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Araştırma
Research

CHEMICAL COMPOSITION AND *IN VITRO* GAS PRODUCTION KINETICS OF SOME TREE LEAVES OBTAINED IN THE MEDITERRANEAN REGION OF TURKEY

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ABSTRACT: This study was carried out to determine the potential nutritive value and *in vitro* gas production (IVGP) kinetics of some tree leaves. In this study four different tree foliages (orchid tree -*Bauhinia purpurea* L. *(Fabaceae)*, eucalyptus -*Eucalyptus camaldulensis,* yellow oleander tree -*Thevetia peruviana* and pepper tree -*Schinus molle*) were used. The gas production of leaves over time was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h after incubation. The results of the present study suggested that there were differences among the tree leaves in terms of feed value, IVGP and IVGP kinetics such as energy value and organic matter digestibilities (P<0.01). The leaves of yellow oleander tree had the lowest neutral detergent fiber (NDF), acid detergent fiber (ADF) and condensed tannin (CT) content (P<0.01). Also, IVGP of the leaves from yellow oleander tree was highest throughout the incubation period (P<0.01). In contrast, the lowest total gas production (96 h) was obtained from the fermentation of the leaves of orchid tree. Low fibre and condensed tannin contents of leaves of yellow oleander tree would probably increase the voluntary intake and digestibility of these leaves by small ruminants.
Keywords: Leaves, roughage, *in vitro* gas production, energy value, organic matter digestibility

TÜRKİYE'NİN AKDENİZ BÖLGESİNDEN ELDE EDİLEN BAZI AĞAÇ YAPRAKLARININ KİMYASAL BİLEŞİMİ VE *IN VITRO* GAZ ÜRETİM KİNETİKLERİ

ÖZET: Bu çalışma bazı ağaç yapraklarının potansiyel yem değeri ve in vitro gaz üretim (IVGÜ) kinetiklerini belirlemek amacıyla yürütülmüştür. Çalışmada dört farklı ağaç yaprağı (orkide - *Bauhinia purpurea* L. *(Fabaceae),* okaliptus -*Eucalyptus camaldulensis,* sarı zakkum -*Thevetia peruviana* ve karabiber -*Schinus molle*) kullanılmıştır. Yaprakların 3, 6, 9, 12, 24, 48, 72 ve 96 saatlik inkübasyonlar sonrası gaz üretimleri kayıt edilmiştir. Çalışmadan elde edilen sonuçlar ağaç yaprakları arasında yem değerleri, IVGÜ, İVGÜ kinetikleri, enerji değeri ve organik madde sindirilebilirlikleri bakımından farklılıklar göstermiştir (P<0.01). En düşük kondanse tanen (KT), nötral çözücülerde çözünmeyen lifli bileşikler (NDF) ve asit çözücülerde çözünmeyen lifli bileşikler (ADF) içerikleri sarı zakkum ağacı yaprağında bulunmuştur (P<0.01). Ayrıca, sarı zakkum ağacı yaprakları inkübasyon periyodu süresince en yüksek IVGÜ değerlerine sahip olmuştur (P<0.01). Ancak, en düşük toplam gaz üretimi (96 saat) orkide ağacı yaprakları fermentasyonundan oluşmuştur. Sarı zakkum ağacı yapraklarının düşük lif ve kondanse tanin içeriği, muhtemelen küçük baş hayvanların bu yaprakları gönüllü tüketimlerini ve sindirilebilirliğini artıracağı düşünülmektedir.
Anahtar Sözcükler: Yapraklar, kaba yem, in vitro gaz üretimi, enerji değeri, organik madde sindirilebilirliği

1. INTRODUCTION

Grass and pasture, forage plants and field crop residues are mainly traditional forage sources in the Mediterranean region of Turkey. Due to the shortage of grass and pastures and the inadequacy of forage plants production, there are some problems in supply of forage requirements.

There are important forage sources such as trees and shrubs in the natural vegetation of Mediterranaen countries. Foliages and fruits of trees and shrubs are important forages sources for the nutrition of mainly goat, sheep, cattle, deer and wild animals (Louhaichi et al., 2009). It is known that tree and shrubs play an important role in ruminant nutrition by meeting the forage requirements of ruminant. 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 the periods of food scarcity by the environmental changes in the future (Atasoglu et al., 2010). The relevance of evaluating nutritional value of trees is evident as their foliage makes an important contribution to the protein and energy nutrition of browsing ruminants. However, the use of tree and shrub leaves by herbivores is often restricted by defending or deterring mechanisms related to their high tannin contents. Ruminants have the ability to tolerate higher tannin contents than non-ruminants (Sindhu et al., 2002).

The *in vitro* gas production system is a useful and reliable tool to evaluate feedstuffs for ruminants. The aim of this study was to determine the potential nutritive value, gas production kinetics and estimated parameters such as metabolisable energy and organic matter digestibility of some tree leaves by using *in vitro* gas production technique.

2. MATERIALS AND METHODS

The leaves of some trees were harvested in mid August 2008 from the city of Adana in the South of Turkey. The area is located at an altitude of 28 m above sea level. In this study, four leaf samples (orchid tree- *Bauhinia purpurea L. (Fabaceae)* , eucalyptus-*Eucalyptus camaldulensis*, yellow oleander tree- *Thevetia peruviana* and pepper tree- *Schinus molle*) were used. The leaves were harvested by hand from at least 10 different trees, pooled and oven dried at 60˚C for 48 h. Afterwards, the leaves were milled in a hammer mill through a 1 mm sieve for chemical analysis and *in vitro* gas production assays. Dry matter (DM) ash, ether extract contents and nitrogen (N) contents were determined according to AOAC (1998) procedure. Crude protein was calculated as N x 6.25. Neutral detergent fibre (NDF), acid detergent fibre (ADF) analysis were based on the methods of Van Soest et al. (1991) using ANKOM fiber analyzer. Condensed tannin was determined by butanol-HCl method as described by Makkar et al. (1995).

Three infertile Holstein cows with ruminal cannulas (average live weight 650 kg) were used in *in vitro* gas production technique. Approximately 200 mg (dry matter basis) of samples were weighed in triplicate into 100 ml calibrated glass syringes following the procedures of Menke and Steingass (1988). Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Five repetitions of each sample were used in *in vitro* gas production technique. Rumen fluid was obtained from the fistulated Holstein cows fed twice daily (08.30-16.30) with a diet containing corn silage (60%) and concentrate (40%). Organic matter digestibility (OMD) (Menke et al. 1979), metabolisable energy (ME) (Menke et al. 1979) and net energy lactation (NEL) (Menke and Steingass, 1988) contents of the samples were estimated.

Completely Randomised Design was used to compare gas production and gas production kinetics using General Linear Model (GLM) of SPSS (SPSS version 10.0) package programmes. Significance between individual means was identified using the Duncan's multiple comparation test.

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where;

Y_{ij} : The observed value of jth repetition of ith leaves,

μ : General population mean,

α_i : The effect of ith leaves,

e_{ij} : Error term of jth repetition of ith leaves

3. RESULTS AND DISCUSSION

Chemical composition of the tree leaves was given in Table 1. The findings of the present study indicated that the leaves of yellow oleander tree had the highest ash content and the lowest NDF, ADF and CT content (P<0.01).

The effects of tree species on *in vitro* gas production of the leaves were given in Table 2. The leaves of yellow oleander tree had higher *in vitro* gas production levels compared to other leaves (P<0.01). It had a lower CT content. The findings in this experiment supported the fact that the antinutritive factors like tannins may also contribute to reduction of microbial activity and *in vitro* gas production (insert reference). The leaves of orchid tree had lower *in vitro* total gas (96 h) production (P<0.01). They possessed the highest NDF and ADF content (P<0.01).

The effect of tree species on *in vitro* gas production kinetics, estimated energy values and organic matter digestibilities of different tree leaves were given in Table 3. However, the leaves of eucalyptus tree which had high "a value", had the lowest "b value" (P<0.01). The gas production rates (c value) of the leaves of yellow oleander tree were significantly higher than the others (P<0.01). The leaves of yellow oleander tree had the highest OMD, ME and NEL (P<0.01).

The leaves of yellow oleander tree had the lower tannin contents than the others and also caused higher *in vitro* gas production level. However, lower crude protein contents should be taken into consideration when used for ruminant nutrition. Chemical composition of the leaves plays a crucial role in the extent to which they are utilized by goats and sheep. In this study, the leaves of orchid tree and eucalyptus tree had higher crude protein (CP) content and lower ether extract (EE) content. The fibre content of roughages is one of the crucial factors determining the digestibility and intake. Low fibre content of yellow oleander tree leaves would probably increase the voluntary intake and digestibility of these leaves by small ruminants. El-Hassan et al. (2000) reported that the fibre of browse leaves was more digestible than that of mature grasses and crop residues.

Tannins 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 reduction protein degradation in the rumen are reported (Barry, 1987; Muller-Harvey, 2006). In the present study, the leaves of different tree species significantly influenced the CT content of the foliages. The CT content of the leaves of yellow oleander tree was lower than the nutritional critical levels of 2-4 % on DM basis (Barry, 1987; Barry and McNabb, 1999),

Table 1 The effect of tree species on chemical compositions of leaves (g/kg DM)

Species	DM	CP	EE	Ash	NDF	ADF	CT
	g/kg	g/kg DM					
Orchid tree	358.3	130.3±2.83[1a]	16.2±3.78[b]	56.5±0.00[d]	456.3±28.84[a]	438.1±13.86[a]	92.6±12.96[a]
Eucalyptus	316.7	118.1±7.78[a]	8.0±5.3[b]	85.4±0.35[b]	411.3±0.75[b]	388.2±12.36[b]	63.2±3.37[b]
Yellow oleander tree	322.9	86.5±5.66[b]	56.0±10.6[a]	122.2±0.67[a]	288.3±16.46[d]	243.0±12.79[d]	16.7±0.95[c]
Pepper tree	314.8	96.05±2.62[b]	49.3±3.72[a]	82.0±0.45[c]	323.3±12.85[c]	316.9±21.03[c]	105.6±8.49[a]
Significant level	0.533	0.00	0.00	0.00	0.00	0.00	0.00

[1] Column ([a, b, c]) means with common superscript do not differ, DM: Dry matter, CP: Crude protein, EE: Ether extracts, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, CT: condensed tannin, P<0.05.

Table 2. The effect of tree species on *in vitro* gas production of the tree leaves

Species	Incubation time (hour)							
	3	6	9	12	24	48	72	96
Orchid tree	2.6±0.81[1b]	6.5±1.44[c]	11.7±1.72[b]	15.3±1.95[b]	21.6±1.65[b]	25.3±1.5[b]	26.8±1.71[c]	27.4±1.27[c]
Eucalyptus	4.6±0.28[a]	8.7±0.59[b]	11.3±0.66[b]	13.6±0.62[bc]	18.6±1.53[c]	24.8±0.6[b]	29.2±0.47[b]	31.2±0.26[b]
Yellow oleander tree	4.7±0.75[a]	11.0±0.91[a]	16.8±0.93[a]	24.2±0.95[a]	38±1.14[a]	45.5±1.08[a]	49.4±1.08[a]	51.4±1.34[a]
Pepper tree	2.7±0.44[b]	6.1±0.34[c]	9.2±0.50[c]	11.5±0.47[c]	18.5±0.26[c]	25.2±1.01[b]	28.7±0.63[b]	29.6±0.42[b]
Significant level	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[1]Column ([a, b,c]) means with common superscript do not differ, P<0.05

Table 3. The effect of tree species on in vitro gas production kinetics of the tree leaves

Species	a	b	a+b	c	OMD	ME	NE_L
Orchid tree	-4.0±0.82[1c]	30.9±1.65[b]	26.8±1.33[c]	0.08±0.007[a]	40.3±1.46[b]	5.9±0.22[b]	3.0±0.17[b]
Eucalyptus	3.2±1.03[a]	28.7±0.33[c]	31.9±0.70[b]	0.03±0.004[c]	37.3±1.36[c]	5.4±0.21[c]	2.6±0.15[c]
Yellow oleander tree	-4.8±0.71[c]	55.2±0.65[a]	50.5±1.18[a]	0.06±0.00[b]	53.4±1.02[a]	8.0±0.16[a]	4.9±0.12[a]
Pepper tree	-0.4±0.35[b]	30.6±0.26[b]	30.2±0.61[b]	0.04±0.00[c]	36.2±0.23[c]	5.3±0.04[c]	2.9±0.03[b]
Significant level	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[1]Column ([a, b,c]) means with common superscript do not differ, a: the gas production from the immediately soluble fraction (ml), b: the gas production from the insoluble fraction (ml), a+b: potential gas production (ml), c: the gas production rate constant for the insoluble fraction (ml/h), OMD: organic matter digestibility (%), ME: metabolisable energy (MJ/kg DM), NE_L: net energy lactation (MJ/kg DM), P<0.05.

but the other samples were quite higher than the critical levels. The CT content of the foliages increased from early May up to mid October (Atasoglu et al., 2010). In this study tree leaves were collected in mid August which is considered as optimal for the harvest of the leaves.

Some studies showed that high consumption of leaves may have a detrimental effect on feed intake and digestibility of some nutriets in diets (Aganga and Tshwenyane, 2003). Therefore, care should be taken when tree leaves are included in ruminant diets.

In the present study, generally IVGP of the leaves from yellow oleander tree was highest throughout the incubation period. In contrast, the lowest total gas production (96 h) was obtained from the fermentation of the leaves of orchid tree. Low NDF, ADF and low tannin content of the leaves from yellow oleander tree can partly explain the observed high *in vitro* gas production. *In vitro* methods have been successfully used for the prediction of ME and OMD contents of ruminants (Getachew et al., 1998). Significant differences were found among the trees in terms of ME, NEL and OMD values of the leaves studied in the current study. Kamalak et al. (2005) reported that high CP content and lower fibre fractions to be partly responsible for high ME and OMD values. But, the leaves of orchid tree and eucalyptus tree which have high CP content, had lower *in vitro* gas production levels in this study.

4. CONCLUSION

The research has shown that there were differences among the tree leaves in terms of feed value, in vitro gas production (IVGP), and IVGP kinetics such as energy value and organic matter digestibilities (P<0.01). Also, the leaves of yellow oleander tree have been observed to have the lowest neutral detergent fiber (NDF), acid detergent fiber ADF and condensed tannin content (P<0.01), while, IVGP of the yellow oleander tree leaves was the highest throughout the incubation period (P<0.01). On the other hand, the lowest total gas production (96 h) was obtained from the fermentation of the leaves of orchid tree. Low fibre and condensed tannin contents of leaves of yellow oleander tree was likely to increase the voluntary intake and digestibility of these leaves by small ruminants. Therefore, it could be concluded that the foliage from yellow oleander tree have the nutritional potential for the maintenance of goat herds (Kilic et al., 2010).

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