Effect of maturity stage on the nutritive value of birdsfoot trefoil 
(Lotus corniculatus L) hays

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Abstract

The aim of this study was to determine the effect of maturity stage on the chemical composition, gas production kinetics, organic matter digestibility (OMD) and metabolizable energy (ME) content of Lotus corniculatus hay. Gas production were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h and their kinetics were described using the equation $p = a + b(1-e^{-ct})$. Maturity had a significant effect on the chemical composition, gas production kinetics, OMD and ME content of Lotus corniculatus hay. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents of Lotus corniculatus hay increased with increasing maturity whereas the crude protein (CP) and ether extract (EE) contents decreased. The crude protein content of Lotus corniculatus hay ranged from 14.96 to 17.63 %. NDF, ADF and ADL contents ranged from 31.59 to 37.62 %, 25.73 to 31.06 % and 14.49 to 17.75% respectively.

The gas production kinetics, OMD and ME content of Lotus corniculatus decreased with increasing maturity. The OMD and ME contents of Lotus corniculatus hay ranged from 68.61 to 74.45%, 10.18 to 11.03 MJ/kg DM respectively. The gas production kinetics, OMD and ME content were negatively correlated with NDF, ADF and ADL content of Lotus corniculatus hay whereas the same parameters were positively correlated with CP of Lotus corniculatus hay. It was concluded that Lotus corniculatus hay will provide more CP and ME energy for ruminants when harvested or grazed at vegetative and flowering stage.

Key words: Birdsfoot trefoil, Lotus corniculatus, Chemical composition, Maturity, Digestibility, Metabolizable Energy

Introduction

Birdsfoot trefoils (Lotus corniculatus L.) is a drought resistant forage legume plant which tolerates low soil fertility and can be used for grazing in many countries (Beuselinck and Grant, 1995; Foo et al., 1996). Lotus corniculatus is of interest because of its condensed tannins and its effects on animal production. Condensed tannins in Lotus corniculatus may
form stable complexes with dietary protein in the rumen, thereby increasing the proportion
of bypass protein (Hedqvist et al., 2000). Although some researches have been carried out by
several researches in the most parts of world (Min et al., 2001; Hedqvist et al., 2000; Ramirez-
Restrepo et al., 2004; 2005) there is limited report on the nutritive value of Lotus corniculatus in Turkey. Accurate prediction of forages quality during the growth cycle would allow targeting of harvest or grazing to desired levels of nutritive composition to meet specific animal requirements (Valente et al., 2000). The chemical composition and in vitro gas production technique were widely used to evaluate the nutritive value of forages used in ruminant nutrition (Evitayani et al., 2004; Larbi et al., 1998; Tolera et al., 1997).

The aim of this study was to determine the effect of maturity stage on the nutritive value of
Lotus corniculatus hay in terms of chemical composition, gas production kinetics, OMD and
ME content using in vitro gas production technique.

Materials and Methods

Hay samples
Birdsfoot trefoil plants at vegetative, flowering, and mature stages were hand harvested from
at least three replicate plots established in the experimental field in Bursa, Turkey, in 2005. The experimental design was a randomized complete block design with three replications. Samples were shade-dried and representative dry samples from each plot was taken to laboratory and milled in a hammer mill through a 1 mm sieve for subsequent analysis.

Chemical analysis
Ash content was determined by igniting the dry samples in muffle furnace at 525 °C for 8 h. Nitrogen (N) content was measured by the Kjeldhal method (AOAC, 1990). Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and Acid detergent lignin (ADL) content was determined by the method of Van Soest et al. (1991). Condensed tannin (CT) was determined by butanol-HCl method as described by Makkar et al. (1995). All chemical analyses were carried out in triplicate.
Crude protein (CP) was calculated as N X 6.25. Ether extract (EE) was determined by the

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%). Samples were incubated in vitro rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). 0.200 g dry weight of the sample was weighed into calibrated glass syringes of 100 ml. The syringes were prewarmed at 39 °C before the injection of 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. Readings of gas production recorded before incubation (0) and 3, 6, 12, 24, 48, 72 and 96 h after incubation. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979):
\[ y = a + b (1-e^{-ct}) \]

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 metabolizable energy (ME) (MJ/kg DM) content of *Lotus corniculatus* hay samples was calculated using equation of Menke *et al.* (1979) as follows:

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

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

Organic matter digestibility (OMD) (%) of *Lotus corniculatus* hay samples was calculated using equation of Menke *et al.* (1979) as follows:

\[ \text{OMD (%) = 14.88 + 0.889GP + 0.45CP + 0.0651 XA} \]

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

**Statistical analysis**

One-way analysis of variance (ANOVA) was carried out to compare chemical composition, gas production kinetics, OMD and ME content of *Lotus corniculatus* hay with stage as the main factor using 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. A simple correlation analysis was used to establish the relationship between chemical composition and gas production kinetics or OMD and ME content of *Lotus corniculatus* hay.

**Results and Discussion**

**Chemical composition**

Chemical compositions of *Lotus corniculatus* hay harvested at three different maturity stages are given in Table 1. Maturity stage had a significant \( P<0.001 \) effect on the chemical composition of *Lotus corniculatus* hay except for CT.
### Table 1. The effect of maturity stage on the chemical composition (% of DM) of *Lotus corniculatus* hay.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vegetative</th>
<th>Flowering</th>
<th>Mature</th>
<th>SEM</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>7.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.045</td>
<td>***</td>
</tr>
<tr>
<td>EE</td>
<td>5.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.140</td>
<td>***</td>
</tr>
<tr>
<td>CP</td>
<td>17.63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.120</td>
<td>***</td>
</tr>
<tr>
<td>NDF</td>
<td>31.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.118</td>
<td>***</td>
</tr>
<tr>
<td>ADF</td>
<td>25.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.084</td>
<td>***</td>
</tr>
<tr>
<td>ADL</td>
<td>14.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.115</td>
<td>***</td>
</tr>
<tr>
<td>CT</td>
<td>2.27</td>
<td>2.19</td>
<td>2.05</td>
<td>0.089</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means within the same row with various superscripts are significant. SEM = Standard error mean, Sig = significance level, NS = Non-significant, ***P<0.001

Ash and EE contents ranged from 6.45 to 7.03% and 4.33 to 5.18%, respectively. These results are consistent with Niezen *et al.* (2002) who found that the ash content and EE content of *Lotus corniculatus* hay ranged from 7.1 to 8.1 and 3.8 to 4.1%, respectively.

CP content of *Lotus corniculatus* hay ranged from 14.96 to 17.63%. CP content of *Lotus corniculatus* is in agreement with findings of Ramirez-Restrepo *et al.* (2006) who found that CP content of *Lotus corniculatus* ranged from 10.56 to 21.93% and decreased with increasing maturity. On the other hand, CP content of *Lotus corniculatus* hay harvested at different maturity stage was lower than those reported by Niezen *et al.* (2002) who found that CP content of *Lotus corniculatus* obtained in summer and autumn ranged from 21.9 to 22.6%, respectively. The differences may be possibly due to growing conditions and fertilization applied.

CP content of *Lotus corniculatus* hay harvested at early flowering stage was significantly (P<0.001) higher than those of flowering and mature stages. This result is also consistent with finding of Kamalak *et al.* (2005a, b) who found that the CP contents of *Gundelia tuonefortii* and *Sinapsis arvensis* decreased with advancing maturity. The decrease in CP concentration with advancing maturity occurs both because of decrease in protein in leaves and stems, and because stems, with their lower protein concentration, make up a larger portion of the herbage in more mature forage (Buxton, 1996). The average decreases in CP concentration with advance in maturity for several forages averaged 1 g kg<sup>-1</sup> d<sup>-1</sup> in data reported by Minson (1990).

EE content decreased with increased maturity. EE content of *Lotus corniculatus* hay harvested vegetative stage was significantly (P<0.001) higher than those of flowering and mature stages. This is not in agreement with finding of Kamalak *et al.* (2005a) who found...
that EE content of *Gundelia tuonefortii* hay increased with increasing maturity.

NDF, ADF and ADL contents of *Lotus corniculatus* hay were significantly increased with advancing maturity. NDF, ADF and ADL contents ranged from 31.59 to 37.62 %, 25.73 to 31.06 % and 14.49 to 17.75% respectively. NDF and ADF contents of *Lotus corniculatus* hay are comparable with findings of Niezen *et al.* (2002) who reported that NDF and ADF contents harvested in summer and autumn ranged from 26.9 to 31.6 and 20.3 to 25.7%, respectively.

NDF, ADF and ADL contents of *Lotus corniculatus* hay harvested at vegetative stage was significantly (P<0.001) lower than those of flowering and mature stages. This result is consistent with finding of Kamalak *et al.* (2005a, b) who found that NDF and ADF contents of *Gundelia tuonefortii* and *Sinapsis arvensis* were increased with increasing maturity.

CT content of *Lotus corniculatus* hay ranged from 2.05 to 2.27%. This result is consistent with findings of Ramirez-Restrepo *et al.* (2006) and Niezen *et al.* (2002) who found that CT content of *Lotus corniculatus* ranged from 0.83 to 2.87% and 1.6 to 5.5%, respectively. Some reports have clearly suggested that the low level CT in *Lotus corniculatus* hay improved the efficiency of protein digestion in ruminants (Waghorn *et al.*, 1987) and animal production (Wang *et al.*, 1996; Ramirez-Restrepo *et al.*, 2005) since CT in plants can form indigestible complexes with dietary protein in the rumen (pH range 3.5-7.0), thereby increasing the proportion of bypass protein (Hedqvist *et al.*, 2000). The tannin-protein complex is dissociated at the lower pH in the abomasum, resulting in a higher absorption of dietary amino acids from duodenum (Douglas *et al.*, 1995). Some reports have also suggested that condensed tannin may act directly as anthelmintics against parasitic nematodes or indirectly by improving nitrogen supply (Niezen *et al.*, 1995; Robertson *et al.*, 1995; Butter *et al.*, 1998).

**Gas production and estimated parameters**

Gas production of *Lotus corniculatus* hay harvested at different maturity stages is presented in Figure 1. Gas production data are given in Table 2. Gas produced after 96 h incubation ranged between 72.3 and 76.7 ml per 0.200 g of substrate. As can be seen from Figure 1, gas production of *Lotus corniculatus* hay decreased with increased maturity. This result is in consistent with findings of Kamalak *et al.* (2005a, b), Zinash *et al.* (1996) and Lee *et al.* (2000) who reported a decrease in gas production with increased maturity.

Gas production of *Lotus corniculatus* hay harvested at vegetative stage was considerably higher than mature stage. The gas production kinetics, OMD and ME are given in Table 2. The gas production rate (c) of *Lotus corniculatus* hay decreased with increased maturity. There were also significant decrease in gas production (b) from insoluble fraction and potential gas production (a+b) with increased maturity.

OMD and ME content of *Lotus corniculatus* hay ranged from 68.61 to 74.45%, 10.18 to 11.03 MJ/kg DM, respectively. These results are consistent with findings of Ramirez-Restrepo *et al.* (2006) who found that the OMD and ME content of *Lotus*
corniculatus hay decreased with increasing maturity and ranged from 62.9 to 74.0%, 9.7 to 11.2 MJ/kg DM, respectively. These results are also consistent with findings of Niezen et al. (2002) who found that OMD and ME content of Lotus corniculatus hay summer and autumn ranged from 71.5 to 72.6%, 10.7 to 11.6 MJ/kg DM, respectively.

![Gas production vs Incubation time](image)

**Figure 1.** The effect of maturity stage on the *in vitro* gas production of *Lotus corniculatus* hay

NDF, ADF and ADL contents increased with increased maturity (Table 1). An increase in NDF, ADF and ADL contents resulted in the lower gas production (b) from insoluble fraction and potential gas production (a+b) with increased maturity.

Gas production kinetics (c, a, b (a+b)), ME and OMD were negatively correlated with cell wall contents (NDF, ADF and ADL; Table 3). This result is consistent with findings reported by Kamalak et al. (2005 a, b), Ndlovu and Nherera (1997), Larbi et al. (1998), and Abdulrazak et al. (2000).
Table 2. The effect of maturity stage on the gas production kinetics, organic matter digestibility (%) and metabolizable energy (MJ / kg DM) of *Lotus corniculatus* hay

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vegetative</th>
<th>Flowering</th>
<th>Mature</th>
<th>SEM</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>c</td>
<td>6.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.077</td>
<td>***</td>
</tr>
<tr>
<td>a</td>
<td>2.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.141</td>
<td>***</td>
</tr>
<tr>
<td>b</td>
<td>70.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.425</td>
<td>***</td>
</tr>
<tr>
<td>a+b</td>
<td>73.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.415</td>
<td>***</td>
</tr>
<tr>
<td>OMD</td>
<td>74.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.499</td>
<td>***</td>
</tr>
<tr>
<td>ME</td>
<td>11.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.076</td>
<td>***</td>
</tr>
</tbody>
</table>

<sup>c</sup> = gas production rate (%), <sup>a</sup> = gas production (mL) from quickly soluble fraction, <sup>b</sup> = gas production (mL) from the insoluble fraction, <sup>a+b</sup> = potential gas production (mL). Means within the same row with various superscripts are significant. SEM=Standard error mean, Sig. - significance level, ***P<0.001.

Table 3. Correlation coefficient (r) of relationship of chemical composition with gas production kinetics or organic matter digestibility (%) and metabolizable energy (MJ / kg DM)

<table>
<thead>
<tr>
<th>Ash</th>
<th>CP</th>
<th>EE</th>
<th>NDF</th>
<th>ADF</th>
<th>ADL</th>
<th>CT</th>
</tr>
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<tbody>
<tr>
<td>c</td>
<td>0.649&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.917&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.726</td>
<td>-0.942&lt;sup&gt;***&lt;/sup&gt;</td>
<td>-0.931&lt;sup&gt;***&lt;/sup&gt;</td>
<td>-0.889&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>a</td>
<td>0.884&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.859&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.199&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>-0.667</td>
<td>-0.734&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.506&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>b</td>
<td>0.441&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.694&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.791&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.884&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.819&lt;sup&gt;**&lt;/sup&gt;</td>
<td>-0.891&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>a+b</td>
<td>0.639&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.847&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.730&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.916&lt;sup&gt;***&lt;/sup&gt;</td>
<td>-0.915&lt;sup&gt;***&lt;/sup&gt;</td>
<td>-0.908&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>OMD</td>
<td>0.646&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.893&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.781&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.949&lt;sup&gt;***&lt;/sup&gt;</td>
<td>-0.946&lt;sup&gt;***&lt;/sup&gt;</td>
<td>-0.931&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>ME</td>
<td>0.630&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.882&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.788&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.945&lt;sup&gt;***&lt;/sup&gt;</td>
<td>-0.939&lt;sup&gt;***&lt;/sup&gt;</td>
<td>-0.932&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>c</sup> = gas production rate (%), <sup>a</sup> = gas production (mL) from quickly soluble fraction, <sup>b</sup> = gas production (mL) from the insoluble fraction, <sup>a+b</sup> = potential gas production (mL), * P<0.05, **P<0.01, ***P<0.001, NS = Non-significant.

Early stage of growth, all parts of plants are highly digestible, but that during stem elongation and flowering there is a more rapid decline in digestibility of stem than of leaf (Terry and Tilley, 1964). The decline in digestibility can results from the interaction of factors such as increased fiber concentration in plant tissues (Wilson *et al*., 1991), increased lignification during plant development (Morrison, 1980) and different leaf/stem ratio (Hides *et al*., 1983).
Conclusion

The maturity stage had a significant effect on the chemical composition, in vitro gas production kinetics and organic matter digestibility and metabolizable energy contents of *Lotus corniculatus* hay. Cell wall contents of *Lotus corniculatus* hay increased with increasing maturity whereas the crude protein (CP) decreased with increased maturity. As a result of these the organic matter digestibility and metabolizable energy content decreased with increased maturity. It was concluded that *Lotus corniculatus* hay will provide more CP and ME energy for ruminants when harvested or grazed at vegetative and flowering stage.

References


