into a screw-capped test tube containing 5 ml of formalinized physiological saline (containing 20 ml formaldehyde in 100 ml distilled water). Thereafter, two drops of brilliant green dye (2 g brilliant green and 2ml glacial acetic acid diluted to 100 ml with distilled water) was added to the test tube, mixed thoroughly and allowed to stand overnight at room temperature. Total and differential counts of protozoa were made in 30 microscopic fields at a magnification of 20× in a Haemocytometer (Neubauer improved, Marienfeld, Germany). Rumen digesta (50 g) for analysis of microbial populations were collected from the mid rumen. The anaerobic techniques of Hungate (1969) as modified by Bryant (1972) were used for the growth of organisms and preparation of the media. First, Hungate tubes containing media and Whatman no. 1 filter paper, as the sole source of carbohydrate for growing cellulolytic bacteria, and Hungate tubes containing media and gelatin powder, as the protein source, were prepared. Then, rumen fluid was diluted and inoculated to the tubes. Cultures were grown at 39°C for 14 days. Cellulolytic and proteolytic bacteria were also enumerated in broth medium using the MPN procedure described by Dehority *et al.* (2003).

Analytical Methods

The fresh oak leaves were analyzed according to AOAC (1990) for dry matter (DM, method 930.15), crude fat, ash (method 924.05) and N (method 984.13). Ash-free neutral detergent fiber (NDFom) was determined, without sodium sulphite in the ND, according to Van Soest *et al.* (1991). ADFom (method 973.18; AOAC 1990) was determined and expressed exclusive of residual ash. Nitrogen in feed and urine was determined by the Kjeldahl. The dilution effect was taken into account in calculating the N content of urine. Total phenolics (TP) was measured using the Folin Ciocalteau method (Makkar, 2000). Total tannin (TT) was determined after adding insoluble PVPP (polyvinylpyrrolidone) and reacting with Folin Ciocalteau reagent on the basis of the difference between TP and NTP (Makkar, 2000). Tannic acid (Merck GmbH, Darmstadt, Germany) was used as the standard to express the amount of TP and TT. To estimate CT and HTs, random air dried leaf samples were ground to pass 1mm sieve before chemical analysis. The CT standard was separated from non-tannin phenolics using Sephadex LH20 as described by Hagerman and Butler (1989). HTs were analysed using Rhodanine assay according to Makkar (2000). The results were expressed as gallotannin. Urinary purine derivatives were estimated by spectrophotometric method (Chen and Gomes, 1995). Allantoin was determined in urine by colorimetric method after conversion of allantoin to phenyl hydrazone at 522 nm. The combined concentration of xanthine and hypoxanthine were assayed by their conversion to uric acid with xanthine oxidase at 293 nm. The uric acid was measured from the reduction in O.D. at 293 nm following conversion of uric acid to allantoin with uricase. The purine derivates (PD) excreted in a day was used in the iteration process to calculate the microbial protein supply as described (Chen and Gomes, 1995) as given below:

$$Y = 0.84X + (0.150W^{0.75}\ e^{-0.25x})$$

Where, Y is the urinary PD excretion as mmol/day; X the absorbed exogenous purine as mmol/day; W the live weight. The calculation of X based on the above non-linear equation can be performed by means of the Newton–Raphson iteration process as given below:

$$\frac{f(Xn)}{f'(Xn)}\ X_{n}- \ = X_{(n+1)}$$

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Where, $f(X) = 0.84X + 0.150W^{0.75} e^{-0.25x} - Y$; and the derivative of

$f(X) = f_(X) = 0.84X - 0.038W^{0.75} e^{-0.25x}$

For the samples, gas production kinetics, ME and OM digestibility (OMD) were determined as described by Menke and Steingass (1988) and Makkar (2004). Samples of rumen fluid were collected from three rumen-canulated sheep fed twice daily a diet containing lucerne hay (650 g/kg) plus concentrate mixture (350 g/kg) prior to their morning feeding, strained through two layers of cheesecloth, transferred into pre-warmed CO_2-filled thermos bottles and the fluid samples were combined prior to *in vitro* fermentation. The temperature of the rumen fluid was maintained at 39 ˚C throughout the preparation of the incubation medium. Syringes were pre-warmed (39 ˚C) for 1 h before addition of 30 ml of rumen buffer mixture into each syringe, and incubated in a water bath maintained at 39±0.1 ˚C as described by Menke and Steingass (1988). Samples (200±0.20 and 375±0.20 mg) were incubated in 30 ml of incubation medium (Makkar, 2004). Analyses were completed in triplicate with readings of gas production recorded after incubation. Differences in the composition and activity of rumen fluid inoculum without substrate was controlled by parallel measurements within incubation of buffered ruminal fluid (Blank test Gb0) and incubation of a standard hay meal (200 mg DM; Hohenheim hay standard), which should give a mean gas production of 44.16 ml at 24 hours (GbH). From these measurements, each series of determinations was corrected using 44.16/(GbH-Gb0). Cumulative gas production data were fitted to the exponential equation:

$$Y = b\,(1 - e^{-ct})$$

Where *Y* is the gas produced at *t* time, *b* is the gas production after 120 h from the insoluble but fermentable fraction (ml/g OM), *c* the gas production rate constant for *b* and *t* the incubation time.

The organic matter digestibility (OMD) (g kg^{-1} DM) and metabolisable energy (ME) (MJ kg^{-1} DM) in Oak leaves and alfalfa were estimated by equations of Menke and Steingass (1988), based on 24 h gas production (Gas, ml) and CP content (g kg^{-1} DM) as:

OMD (g kg^{-1} OM) = 148.8 + 8.89 GAS + 4.5 CP + 0. 651 XA and

ME (MJ kg^{-1} DM) = 2.20 + 0.136 GAS + 0.057 CP + 0.0029 CP^2.

Where OMD is OM digestibility, ME is metabolisable energy; CP is crude protein in g 100 g^{-1} DM; XA ash in g 100 g^{-1} DM; and GAS is the net gas production (ml) for 200 mg of sample.

Statistical Analysis

Protozoa population counts were transformed ($\log_{10}$) before statistical analysis. The three-tube MPN tables were used to estimate the number of cellulolytic and proteolytic bacteria in medium (Dehority, 2003). All data was analyzed using the Statistical Analysis System (2001). Data obtained from in vivo digestibility were analyzed as a 3X3 change over design using a general linear model. Multiple comparisons among means were performed with the Duncan method.

$$Y_{ijk} = \mu + T_i + P_j + A_k + e_{ijk}$$

$\boldsymbol{Y_{ij}}$: is observation; $\boldsymbol{\mu}$: is general mean; $\boldsymbol{T_i}$: is treatment P_j: is period; A_k: is animal and e_{ijkj}: the standard error term common for all observations.

RESULTS

Nutrients Digestibilities

Incorporation of oak leaves significantly (P<0.05) reduced the DMD, OMD, CPD and NDFD of the OL and OL+PEG diets in comparison to the control diet (Table 3).

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Table 3. Mean values for DMD, OMD, CPD, NDFD, ruminal pH, ammonia-N (mg/dl) and microbial nitrogen (g/d), in sheep fed the experimental diets.

	Diets				
	Control	OL	OL+PEG	SEM	Sig.
DMD [a]	682[a]	495[b]	498[b]	20.3	*
OMD [b]	719[a]	506[b]	521[b]	9.75	*
CPD [c]	726[a]	630[b]	754[a]	12.56	*
NDFD [d]	656[a]	478[b]	480[b]	10.50	*
pH	6.27	6.26	6.41	0.05	ns
NH_3-N [e]	18.99	16.57	22.43	2.61	ns
Purine derivatives					
Allantoin	4.22[b]	3.77[c]	5.34[a]	0.13	*
Uric acid	0.45	0.35	0.42	0.059	ns
Xanthine and hypoxanthine	0.074	0.056	0.065	0.007	ns
N gd^{-1}	3.15[b]	2.46[c]	4.4[a]	1.04	*
Endogenous faecal nitrogen loss	0.83[c]	2.6[a]	1.8[b]	0.10	*
Faecal nitrogen	3.90[b]	5.70[a]	3.50[b]	0.35	*

[a] dry matter digestibility (gkg^{-1} DM); [b] organic matter digestibility (gkg^{-1} DM); [c] crude protein digestibility (gkg^{-1} DM); [d]: Neutral detergent fiber digestibility; [e]: ammonia-N ($mgdl^{-1}$) and N gd^{-1}: g nitrogen production in rumen per day, Mean values in rows which do not have a common superscript letter are significantly different (P<0.05).

Adding PEG had no influence on the OMD and NDFD, but increased CPD (P<0.05).

Ruminal Parameters

The ruminal pH and ammonia concentration were not affected by the diet. The ammonia concentration increased during feeding period, reaching its peak 1 h after feeding, and decreased 5 h later. Urinary purine derivatives such as allantoin, uric acid, xanthine and hypoxanthine were estimated to determine the microbial nitrogen supply from rumen (Table 3). Allantoin excretion was less (P<0.05) on OL diet than the other 2 diets. However, the concentration of xanthine, hypoxanthine and uric acid were not affected by the diet. Adding PEG to the diet significantly (P<0.05) increased the urinary allantoin content. Consequently, microbial N concentration in OL diet was the lowest and in OL+PEG was the highest i.e., the increase was predominantly in allantoin concentration.

Enumeration of Rumen Protozoa and Bacteria

Total protozoa, *Isotricha, Diplodinium* and *Eudiplodinium* population declined in sheep fed OL diet in comparison to those fed the control diet (Table 4). However, the population of *Entodinium* genera (predominate protozoa), *Dasytricha, Metadinium* and *Ophrioscolex* were not affected by feeding oak leaves. When PEG was added to OL diet, inconstant results in the population of different protozoa genera were obtained.

Also, when sheep were fed OL diet, cellulolytic and proteolytic bacteria decreased significantly (P<0.05) in comparison to those fed the control diet, while the number of these bacteria increased (P< 0.05) after incorporation of PEG into the OL diet.

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Table 4. Number of protozoa and bacteria (log_{10}/g digesta) in the rumen samples of sheep fed the experimental diets.

	Diets				
	Control	OL	OL+PEG	SEM	Sig.
Protozoa					
Total	6.00^a	5.74^b	5.81^b	0.059	*
Entodinium	5.74	5.66	5.73	0.092	ns
Dasytricha	5.1	3.81	3.86	0.602	ns
Isotricha	4.85^a	1.57^b	0.00^c	0.52	*
Diplodinium	4.8^a	0.00^b	0.00^b	0.509	*
Eudiplodinium	2.61^a	0.00^b	0.00^b	0.375	*
Metadinium	2.61	1.57	0.00	0.712	ns
Ophrioscolex	1.62	0.52	0.52	0.606	ns
Bacteria					
Cellulolytic bacteria	7.68^a	5.85^c	7.27^b	0.052	*
Proteolytic bacteria	7.68^a	6.51^b	7.49^a	0.092	*

Mean values in rows which do not have a common superscript letter are significantly different ($P<0.05$). ns: not significant.

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DISCUSSION

Chemical Composition

Crude protein in oak leave was 116 g kg^{-1} DM (Table 1) and either comparable (Yildiz *et al*., 2005) or 18 to 30% higher (Kamalak, 2004; Ben Salem *et al*., 2005) or 5.7% lower (Yousef Elahi and Rouzbehan, 2007) than the previous studies. This could be due to variation in the species, age, microenvironment, etc., (Kamalak, 2004).

Similarly, OMD and ME content of oak leaves in the present study were 400 g/kg DM and 6.28 MJ/kg DM respectively, and either comparable (Sing *et al*., 1996) and was 14% less than earlier estimates (Yousef Elahi and Rouzbehan, 2008). The disagreement between the results of this study and others may be due to species variation (Kamalak, 2004) and different levels of CP, which are vital substrates for ruminal microorganism's growth (Van Soest, 1994).

Nutrients Digestibilities

The DMD, OMD, CPD and NDFD were significantly lower in sheep fed the OL diet than those fed the control diet. Many workers have reported that HTs may reduce cell-wall digestibility by direct inhibition of microorganisms and binding microbial enzymes and/or forming indigestible complexes with cell wall carbohydrates (Silanikove *et al*., 1996). This finding is in agreement with earlier observations (Sing *et al*., 1996) but disagree with contrary reported by Bhatta *et al*., (2005). The addition of PEG has been used to neutralize the negative effect of tannin in tanniniferous feeds (Makkar, 2003). However, in the present study, addition of PEG had no effect on DMD, OMD and NDFD, which may be due to presence of HTs and HTs-protein complexes as NDF in faeces, thus compromising NDF digestibility (Table 3). These results are in agreement with earlier studies conducted on sheep fed with *Quercus coccifera* L. containing 43 g tannin/kg DM (Ben Salem *et al*., 2005) and

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Quercus hartwissiana with 63 g tannin kg^{-1} DM and 11 g HTs kg^{-1} DM (Yildiz *et al*., 2005). In contrast, Ben Salem *et al*. (2003) have shown that NDFD increased in goats fed to *Quercus coccifera* L. containing 34.8 g tannin kg^{-1} DM (21 g tannin/day) and 15 g PEG day^{-1}. Discrepancy between Ben Salem *et al*. (2003) conclusions and our findings may be ascribed to the difference in the plant material used, concentration and structure of tannins. Tannins (condensed or hydrolysable) lead to formation of complexes mainly with proteins and, to a lesser extent, with polysaccharides, limiting their availability to animals (Makkar, 2003). In the present study, the increase in CP digestibility following PEG intake support the findings that tannins may reduce CPD. A similar effect was also seen in the study of Yildiz *et al*. (2005) who observed decreases in N digestibility of sheep fed oak leaves. In contrast, Bhatta *et al*. (2005) noted that CP digestibility was not reduced when sheep were fed *A. nilotica* foliage, in spite of the presence of HTs in this feed. They suggested that the absence of negative effects of HTs on N digestibility could be attributed to the quantity of HTs ingested *i.e.* 1.8% DM. Barry *et al*. (1986) reported that less than 4% of tannin in the ration was beneficial to ruminants.

Ruminal Parameters

In all diets, pH values were within the normal range. Tannins have been reported to have mixed effect on rumen pH such as decrease (Osakwe *et al*., 2004; Yanez Ruiz *et al*., 2004), or increase (Ben Salem *et al*., 1999) or have no effect on ruminal pH (Sliwiniski *et al*., 2002; Yildiz *et al*., 2005).

The ruminal ammonia values for all diets were within the normal range (85-300mg/l) of rumen fluid (McDonald *et al*., 1995). Ruminal ammonia was not significantly different in all diets, but there was a decrease in OL diet in comparison to the control diet (Figure 1). Many authors have indicated that the principal effects of tannins in ruminal fermentation include a reduction in proteolysis of dietary protein and, subsequently, lower concentrations of ammonia in rumen fluid (Frutos *et al*., 2004; Mueller-Harvey, 2006). Formation of HTs and proteins complex depends on pH, preferably at a pH of 3–4, but still occurring at typical rumen fluid conditions (Sliwiniski *et al*., 2002). However, inclusion of PEG increased ruminal ammonia concentration, although not significantly, which may indicate more ruminal fermentation of the dietary protein than those without PEG (Makkar, 2003; Yildiz *et al*., 2005). Hydrolysable tannins may have a less damaging effect on protein digestion than condensed tannin because tannins-protein complex may hydrolyze in the acidic gastric environment and release the bound proteins. This would explain the limited increase in rumen ammonia by binding tannins in *Quercus* with PEG.

The daily excretion of allantoin was negatively influenced by tannins in OL diet, which may be due to the decline in microbial population in the rumen. It can be suggested that HTs in OL diet caused a decline in the microbial protein production in the rumen. This decline could be due to the high concentration of HTs in OL that lowers the amount of truly degraded substrate in the rumen (Makkar, 2003), leading to a reduction in the growth of ruminal microorganisms (McSweeney *et al*., 2001a).

In contrast**,** Yildiz *et al*. (2005) found that microbial flow from the rumen was not affected when sheep fed low level of HTs (185 or 370 g d^{-1} of oak leaves containing 11 g HTs kg^{-1} DM) and suggested that tannins channel higher proportion of available nutrients to microbial mass synthesis and less to short chain fatty acids production. The addition of PEG has neutralized the negative effect of HTs and increased the microbial protein yield that may be due to the improvement in nitrogen availability in rumen (Ben Salem *et al*., 2005). In line with our findings, Yildiz *et al*. (2005) illustrated

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that PEG improved the ruminal microbial protein in sheep fed oak leaves.

Enumeration of Rumen Protozoa and Bacteria

The total protozoa, *Isotricha, Diplodinium* and *Eudiplodinium* population were reduced in sheep fed OL diet in comparison to those fed the control diet, probably due to the presence of tannins (Vaithiyanathan *et al*., 2007). However, the population of *Entodinium* genera, *Dasytricha, Metadinium* and *Ophryoscolex* were not affected by feeding oak leaves. No conclusive explanation could be made from earlier studies about the effect of tannins on protozoa population in rumen (Chiquette *et al*., 1989; Sliwiniski *et al*., 2002), because of variation in the diet type, tannins level, species, individual animal differences and sampling methods, (Yanez Ruiz *et al*. 2004). Results were inconsistent even with feeding OL and PEG (McSweeney *et al*., 2001a; Monforte-Briceno *et al*., 2005; Mojahed, *et al*., 2000; Yanez Ruiz *et al*., 2004). The absence effect of PEG to neutralize tannin defaunating influence may be due to presence of saponin in oak leaves. In this study, saponin concentration was not measured, but Arramon *et al*. (2002) and Romussi *et al*. (1994) have shown that this plant contains saponin. Saponins from different sources have been found to have antiprotozoal activity and have been suggested as possible defaunating agents (Wallace *et al*., 1994). However, feeding of PEG had both beneficial and adverse effects in ruminants. Apart from the concentration of tannins, their nature also influences the response of animals to PEG incorporation. Moreover, whether the PEG fed to the animals can bind all tannins present in the diet to make them inert is yet to be known (Makkar, 2003).

In sheep fed oak leaves, the reduction in cellulolytic and proteolytic bacteria was probably due to the presence of tannins, since the number of these organisms was increased when PEG was added to OL diet. A reduction in the cellulolytic and proteolytic population on feeding of OL diet could be explained by several mechanisms including (1) direct inhibition of these bacteria through tannin interactions with its cell wall and secreted catabolic enzymes, (2) reduced substrate availability due to binding of tannin with nutrients (McSweeney *et al*., 2001b). In sheep fed oak leave plus PEG, the population of both cellulolytic and proteolytic bacteria increased, which is consistent with the findings of McSweeney *et al*. (2001b) and Min *et al*. (2002), respectively.

CONCLUSIONS

Diet containing *Q. Libani* leaves had lower ruminal fermentability than the diet containing alfalfa and supplementation of PEG in OL diet improved the fermentability. However, the inclusion of 80 g of this supplement per day is not an appropriate amount in terms of cost**:**benefit analysis under Iranian condition. Therefore, **t**here is a need to explore cheap PEG-like compounds tannin-complexing agents for enhancing utilization of tanniniferous feeds.

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تاثیر تانن برگ بلوط بر تخمیر شکمبه گوسفند

م. ج. ابرقویی، ی. روزبهان، و د. علیپور

چکیده

این مطالعه در قالب طرح گردان با استفاده از ۶ راس گوسفند نر اخته شده با میانگین وزن ۶۱/۸ کیلوگرم در ۳ دوره ۲۸ روزه برای بررسی اثرات تانن موجود در برگ بلوط بر روی پارامترهای شکمبه انجام گرفت. . مقدار۸۰ گرم پلی‌اتیلن‌گلیکول (PEG) به ازای هر راس گوسفند در روز برای خنثی کردن اثرات تانن استفاده گردید. سه جیره آزمایشی شامل: شاهد (یونجه خشک، جو، سبوس گندم و کاه گندم)؛ OL: (برگ بلوط، جو، سبوس گندم و اوره)؛ OL+PEG: (جیره OL بعلاوه PEG) بود. حیوانات در قفسهای انفرادی جداگانه قرار داده شدند. قبل از شروع دوره اندازه گیری، ۲۱ روز آداپتاسیون انجام گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و پارامترهای شکمبه (pH، آمونیاک، باکتریها و پروتوزوآ) و سنتز پروتئین میکروبی به وسیله مشتقات پورینی ادرار اندازه‌گیری شد. برگ بلوط باعث کاهش قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF گردید (P<۰/۰۵) و استفاده از PEG فقط قابلیت هضم پروتئین را افزایش داد. مقادیر pH و آمونیاک شکمبه در دامنه استاندارد شکمبه بودند و در بین تیمارهای آزمایشی تفاوت معنی‌داری نداشتند (P>۰/۰۵). تانن قابل هیدرولیز موجود در برگ بلوط مقدار آلانتوئین دفعی از طریق ادرار را کاهش داد و با دادن PEG این مقدار افزایش یافت. تفاوت معنی‌داری بین تیمارها برای مقدار اسید اوریک، گزانتین و هیپوگزانتین مشاهده نشد (P>۰/۰۵). تیمار OL تولید نیتروژن میکروبی را کاهش داد، در حالی که اضافه کردن PEG آن را بهبود بخشید. تیمار OL باعث کاهش تعداد کل پروتوزآ شد، که با افزودن PEG نیز تغییر معنی‌داری مشاهده نشد (P>۰/۰۵). استفاده از برگ بلوط باعث کاهش تعداد باکتری های سلولیتیک و پروتئولیتیک (بر مبنای لگاریتم۱۰) گردید (P<۰/۰۵). با اضافه کردن PEG این جمعیت‌ها افزایش یافت. در کل، این داده‌ها پیشنهاد می‌دهد که تیمار برگ بلوط در مقایسه با یونجه باعث کاهش تخمیر شکمبه گردید و اضافه کردن پلی‌اتیلن‌گلیکول قابلیت تخمیر شکمبه را بهبود بخشید.

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