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RESEARCH ARTICLE

Effect of Species on Nutritive Value of Mulberry Leaves

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Summary

The aim of this study was to determine the effect of species on the nutritive value of *Mulberry* leaves using chemical composition and *in vitro* gas production technique. The leaves of four different mulberry species were collected from 10 different trees. Gas productions of *Mulberry* leaves were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times and their gas production kinetics were described using the equation $y = A (1\text{-}exp^{-ct})$. Species had a significant effect on the chemical composition, *in vitro* gas production, metabolisable energy (ME) and organic matter digestibility (OMD) of *Mulberry* leaves. Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash and condensed tannin (CT) contents of mulberry leaves ranged from 25.97% to 46.27%, 11.75% to 23.72%, 19.38% to 33.33%, 17.33% to 26.06%, 15.40% to 22.3% and 0.47% to 0.76% respectively. The NDF and ash contents of *Morus rubra* were significantly (P<0.001) higher than those of other mulberry species whereas ADF and CP contents of *Morus alba* pendula were significantly (P<0.001) higher then those of other mulberry species. On the other hand the DM content of *Morus alba* was significantly (P<0.001) higher than those of others. Almost at all incubation times, gas productions of *Morus nigra* and *Morus alba* pendula were significantly (P<0.001) higher than those of *Morus alba* and *Morus rubra*. The values for ME, OMD and potential gas production (A) of *Morus alba pendula* were significantly (P<0.001) higher than those of the other mulberry species whereas the gas production rates of *Morus alba pendula* and *Morus alba* were significantly (P<0.001) higher than those of *Morus rubra* and *Morus nigra*. The time to produce 50% and 95% of total gas production (t_{50} and t_{95}) of *Morus alba pendula* and *Morus alba* were significantly (P<0.001) higher than those of *Morus rubra* and *Morus nigra*. In conclusion, species had a significant effect on the nutritive value of mulberry leaves. Although there were marked differences among species in chemical composition and some estimated parameter of mulberry leaves, it can be said that mulberry leaves had high CP content and were quite digestible.

Keywords: *Mulberry leaves, Nutritive value, Chemical composition, Condensed tannin, In vitro gas production, Digestibility*

Dut Yapraklarının Besleme Değerine Türün Etkisi

Özet

Bu çalışmanın amacı, dut yapraklarının besleme değerine türün etkisini kimyasal bileşimi ve *in vitro* gaz üretim tekniği kullanarak belirlemektir. Dört farklı dut türü kullanılmış olup her türden on ağaçtan yapraklar toplanmıştır. Dut yapraklarının *in vitro* gaz ölçümleri fermentasyonun 0, 3, 6, 12, 24, 48, 72 ve 96. saatlerinde yapılmıştır. Gaz üretimine ait parametreler $y = A (1\text{-}exp^{-ct})$ üssel fonksiyonu kullanılarak belirlenmiştir. Dut yapraklarının kimyasal bileşimine, in vitro gaz üretimine, metabolik enerji değerine (ME) ve organik madde sindirim derecesine (OMSD) türün önemli derecede etkisi vardır. Dut yapraklarının kuru madde (KM), ham protein (HP), nötral deterjan fiber (NDF), asit deterjan fiber (ADF) ve kondense tanen (KT) içeriği sırasıyla, %25.97 ile %46.27, %11.75 ile %23.72, %19.38 ile %33.33, %15.40 ile %22.3 ve %0.47 ile %0.76 arasında değişmiştir. *Morus rubra* yaprağının NDF içeriği diğer dut türlerinden daha yüksek bulunurken, *Morus alba pendula* yaprağının ADF ve HP içeriği diğer dut türlerinden yüksek bulunmuştur. Diğer taraftan *Morus alba* yaprağının KM içeriği diğer dut türlerinden daha yüksek bulunmuştur. Hemen hemen bütün inkübasyon zamanlarında *Morus nigra* ve *Morus alba pendula* yapraklarından üretilen gaz miktarı *Morus alba* ve *Morus rubra* yapraklarından daha fazla olmuştur. *Morus alba pendula* yapraklarının ME, OMSD ve potansiyel gaz üretim değeri (A) diğer dut türlerinden daha yüksek bulunurken *Morus alba pendula* ve *Morus alba* yapraklarının gaz üretim hızı (c) *Morus rubra* ve *Morus nigra* yaprakların gaz üretim hızından daha yüksek bulunmuştur. *Morus alba pendula* ve *Morus alba* yapraklarının fermantasyonu sonucu açığa çıkan gazın %50 ve %95'nin üretilmesi için geçen süre (t_{50} ve t_{95}) *Morus rubra* ve *Morus nigra* yapraklarından daha yüksek bulunmuştur. Sonuç olarak, dut yaprakların besleyici değeri üzerinde türün önemli etkisi vardır. Türler arasında bileşim ve bazı tahmin edilen parametreler bakımından belirli farklar olmasına rağmen dut yapraklarının yüksek protein içeriğine sahip ve oldukça sindirilebilir olduğu söylenebilir.

Anahtar sözcükler: *Dut yaprağı, Besleme değeri, Kimyasal bileşim, Kondense tanen, İn vitro gaz üretimi, Sindirim derecesi*

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INTRODUCTION

Mulberry tree can grow in a wide range of climatic, topographical and soil conditions [1]. It is well known that mulberry leaves are traditionally used for feeding the silkworm in the most parts of world. Mulberry fruits are also eaten fresh as well as dried forms and consumed in marmalades, juices, liquors, natural dyes and cosmetic industries [2]. Although there are many mulberry species, the Anatolia region of Turkey has growing conditions suitable for cultivating high quality mulberry fruits, mainly *Morus alba, Morus nigra* and *Morus rubra* [3].

Recently locally available mulberry tree leaves have received increasing attention as source of feed or feed ingredients to meet ruminant and some other simple stomach animals requirements for growth and production during the season when there is a shortage of adequate and good quality feed or feed ingredients [4-8].

Although there are a lot of information about the chemical composition and nutritive value of *Morus alba* and its varieties, the information about chemical composition and nutritive value of other mulberry species such as *Morus nigra* and *Morus rubra* is limited. In addition, there is no available information about the biomass production of mulberry leaves as a feed source for ruminant animals in Turkey. Recently some researches suggested that chemical composition and *in vitro* gas production technique can be used to determine the nutritive value of previously uninvestigated plants [9-14]. Therefore the aim of the current study was to provide a further contribution to knowledge about the nutritive value of some mulberry species grown in Turkey using chemical composition and *in vitro* gas production technique.

MATERIAL and METHODS

Leaves of four different mulberry species (*Morus nigra, Morus alba, Morus rubra* and *Morus alba pendula*) from 10 different mulberry trees were hand harvested on the 21st June 2011 in the same area in Kahramanmaraş in Turkey. Mulberry leave samples were shade dried and representative dry samples from each species was taken to laboratory and milled in a hammer mill through a 1 mm sieve for subsequent analysis. Dry matter (DM) was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method [15]. Crude protein was calculated as N X 6.25. Neutral detergent fiber (NDF) and ADF contents were determined by the method Van Soest et al.[16]. Condensed tannin was determined by butanol-HCl method as described by Makkar et al.[17].

Mulberry leave samples (0.200 g DM) milled through a 1 mm sieve were incubated *in vitro* with diluted rumen fluid (10 ml rumen fluid + 20ml culture medium) in triplicate calibrated glass syringes of 100 ml following the procedures of Menke et al.[18]. Rumen fluid was obtained from two cows fed a daily ration containing maize silage and concentrates. The cows had free access to water throughout the experiment. Rumen samples was collected before the morning meal in the thermos flaks and taken immediately to the laboratory where it was strained through 4 layers of cheesecloth and kept at 39°C. The rumen fluid was flushed with CO_2. The rumen fluid was added to buffered mineral solution in the ratio of 1:2 respectively. 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. Gas production values were recorded at 0, 3, 6, 12, 24, 48, 72 and 96 h after fermentation and corrected for blank incubation. Cumulative gas production data were fitted to non-linear exponential model as: $Y = A\ (1 - \exp^{-ct})$.

Where *Y* is gas production at time't', *A* is the potential gas production (ml/200 mg DM), c is the gas production rate (h^{-1}) and *t* is the incubation time (h).

Time (h) to produce 50 and 95 % of potential gas production using the equation suggested by Şahin et al.[19].

$t_{50}=0.693/c$

$t_{95}=2.996/c$

ME (MJ/kg DM) content of mulberry leaves was calculated using the equation of Menke et al.[18] as follows:

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP, where GP = 24 h net gas production (ml/200 mg); CP = crude protein.

Organic matter digestibility (%) of mulberry leaves was calculated using the equation of Menke et al.[18] as follows:

OMD (%) = 14.88 + 0.889GP + 0.45CP +0.0651 XA, where XA: ash content (%).

One-way analysis of variance (ANOVA) was used to determine the effect of species on the chemical composition, gas production kinetics, and some estimated parameters such as ME and OMD of mulberry leave. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $P<0.05$.

RESULTS

The effect of species on the chemical composition of mulberry leaves is given in *Table 1*. The species has significant ($P<0.001$) effect on the chemical composition of mulberry leaves. Dry matter, CP, NDF, ADF, ash and CT contents of mulberry leaves ranged from 25.97% to 46.27%, 11.75% to 23.72%, 19.38% to 33.33%, 17.33% to 26.06%, 15.40% to 22.3% and 0.47% to 0.76 % respectively. The NDF and ash contents of *Morus rubra* were significantly ($P<0.001$) higher than those of other mulberry species whereas ADF and CP contents of *Morus alba pendula* were significantly ($P<0.001$) higher then those of other mulberry species. On the other

Table 1. *The effect of species on the chemical composition of mulberry leaves*
Tablo 1. *Dut yaprağının bileşimi üzerine türün etkisi*

Nutrients (%)	Species				SEM	Sig.
	M. nigra	*M.alba*	*M. rubra*	*M. alba pendula*		
DM	42.20^{b}	46.27^{a}	37.36^{c}	25.97^{d}	0.727	***
CP	16.06^{c}	18.73^{b}	11.75^{d}	23.72^{a}	0.293	***
NDF	22.08^{c}	19.38^{d}	33.33^{a}	29.53^{b}	0.433	***
ADF	19.46^{c}	17.33^{d}	24.06^{b}	26.06^{a}	0.165	***
Ash	17.50^{b}	15.40^{c}	22.36^{a}	17.70^{b}	0.184	***
CT	0.74^{a}	0.76^{a}	0.47^{b}	0.67^{a}	0.059	***

^{abc} *Row means with common superscripts do not differ (P>0.05); s.e.m. * standard error mean; Sig. * significance level;* ***DM*** *- Dry matter,* ***CP*** ** Crude protein,* ***NDF*** *– Neutral detergent fiber,* ***ADF*** ** Acid detergent fiber,* ***CT*** ** Condensed tannin, CP, NDF, ADF and CT were given as % of DM*

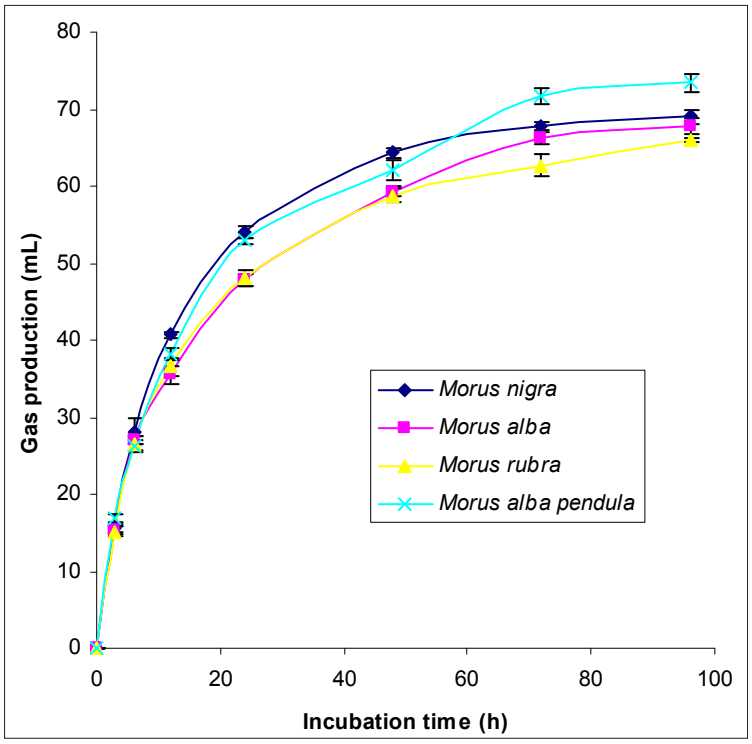

Fig 1. The effect of species on gas production values of mulberry leaves

Şekil 1. Dut yaprağının gaz üretim değerleri üzerine türün etkisi

Table 2. *The effect of species on the gas production characteristics, metabolisable energy and organic matter digestibility of mulberry leaves*
Tablo 2. *Dut yaprağının gaz üretim özelliklerine, metabolik enerji ve organik madde sindirim derecesi üzerine türün etkisi*

Parameters	Species				SEM	Sig.
	M. nigra	*M.alba*	*M. rubra*	*M. alba pendula*		
c	0.078^{a}	0.068^{b}	0.074^{a}	0.065^{b}	0.002	***
A	67.22^{b}	65.01^{bc}	62.63^{c}	70.16^{a}	0.771	***
t_{50}	8.84^{b}	10.19^{a}	9.38^{b}	10.57^{a}	0.324	***
t_{95}	38.25^{b}	44.54^{a}	40.54^{b}	45.72^{a}	1.398	***
ME	10.46^{b}	9.77^{c}	9.41^{d}	10.74^{a}	0.017	***
OMD	71.28^{b}	66.83^{c}	64.38^{d}	73.70^{a}	0.135	***

^{abc} *Row means with common superscripts do not differ (P>0.05); s.e.m. - standard error mean; Sig. - significance level;* ***NS*** *- Non-significant,* ***c*** *- gas production rate (%);* ***A*** *- potential gas production (mL),* ***ME*** *- Metabolisable energy (MJ /Kg DM);* ***OMD*** *- Organic matter digestibility %,** P < 0.01, *** P<0.001*

hand the DM content of *Morus alba* was significantly (P<0.001) higher than those of others.

The effect of species on gas production at different time intervals is given in *Fig. 1*. The species had also significant (P<0.001) effect on the gas production and estimated parameters. Almost at all incubation times, gas productions of *Morus nigra* and *Morus alba pendula* were significantly (P<0.001) higher than those of *Morus alba* and *Morus rubra*.

The effect of species on gas production kinetics, ME, OMD of mulberry leaves is given in *Table 2*. The species had a significant (P<0.001) effect on the gas production kinetics (c, A, t_{50} and t_{95}) and estimated parameters such as ME and OMD of mulberry leaves.

The ME, OMD and A values of *Morus alba pendula* were significantly (P<0.001) higher than those of the other mulberry species whereas the gas production rate of *Morus alba pendula* and *Morus alba* were significantly (P<0.001) higher than those of *Morus rubra* and *Morus nigra*. The time to produce 50% and 95% of total gas production (t_{50} and t_{95}) of *Morus alba pendula* and *Morus alba* were significantly (P<0.001) higher than those of *Morus rubra* and *Morus nigra*.

DISCUSSION

The species had a significant (P<0.001) effect on the chemical composition on the mulberry leaves. Except for *Morus rubra* the crude protein contents of mulberry species is excess of those proposed as the minimum requirements for lactation (12% of DM) and growth (11.3% of DM) in ruminants [20]. Therefore mulberry leaves investigated in the current study have the potential for ruminant animals to meet the protein requirements during the critical periods when there is a shortage of high quality forages. The crude protein content of *Morus alba* obtained in the current study was lower than that reported by Vu et al.[8] who reported that CP content of Morus Alba was 22.3% of DM. However the crude protein content of *Morus alba pendula* was similar to that reported by Vu et al.[8]. Although the NDF content of *Morus alba* obtained in the current study was lower than that reported by Vu et al.[8], the ADF content of *Morus alba* was similar to that reported by Vu et al.[8]. On the hand the crude protein content of *Morus nigra* obtained in the current study was similar to that reported by Malik et al.[21]. The reasons why there are differences in the chemical compositions of mulberry species among the different studies are possibly due to differences in species, growing conditions, soil and harvesting time of leaves. Yao et al.[22] suggested that Mulberry strains and harvesting time had a significant effect on the chemical composition.

As can be seen from *Table 1* CT contents of mulberry species are considerable lower than 1% of DM. Possibly low level of condensed tannin is one of the reasons why mulberry leaves is used for the silkworm feeding. On the other hand, it was reported that high level of CT in forages may adversely affect of the microbial and enzyme activities [23-26]. However, in the current study, the CT contents of Mulberry leaves were lower than those considered detrimental to ruminant animals.

Species had a significant (P<0.001) effect on the gas production and estimated parameters of mulberry leaves. The differences in the gas production and estimated parameters may possible be associated with chemical composition of mulberry leaves. The potential gas production (A) of Morus spices obtained in the current study were considerably higher than those reported by Yao et al.[22] whereas the gas production rate of mulberry leaves was similar to that reported by Yao et al.[22] who reported that the strain and harvesting time had a significant effect on the gas production kinetics.

On the other hand Yao et al.[22] suggested that the strain and harvesting time had a significant effect on the OMD of Mulberry leaves. The OMD values of Mulberry species obtained in the current study were similar to that reported by Yao et al.[22] who found that the OMD values of different mulberry strains ranged from 65.9 to 72.9%.

It would be more informative to give more information about the mulberry leaves which is harvested at different growing periods in year. However in the current experiment, mulberry leaves was considered as source of feed or feed ingredients to meet ruminant animals requirements for growth and production during the season when there is a shortage of adequate and good quality feed or feed ingredients. There are two reasons why mulberry leaves were harvested in June. Firstly the feed shortages for ruminant animals start in June in the South of Turkey. Secondly harvest of mulberry fruit finishes until June as mentioned before mulberry fruits are consumed by people. Therefore mulberry leaves from different species were collected in June after harvest of fruit to prevent the yield of mulberry fruit.

Although the mulberry trees is well grown in the most parts of Turkey there is no information about the biomass production of mulberry leaves as a feed source for ruminant animals. It would be more useful if further studies should be focused on the biomass production potential of leaves of mulberry leaves in the Turkey. The use of mulberry leaves is unlikely to be practical in the intensive conditions for ruminant animals whereas in the extensive conditions, the cut and carry feeding system can be used to feed ruminant animals with mulberry leaves. It is well known that the cut and carry feeding system is used for oak and strawberry tree leaves to meet the small ruminant animals throughout the Mediterranean.

In conclusion, species had a significant effect on the nutritive value of mulberry leaves. Although there were marked differences among species in chemical composition and some estimated parameter of mulberry leaves, it can be said that mulberry leaves had high CP content and was quite digestible.

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