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Investigation of Potential Nutritive Values of Some Tree Leaves and Its Extracts by Using In Vitro Gas Production

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ABSTRACT
This study was performed to assess the nutritional value of specific tree species (laurus nobilis, albizia julibrissin, glycyrrhiza glabra, salix alba, robinia pseudoacacia, liquidambar orientalis, juniperus communis, quercus coccifera, cedrus libani, arbutus andrachne) growing in different regions of Kahramanmaras, besides the gas production of the leaves and their extracts at various dose levels (0.6, 1.2 and 1.8 mL). The ADF and NDF contents were differed between 16.20% - 32.47% and 28%-49.66%, respectively. Liquidambar orientalis leaves had the highest values for both characteristics, whereas Salix alba leaves had the lowest values. The CP value, varied between 7.94% and 25.94%. Liquidambar orientalis leaves had the highest concentration of condensed tannins, 16.19%, and Albizia julibrissin leaves had the lowest concentration, 2.12%. ME and OMD values ranged from 6.72 to 10.24 MJ kg^{-1} and 43.68 to 65.72%, respectively. The GP content of the samples varied between 22.25-40.03 mL 200^{-1} mg(DM). According to the study's various dose, GP and CH4 production significantly increased when compared to the control group. The GP of leaf extracts for the control group was 44.89 mL, and doses at, 0.6, 1.2, and 1.8 mL were found; 51.05-105.96 mL, 52.71-106.26 mL, and 47.33-106.85 mL correspondingly. Methane production (%) concentration for the control group were 16.54%, and at 0.6, 1.2 and 1.8 mL doses were observed 16.64%-34.40%, 22.44%-34.80% and 18.41%-31.46% respectively. Significant relationships between CH_4 production, ADF, and NDF have been found.

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Bazı Ağaç Yaprakları ve Ekstraktlarının Potansiyel Besin Değerlerinin İn Vitro Gaz Üretim Yöntemiyle Araştırılması

ÖZET
Bu araştırma, Kahramanmaraş'ın farklı bölgelerinde yetişen (defne, gülibirşim, meyan, söğüt, akasya, sığla, ardıç, meşe, sedir ve sandal) ağaç türlerinin besin değerleri ile yaprak ve ekstraktlarının farklı doz seviyelerinde (0.6, 1.2 ve 1.8 mL) gaz üretimini belirlemek amacıyla yapılmıştır. ADF ve NDF içerikleri sırasıyla %16.20-%32.47 ve %28-%49.66 değerleri arasında farklılık göstermiştir. Her iki özellik açısından (ADF, NDF) en yüksek değerler sığla, en düşük değerler ise söğüt yapraklarında bulunmuştur. HP değeri ise %7.94 ile %25.94 arasında değişmiştir. Kondanse tanen içerikleri en yüksek %16.19 ile sığla yapraklarında, en düşük değeri ise %2.12 ile gülibirşim yapraklarında bulunmuştur. Besin değerleri, metabolik enerji (ME) ve organik madde sindirim derecesi (OMD) sırasıyla 6.72-10.24 MJ kg^{-1} ve %43.68-%65.72 arasında değişmektedir. Numunelerin gaz üretim (GÜ) içeriği 22.25-40.03 mL 200^{-1} mg (KM) arasında değişmiştir. Çalışmada kullanılan farklı doz, kontrol grubuna kıyasla hem GÜ'nde hem de CH_4 üretiminde önemli artışlar bulunmuştur. Kontrol grubunun GÜ'i 44.89 mL iken, 0.6, 1.2 ve 1.8 mL'deki dozların GÜ'leri sırasıyla 51.05-105.96 mL, 52.71-106.26 mL ve 47.33-106.85 mL arasında değişiklik göstermiştir. Kontrol grubu için CH_4 üretimi (%) konsantrasyonu %16.54 olup, 0.6, 1.2 ve 1,8

Zootekni

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Anahtar Kelimeler
Ağaç yaprakları
Ekstraktlar
Enterik metan üretimi
Metabolize enerji
Sindirilebilirlik

mL dozlarında sırasıyla %16.64-%34.40, %22.44-%34.80 ve %18.41-%31.46 arasında farklılık gözlenmiştir. CH_4 üretimi ile ADF ve NDF arasında anlamlı ilişkiler olduğu tespit edilmiştir.

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INTRODUCTION

The world population and food consumption continue to increase rapidly, it is thought that animal foods required for nutrition can be met less due to current speed. In Türkiye, the degradation of natural pasture lands by man, the inability to meet the roughage needs adequately, feeding animals directly from some plant products, and the inefficient use of potential human foods are seen as the main problems.

Thanks to their ability to turn into plant matter into the food sources, ruminants are improving the nutritional standard of societies (Anonymous, 2011). Its have always contributed greatly to the well-being of societies by providing food, fuel (manure), fertilizer and other products and services. These animals in another word can be called also renewable living resources (Steensland and Zeigler, 2021). A wide variety of plant materials not included in the diets of livestock systems are used in the feeding of cattle and small ruminants when they encounter feed gaps (Salem, 2010). Some varieties of tree leaves are traditional fodder for sheep and goats (Muwanika et al., 2018; Nsubuga et al., 2019). And some varieties are also fed to cattle and buffaloes. Leaves of different species have different nutritional properties. Generally, the leaves in the early stages of growth contain a fairly high content of CP and a relatively low fiber content. As plant maturation progress, there is a gradual decrease in protein content and an increase in crude fiber (Singh, 2019).

Small ruminant livestock breeders in some regions actually use a range of tropical plants to feed their animals daily, especially during the dry season and are therefore currently actually unwittingly reducing enteric CH_4 emissions (Anonymous, 2019a; Anonymous, 2019b). It is the responsibility of researchers to explain this to academia and the public. There is a grand possibility to reduce enteric CH_4 releases in the livestock in such countries, as well as opportunities to improve the productivity. Countries that are included in the UNFCCC can encourage the widespread use of CH_4 reduction practices by providing various supports to producers who switch to CH_4 abatement practices (Herrero et al., 2016; Salmon et al., 2020; Ku-Vera et al.,2020).

The livestock sector is also the source of emissions of CH_4 and CO_2 gases, which pollute the atmosphere and contribute to the greenhouse effect. This sector requires significant natural resources and takes an important place in global greenhouse gases (GHG) emissions. The most important GHG from animal husbandry are methane and nitrous oxide. Therefore, strategies and studies to reduce methane emissions are important. Methane production is an important formation both because it is approximately 23 - 25 times more effective than CO_2, which contributes to global warming, and because it causes yields to lose 2 - 12% energy in ruminant feeding (Patra, 2012; Singh et al., 2012; Ramin and Huhtanen, 2013; Piñeiro-Vázquez et al., 2015; Haque, 2018).

The gradual restriction of chemical additives in animal feed directs producers and investigators to alternative natural products in animal nutrition. These natural products allow them to be resistant to predators and pathogens thanks to their plant secondary metabolites (saponins, condensed tannins, alkaloids, lignin, antioxidants, and essential oils). Due to its highly effective anti-microbial activities, these compounds can be used in a specific inhibition on a microbial group belonging to the ecosystem that performs fermentation in the rumen (Benchaa et al., 2008). Methanogenesis reduces the efficiency of the use of nutrients in ruminant animals. Therefore, reducing CH_4 production of the ruminal microbial ecosystem and converting to the energy exceeded by CH_4 gas into efficiency is extremely important for animal feeding (Haque, 2018).

Previously, in order to increase the production efficiency of ruminants; feed additives such as ionospheres, antibiotics, and CH_4 inhibitors have been used in animal feeds to improve rumen fermentation. Whereas, most of such supplements are not used always due to poisonous effect issues and possible rumen microbial modification. In addition, using of these chemicals in animal feeds has been restricted in the EU since 2006 owing to the risk of the residuum in livestock products and a potential adverse effects on human health (Jouany and Morgavi, 2007; Demirtas et al., 2018).

Inclusion of plant materials containing plant secondary metabolities (PSM) such as saponins or phenolic compounds appears to be a substitute feeding strategy to reduce CH_4 release in ruminants because many plants are rich in their secondary metabolites. In

addition, it has been reported that these needs can be met by substitution of alternative feed materials such as plants, shrubs and trees in order to meet the vital and production needs of small ruminants during periods of feed gap (Bodas et al., 2012; Ebrahim and Negussie, 2020).

The aim of this study is to determine nutritive values of some tree leaves, and also the GP that will be formed as a result of rumen fermentation of leaves and their extracts added at different dose levels. These mentioned tree leaves are chosen cause of they are especially used in the feeding of ovine by the farmers in Kahramanmaraş region. Besides that, in order to meet the roughage needs of ruminant animals during the harsh and cold winter months, it will be investigated whether this need can be met when using with those tree leaves grown in the region as a substitute (Akcil and Denek, 2013; Kamalak et al., 2015).

MATERIAL and METHOD

Feed samples of the study were supplied from different locations (37°61'94.0"N, 36°80'68.3"E, 37°34'00.7"N 36°34'38.0"E, 37°58'24.6"N, 36°86'66.0"E) of Kahramanmaras in the East-Mediterranean region of Türkiye in late May 2019 (spring season), when leaves were fresh. Each leaf sample was collected from 15-20 different tree species in the specified coordinates, then they were mixed for extraction and further use in the Animal Nutrition Laboratory at Kahramanmaras Sutcu Imam University. The leaves were arranged and then put into the oven for determination of dry matter content. The remaining portions of leaves were left to dry in shade. The plant materials were ground with a 1 mm diameter screen mill for chemical and in vitro analysis. Dry matter (DM), ether extracts (EE), crude protein (CP), crude ash (CA) and organic matter (OM) contents of samples were determined according to AOAC, 1990 procedures. Plant cell wall structures; acid detergent fiber (ADF) and neutral detergent fiber (NDF) were made according to the method reported by Van Soest et al., 1991 and condensed tannins (CT) contents of leaves were made according to the method reported by Makkar et al., 1995.

In Vitro Gas Production of Leaves

The rumen fluid used for in vitro gas production analysis was obtained from 3 awassi rams which were 2 years old (Ethics Committee Report No: 2020/09-01). These rams were fed twice daily with a diet mixed of 60% roughage (alfalfa hay) and 40% grain (barley). Leaf samples were weighed 200 mg in four repetitions (n=4), placed in glass syringes together with 30 mL of mixture solution (10 mL of rumen fluid + 20 mL of buffer solution) and incubated in a water bath at 39 ºC. And then gas productions were carried out after 24 hours of incubation (Menke et al., 1979). Total gas obtained from samples was determined by subtracting the gas volume obtained from 4 repetitions of control tests of the measurements. And then the gas formed in glass syringes into the plastic syringes was transferred. After 24 hours of fermentation, the CH_4 concentration of the total gas transferred to plastic syringes was measured with an infrared CH_4 analyzer device (Sensor Europe GmbH, Erkrath, Germany) as mL and (%) percent (Goel et al., 2008).

Extraction of Leaves and In Vitro Gas Production of Dose Levels

Leaves were chopped freshly (1-2 cm) and immediately extracted in a 10 g leaf+80 mL solvent mixture. The mixture of solvents contained 10 mL of methanol (99.8 100^{-1}), 10 mL of ethanol (99 100^{-1}) and 80 mL of distilled water (Cedillo et al., 2014). According to former studies, it was considered that these solvents would not have an effect on fermentation. Plant materials were added to 250 mL closed flasks as 10g leaf + 80 mL solvent individually and then put on a hot plate, set at 25 - 30 ºC mixed for 24 hours. After this process, the solid and liquid parts were separated from each other by filtration through Whatman filter paper (No: 1). Finally, it was stored at +4 ºC for later use in the in vitro analysis. In order to prepare in vitro gas production analysis; 200 mg of alfalfa hay was weighed and added with 30 mL of mixed solution into the glass syringes. After that stored extracts were transferred by injectors to glass syringes at dose levels of 0.6, 1.2 and 1.8 mL respectively. And finally, without delay, those syringes has shaken fastly then directly put for incubation at 39 °C in a water bath. And then gas productions were carried out after 24 hours of incubation (Menke et al., 1979). Total gas obtained from samples was determined by subtracting the gas volume obtained from 4 repetitions of control tests of the measurements. And then the gas formed in glass syringes into the plastic syringes was transferred. After 24 hours of fermentation, the CH_4 concentration of the total gas transferred to plastic syringes was measured with an infrared CH_4 analyzer device (Sensor Europe GmbH, Erkrath, Germany) as mL and (%) percent (Goel et al., 2008).

Estimation of Tree Leaves Fermentation Parameters

Organic matter digestibility (OMD) of tree leaves was determined by using at 24th hour in vitro gas production, CP and CA values which are given in the below equation (Menke, 1988).

OMD (%) =14.88+0.8893*GP+0.448*CP+0.00651*CA (Eq.1)

The metabolic energy (ME) content of the feedstuffs was estimated by using in vitro gas production, CP and EE values of analysis which are given in the below equations (Bllümmel and Ørskov, 1993).

ME (MJ/kg DM)= 2.2+ 0.13576*GP+0.057*CP

$+0.02859*EE^2$ (Eq.2)

OMD: Organic Matter Digestibility

GP: Gas Production (where 24h net GP of mL 200^{-1} mg DM)

CP: Crude Protein (% DM)

A: Ash (% DM)

EE: Ether Extract (% DM).

Statistical Analysis

Normality tests were applied in order to statistically evaluate chemical composition, total gas production and CH_4 (mL and %) concentration of leaves. As the statistical analysis showed normal distribution, it was subjected to analysis of variance (ANOVA). Since the analysis of variance is homogeneous, the differences between treatment means were examined by Tukey multiple comparison tests (Pearse, 1966) through SPSS 25.0 software (Anonymous, 2017). The differences between the means were evaluated at the $p<0.05$ level.

RESULTS and DISCUSSION

The nutritional compositions of the tree leaves used in this study were investigated in terms of DM, CA, OM, ADF, NDF, EE, CP, and CT analysis, obtained results were given in Table 1. It is mentioned that in the literature; factors such as the use of different consumables, different processing, measurement method, growth stage, ripening level, drying method, climatic conditions, seasonal changes, geographical and soil properties are indicated to be effective in the formation of chemical composition (Kilic, 2010). Since the chemical composition is one of the most important indicators of the nutritional value of feeds, it can be predicted that different chemical compositions lead to differences in nutritional values index.

The DM of tree leaves content were ranged between 24.28 - 53.67 (DM %), the highest DM value was found in J.communis and the lowest was in A. julibrissin leaves (Table 1). The cell wall structures of the tree leaves ranged from 16.20% to 32.47% in terms of ADF. L. orientalis was found at the highest rate in terms of ADF content of 32.47%, and S.alba was found at the lowest rate with 16.20%. Another cell wall structure, NDF, varied in content from 28.69% to 49.66%. The highest NDF rate was found in L. orientalis and J. communis with 49.66%, 48.55% respectively, and the lowest rate was found in G. glabra leaves with 28.69%. Comparing the data obtained from the cell wall structure elements of tree leaves belonging to ADF and NDF with other studies; in harmony with the findings of some researchers (Kamalak et al., 2005; Kilic, 2010; Boga, 2014) while some found their findings (Kamalak et al., 2011; Kara et al., 2015) was to be different.

The plant cell wall that forms the structure of the feed samples is asked to be low in ADF (Van Soest, 1994). When this recommendation is taken into account, the lowest ADF content of the study was found in S.alba leaves, so it is understood that the animal feeding value is more important than others. Since fibrous feeds with less than 45% (in DM) of NDF content are in the class of high-quality roughage, tree leaves examined in the current study can be considered high-quality roughage feed sources. A diet with high NDF ratio can reduce the dry matter intake (DMI). However, tree leaves with low NDF content in rumen make the feed break down, ingest and digest more quickly than in the meadow.

EE content of tree leaves obtained in the study differed between 3.52% - 13.90%. In terms of EE amount, the lowest rate was found in Q. coccifera structures with 3.52%, and the highest rate was found in G. glabra with 13.90%.

CP content of leaves was found between 7.94% - 25.94%. And the highest value was found in A. julibrissin leaves. The CP content of ruminants should be at least 10% in their diets, it has been reported from previous studies that is below this amount will cause the microbial activities in the rumen to work limitedly and therefore the desired yield will not be achieved. In this research, as can be seen from the following in Table 1, only A. andrachne, J. communis and C. libani leaves were found to be below 10% CP. Moreover, CP content of these samples was found to be higher than the value of ruminants need at least 7% CP to provide the ammonia needed by rumen micro-organisms to optimally support microbial growth. The protein requirement for the maintenance of lactating sheep is 7 - 9% in the diet and 10 - 12% crude protein for the lactation period (Tatliyer et al., 2019; Kaya, 2021). Although the CP content of these tree leaves meets some needs of ruminants, the diets provided to animals should be supplemented with an additional protein source, since it is not at a level to meet production periods (Tatliyer et al., 2019). Therefore, it is thought that the protein content of the leaves obtained from J. communis, C. libani and A. andrachne is at a level to meet the protein requirement of the sheep maintenance, but not sufficient for milk yield. Additionally, it has been reported by many researchers that diets containing more than 5% CT reduce nutrient digestion and feed consumption, and that condensed grain forms a compound with proteins and inhibits the activity of microorganisms and enzymes (Makkar et al., 1989; Silanikove et al., 1994; Tatliyer et al., 2019). So that, whenever L. orientalis, C.libani, R. pseudoacacia, S. Alba and J. Communis leaves provided to diet of ruminants, it should be taken into consideration of their CT contents.

The GP, CH_4 production (mL and %) resulting from fermentation of tree leaves analyzed in this study by in vitro gas production method at 24-hour incubation,

metabolic energy (ME), and organic matter digestivity (OMD) are given in Table 2. As a result of the incubation of the tree leaves investigated, significant differences were found between the values of GP, CH_4 (mL and %), ME, and OMD ($p<0.001$). GP of matters was ranged between 22.25 mL - 40.03 mL. The highest production was found in A. julibrissin, and the lowest in G. glabra leaves. Likewise, CH_4 productions (mL) formed as a result of 24-hour incubations of tree leaves changed between 1.50 - 4.98 mL, where the lowest CH_4 production was found in G. glabra as 1.50 mL, and the highest production was found in A. julibrissin. The other parameter, CH_4 percentage (%) productions varied between 6.76% - 12.46% within incubating for 24 hours in terms of fermentation. OMD calculated by using gas values and chemical components produced as a result of in vitro incubation of these tree leaves, and it was found between 43.68 - 65.72%. The highest OMD was found in A. julibrissin, and the lowest was in L. orientalis leaves. In terms of ME values of tree leaves, it was ranged from 6.72

Table 1. Chemical composition of Tree Leaves (% DM)
Çizelge 1. Ağaç Yapraklarının Kimyasal Bileşimi (% KM)

T. Species	DM	CA	OM	ADF	NDF	EE	CP	CT
L. nobilis	40.21±0.30^{c}	4.12±0.03^{g}	95.88±0.03^{a}	26.65±0.17^{c}	42.23±0.37^{c}	6.57±0.14^{c}	10.94±0.05^{f}	5.77±0.29^{de}
A. julibrissin	24.28±0.06^{f}	5.57±0.12^{de}	94.43±0.12^{cd}	18.81±0.15^{f}	31.25±0.02^{d}	3.85±0.07^{e}	25.94±0.05^{a}	2.12±0.06^{f}
G. glabra	33.60±0.23^{e}	5.22±0.05^{e}	94.78±0.05^{c}	19.52±0.06^{ef}	29.36±0.79^{e}	13.90±0.34^{a}	23.75±0.05^{b}	6.83±0.43^{d}
S. alba	37.23±0.20^{d}	7.68±0.15^{b}	92.32±0.15^{f}	16.20±0.08^{g}	28.69±0.22^{e}	4.74±0.15^{d}	18.75±0.05^{d}	13.83±0.25^{b}
R.pseudoacacia	36.92±0.59^{d}	6.75±0.05^{c}	93.25±0.05^{e}	19.83±0.14^{e}	29.68±0.19^{e}	5.16±0.01^{d}	19.81±0.05^{c}	14.14±0.42^{b}
L.orientalis	43.40±0.28^{b}	5.61±0.03^{d}	94.39±0.03^{d}	32.47±0.22^{a}	49.66±0.23^{a}	5.26±0.12^{d}	10.25±0.05^{g}	16.19±0.15^{a}
J. communis	53.67±0.34^{a}	8.55±0.05^{a}	91.45±0.05^{g}	25.31±0.24^{d}	48.55±0.13^{a}	7.38±0.10^{b}	8.63±0.05^{i}	12.31±0.28^{c}
Q. coccifera	36.23±0.19^{d}	4.37±0.04^{fg}	95.63±0.04^{ab}	24.84±0.30^{d}	44.96±0.10^{b}	3.52±0.08^{e}	12.25±0.05^{e}	5.04±0.43^{e}
C. libani	36.65±0.21^{d}	4.27±0.03^{g}	95.73±0.03^{a}	30.49±0.16^{b}	45.17±0.07^{b}	6.36±0.13^{c}	7.94±0.05^{l}	15.12±0.07^{ab}
A. andrachne	39.45±0.38^{c}	4.75±0.05^{f}	95.25±0.05^{b}	26.38±0.20^{c}	41.37±0.05^{c}	3.79±0.53^{e}	9.75±0.05^{h}	5.08±0.28^{e}
SEM	1.130	0.050	0.050	0.937	1.488	0.537	1.447	0.911
SE	0.443	0.108	0.108	0.270	0.435	0.213	0.070	0.423
Sig.	**	**	**	**	**	**	**	**

DM: dry matter, A: crude ash, OM: organic matter, ADF: asit detergent fiber, NDF: neutral detergent fiber, EE: ether extracts, CP: crude protein, CT: condensed tannins, SEM: Stnd. Error of Means, Stnd. of Error Sig.: Significancy, ** : ($p<0.001$)

MJ kg^{-1} (in DM) to 10.24 MJ kg^{-1}. According to this feature, the lowest ME content was found in Q. coccifera with 6.72 MJ kg^{-1} and the highest ME value was found in A. julibrissin with 10.24 MJ kg^{-1}. The probability of the feeds used in ruminant feeding being anti-methanogenic can be determined based on the percent (%) methane content of the gas produced by fermentation and it's reported that there are three groups that can be divided into feeds with anti-methanogenic potential. These groups are respectively, low (>11 to <14), medium (>6 to <11), and high (>0 to <6) (López et al., 2010). According to this consideration, it is understood that the leaves of G. glabra, S. alba, Q. coccifera, L.orientalis, C. libani, A. andrachne, and R. pseudoacacia tree leaves in the study may have anti-methanogenic properties. Moreover, it can be said that none of these tree species leaves are highly anti-methanogenic, especially J. communis, L. nobilis and A. julibrissin leaves have low anti-methanogenic properties according to López et al., 2010.

As Cheema et al., (2014) reported that the ME contents of four different tree leaves varied between 5.77 and 9.07 MJ kg^{-1} in DM. The ME content of A. procera leaves was found to be 7.57 MJ kg^{-1} in DM, and the ME content of A. julibrissin leaves in this study was found to be 10.24 MJ kg^{-1} in DM, higher than the value of the same tree leaves reported by Cheema et al., (2014). These different results; It is predicted that it is caused by different tree species belonging to the same genus but not the same harvest time and chemical composition. To having a higher ME usability value of A. julibrissin tree leaves than the other species may be explained by DM digestibility and its better CP quality. In addition, diversity in the chemical composition of tree leaves may be due to the different geographical distribution, climate, and maturity of plant species (Kilic, 2010). In line with previous studies, the nutritive values of these species provide a good alternative fibrous feed sources for small ruminants. Moreover, these species can be supplied to ruminants as substitute feed by paying attention to the amount in feed gaping periods.

The correlation coefficients (r) consisting of the estimations of some parameters such as chemical composition, GP, CH_4 production (mL and %), metabolizable energy contents, and OMD of tree leaves are given in Table 3. The GP was positively correlated with CH_4 (r=0.899 and r=0.611), ME (r=609), and OMD (r=0.901) ($p<0.01$). The CH_4 (mL) was positively correlated with ME (r=0.515) and OMD (r=0.789) ($p<0.01$). In this study; It was found that ADF and NDF showed a negative correlation ($p<0.001$) with GP. The negative correlation between GP and ADF or NDF

may be due to reduced microbial activity resulting from increasingly unfavorable environmental conditions.

Table 2. In vitro gas production and estimated digestion values of some tree leaves
Çizelge 2. Bazı ağaç yapraklarının in vitro gaz üretimi ve tahmini sindirim değerleri

Tree Species	GP (mL)	CH_4 (mL)	CH_4 (%)	OMD (%)	ME (MJ kg^{-1})
Laurus nobilis	26.79±1.36^{b-d}	3.04±0.13^{ab}	11.36±0.34^{ab}	46.28±1.23^{d-f}	7.81±0.20^{d-f}
Albizia julibrissin	40.03±0.76^{a}	4.98±0.09^{a}	12.46±0.24^{a}	65.72±0.75^{a}	10.24±0.13^{a}
Glycyrrhiza glabra	22.25±0.38^{d}	1.50±0.04^{e}	6.76±0.06^{e}	48.71±0.31^{c-f}	9.73±0.05^{ab}
Salix alba	30.95±0.76^{b-d}	2.34±0.10^{cd}	7.56±0.20^{de}	55.81±0.58^{bc}	8.68±0.07^{b-d}
Robinia pseudoacacia	32.09±1.00^{ab}	3.50±0.19^{b}	10.89±0.36^{b}	56.68±0.92^{b}	8.98±0.14^{bc}
Liquidambar orientalis	24.90±1.13^{b-d}	2.29±0.11^{c-e}	9.21±0.05^{c}	45.27±1.02^{ef}	7.25±0.14^{ef}
Juniperus communis	32.09±0.38^{ab}	3.56±0.14^{b}	11.11±0.38^{b}	52.85±0.37^{b-d}	8.70±0.08^{b-d}
Quercus coccifera	23.01±4.36^{cd}	1.91±0.38^{de}	8.31±0.22^{cd}	43.68±3.89^{f}	6.72±0.60^{f}
Cedrus libani	28.30±1.13^{b-d}	2.64±0.13^{cd}	9.32±0.08^{c}	46.39±0.99^{d-f}	7.77±0.15^{d-f}
Arbutus andrachne	32.46±1.00^{ab}	3.00±0.05^{bc}	9.27±0.27^{c}	51.21±0.87^{b-e}	7.95±0.17^{c-e}
SE	2.319	0.232	0.354	2.071	0.324
Sig.	**	**	**	**	**

$^{a-f}$ Means with different symbols in the same column are significantly different from each other. SE: Standard Error. Sig.: Significancy. **: P<0.001. GP: Gas production at 24h of incubation as mL/0.2g DM. CH_4: in vitro methane production at 24h as mL/0.2g DM. OMD: Organic matter digestibility. ME: Metabolizable energy (MJ/kg DM)

Table 3. Correlations between nutritive values and in vitro incubation parameters of leaves
Çizelge 3. Yaprakların besin değerleri ile in vitro inkübasyon parametreleri arasındaki korelasyonlar

	GP (mL)	CH_4 (mL)	CH_4 (%)	DM (%)	CA (%)	OM (%)	ADF (%)	NDF (%)	EE (%)	CP (%)	CT (%)	ME (%)
CH_4 (mL)	0.899**	1										
CH_4 (%)	0.611**	0.885**										
DM (%)	-0.226	-0.192	1	1								
CA (%)	-0.433**	-0.422*	0.005	0.079	1							
OM (%)	0.342*	0.235	-0.395*	0.440**	0.028	1						
ADF (%)	-0.342*	-0.235	0.092	-.440**	-0.028	-1**	1					
NDF (%)	-0.324*	-0.178	-0.092	0.474**	-0.129	-.424**	0.424**	1				
EE (%)	-0.277	-0.122	0.092	0.644**	-0.214	-0.194	0.194	0.901**	1			
CP (%)	0.273	0.220	0.144	-.726**	0.247	0.156	-0.156	-0.833**	-.889**	1		
CT (%)	-0.167	-0.250	-0.024	0.506**	0.037	0.441**	-.441**	0.283	0.198	-0.336*	1	
ME (%)	0.609**	0.515**	-0.190	-0.412*	0.368*	0.369*	-0.369*	-0.681**	-.711**	0.773**	-0.226	1
OMD(%)	0.901**	0.789**	0.211	-0.411*	-0.213	0.457**	-.457**	-0.648**	-.605**	0.643**	-0.203	0.826**

*:Correlation is significant at level of 0.05, **:correlation is significant at level of 0.01

The effect parameters on gas production, methane production mL, and percent (%)CH_4 production by dosage levels of 0.6, 1.2, and 1.8 mL of extracts are given in Table 4. Dosage levels of the extracts during the 24-hour incubation period; GP, CH_4 (mL and %) values were found significant (P<0.001).

The GP of the extractions was found to be 44.89 mL for the control group, and at 0.6, 1.2, and 1.8 mL dosage levels were ranged between 51.05 - 105.96 mL, 52.71 - 106.26 mL, and 47.33 - 106.85 mL, respectively. The CH_4 productions (mL) of the extractions was found to be 7.41 mL for the control group, and at 0.6, 1.2, and 1.8 mL dosage levels were ranged between 8.56 - 25.11 mL, 12.27 - 24.48 mL, and 8.71 - 25.72 mL, respectively. The used extractions in the study significantly increased both GP and CH_4 production compared to the control. And the CH_4 productions (%) of the extractions was found to be 16.54% for the control group, and at 0.6, 1.2, and 1.8 mL dosage levels were ranged between 16.64 - 34.40%, 22.44 - 34.80%, and 18.41 - 31.46%, respectively (Table 4). In this study, it was observed that the production of CH_4 (mL and %) showed a proportionally higher increase than the GP. Patra et al., (2006) obtained similar results with different plant extracts in their study. They reported that the GP and CH_4 production increases could be due to the water-soluble sugar found in the extracts. On the other hand, the possibility that the methanol and ethanol mixed solution used in the research may have an effect on gas and methane production was not mentioned.

In contrast to this study, Oh et al., (2017) have found GP at higher dosage levels (1.6%, 3.2%, and 6.4%) to be lower than the lowest dosage level (0.8%) compared to the control group (0%). As the dose amount has increased, the CH_4 production has also been found lower than the control group.

Table 4. Effects of tree leaf extracts at 0.6, 1.2 and 1.8 mL doses on rumen fermentation parameters
Çizelge 4. Yaprak ekstraktlarının 0.6, 1.2 ve 1.8 mL doz seviyelerinde rumen fermantasyon parametreleri etkileri

Tree species	Dosage level (mL)	GP (mL)	CH_4 (mL)	CH_4 (%)
Laurus nobilis	Ln-0.6	51.05±1.50^{i-k}	8.56±0.96^{k-l}	16.64±1.38^{m}
	Ln-1.2	52.71±0.66^{i-k}	12.27±0.51^{i-k}	23.26±0.77^{h-k}
	Ln-1.8	47.33±0.01^{j-k}	8.71±0.33^{k-l}	18.41±0.70^{l-m}
Albizia julibrissin	Aj-0.6	54.49±0.55^{g-k}	11.58±0.60^{j-l}	21.24±0.90^{j-m}
	Aj-1.2	60.22±0.98^{e-i}	17.58±0.38^{f-h}	29.21±0.74^{c-f}
	Aj-1.8	54.14±0.29^{g-k}	11.95±0.50^{j-l}	22.07±0.83^{j-l}
Glycyrrhiza glabra	Gg-0.6	59.88±0.55^{e-i}	16.80±0.40^{g-i}	28.06±0.57^{e-h}
	Gg-1.2	53.57±0.55^{h-k}	13.25±0.31^{h-k}	24.73±0.38^{f-k}
	Gg-1.8	106.85±0.89^{a}	25.72±0.21^{a}	24.08±0.36^{g-k}
Salix alba	Sa-0.6	105.96±2.32^{a}	23.60±0.72^{a-c}	22.27±0.37^{j-l}
	Sa-1.2	106.26±2.43^{a}	24.48±0.95^{ab}	23.02±0.62^{i-l}
	Sa-1.8	102.71±2.58^{ab}	21.08±1.92^{a-g}	20.43±1.41^{k-m}
Robinia pseudoacacia	Rp-0.6	106.26±1.22^{a}	23.93±0.33^{ab}	22.53±0.25^{i-l}
	Rp-1.2	101.82±1.12^{ab}	22.87±0.98^{a-e}	22.44±0.77^{j-l}
	Rp-1.8	95.60±3.15^{b}	20.27±1.14^{b-g}	21.16±0.61^{j-l}
Liquidambar orientalis	Lo-0.6	64.02±1.66^{c-e}	16.32±0.67^{g-j}	25.46±0.38^{e-k}
	Lo-1.2	65.09±1.57^{c-e}	18.39±0.79^{e-g}	28.24±0.92^{c-g}
	Lo-1.8	62.15±2.84^{d-h}	17.96±0.83^{f-h}	28.92±0.64^{c-f}
Juniperus communis	Jc-0.6	63.22±1.76^{d-g}	16.26±0.35^{g-j}	25.79±0.96^{e-k}
	Jc-1.2	66.42±0.62^{c-e}	18.85±0.52^{c-g}	28.39±0.81^{cd-g}
	Jc-1.8	66.15±1.44^{c-e}	18.75±0.40^{d-g}	28.37±0.65^{c-g}
Quercus coccifera	Qc-0.6	63.75±1.40^{c-f}	18.05±0.37^{e-h}	28.32±0.25^{c-g}
	Qc-1.2	65.88±0.75^{c-e}	18.71±0.54^{e-g}	28.44±1.11^{c-g}
	Qc-1.8	66.76±1.38^{c-e}	18.27±1.28^{e-g}	27.29±1.45^{d-i}
Cedrus libani	Cl-0.6	68.76±2.60^{c-e}	23.59±2.21^{a-d}	34.09±1.89^{ab}
	Cl-1.2	64.01±3.93^{c-e}	20.85±1.80^{b-g}	32.42±1.03^{a-c}
	Cl-1.8	63.01±2.06^{c-e}	18.76±0.94^{d-g}	29.83±1.56^{b-e}
Arbutus andrachne	Aa-0.6	73.01±1.56^{c}	25.11±0.53^{ab}	34.40±0.43^{ab}
	Aa-1.2	70.76±1.25^{cd}	24.63±0.62^{ab}	34.80±0.29^{a}
	Aa-1.8	70.26±0.25^{cd}	22.10±0.34^{a-f}	31.46±0.58^{a-d}
SEM		1.728	0.474	0.460
SE		2.319	0.232	0.354
Sig.		**	**	**

$^{a-k}$ Means with different symbols in the same column are significantly different from each other. SEM: Standard Error of Means. SE: Standard Error Sig.: Significancy. **: P<0.001. GP: Gas production at 24h of incubation as mL/0.2g DM. CH_4: in vitromethane production at 24h as mL/0.2g DM.

It's possible that a small amount of solvent residue left over from the extraction process are what is causing the increase in CH_4 production. Archeas living on protozoa produce CH_4 both directly and indirectly by using methanol and H2 together (Johnson and Johnson, 1995; Knapp et al., 2014). It is estimated that the dissolution of leaves in mixed solution (ethanol, methanol and distilled water) caused a very rapid increase in the number of archeas (methanogens) at the beginning of incubation under the influence of methanol. Shakeri, P., et al., (2017) applied ethanol and methanol separately, but the results were found like in the study of Patra et al., (2006), an increase in GP was observed with the increase in dose level. In line with the findings of the aforementioned researchers, neither the production of GP nor CH_4 was decreased by this investigation. Additionally, the variations in the phenolic compound structures, the phenolic source activity, and the extract dose levels may all contribute to the variances across these findings (Makkar, 2003; Rochfort et al., 2008; Jayanegara et al., 2011).

There are numerous factors that affect determining the amount of gas production; such as environment, maturation level, plant secondary metabolites (saponin, essential oils and flavonoids) and harvest time. The effectiveness of plant or plant extracts with a high content of saponin, flavonoids, and grain varies depending on the source, type, and level of secondary

metabolites contained in it (Patra et al., 2006). Blümmel and Ørskov, (1993) have claimed that gas production is associated with the production of volatile fatty acids and that although fermentation end products are more closely related to gas production, fermentation and gas production increase with the increase of the substrate surface. Gas production is a function of decomposing carbohydrates. Therefore, the amount of gas production depends on the content and structure of carbohydrates. The speed and quantity of gas production of some shrubs have decreased with the increase of lignin and its grain content. In addition, high cell wall (ADF and NDF) and CT content may reduce the breakdown of nutrients by ruminant animal micro-organisms, resulting in low gas production (Bllümmel and Ørskov, 1993).

Correlation coefficients consisting of chemical compositions of leaves and gas production parameters of extracts are given in the Table 5. The correlations between GP and CP, CA, CT, and cell wall structures (NDF and ADF) have been found significant. This result was in harmony with the data reported by some researchers (Gemeda and Hassen, 2015; Boga et al., 2020), and was different from the results obtained from the studies of the other researchers (Canbolat, 2012). There was also, a non-significant correlation was observed between EE and GP, in terms of this feature, it was in coherence with the studies reported by other researchers (Gemeda and Hassen, 2015; Boga et al., 2020). The cell wall structures (ADF and NDF) were found to be negatively correlated with GP ($P < 0.001$). The negative correlation between GP and these properties may be due to the decrease in microbial activity resulting from increasingly unfavorable environmental conditions. In addition, CP which is one of the factors limiting microbial growth showed positive correlations with GP, and negative correlations with CH_4 production values (mL and %). NDF and ADF contents of leaves are more effective than other properties in determining gas and methane production in general, as they constitute the fibrous parts of plants. Some researchers reported that to reduce CH_4 production have suggested that a decrease in CH_4 production, while others an increase, occurs when plant origin oils are mixed into the ration.

Table 5. Correlation coefficients (r) between the chemical composition of leaves and the gas parameters of its extracts

Çizelge 5. Yaprakların kimyasal bileşimi ile ekstraktlarının gaz parametreleri arasındaki korelasyon katsayıları

	GP (mL)	CH_4 (mL)	CH_4 (%)	DM (%)	CA (%)	OM (%)	ADF (%)	NDF (%)	EE (%)	CP (%)
CH_4 (mL)	-0.752**	1								
CH_4 (%)	-0.159^{NS}	0.524**	1							
DM (%)	-0.018^{NS}	0.086^{NS}	0.147^{NS}	1						
CA (%)	0.486**	0.242**	-0.225*	0.442**	1					
OM (%)	-0.486**	-0.242**	0.225*	-0.0442**	-1**	1				
ADF (%)	-0.514**	-0.117^{NS}	0.472**	0.464**	-0.421**	0.421**	1			
NDF (%)	-0.535**	-0.157^{NS}	0.444**	0.646**	-0.194*	0.194*	0.899**	1		
EE (%)	-0.038^{NS}	-0.096^{NS}	-0.093^{NS}	0.078^{NS}	0.027^{NS}	-0.027^{NS}	-0.129^{NS}	-0.214*	1	
CP (%)	0.288**	-0.061^{NS}	-0.440**	-0.727**	0.156^{NS}	-0.156^{NS}	-0.829**	-0.890**	0.248**	1
CT (%)	0.425**	0.368**	0.042^{NS}	0.492**	0.442**	-0.442**	0.289**	0.199*	0.037^{NS}	-0.336**

*:Correlation is significant at $p<0.05$ value,**: Correlation is very significant at $p<0.01$ value, NS: Not significant

CONCLUSION

The chemical composition of tree leaves, gas production, methane production, digestion degree, and ME values varied depending on the species and harvest time. The entirety of these tree leaves have the capacity to satisfy the maintenance, production, protein, and metabolic energy requirements of ruminant animals, to sum it succinctly. Because of their CT concentration, which may reduce DMI, it is important to examine how many of these tree (*S. alba, R. pseudoacacia, L. orientalis, J. communis, C. libani*) leaves are given to ruminants. These tree leaves can also be used as a substitute for other fibrous feed to enhance rumen digestion and to fill feed gaps. It has been found that, in contrast to the literature, the tree leaves and extracts described do not significantly lower the gas production. Consequently, it was determined that they lacked anti-methanogenic qualities. The study would have been more thorough if assessments of the nutrient composition of tree leaves, determination of volatile fatty acids, and other plant secondary metabolites, as well as in vivo application of in vitro gas production, had all been done. These samples should be confirmed using in vivo techniques, and the concentration of each volatile fatty acid produced as a result of fermentation should also be established, in order to completely understand whether these samples have anti-methanogenic capabilities.

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The contribution of the authors to this article is equal.

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Authors have declared no conflict of interests.

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