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

M. J. Abarghuei¹, Y. Rouzbehan¹*, and D. Alipour²

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 leaf, 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

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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 \textit{Q. libani} was nutritionally the best among \textit{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 \textit{Q. libani} on the ruminal parameters (pH, ammonia concentration, microbial protein, bacterial and protozoa counts) of Ghezal sheep. We also examined the effects on the ruminal parameters of PEG treatment in deactivating tannins in \textit{Q. libani}.

**MATERIAL AND METHODS**

**Oak Leave and alfalfa**

Oak leaves (\textit{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.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Dry matter</th>
<th>Crude protein</th>
<th>Crude fat</th>
<th>ADFom</th>
<th>ME</th>
<th>OMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oak leave</td>
<td>959</td>
<td>116</td>
<td>27</td>
<td>316</td>
<td>6.3</td>
<td>400</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>960</td>
<td>150</td>
<td>28</td>
<td>341</td>
<td>8.7</td>
<td>539</td>
</tr>
</tbody>
</table>

ADFom: acid detergent Fiber; GP: gas production; ME: metabolisable energy and OMD: organic matter digestibility. OMD was measured using in vitro gas production method.
Table 2. Ingredients and nutrient composition (g kg$^{-1}$ DM) or as stated for the experimental diets given to sheep.

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

$^a$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
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 derivatives (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:

\[ \frac{f'(X_n)}{f''(X_n)} X_{n+1} = X_n - X_{n+1} \]

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Where, \( f(X) = 0.84X + 0.150W \cdot Y \); and the derivative of 
\( f(X) = f'(X) = 0.84X - 0.038W \cdot e^{-0.25x} \).

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)
before 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 \( 39^\circ C \)
throughout the preparation of the incubation medium. Syringes were pre-warmed (\( 39^\circ C \))
for 1 h before addition of 30 ml of rumen buffer mixture into each syringe, and
incubated in a water bath maintained at \( 39\pm0.1^\circ C \) 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:

\[
Y = b \left(1 - e^{-ct}\right)
\]

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:

\[
\text{OMD} \text{ (g kg}^{-1}\text{ OM)} = 148.8 + 8.89 \text{ GAS} + 4.5 \text{ CP} + 0.651 \text{ XA and}
\]

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

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
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.

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

\( Y_{ij} \): is observation; \( \mu \): is general mean; \( T_i \):
is treatment; \( P_j \): is period; \( A_k \): is animal and \( e_{ij} \): 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).
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.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Control</th>
<th>OL</th>
<th>OL+PEG</th>
<th>SEM</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD (^a)</td>
<td>682</td>
<td>495</td>
<td>498</td>
<td>20.3</td>
<td>*</td>
</tr>
<tr>
<td>OMD (^b)</td>
<td>719</td>
<td>506</td>
<td>521</td>
<td>9.75</td>
<td>*</td>
</tr>
<tr>
<td>CPD (^c)</td>
<td>726</td>
<td>630</td>
<td>754</td>
<td>12.56</td>
<td>*</td>
</tr>
<tr>
<td>NDFD (^d)</td>
<td>656</td>
<td>478</td>
<td>480</td>
<td>10.50</td>
<td>*</td>
</tr>
<tr>
<td>pH</td>
<td>6.27</td>
<td>6.26</td>
<td>6.41</td>
<td>0.05</td>
<td>ns</td>
</tr>
<tr>
<td>NH(_3)-N(^e)</td>
<td>18.99</td>
<td>16.57</td>
<td>22.43</td>
<td>2.61</td>
<td>ns</td>
</tr>
</tbody>
</table>

Purine derivatives
- Allantoin: 4.22 \(^b\), 3.77 \(^c\), 5.34 \(^a\), 0.13 \(*\)
- Uric acid: 0.45, 0.35, 0.42, 0.059 \(\text{ns}\)
- Xanthine and hypoxanthine: 0.074, 0.056, 0.065, 0.007 \(\text{ns}\)
- 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 \(^c\), 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.
Table 4. Number of protozoa and bacteria (log_{10}/g digesta) in the rumen samples of sheep fed the experimental diets.

<table>
<thead>
<tr>
<th>Prototaxa</th>
<th>Control</th>
<th>OL</th>
<th>OL+PEG</th>
<th>SEM</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Entodinium</td>
<td>5.74</td>
<td>5.74b</td>
<td>5.81b</td>
<td>0.059</td>
<td>*</td>
</tr>
<tr>
<td>Dasytricha</td>
<td>5.1</td>
<td>3.81</td>
<td>3.86</td>
<td>0.602</td>
<td>ns</td>
</tr>
<tr>
<td>Isotricha</td>
<td>4.85a</td>
<td>1.57b</td>
<td>0.00c</td>
<td>0.52</td>
<td>*</td>
</tr>
<tr>
<td>Diplodinium</td>
<td>4.89b</td>
<td>0.00b</td>
<td>0.00b</td>
<td>0.509</td>
<td>*</td>
</tr>
<tr>
<td>Eudiplodinium</td>
<td>2.61a</td>
<td>0.00b</td>
<td>0.00b</td>
<td>0.375</td>
<td>*</td>
</tr>
<tr>
<td>Metadinium</td>
<td>2.61</td>
<td>1.57</td>
<td>0.00</td>
<td>0.712</td>
<td>ns</td>
</tr>
<tr>
<td>Ophrioscole</td>
<td>1.62</td>
<td>0.52</td>
<td>0.52</td>
<td>0.606</td>
<td>ns</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulolytic bacteria</td>
<td>7.68a</td>
<td>5.85c</td>
<td>7.27b</td>
<td>0.052</td>
<td>*</td>
</tr>
<tr>
<td>Proteolytic bacteria</td>
<td>7.68a</td>
<td>6.51b</td>
<td>7.49a</td>
<td>0.092</td>
<td>*</td>
</tr>
</tbody>
</table>

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

DISCUSSION

Chemical Composition

Crude protein in oak leaves 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 Rouzbehian, 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 Rouzbehian, 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
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...
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, there is a need to explore cheap PEG-like compounds tannin-complexing agents for enhancing utilization of tanniniferous feeds.

REFERENCES

تأثیر تانر بر گل‌بوط بر تخمیر شکم‌های گوسفند
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چکیده
این مطالعه در قالب طرح گردان با استفاده از ۶ راس گوسفنده نر اخت تهدره با میانگین وزن ۹۱/۸ کیلوگرم در ۳ دوره ۲۰ روزه برای برسی اثرات تانر موجود در گل‌بوط بر روی پارامترهای شکم‌های
اجام گرفت. مقدار ۵۰ گرم پیلی اتانول گلیکول (PEG) اضافه سه مقدار یک آزمایش شامل شاهد (سناره خشک‌کن جو، سوسس گندم و کاه گندم)، (برگ گل‌بوط، جو سوسس گندم و اوره) (چرخه OL+PEG)، (برگ گل‌بوط و اروره) (OL) بدون پیلاتیورا. حیوانات در فضه‌ای انفرادی جداگانه قرار داده شدند. قبل از شروع دوره اندازه گیری، ۲۱ روز آداباتسیون اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهنین اولین اندازه گرفت. در هر دوره قابلیت هضم ماده خشک، ماده آلی، پروتئین خام، NDF و آهن