Effect of polyethylene glycol on in vitro gas production, metabolizable energy and organic matter digestibility of Quercus cerris leaves

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Abstract

In this study the effects of Polyethylene glycol (PEG) on in vitro gas production, organic matter digestibility (OMD) and metabolizable energy (ME) contents of Quercus cerris leaves were investigated. Gas production was measured at 3, 6, 12, 24, 48, 72 and 96 hours in the presence (15, 30, 60 and 90 mg) and in the absence of PEG (MW 8000), and gas production kinetics were estimated using the equation y = a + b (1 - e^-ct).

PEG had a significant effect on in vitro gas production, OMD and ME. The OMD and ME contents of Quercus cerris leaves increased with increased level of PEG. The mean increase in OMD per mg PEG supplementation was 0.121 digestibility units although the mean increase in ME per mg PEG supplementation was 0.0185 metabolizable energy units.

In vitro gas production showed positive responses to incubation of Quercus cerris leaf samples with tannin binding agent (PEG) in comparison to non-treated samples. The improvement in gas production, OMD and ME depended on the level of PEG supplementation. The improvement in gas production, OMD and ME with PEG emphasizes the negative effect of tannins on digestibility. However PEG supplementation to improve the nutritive value of tannin containing tree leaves should be further analysed in detail whether or not it is economical before large scale implementation.

Key Words: Digestibility, gas production, in vitro, metabolizable energy, polyethylene glycol

Introduction

Oak leaves and twigs are often grazed by animals or lopped to use as livestock fodder during lean periods (Singh et al 1996). Approximately 7 million ha of forest in Turkey were covered by oak trees (Kayacik 1996). Oak leaves contain a considerable amount of condensed tannins (Kamalak et al 2004). Condensed tannins are phenolic compounds of
widespread occurrence in higher plants. They are heterogeneous in composition, and their chemical nature is not known in all cases (Barroso et al 2001). The use of tree and shrub leaves by herbivores may be restricted by defending or deterring mechanisms related to high tannin content (Provenza 1995). Tannins act within the animal's digestive tract by binding to the substrate to be digested (usually proteins, carbohydrate, lipids), inhibiting digestive enzymes or exerting anti-microbial effects (Scalbert 1991). However, PEG can form a stable complex with tannins thereby preventing the binding between tannins and proteins (Bandran and Jones 1965). Therefore, PEG has been widely used to reduce the detrimental effect of condensed tannin in ruminant diets (Pritchard et al 1998; Barry 1989; Silanikova et al 1994; Jones et al 2000).

The aim of this study was to determine the effect of PEG on in vitro gas production kinetics, OMD and ME of *Quercus cercis* leaves.

**Materials and Methods**

**Forage Samples**

Leaves from *Quercus cercis* were harvested in June, 2004 from the city of Kahramanmaraş, in the south of Turkey. The area is located at altitude of 630 m above sea level. The mean annual rainfall and temperature are 858 mm and 16.2 °C respectively. Leaves were hand harvested from at least 10 different trees, then pooled and oven dried at 60°C for 48 h (Abdulrazak et al 2000).

**Chemical Analysis**

Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in a muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC 1990). Crude protein was calculated as N X 6.25. Acid detergent fiber (ADF) content and neutral detergent fiber (NDF) content of leaves were determined using the method described by Van Soest et al (1991). Condensed tannin was determined by butanol-HCl method as described by Makkar et al (1995). Mimosa tannin (MT; Hodgson, England) was used as an external standard. All chemical analyses were carried out in triplicate.

**In vitro gas production**

Rumen fluid was obtained from two fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988) as follows. 0.200 g dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 ml in the presence (15, 30, 60 and 90 mg) and in the absence of PEG (MW 8000). The syringes were pre-warmed at 39°C before injecting 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Gas production was measured as the volume of gas.
in the calibrated syringes and was recorded before incubation (0) and 3, 6, 12, 24, 48, 72 and 96 hours after incubation. Total gas values were corrected for blank incubation which contained only rumen fluid. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979)

\[
y = a + b \left(1 - \exp^{-ct}\right)
\]

Where:

- \(a\) = the gas production from the immediately soluble fraction (ml)
- \(b\) = the gas production from the insoluble fraction (ml)
- \(c\) = the gas production rate constant for the insoluble fraction (b)
- \(t\) = incubation time (h)
- \(y\) = gas produced at time 't'

The OMD of forages was calculated using equations of Menke et al (1979) as follows:

\[
\text{OMD} (%) = 14.88 + 0.889 \text{GP} + 0.45 \text{CP} + \text{XA}
\]

Where:

- \(\text{GP}\) is 24 h net gas production (ml/200 mg),
- \(\text{CP}\) = Crude protein (%)
- \(\text{XA}\) = Ash content (%)

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

Where:

- \(\text{GP}\) is 24 h net gas production (ml/200 mg),
- \(\text{CP}\) = Crude protein

**Statistical Analysis**

One-way analysis of variance (ANOVA) was carried out to compare gas production kinetics, OMD and ME values using the General Linear Model (GLM) of Statistica for windows (1993). Significance between individual means was identified using the Tukey's multiple range test (Pearse and Hartley 1966). Mean differences were considered significant at \(P<0.05\). Standard errors of means were calculated from the residual mean square in the analysis of variance.

**Results and Discussion**
The chemical composition of *Quercus cerris* leaves (Table 1) is consistent with that reported by Kamalak et al (2004).

**Table 1.** The chemical composition of *Quercus cerris* leaves

<table>
<thead>
<tr>
<th>Constituents</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>946</td>
</tr>
<tr>
<td><strong>As g/kg of DM</strong></td>
<td></td>
</tr>
<tr>
<td>Crude protein</td>
<td>84</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>435</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>360</td>
</tr>
<tr>
<td>Ash</td>
<td>55</td>
</tr>
<tr>
<td>Condensed tannin</td>
<td>42</td>
</tr>
</tbody>
</table>

There was considerable increase in gas production when the oak leaves were incubated in the presence of PEG (Figure 1). The increase in cumulative volume of gas production depended on the level of PEG supplementation. This result is in agreement with findings of Seresinhe and Iben (2003) and Tedonkeng et al (2004).

**Figure 1.** The effect of Polyethylene glycol on gas production

The gas production kinetics, are given in Table 2. The PEG supplementation had also a significant effect on the estimated parameters of OMD and ME (Table 2). PEG supplementation increased the gas production from the insoluble fraction (b) whereas PEG supplementation had no effect on the gas production from the immediately soluble fraction (a), and the gas production rate (c). On the other hand there were significant increases in the OMD and ME content of the oak leaves. These results are in agreement with the findings of Getachew et al (2001), Getachew et al (2002) and, Seresinhe and Iben (2003). PEG, a non-nutritive synthetic polymer, has a high affinity to tannins and makes tannins inert by forming tannin PEG complexes (Makkar et al 1995). PEG also can also liberate protein from the preformed tannin-protein complexes (Barry et al 1986). The increase in the gas production in the presence of PEG is possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen. McSweeney et al (1999) showed that addition of PEG caused a significant and marked increase in the rate and extent of ammonia production.

**Table 2.** The parameters estimated from the gas production of *Quercus cerris* leaves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Estimated Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>0 PEG</td>
<td>2.40</td>
</tr>
<tr>
<td>Treatment</td>
<td>Gas Production (a)</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------</td>
</tr>
<tr>
<td>15 PG</td>
<td>3.24</td>
</tr>
<tr>
<td>30 PEG</td>
<td>2.73</td>
</tr>
<tr>
<td>60 PEG</td>
<td>2.47</td>
</tr>
<tr>
<td>90 PEG</td>
<td>3.07</td>
</tr>
<tr>
<td>SEM</td>
<td>0.320</td>
</tr>
<tr>
<td>Sig.</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means within the same column with differing superscripts are significantly different.

*** P<0.001, NS- Non-significant. SEM: Standard error mean, Sig: Significance level

a = the gas production from the immediately soluble fraction (ml),
b = the gas production from the insoluble fraction (ml),
c = the gas production rate constant for the insoluble fraction (b),
a+b: Potential gas production,
ME: Metabolizable energy, OMD: Organic matter digestibility

The mechanism of dietary effects of tannins may be understood by their ability to form complex with proteins. Tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh 1984). Tannin can adversely affect the microbial and enzyme activities (Singleton 1981; Lohan et al 1983; Barry and Duncan 1984; Makkar et al 1989).

The improvement in gas production, OMD and ME with PEG emphasizes the negative effect tannins may have on digestibility. PEG, a non-nutritive synthetic polymer, has a high affinity to tannins and makes tannins inert by forming tannin PEG complexes (Makkar et al 1995). PEG also can also liberate protein from the preformed tannin-protein complexes (Barry et al 1986).

As can be seen from Figure 2, the OMD of tannin-containing tree leaves increased with increased level of PEG. The mean increase in OMD per mg PEG supplementation was 0.121 digestibility units.

Figure 2. The relationship between dose of PEG supplementation and organic matter digestibility

As can be seen from Figure 3 ME of tannin containing tree leaves increased with increased level of PEG. The mean increase in ME per mg PEG supplementation was 0.0185 metabolizable energy units.

Figure 3. The relationship between dose of PEG supplementation and estimated metabolizable energy
In this experiment the results of PEG did not reach the optimum. Therefore the linear part of the curve was obtained. It does not mean that all the sample incubated with rumen fluid is digestible when sufficient PEG is added. This is not the case. However further study is required to identify the optimum as the relationship will be curvilinear.

All tannin-containing leaves do not give similar responses to PEG supplementation. The gas production from *Calliandra calothyrsus* was linearly increased with increased levels of PEG whereas gas production from *Gliricidia sepium* was curvilinearly increased with increased levels of PEG (Serresinhe and Iben 2003), possibly due to differences in chemical composition of tannins in the leaves.

The results of this experiment support the fact that PEG can be added to tannin-containing plant material in *in vitro* fermentation systems to demonstrate the nutritional importance of tannins on organic matter digestibility and to measure nutritive value of the forage after neutralization (Makkar et al 1995; McSweeney et al 1999; Getachew et al 2001). However there is a lack of information about feasibility of using PEG in tannin-rich diets for ruminants. PEG supplementation to improve the nutritive value of *Quercus cerris* leaves should be further analysed in detail whether or not it is economical due to high price of PEG, before large scale implementation. However, Makkar (2003) reported that some other substances such as wood ash, NaOH and urea can be used instead of PEG.

**Conclusion**

- PEG supplementation had a significant effect on the gas production, OMD and ME content of *Quercus cerris* leaves. The improvement in gas production, OMD and ME depended on the level of PEG.
- PEG supplementation to improve the nutritive value of tannin-containing tree leaves should be evaluated in animal response trials to ascertain whether or not it is economical before large scale implementation.

**References**


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