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Araştırma Makalesi

Potential Nutritive Value of Browse Foliages from *Pinus pinaster, Prunus amygdalus* and *Ulmus glabra*

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Abstract

The aim of the current study was to determine the influence of species and harvest stage on the potential nutritive value of tree foliages using the chemical composition and *in vitro* gas production technique. The foliages were harvested in early May, mid July, early September and mid October of 2006 and analysed for their chemical composition. Gas production was recorded at 0, 3, 6, 12, 24, 48, 72 and 96 h after incubation. Chemical composition of the foliages differed significantly among the species and harvest stages. The crude protein content ranged from 73.6 g kg^{-1} DM in *Pinus pinaster* to 124.5 g kg^{-1} DM in *Ulmus glabra*. Total cell wall contents of *Pinus pinaster* (530.0 g kg^{-1} DM) and *Ulmus glabra* (413.3 g kg^{-1} DM) were higher that that of *Prunus amygdalus* (326.4 g kg^{-1} DM). Among the foliage species tested, *Pinus pinaster* had the highest content of condensed tannins with 167.1 g kg^{-1} DM, followed by *Ulmus glabra* (157.7 g kg^{-1} DM) and *Prunus amygdalus* (61.9 g kg^{-1} DM). Significant differences were found in gas production and ruminal fermentation parameters among the browse foliage species and harvest stages. *Prunus amygdalus* generally produced the highest gas volume, which was followed by *Ulmus glabra* and by *Pinus pinaster*, respectively. Gas production from the foliages in mid October was generally greatest, which was followed by in mid September and by in both early May and mid July.

It can be concluded that *Prunus amygdalus* and *Ulmus glabra* have the nutritional potential for goats and sheep when browsed alone or in combination. On the contrary, *Pinus pinaster* is unlikely to maintain nutritional status of small ruminants and should be supplemented with N for better utilisation.

Key words: Condensed tannin, crude protein, goat, roughage, sheep

Pinus pinaster, Prunus amygdalus ve *Ulmus glabra* Yapraklarının Potansiyel Besleme Değerinin Saptanması

Özet

Kimyasal kompozisyon ve *in vitro* gaz üretim tekniği kullanarak tür ve hasat zamanının ağaç yapraklarının besleme değeri üzerine etkilerinin araştırılması bu çalışmanın amacını oluşturmuştur. Ağaç yaprakları erken Mayıs, Temmuz ortası, erken Eylül ve Ekim ortasında hasat edilmiş ve kimyasal kompozisyonları belirlenmiştir. Inkübasyonun 0, 3, 6, 12, 24, 48 ve 96 saatlerindeki gaz üretim değerleri saptanmıştır. Yaprakların kimyasal kompozisyonu tür ve hasat zamanına bağlı olarak önemli düzeyde değişim göstermiştir. Ham protein içeriği 73.6 g kg^{-1} KM (*Pinus pinaster*) ile 124.5 g kg^{-1} KM (*Ulmus glabra*) arasında değişim göstermiştir. *Pinus pinaster* (530.0 g kg^{-1} KM) ve *Ulmus glabra* (413.3 g kg^{-1} KM) toplam lif içerikleri *Prunus amygdalus*'tan (326.4 g kg^{-1} KM) yüksek bulunmuştur. Test edilen türler arasında en yüksek kondense tanen içeriği *Pinus pinaster* yapraklarında (167.1 g kg^{-1} KM), daha sonra *Ulmus glabra* (157.7 g kg^{-1} KM) ve *Prunus amygdalus* (61.9 g kg^{-1} KM) yapraklarında saptanmıştır. Yaprakların gaz üretimi ve *in vitro* fermantasyon parametreleri tür ve hasat zamanına bağlı olarak önemli düzeyde değişim göstermiştir. En yüksek gaz üretimini *Prunus amygdalus* yaprakları sağlamıştır. Bunu sırasıyla *Ulmus glabra* ve *Pinus pinaster* takip etmiştir. Erken Ekim ortasında hasat edilen yapraklar en yüksek gaz üretimini sağlarken bunu sırasıyla Eylül ortası, erken Mayıs ve Temmuz ortasında hasat edilen yapraklar takip etmiştir.

Prunus amygdalus ve *Ulmus glabra* yapraklarının tek başına veya karışık otlatılmaları durumunda keçilerin ve koyunların beslenmesi açısından potansiyel besleme değerine sahip oldukları sonucuna varılmıştır. Buna karşın, *Pinus pinaster* yapraklarının küçükbaş hayvanların besleme durumunu desteklemesi muhtemel değildir ve kullanım etkinliğini artırmak amacıyla N ile desteklenmelidir.

Anahtar kelimeler: Kondense tanen, ham protein, keçi, kaba yem, koyun

Introduction

There is growing body of evidence that the frequency of severe environmental events such as, forest fires, floods, landslides, droughts, desertification, heat weaves and water scarcity will increase in the near future as a consequence of global warming. Some parts of the Mediterranean have been reported to face even more severe environmental problems affecting the land stability, its potential to produce food and water resources (Rubio, 2009). Consequences of severe environmental changes will have an impact not only on the ecological balance of the earth but also on economical, social and cultural structures of the societies in these regions. The sustainability of animal-based agricultural systems such as ruminants would possibly be under the high risk of degradation by the environmental changes in the future. It is highly probable that the feeding of ruminants will be less dependent on feedstuffs derived from the cultivation of plants, but more on natural resources such as forested ranges, savannas and shrublands during periods of food scarcity.

Trees are important components of woodlands and fill in a significant niche in habitat through wild life reservoir, soil fertility maintenance, water conservation, landscaping and production of food and fuel wood for man (Depommier, 2003; Papachristou et al., 2005). They also provide green fodder year-round (evergreen species) or at critical times of the year (deciduous species) for ruminants and there is renewed research interest in woody species owing to their important role in sustainable production systems (Papanastasis et al., 2008). *Pinus pinaster, Prunus amygdalus* and *Ulmus glabra* are the main components of the natural pastures, which are preferentially browsed by goats in Çanakkale province. However, their nutritive value as fodder for small ruminants is not known. The aim of this study was to investigate the influence of species and harvest stage of the trees on the chemical composition and *in vitro* fermentation characteristics of foliages.

Materials and methods

Foliage samples

Nutritive value of foliages from the tree species; *Pinus pinaster, Prunus amygdalus and Ulmus glabra* that are well adapted to the climate of Çanakkale Province in Turkey was investigated in this study. The selected tree species are the main components of a woody pasture and largely browsed by goats in the area. Foliages were collected by hand from 4 different trees, which were the representatives of the grazing area in early May, mid July, early September and mid October of 2006. The area was located at an altitude of 36 m above sea level with and annual rainfall and temperature of 505 mm and 14.8 ^{o}C, respectively.

Chemical analysis

The samples were dried on the bench in the laboratory and ground to pass through 1.0 mm sieve for subsequent analyses. All the samples were analyzed for crude protein (CP; method 990.03), ether extract (EE; method 2003.05) and ash (method 942.05) according to the standard methods of AOAC (2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) analyses were carried out as described by Van Soest et al. (1991). Heat stable amylase was not used in NDF determination and NDF was expressed with residual ash. Condensed tannin (CT) was determined by butanol-HCl method as described by Makkar et al. (1995). All chemical analyses were carried out in quadruplicate.

In vitro incubations

Rumen fluid was obtained from two fistulated sheep fed twice daily with a diet consisting of alfalfa hay (60%) and concentrate (40%). About 200 mg of sample were incubated *in vitro* with 30 ml of rumen fluid-buffer mixture (ratio of 1:2) in calibrated glass syringes in a water bath kept at 39.0 °C in triplicate following the procedures of Menke et al. (1979). Blanks with buffered rumen fluid were also included in the incubations. Gas production after 3, 6, 12, 24, 48, 72 and 96 h of incubation was recorded. Cumulative gas production data were fitted to the exponential equation: $y = a + b\ (1\text{-}exp^{-ct})$ (Ørskov and McDonald, 1979),

Where y is the gas production at time t; a is the gas production from the immediately soluble fraction (ml), b is the gas production from the insoluble fraction (ml), c is the gas production rate constant, a + b = the potential gas production (ml), t = incubation time (h).

The metabolisable energy (ME; MJ kg^{-1} DM) of foliages was calculated using equations of Menke et al. (1979) as follows:

$$ME\ (MJ\ kg^{-1}\ DM) = 2.20 + 0.136\ GP + 0.057\ CP$$

$R^2 = 0.94$

Where, GP is 24 h net gas production (ml/200 mg),
CP = Crude protein (%)

The *in vitro* organic matter digestibility (IVOMD) of foliages was calculated using equations of Menke et al. (1979) as follows:

IVOMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651XA

Where, GP is 24 h net gas production (ml/200 mg),
CP = Crude protein (%)
XA = Ash content (%)

Statistical analysis

Data on chemical composition, *in vitro* gas production kinetics, OMD and ME contents of tree leaves were subjected to the two way of ANOVA using GLM of SPSS for Windows (2002), and were analyzed based on the statistical model : Y_{ij}: = μ_{ij} + S_i + P_j + $(S \times P)_{ij}$ + e_i. Where, Y_{ij} is the general observation on chemical composition, *in vitro* gas production kinetics, OMD and ME contents, S_i the *i*th effect of species on the observed parameters and P_j the *j*th effect of harvest stage on the observed parameters. The $(S \times P)_{ij}$ term represents *i*th and *j*th interaction effects of species and harvest stage on chemical composition, *in vitro* gas production kinetics, OMD and ME contents, and e_i the standard error term common for all observations. Significance between individual means was identified using the Duncan test. 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

The chemical composition of the tree foliages harvested at different vegetative stages in the present study is presented in Table 1 and showed significant variations (Table 1). The CP content ranged from 73.6 g kg^{-1} DM in *Pinus pinaster* to 124.5 g kg^{-1} DM in *Ulmus glabra*. The EE content of *Ulmus glabra* was significantly lower than that of *Pinus pinaster* and *Prunus amygdalus*. Total cell wall contents of *Pinus pinaster* (530.0 g kg^{-1} DM) and *Ulmus glabra* (413.3 g kg^{-1} DM) were higher that that of *Prunus amygdalus* (326.4 g kg^{-1} DM). The ADF content differed significantly, which was highest in *Pinus pinaster* with 388.0 g kg^{-1} DM, followed by *Ulmus glabra* with 193.8 g kg^{-1} DM and by *Prunus amygdalus* with 170.6 g kg^{-1} DM (Table 1). Ash content of the foliage species varied from 39.2 g kg^{-1} DM in *Pinus pinaster* to 142.5 g kg^{-1} DM in *Ulmus glabra*. Among the foliage species tested, *Pinus pinaster* had the highest level of CT with 16.7%, followed by *Ulmus glabra* (15.8%) and *Prunus amygdalus* (6.2%).

The CP content was highest in early May with 12.7% on DM basis then decreased to 8.2% in early September, and then increased again up to 9.4% in mid October (Table 2). In spite of numerical differences, harvest stage did not result in any difference in EE content. The total cell content ranged significantly from 37.0% in early May to 45.9% in mid October. On the other hand, the ADF and ADL contents of the foliages did not vary among the harvest stages (P>0.05). The ash content of the foliage was lowest in early May with 7.3% and gradually increased up to 12.9% on DM basis in mid October. A similar trend was observed in CT content, which increased from 10.2% in early May up to 16.6% on DM basis in mid October (Table 1).

Table 1. The effect of species and harvest stage on the chemical composition of tree foliages

	Chemical Constituents						
Species	CP (g kg^{-1} DM)	EE (g kg^{-1} DM)	NDF (g kg^{-1} DM)	ADF (g kg^{-1} DM)	ADL (g kg^{-1} DM)	Ash (g kg^{-1} DM)	CT (g kg^{-1} DM)
Prunus amygdalus	111.9b	88.6b	326.4a	170.6a	77.8a	118.8b	61.9a
Pinus pinaster	73.6a	114.6b	530.0b	388.0c	79.8a	39.2a	167.1c
Ulmus glabra	124.5b	54.2a	413.3b	193.8b	139.9b	142.5c	157.7b
SEM	3.99	8.18	10.07	5.01	3.33	2.96	2.84
Harvest stage							
Early May	127.2b	88.0	370.3a	253.0	107.5	73.0a	102.0a
Mid July	95.6a	83.8	451.7bc	255.0	99.5	91.3b	106.4a
Early September	82.0a	114.7	411.1ab	262.2	99.8	90.3b	141.1b
Mid October	94.4a	77.0	459.1c	255.9	96.5	128.6c	166.2c
SEM	4.61	9.45	11.63	5.71	3.85	3.42	2.46
Treatment effects							
Species	***	***	***	***	***	***	***
Harvest stage	***	NS	***	NS	NS	***	***
Interaction	**	NS	**	***	***	***	***

Row means with common superscripts do not differ (P>0.05), CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin, CT: Condensed tannin, SEM: Standard error of mean; ** P<0.01, *** P<0.001, NS: Non-significant at P>0.05

In vitro gas production characteristics, ME and IVOMD of the browse foliages of the tree species over the vegetative stage is presented in Table 2. Significant differences were found in gas production and ruminal fermentation parameters among the browse foliage species and harvest stages. Gas production from the fermentation of the three foliages was comparable after 3 h of incubation. However, gas production differed among the foliage species after 6 h up to 96 h of the incubation. *Prunus amygdalus* generally produced the highest gas volume, which was followed by *Ulmus glabra* and by *Pinus pinaster*, respectively (Table 2). Gas production from the immediately soluble fraction (a) was highest in *Ulmus glabra*, followed by *Pinus pinaster* and *Prunus amygdalus*. On the other hand, *Prunus amygdalus* was the foliage species with the highest gas production from the insoluble fraction (b). Rate of gas production was greatest in *Prunus amygdalus* and lowest in *Ulmus glabra*. *Prunus amygdalus* had the greatest ME content and OMD; whereas *Pinus pinaster* had the lowest values (Table 2).

Gas production parameters except for rate of gas production differed among the foliage species throughout the incubation. Gas production from the foliages in mid October was generally greatest, which was followed by in mid September and by in both early May and mid July, respectively (Table 2). ME content of the foliages ranged from 9.21 MJ kg^{-1} DM in mid July to 10.21 MJ kg^{-1} DM in mid October. IVOMD followed a pattern similar to ME content of the foliages.

Discussion

Chemical composition of the foliage species plays a crucial role in the extent to which they are utilised by goats and sheep (Osuga et al., 2008). *Prunus amygdalus* and *Ulmus glabra* can be regarded as intermediate quality roughages since they contain over 80 g CP kg^{-1} DM and less than 500 g NDF kg^{-1} DM (Leng, 1990) during the most part of grazing period and can provide sufficient ammonia for the growth of microorganisms in the rumen (Kamalak et al., 2005). They also have the potential to meet the requirements of small nutrients when they are the basal diet especially during the critical periods of the year, such as in summer when herbaceous vegetation is dry (Papanastasis et al., 2008). On the other hand, *Pinus pinaster* can be classified as low quality forage due its low CP and high NDF content (Table 1) and can not maintain sufficient ammonia concentrations for microbial activity in the rumen. This foliage species should be supplemented with nitrogen when it is the only feed source.

The fibre content of roughages is one of the crucial factors determining the digestibility and intake (Van Soest, 1994). Low fibre content of foliages such as *Prunus amygdalus* and *Ulmus glabra* would probably increase the voluntary intake and digestibility of these foliages by small ruminants. El Hassan et al. (2000) also demonstrated that the fibre of browse foliages was more digestible than that of mature grasses and crop residues. Hence, the use of these foliages as supplements in sheep and goats may improve the utilisation of poor quality pastures or crop residues as the basal diet by providing non-structural carbohydrates, rumen degradable N or highly colonised fibre source to inoculate rumen bacteria onto the less digestible fibre (Leng, 1990).

Tannins are complex polyphenolic compounds present in plants. The issue of tannins in animal nutrition is controversial. They are able to bind proteins and to form complexes with carbohydrates, thereby reducing the digestibility and utilization of nutrients in the gut (Kumar and Vaithiyanathan, 1990) when high amounts (>40 g kg^{-1} DM) are consumed by the animals (Barry and McNabb, 1999). On the contrary, beneficial effects of tannins such as suppression of bloat and protection of dietary protein in the rumen are reported (Mueller-Harvey, 2006). In the present study, species and harvest stage significantly influenced the CT content of the foliages (Table 1). The CT content of *Pinus pinaster* and *Ulmus glabra* was quite higher than the nutritional critical levels of 3-4 % on DM basis (Barry and McNabb, 1999). In contrast, *Prunus amygdalus* had a reasonable CT content of 61.9 g kg^{-1} DM, which is lower than the detrimental levels of >70 g kg^{-1} DM. The CT content of the foliages increased from 102 g kg^{-1} DM in early May up to 166.2 g kg^{-1} DM in mid October (Table 1), which coincides with the progression of vegetation leading to maturation in plants (Cabiddu et al., 2000). However, it should be born in mind that the nutritional significance of tannins depends on several factors such as the intake level and biological activity of tannins, ruminant species (sheep vs. goat) and the use of polyethylene glycol (Decandia et al., 2008).

The *in vitro* gas production system is a reliable tool to evaluate feedstuffs for ruminants since gas production is reported to be highly correlated with microbial protein synthesis and *in vitro* digestibility (Getachew et al., 1998). The effect of foliage species and harvesting stage on *in vitro* gas production and their kinetics is given in Table 2. The foliage species and harvest stage resulted in significant effects *on in vitro* gas production and their kinetics. Generally gas production of the foliage from

Table 2. The effect of species and harvest stage on the gas production kinetics, metabolisable energy and organic matter digestibility of tree foliages

	Gas production parameters over incubation period												
Species	3 h (ml)	6 h (ml)	12 h (ml)	24 h (ml)	48 h (ml)	72 h (ml)	96 h (ml)	c	a (ml)	b (ml)	a+b (ml)	ME (MJ kg^{-1} DM)	OMD (%)
Prunus amygdalus	15.26b	28.55c	42.23c	53.44c	63.30c	67.25c	68.98c	0.078c	1.46a	65.06c	66.51c	10.10c	68.20c
Pinus pinaster	14.64a	24.71a	36.59b	47.90a	56.31a	61.20a	64.20a	0.073b	2.14b	59.05a	61.20a	9.12a	61.02a
Ulmus glabra	14.82ab	25.70b	35.57a	48.95b	59.00b	65.32b	67.46b	0.061a	2.90c	62.34b	62.25b	9.61b	65.26b
SEM	0.168	0.248	0.230	0.205	0.489	0.236	0.271	0.001	0.084	0.241	0.251	0.027	0.206
Harvest stage													
Early May	13.34a	24.02a	35.56a	47.23a	56.01a	60.28a	61.85a	0.0709	1.555a	58.48a	60.03a	9.34b	63.06b
Mid July	13.86a	25.42b	35.72b	47.68a	56.38a	60.77a	63.54b	0.074	2.008b	58.84a	60.85a	9.21a	62.17a
Early September	15.46b	27.32c	39.17b	50.55b	61.98b	66.33b	68.33c	0.0696	2.516c	63.47b	65.98b	9.72c	65.31c
Mid October	16.96c	28.54d	42.06c	54.92c	63.78b	70.98c	73.82d	0.0700	2.605c	67.80c	70.42c	10.21d	68.78d
SEM	0.194	0.286	0.266	0.237	0.564	0.272	0.313	0.010	0.097	0.278	0.298	0.023	0.179
Treatment effects													
Species	***	***	***	***	***	***	***	***	***	***	***	***	***
Harvest stage	***	***	***	***	***	***	***	NS	***	***	***	***	***
Interaction	***	***	***	***	***	***	***	***	***	***	***	***	***

Row means with common superscripts do not differ ($P>0.05$), a: Gas production from the immediately soluble fraction, b: Gas production from the insoluble fraction, c: Gas production rate constant, a+b: Potential gas production, ME: Metabolisable energy, OMD: Organic matter digestibility, SEM: Standard error of mean; *** $P<0.001$, NS: Non-significant at $P>0.05$

Prunus amygdalus was highest throughout the incubation period. In contrast, the lowest gas production was obtained from the fermentation of the foliages from *Pinus pinaster*. The high extent of gas production may be attributed to higher nutrient availability in *Prunus amygdalus* than in *Ulmus glabra* and *Pinus pinaster*, resulting in the fermentation of OM to produce short chain fatty acids and hence high gas volumes (Osuga et al., 2008) or a possible adaptation of microorganisms to structural carbohydrates and tannins (Ammar et al., 2005). High non-structural constituents and low tannin content of *Prunus amygdalus* can partly explain the observed high gas production. Potential gas production of the foliage species tested in the present study was higher than that of *Carpinus betilus* and *Juniperus communis*, but lower than that of *Glycrrhiza glabra* L, *Arbutus andrachne*, *Quercus libari* L and *Pistica lentiscus* (Kamalak et al., 2005).

In vitro methods have been successfully used for the prediction of ME and IVOMD contents of ruminants (Gatachew et al., 1998). The use of cumulative gas production derived from the fermentation of feedstuffs with buffered rumen fluid *in vitro* was proposed by Menke et al. (1979) and is widely used for the estimation of IVOMD and ME of roughages and concentrates. Significant differences were found among the species and harvest stage in terms of ME and IVOMD values of the foliages studied in this study (Table 2). The differences can be attributed to variations in CP and NDF contents of the foliages. High CP content and lower fibre fractions are reported to be partly responsible for high ME and IVOMD values (Kamalak et al., 2005; Decandia et al., 2008). However it is indicated that N-linked to acid detergent fibre, which is quite high in shrubs, has an important effect on OMD (Decandia et al., 2008). The ME contents of the foliage species were roughly comparable to *Glycrrhiza glabra* L, *Arbutus andrachne, Quercus libari* L and *Pistica lentiscus* and were higher than *Carpinus betilus* and *Juniperus communis* reported by Kamalak et al. (2005). Similarly, the IVOMD of the foliages were quite higher than that of the spontaneous species such as *Pistacia lentiscus* (Ammar et al., 2005) or were comparable to that of the cultivated species such as *Robinia pseudoacacaia* (Papachristou and Papanastasis, 1994).

The results of the present study suggest that *Prunus amygdalus* and *Ulmus glabra* have the nutritional potential for maintenance and lactation requirements of goats and sheep when browsed alone or in combination and can be used to supplement low quality mature pastures and crop residues. On the contrary, *Pinus pinaster* is unlikely to maintain nutritional status of small ruminants and should be supplemented with N for better utilisation. The presence and browsing of variety of species may be crucial for alleviating feed shortages during the critical periods of scarcity in food supply. As a conclusion, the maturity stage and species had a significant effect on the potential nutritive value of tree foliages.

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