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Potential nutritive value of some tree leaves commonly used for small ruminant in the Aegean region of Turkey

Hayrullah Bora Ünlü[1], *Çağrı Özgür Özkan*[2], *Adem Kamalak*[2]

[1]Department of Animal Science, Faculty of Agriculture, Ege University, Bornova, Izmir, Turkey; [2]Department of Animal Science, Faculty of Agriculture Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey

Abstract. The nutritional potential of the leaves of 10 different maquis tree species were evaluated in terms of chemical composition, *in vitro* gas and methane production, metabolizable energy (ME) and organic matter digestibility (OMD) in a maquis and mountainous region of western Turkey. Leaves from 10 tree species, *Quercus aucheri*, *Olea europaea*, *Quercus robur*, *Morus alba* L, *Paliurus spina-christi*, *Pistacia terebinthus*, *Punica granatum*, *Pyrus elaeagnifolia*, *Vitis vinifera*, and *Tilia cordata*, were sampled from maquis areas. Tree species had a significant effect on the chemical composition, *in vitro* gas production, methane production (CH_4), ME, and OMD ($P<0.05$). The results showed large variation in the contents of all examined nutritional components, *in vitro* gas and methane production, ME and OMD among the leaves of these tree species. The dry matter (DM), ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and condensed tannin (CT) ranged from 24.50 to 60.88%, 1.98 to 5.21%, 7.81 to 21.44%, 22.29 to 58.15%, 13.55 to 44.12% and 1.15-1.59% respectively. *In vitro* gas production, methane production, ME and OMD ranged from 20.88 to 45.05 ml, 1.97 to 6.19 ml, 5.60 to 9.46 MJ/kg DM and 41.81 to 71.59% respectively.

Species had a significant effect on the chemical composition, gas and methane production, ME, and OMD of tree leaves. The current study not only provides information about the chemical composition but also ME and OMD of tree leaves, making possible accurate formulation for ruminant animals grown in areas where tree leaves could be used as supplementary feeds for low-quality forages or substrate deficits. In particular, the nutritional value of the leaves of *Morus alba* L., *Vitis vinifera*, *Pistacia terebinthus*, and *Paliurus spina-christi* may offer considerable potential as high-quality forages and increase the economic profitability of ruminants during critical periods in some semi-arid, arid, and maquis regions of Turkey. All tree leaves except for *Olea europaea* and *Morus alba* L. have a low or moderate potential and seems to be used as alternative feedstuffs to mitigate the enteric methane production. However, *in vivo* experiment is required to test the anti-methanogenic potential of tree leaves.

Key words: tree leaves; potential nutritive value; digestibility; condensed tannin; methane

Introduction

Turkey and other developing countries have a serious shortage of quality forage feeds for animal production in semi-arid and mountainous regions. In these regions, the feeding of animals is mainly done by grazing in natural pastures and mountainous and maquis areas (1). However, the vegetation cover and productivity of rangelands have gradually decreased due to seasonal or climatic limitations such as repeated droughts and excessive grazing in the last decade (1). Over time, in these areas, edible perennial herbs, legumes with high protein contents, and other forage plants have been replaced by species that are not consumed by animals. When poor pasture opportunities exist or forage opportunities are limited, animal producers use tree leaves or shrubs to meet their animals' nutritional requirements. Not only in Turkey but also in many parts of the world, and especially in developing countries, problems experienced in accessing roughage due to climatic changes and other limiting factors have increased the interest in using tree leaves or shrubs in ruminant feeding. In such cases, the leaves of trees and shrubs are evaluated as important nutritional resources for ruminant animals, especially in dry land areas, where the available grazing or pasture land with feed resources of very low quality and quantity is not sufficient to meet the nutritional requirements of omnivores such as goats, sheep, and cattle for a part of the year (2, 3).

In the rations of ruminants, the feed value of tree leaves depends primarily on the nutrient content, digestion, and the anti-nutritional content of the leaves. These nutritional values of tree leaves change with the species, maturity stage, season, fertilization, environment, and location (4, 5). Research has shown that the nutrient composition, and especially the levels of anti-nutritional factors such as tannins or other phenolic compounds, plays an important role in animal digestion (6, 7, 8). Because tannins adversely affect potential dry matter intake and nutrient utilization, they are sometimes categorized as anti-nutritional factors; however, tannins at low levels can beneficially influence ruminal fermentation to decrease methane emissions and nitrogen excretion with improved performance (9). On the other hand, in times of feed scarcity such as dry years or poor pasture quality, forages are extremely important for ruminant nutrition in terms of meeting the animals' nutritional requirements such as fiber, energy, protein, and minerals (10). Feeding ruminants with nitrogen-rich tree and shrub leaves as an alternative protein or roughage source also increases ruminal fermentation efficiency and subsequently animal performance, as well as decreasing methane emissions (11). However, the consumption of high-fiber, low-protein roughage by grazing ruminants in pastures is often associated with a significant feed energy loss via heat increase and methane gas production and contributes significantly to global warming (12).

Maquis vegetation and trees have adapted to the western Anatolian region of Turkey climatic conditions and develop in areas up to 800 m above sea level. These plants, having deep root systems, are green most of the year and are fast-growing in character. They have different potential advantages, growing naturally and being highly resistant to changing climatic and weather conditions (2). *Quercus aucheri*, *Olea europaea*, *Quercus robur*, *Morus alba* L., *Paliurus spina-christi*, *Pistacia terebinthus*, *Punica granatum*, *Pyrus elaeagnifolia*, *Vitis vinifera*, and *Tilia cordata* are the common species of maquis communities. Animal breeders in these regions usually feed their animals with the leaves of trees or shrubs found among this vegetation in pastures or mountainous lands. However, there is no systematic research on the use of these tree leaves in ruminant feeding. Therefore, it is extremely important to learn their nutritional compositions and anti-nutritional contents such as condensed tannins, their digestibility, and the methane gas production potential for the use of these tree leaves in more rational ruminant feeding and sustainable animal husbandry. The aim of this study was to determine the nutrient structure, metabolic energy values, *in vitro* digestibility, anti-nutritional contents such as tannins, and methane gas release values of some maquis tree leaves, which is a common vegetation type in western Anatolian region of Turkey. Thus, more rational animal feeding can be achieved by determining which of these tree species are most beneficial.

Materials and methods

Sample Collection

Samples from 10 tree species commonly growing in maquis vegetation zones in the western Aegean region of Turkey were collected for the current study. Leaves from *Quercus aucheri*, *Olea europaea*, *Quercus robur*, *Paliurus spina-christi*, *Morus alba* L., *Pistacia terebinthus*, *Punica granatum*, *Pyrus elaeagnifolia*, *Vitis vinifera*, and *Tilia cordata* were sampled from maquis areas of Menemen (38°58′N, 27°20′E) in Izmir province, Turkey. Leaves of each tree species were collected from six randomly selected trees. Each set of tree leaves thus consisted of six different randomly selected samples; each one was analyzed in three replicates one by one for each parameter. Before chemical analyses, to obtain air-dried samples, all experimental tree leaves were dried at 65 °C in desiccators for 24 hours and then were ground through a 1-mm screen in preparation for chemical analysis and stored at 4 °C in a refrigerator until analysis.

Nutritional composition

Nutrient contents of air-dried samples were analyzed according to the methods reported by the AOAC (13) and all data are presented on dry matter (DM) basis. All samples were analyzed for DM (method 934.01), crude ash (method 942.05), crude protein (CP) (method 990.03), and ether extract (EE) (method 920.39). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the methods of Van Soest (14). The condensed tannin (CT) contents of leaves were analyzed by butanol-HCl method as suggested by (15).

Determination of gas and methane production of tree leaves

An *in vitro* gas production technique (16) was used to determine the gas production and methane production of tree leaves. Two fistulated Awassi sheep were fed with alfalfa hay (800 g) and barley (400 g) to obtain rumen fluid for *in vitro* fermentation. Leaf samples of approximately 200 mg were weighed into 100 ml glass syringes in triplicate, and then 40 ml of the buffered rumen fluid (1:2 V/V) was added to the glass syringes containing leaf samples and these were transferred to a water bath set at 39 °C for 24 h of incubation. The percentages of methane of total gas production of tree leaf samples after 24 h of incubation were determined using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (17). The methane productions of tree leaf samples as ml were calculated as follows:

Methane production (ml) = Total gas production (ml) × Percentage of methane (%)

The ME and OMD of leaf samples were estimated with the equations suggested by (18):

$$\text{ME (MJ/kg DM)} = 2.2 + 0.1357\text{GP} + 0.057\text{CP} + 0.02859\text{EE}^2$$

$$\text{OMD (\%)} = 15.38 + 0.8453\text{GP} + 0.595\text{CP} + 0.675\text{Ash}$$

GP: Gas production of 200 mg of leaf samples at 24 h of incubation (ml), CP: Crude protein (%), EE: Ether extract (%), Ash: Crude ash (%)

Statistical analysis

The statistical analysis of the results included one-way analysis of variance (ANOVA) using general linear models and Duncan's multiple range test, which were applied to the results using IBM SPSS Statistics 25 (19). The model included tree leaf samples as main effects. Differences were considered to be significant based on the P<0.05 level of probability.

Results

The means and standard errors for the nutritional composition and condensed tannins of the leaves of 10 tree species in maquis vegetation zones in the Aegean region of Western Turkey are summarized in Table 1. There were significant (P<0.05) differences among the leaves of the tree species in terms of DM, ash, EE, CP, NDF, ADF, and CT contents. The DM contents of the leaves were within the range of 24.50% to 60.88%. Leaves of *Quercus aucheri* had the highest DM content, while *Vitis vinifera* had the lowest DM content. The overall average means of the ash in leaf samples varied between 3.12% and 12.25%. *Pistacia terebinthus* and *Tilia cordata* had the highest ash content, being equal, and *Paliurus spina-christi* leaves had the lowest ash content. The differences in EE contents of the leaves of the tree species in this study were significant, but the variation was not as high as that seen for other chemical nutritional parameters. While *Paliurus spina-christi* leaves had the highest concentration of EE (5.21%), *Punica granatum* leaves had the lowest (1.98%). The CP contents ranged from 7.81% to 21.44%. The CP content of leaves from *Paliurus spina-christi* was significantly higher (P<0.05) than those of the other tree leaves. In terms of the fiber contents of the leaves, there were considerable variations among species. The NDF and ADF contents of the tree leaves ranged from 22.29% to 58.15% and from 13.55% to 28.06%, respectively. The NDF content of leaves from *Quercus aucheri* was significantly higher than those of other studied trees, while *Morus alba* L. and *Punica granatum* had the lowest NDF contents (22.29% and 22.77%, respectively). The ADF contents ranged from 13.55% to 44.12%. Among the species, the ADF content of

Table 1: The effect of species on the chemical composition (% of DM) of tree leaves

Tree Species	DM	Ash	EE	CP	NDF	ADF	CT
Quercus aucheri	60.88[a]	5.20d[e]	2.85[bc]	7.81[g]	58.15[a]	44.12[a]	7.93[c]
Olea europaea	43.21[c]	6.14[cd]	2.89[bc]	11.54[e]	36.35[d]	30.97[c]	1.59[d]
Quercus robur	54.60[b]	4.85[e]	2.93[bc]	11.90[e]	52.56[b]	38.66[b]	12.10[b]
Paliurus spina-christi	38.28[d]	3.12[f]	5.21[a]	21.44[a]	52.56[b]	18.31[e]	6.49[c]
Morus alba L.	36.34[d]	11.61[a]	2.93[bc]	20.96[a]	22.29[f]	16.86[ef]	1.51[d]
Pistacia terebinthus	54.88[b]	12.25[a]	2.10[c]	14.76[d]	25.06[f]	13.55[g]	6.58[c]
Punica granatum	53.10[b]	6.91[bc]	1.98[c]	16.09[c]	22.77[f]	16.75[ef]	1.15[d]
Pyrus elaeagnifolia	59.94[a]	7.39[b]	3.79[b]	10.36[f]	41.38[c]	31.50[c]	16.63[a]
Vitis vinifera	24.50[e]	7.89[b]	2.92[bc]	20.93[a]	31.22e	16.50[f]	14.80[b]
Tilia cordata	35.56[d]	12.25[a]	2.94[bc]	19.40[b]	36.88[d]	25.58[d]	1.99[d]
SEM	0.88	0.32	0.35	0.21	0.91	0.53	1.01
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[abcdefg] Column means with common superscripts do not differ ($P<0.05$). SEM: Standard error of the mean

Pistacia terebinthus leaves was the lowest, while *Quercus aucheri* had the highest ADF content. The CT contents of the studied tree leaves ranged from 1.15% to 16.63%. The CT contents of *Punica granatum*, *Morus alba* L., and *Olea europaea* were significantly lower than those of other tree leaf samples. On the other hand, leaves from *Pyrus elaeagnifolia* had the highest CT content (16.63%) among the studied samples.

The effects of the plant species on *in vitro* gas and methane production, ME, and OMD were given in Table 2. There were significant differences among the leaves of the tree species in terms of *in vitro* gas, methane production, ME, and OMD ($P<0.05$). Species had a significant effect on *in vitro* gas, methane production, ME, and OMD ($P<0.05$). The gas production from leaves of *Morus alba* L. was significantly higher (51.65%) than those of other selected tree species, while the gas productions of *Quercus robur* and *Quercus aucheri* were significantly lower than those of other samples (20.88% and 21.61%, respectively). Methane production of tree leaves ranged from 1.97 to 7.94 ml. The highest methane production occurred

Table 2: The effect of species on the gas, methane, metabolizable energy, and organic matter digestibility of tree leaves

Tree Species	GP	CH_4 (ml)	CH_4 (%)	ME	OMD
Quercus aucheri	21.61[f]	1.97[e]	9.10[f]	5.60[f]	41.81[g]
Olea europaea	42.49[bcd]	7.94[a]	18.70[a]	8.65[cd]	62.32[e]
Quercus robur	20.88[f]	2.25[e]	10.75[de]	5.74[f]	43.39[g]
Paliurus spina-christi	43.96[bc]	6.19[b]	14.08[b]	9.46[b]	67.40[bcd]
Morus alba L.	51.65[a]	7.85[a]	15.22[b]	10.43[a]	79.35[a]
Pistacia terebinthus	45.05[b]	4.08[d]	9.06[f]	9.17[bc]	70.52[bc]
Punica granatum	39.56[cde]	4.66[cd]	11.78[d]	8.50[cd]	63.39[de]
Pyrus elaeagnifolia	35.90[e]	4.95[c]	13.79[bc]	7.70[e]	57.18[f]
Vitis vinifera	42.49[bcd]	4.27[d]	10.05[ef]	9.18[bc]	71.59[b]
Tilia cordata	37.36[de]	4.57[cd]	12.25[cd]	8.40[de]	66.78[ed]
SEM	1.48	0.23	0.46	0.20	1.25
P	<0.001	<0.001	<0.001	<0.001	<0.001

[abcdefg] Column means with common superscripts do not differ ($P<0.05$). SEM: Standard error of the mean, GP: Gas production (ml) CH_4: Methane (ml or %), ME: MJ/kg DM, OMD: %

with the leaves of *Olea europaea*, while the lowest mean value was seen for *Quercus aucheri* leaves. As a result of microbial fermentation, the percentage of methane of gas production was again the highest in the leaves of *Olea europaea* (18.70%), while the lowest was obtained from *Pistacia terebinthus* and *Quercus aucheri* leaves (9.06% and 9.10%, respectively).

The ME and OMD contents of the studied tree leaves ranged from 5.60 to 10.43 MJ/kg DM and from 41.81% to 79.35% respectively. The highest ME and OMD values were found in the leaves of *Morus alba* L. while *Quercus aucheri* had the lowest ME and OMD values among tree species.

Discussion

There were large differences in DM concentrations among the tree species; leaves from all tree species had DM contents of higher than 35%, while a few had DM contents above 59%. In this study, the DM contents of all tree leaves except those of *Vitis vinifera* were thus seen to offer good potential as quality forage sources for ruminants. The DM contents of these tree species were comparable to those reported by (3, 10, 20) but they were considerably lower than the value reported by (21). These wide variations of the DM contents of the leaves of different tree species could be a result of the species, agro climatic conditions such as time of sampling (August), and agronomic factors such as location of the plants and tree density. (22) indicated that tree leaves containing DM of more than 30% constitute an important, reasonable, and reliable source of DM, together with other nutrients for feeding ruminants.

The ash of the tree leaves varied from 3.12% and 12.25% with large variations found among the species. These findings partially agree with the previously demonstrated results of (3, 9, 10, 20, 21) while they are relatively lower than the values reported for tree species growing in hilly, semi-arid, and arid zones (4, 22, 23). The large variation in ash contents among the tree leaves could be due to intrinsic factors such as the tree species or extrinsic factors such the lower or higher mineral contents of soils of maquis land. (4, 24) likewise suggested that such differences in the ash contents of tree leaves may be attributed to variations in soil mineral profiles. The high ash contents of the tree leaves in the present study support the previously reported results of (4), who stated that tree leaves are important mineral sources for ruminant animals. The EE contents of most of the species were higher than 2% and were generally similar to each other, except for *Paliurus spina-christi* (5.21%). This result is in agreement with the findings of (10) and (9) but in contrast to those of (25). Differences may be due to the species and the region, e.g. semi-arid or tropical regions. The EE content in tree fodder forages was usually low and has been reported to fall between 2.34% and 5.92% (26).

Other than the leaves of *Quercus aucheri*, the CP contents of the other tree species had values above 10% and some of them had values above 20% (*Paliurus spina-christi*, *Vitis vinifera*, and *Morus alba* L.). These results are comparable to those reported by (9, 20, 21). The CP values of the leaves were also similar to the contents reported by (4, 10, 25). CP values tend to vary in the leaves of trees based on the location, age, and species of the tree (26). All of the species studied here had values above the minimum critical levels of CP values (80 g kg^{-1} DM) required for the normal functioning of rumen microorganisms (28). The CP concentrations for grazing species should always be higher than the minimum level of 7-10% DM required for maintenance, optimum rumen function, and feed intake in ruminant livestock (14, 29, 30). Additionally, CP in these amounts will be sufficient to support low to medium levels of production requirements of cattle, sheep, and goats (31, 32). In this study, all tree species, especially *Paliurus spina-christi*, *Morus alba* L., *Vitis vinifera*, and *Tilia cordata*, which had CP contents above 19% DM, can be used for supplemental CP in the current low-quality forage-based diets of livestock in the arid and semi-arid regions of Turkey. The fibrous feeds in these regions are often deficient in CP, particularly during dry seasons. If the leaves are used in rations, not only will livestock productivity improve but the feed costs will also be reduced. Therefore, these tree leaves may be used as potential feed resources, mainly as protein supplements for ruminant animals. In agreement with the findings of this research, (33) indicated that given the high CP content of such leaves, they can be considered as suitable alternative supplemental protein sources for poor-quality pastures or during critical periods of the year when feed shortages occur.

The cell wall contents (NDF and ADF) obtained in the current study are consistent with the findings of the studies carried out by (4, 20, 34). The cell wall contents of *Quercus aucheri* and *Pistacia terebinthus* leaves are significantly higher than those of the other tree species. This could be associated with the tree species and the vegetative stage of the leaves, as fresh leaves have a relatively smaller proportion of cellulosic parts than mature leaves (35). It is well known that the cell wall contents of forages are negatively correlated with palatability and thus the relative preference of the livestock. The tree leaves evaluated in this study can be considered as good-quality roughage sources since fibrous feeds with NDF content of less than 45% DM were classified as high-quality roughage feeds (36). Additionally, the low to moderate NDF and ADF contents of *Morus alba* L., *Punica granatum*, and *Pistacia terebinthus* (22.29%, 22.77%, and 25.06%, respectively) would also improve the voluntary intake and digestibility of poor-quality roughage or rations (30).

The CT contents in the tree leaves showed considerable variation among the species, with *Pyrus elaeagnifolia* having the highest concentration of CT (16.63%) and leaves of *Punica granatum*, *Morus alba* L., *Olea europaea*, and *Tilia cordata* having the lowest CT content (1.15%, 1.51%, 1.59%, and 1.99%, respectively). These differences may be due not only to the plant species but also plant maturity stages (15). In general, most of the CT concentrations of the tree leaves obtained in the present study were comparable to those obtained by (9, 20, 25, 27, 30). However, the CT values obtained for *Punica granatum*, *Morus alba* L., *Olea europaea*, and *Tilia cordata* were lower than previously reported values. The differences among studies in terms of CT contents could be associated with the analytical method used, the nature of tannins in different tree species, plant growth stages, and the influence of soil and climatic factors (29). It must also be kept in mind that the amount of CT in tree leaves tends to be influenced by the level of leaf maturity and the stress or hazards present within the growing conditions to which the plants are exposed. Younger leaves have more CT and other polyphenols compared with older or mature leaves (27), while a low concentration of CT has a beneficial effect on nitrogen utilization due to the protection of proteins against microbial degradation in the rumen (37). The DM intake and the digestibility of the feed decrease when CT rises above 5% of DM in the ration and a decrease was also seen in the efficiency of the utilization of CP in feeds due to the excessive formation of tannin-protein complexes (38). However, dietary CT (>2-3%) were shown to have beneficial effects because they reduced the protein degradation in the rumen by formation of a protein-tannin complex (39). With the exception of four tree species (*Punica granatum*, *Morus alba* L., *Olea europaea*, and *Tilia cordata*), the CT contents of the leaves of the other examined species were higher than 5% DM. Therefore, they should be useful in grazing or in rations, considering the phenolic compounds of the tree species at different periods.

The *in vitro* gas production of feedstuff materials incubated with rumen fluid feedstuff has received much attention as a means of evaluating the nutritional quality of feedstuffs. The gas production ranged widely among the sampled tree leaves. These *in vitro* gas production values are consistent with those previously reported by (9, 21, 40), although *in vitro* gas production values were lower than the those reported by some authors (22, 25, 30, 41). These differences may be due to the chemical composition of tree leaves, such as the CT, CP, NDF, and ADF contents of leaves, as well as the vegetation stage of leaves and also the soil type (21, 40). This result is in agreement with the findings of (42), who concluded that the *in vitro* gas production was negatively correlated with cell wall contents (NDF and ADF). On the other hand, the leaves of *Pistacia terebinthus* and *Quercus aucheri* had lower *in vitro* gas production values than others in this study, while they had relatively low CT contents. Meanwhile, *Pyrus elaeagnifolia* had the highest CT content among the samples; it showed a moderate level of *in vitro* gas production. The results of the current study seem to not confirm earlier reports (41) that concluded that the *in vitro* gas production method is more reliable in detecting the effects of anti-nutrients in feed because these compounds are likely to affect the activity of rumen microbes in a closed system.

This study clearly showed that maquis tree leaves have shown low methane production values. These results are comparable to those previously reported by (9, 12) but are lower than the values demonstrated by (11). Methane is a byproduct of the anaerobic microbial fermentation of carbohydrates in the rumen and its production by ruminants is a natural process that originates in the rumen during digestion (43). It is one of the greenhouse gases, with a global warming potential 23 times that of carbon dioxide (44). Enteric methane production primarily depends on the quantity and quality of the diet, as they affect the rate of fermentation and passage (7). The methane production was due to the higher fermentation potential of the tree leaves, which could be associated with their low CT contents. The lower CT content of these forage species may have minimal inhibitory effects on the rumen microbes that are responsible for the methane production (11). Different sources of tannin extracts have been shown to decrease methane production in both *in vitro* and *in vivo* conditions depending on the doses (45). However, while *Olea europaea* had one of the lowest CT contents among the studied species, it also showed the highest methane production. This finding is in agreement with the previous result reported by (46), who suggested that tannins did not affect methanogenesis by decreasing the methane production. All tannin-containing leaves are not equally effective in decreasing methane production (47).

(48) suggested that percentage of methane in gas production can be used to determine the anti-methanogenic potential of tree leaves. The feedstuffs can be divided in three groups, low potential (percentage of methane between >11% and ≤14%), moderate potential (% methane in gas percentage of methane between >6% and <11%), high potential percentage of methane between >0% and <6%). As can be seen from Table 2, leaves of *Quercus aucheri*, *Pistacia terebinthus* and *Vitis vinifera* had a moderate anti-methanogenic potential since the percentage of methane of leaves of *Quercus aucheri*, *Pistacia terebinthus* and *Vitis vinifera* are between >6% and <11%), On the other hand, leaves of *Quercus robur Paliurus spina-christi*, *Punica granatum*, *Pyrus elaeagnifolia* and *Tilia cordata* had a low anti-methanogenic potential since the percentage of methane leaves of *Quercus robur Paliurus spina-christi Punica granatum Pyrus elaeagnifolia and Tilia cordata* are between >11% and ≤14%). Therefore all tree leaves except for *Olea europaea* and *Morus alba* L. have a low or moderate potential and seems to be used as alternative feedstuffs to mitigate the enteric methane production. However, *in vivo* experiment is required to test the anti-methanogenic potential of tree leaves.

The studied plant species also had significant variations in ME and OMD contents. The ME and OMD contents of the sampled leaves ranged from 5.60 to 10.43 MJ/kg DM and 41.81% to 79.35%, respectively. In the current research, the highest ME content and OMD values were found in *Morus alba L.* leaves, while *Quercus aucheri* had the lowest ME and OMD among the tree leaves. The highest ME and OMD contents being found in *Morus alba* L. leaves regarding the extent of *in vitro* fermentation suggests that this tree and its leaves are of higher nutritional value than the other nine tree species. This may be due to *Morus alba* L. leaves having lower NDF, ADF, CT and high CP concentration, while *Quercus aucheri* has high NDF, ADF and low CP and medium CT concentrations. Furthermore, *Vitis vinifera*, *Pistacia terebinthus*, and *Paliurus spina-christi* had high ME values with highly fermentable OMD and lower methane production. These results are consistent with the findings of (1, 20) who suggested that the increase in CT content decreased the ME and OMD contents of tree leaves. In this study, the CT content was not considered alone; the levels of cell wall contents (NDF and ADF) and the protein contents of the tree leaves were also thought to be jointly positively or negatively effective on ME and OMD. The probable cause of high or low fermentation and ME contents in leaves could be the high fiber content of tree leaves, in agreement with the previously indicated results of (24, 39).

Conclusion

In conclusion, species had a significant effect on the chemical components, gas and methane production, ME, and OMD of tree leaves. The current study not only provides information about the chemical composition but also ME and OMD of tree leaves, facilitating accurate formulations for ruminant animals raised in areas where tree leaves could be used as supplementary feeds for low-quality forages or substrate deficits. In particular, the nutritional value of the leaves of *Morus alba* L., *Vitis vinifera*, *Pistacia terebinthus*, and *Paliurus spina-christi* may offer considerable potential as high-quality forages and increase the economic profitability of ruminants during critical periods in some semi-arid, arid, and maquis regions of Turkey. All tree leaves except for *Olea europaea* and *Morus alba* L. have a low or moderate potential and seem able to be used as alternative feedstuffs to mitigate enteric methane production. However, *in vivo* experiments are required to test the anti-methanogenic potential of tree leaves.

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Correspondence
Hayrullah Bora Ünlü
Department of Animal Science, Faculty of Agriculture, Ege University, 35040, Bornova, Izmir, Turkey
E-mail: hayrullahboraunlu@gmail.com