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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Nutrient Composition and Metabolic Energy of Different Fruit Tree Leaves as Feeding Sources for Ruminants*

Ruminantlar için Besleme Kaynağı Olarak Farklı Meyve Ağacı Yapraklarının Besin Bileşimi ve Metabolik Enerjisi

Filiz KOCADAYIOĞULLARI[1], Mustafa BOĞA[2*], Orhan ERMETİN[3]

Abstract

The need for roughage in the animal nutrition industry has led to the search for various alternative roughage. In this context, as a result of the research, tree leaves have the potential to be considered as a nutritious and balanced forage source for animals. In this study, it was aimed to investigate the usability of apple (*Malus domestica*), apricot (*Prunus armeniaca*), cherry (*Prunus avium*), grape (*Vitis vinifera*), and umber mulberry (*Morus rubra*) leaves in ruminant nutrition. As a result of the research, nutritional contents of tree leaves (dry matter, crude protein, crude ash, crude oil, neutral detergent fiber, acid detergent fiber, acid detergent lignin, tannin), *in vitro* gas, carbon dioxide and methane gas production, *in vitro* gas post-production rumen fluid volatile fatty acid contents were found to be different from each other ($P<0.05$). In addition, although metabolic energy, organic matter digestion, digestible dry matter, dry matter consumption and relative feed values were found to be different in tree leaves ($P<0.05$), the highest values of these parameters were found in mulberry leaves. Metabolic energy (ME-J/kgDM) in grape, mulberry, apple, apricot and cherry is 9.24, 10.69, 10.07, 10.24, 9.33, organic matter digestibility (OMD-%) is 56.49, 58.98, 54.46, 57.34, and 58.31, respectively. has been detected. In the volatile fatty acids in the rumen fluid of the leaves after *in vitro* gas production; Total volatile fatty acid (TVFA-mmol/L) was defined as 69.16, 76.25, 66.04, 68.63, and 65.24, acetic acid (AA-mmol/L) was determined as 44.90, 48.52, 41.93, 46.82, and 43.62, respectively. As a result of our detailed studies, it has been determined that the leaves of various fruit trees stand out with their considerable nutritional content. The most important reason for the differences in the findings is that the nutritional content of tree leaves is different from differs. As a result of our comprehensive study, these nutritional content differences offer significant potential for using leaves of various fruit trees as a source of forage.

Keywords: Forage, Fruit tree leaves, Animal feeding, *In vitro* gas production

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Öz

Hayvan besleme sektöründeki kaba yem ihtiyacı, çeşitli alternatif kaba yem arayışlarına sebep olmuştur. Bu bağlamda, yapılan araştırmalar neticesinde ağaç yaprakları, hayvanlar için besleyici ve dengeli bir kaba yem kaynağı olarak değerlendirilebilecek potansiyele sahiptir. Bu çalışmada, elma (*Malus domestica*), kayısı (*Prunus armeniaca*), kiraz (*Prunus avium*), asma (*Vitis vinifera*), urum dutu (*Morus rubra*) yapraklarının ruminant beslemede kullanılabilirliğini araştırmak amaçlanmıştır. Araştırma sonucunda ağaç yapraklarının besin madde içerikleri (kuru madde, ham protein, ham kül, ham yağ, nötral deterjan lif, asit deterjan lif, asit deterjan lignin, tanen), *in vitro* gaz, karbondioksit ve metan gazı üretimi, *in vitro* gaz üretim sonrası rumen sıvısı uçucu yağ asit içerikleri birbirinden farklı bulunmuştur (P<0.05). Ayrıca ağaç yapraklarında metabolik enerji, organik madde sindirimi, sindirilebilir kuru madde, kuru madde tüketimi, nispi yem değerleri de farklı bulunmakla beraber (P<0.05), söz konusu parametrelere ait en yüksek değerler dut yaprağında bulunmuştur. Asma, dut, elma, kayısı ve kirazda metabolik enerji (ME-J/kgKM) sırasıyla, 9.24, 10.69, 10.07, 10.24, 9.33 olarak, organik madde sindirilebilirliği (OMS-%) 56.49, 58.98, 54.46, 57.34, 58.31, olarak tespit edilmiştir. Yaprakların *in vitro* gaz üretim sonrası rumen sıvısı uçucu yağ asitlerinde toplam uçucu yağ asidi (TUYA-mmol/L) sırasıyla 69.16, 76.25, 66.04, 68.63, 65.24 olarak, asetik asit (AA-mmol/L) 44.90, 48.52, 41.93, 46.82, 43.62 olarak tespit edilmiştir. Yapmış olduğumuz detaylı çalışmalar neticesinde, çeşitli meyve ağaçlarının yapraklarının azımsanmayacak besin içeriğiyle öne çıktığı tespit edilmiştir. Bulgulardaki farklılıkların en önemli nedeni ağaç yapraklarının besin madde içeriğinin birbirinden farklı olmasıdır. Yaptığımız kapsamlı çalışma sonucunda, bu besin içeriği farklılıklarının, çeşitli meyve ağaçlarının yapraklarının kaba yem kaynağı olarak kullanılabilirliği konusunda önemli bir potansiyel sunmaktadır.

Anahtar Kelimeler: Yem, Meyve ağacı yaprakları, Hayvan besleme, *İn vitro* gaz üretimi

1. Introduction

Livestock enterprises incur substantial feed expenses, accounting for 60-70% of operating costs (Boğa and Çevik, 2012; Özdemir and Kaya, 2020; Alçiçek, 2021; Kurt, 2022; Kurt et al., 2022). This situation has led researchers to explore alternative feed raw materials (Özdemir and Kaya, 2020; Kurt, 2022). Roughage is crucial in ruminant nutrition, supporting rumen microbial activities and digestive system functions. Affordable roughage options are preferred because they positively affect product quality and performance (Özdemir and Kaya, 2020). With their favourable protein content, tree leaves can be an alternative roughage source (Canbolat, 2012a; Boğa, 2014; Nabi et al., 2018; Boğa et al., 2022). The protein content is a crucial factor for microbial activities, and the protein ratio in feed should be approximately 10% to sustain microbial activities (Canbolat et al., 2013). The leaves and fruits of the trees are significant in the nutrition of ruminant animals such as goats and sheep (Atalay et al., 2017). As a result of their high nutritional value and positive effects on rumen function, microbial productivity, and body metabolism, tree leaves are increasingly recognized as a potentially high-quality feed source for ruminant animals, especially to provide crude protein and animal nutrition as a source of energy (Kamalak et al., 2005; Rababah et al., 2008). Yet, the utilization of tree leaves by herbivores is frequently hindered by the defence or deterrent mechanisms of plants, which are attributed to their elevated tannin levels, nutrient deficiency, and phenolic substance content (Provenza, 1995; Kılıç and Abdiwali 2016; Olfaz et al., 2018).

Mulberry is a type of fruit belonging to the *Moraceae* family. For thousands of years, it has adapted to tropical, subtropical, and temperate regions of Asia, Europe, North and South America, and Africa. Mulberry, important in Turkey, has been known and grown for over 400 years (Ayaşan and Baylan, 2016; Jan et al., 2021; Ali et al., 2023; Öcalan et al., 2023). Our country ranks 4th in apple production, one of the world's most consumed fruits, and the world's largest apricot producer (Şamlı et al., 2014; Taşova and Dursun, 2023). Turkey is located in a suitable region for viticulture and has a deep-rooted viticulture culture and rich grape gene potential because it is at the intersection of vineyard centers and in the center of the geography where viticulture was first practiced (Güveanateş and Topkaya, 2023).

Tannins are significant limiting factors in using alternative feed sources in animal rations (Herva’s et al., 2003). Excessive tannin consumption in ruminant rations can lead to toxicity, loss of appetite, and constipation. Therefore, when incorporating fruit tree leaves into rations, the tannin content should be adjusted according to the type and age of the animal. Tannins reduce protein breakdown by rumen microorganisms, inhibiting protein digestibility. Consequently, undigested proteins (such as bypass proteins) pass through the rumen and are digested in the small intestine (Boğa et al., 2021). The effects of tannins on ruminants vary depending on the ration content, grain structure, and amount (Ünver et al., 2014). Tree parts such as branches, leaves, and fruits, which contain high levels of tannins and saponins, can reduce methane formation by improving nitrogen metabolism in the rumen (Özdemir and Kaya, 2020).

Methane gas is produced through fermentation in the digestive system of ruminants. Methane production results in a loss of gross energy (2-12%) obtained from feed and contributes to global warming as a harmful greenhouse gas (Johnson and Johnson, 1995; Meral and Biricik, 2013; Şimşek and Kamalak, 2019; Cholewińska et al., 2020; Ungerfeld et al., 2023). The proportion of roughage in the ration increases methane release, whereas an increase in easily degradable carbohydrates decreases it (Kutlu and Serbester, 2014).

Considering the high protein content and potential avoidance of negative effects, different tree leaves can be recommended as alternative feed raw materials. This study aimed to determine tree leaf nutrient content, digestibility, and methane values.

2. Materials and Methods

Feed materials: Five different tree leaf samples (*apple/Malus domestica*, *apricot/Prunus armeniaca*, *cherry/Prunus avium*, *grape/Vitis vinifera*, and *mulberry/Morus rubra*) were collected from the Niğde region to determine their nutrient composition and *in vitro* digestibility. The leaves were collected by hand from different regions of the Central District of Niğde province in June, and samples were obtained from these mixtures by mixing according to species. After the tree leaves were collected, they were brought to the laboratory and left in the shade to dry. After the tree leaves were dried, they were ground in a mill to pass through a 1 mm sieve and prepared for analysis. The study was conducted in 3 repetitions, and the averages were evaluated.

Animal materials: Rumen fluid was obtained from three Kıvırcık rams slaughtered in the slaughterhouse for the application of the *in vitro* gas production technique. An ethics committee decision was not required for the *in vitro* study.

Chemical analysis: The tree leaves were dried at room temperature under shade at Niğde Ömer Halisdemir University, Bor Vocational School Food Processing Laboratory. The dried leaves were ground to pass through a 1 mm sieve and used for analysis.

The leaf dry matter (DM) content was determined by drying in an oven at 105°C for 4 hours, and the crude ash content was determined by burning in a muffle furnace at 550°C for 4 hours. The nitrogen (N) content was determined using the Kjeldahl method, and crude protein was calculated using the Nx6.25 formula (AOAC, 1990). Ether extract analysis was performed according to the method reported in AOAC (1990).

The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents, representing the cell wall components of the feed, were determined using the ANKOM 200 Fiber Analyzer (ANKOM Technology, 2008) following the Van Soest et al. (1991) method. The tannin contents of the leaves were determined using the Folin-Denis method (AOAC, 1990). Gas chromatography was used to determine the total volatile fatty acid (TVFA), acetic acid, propionic acid, and butyric acid contents of the leaves (Wiedmeier et al., 1987), while lactic acid analysis was performed using the spectrophotometric method (Barker and Summerson, 1941).

Application of *in vitro* gas production technique: The "*in vitro* gas production technique" reported by Menke and Steingass (1988) was employed to determine feed raw materials' digestibility and metabolic energy level under *in vitro* conditions. Approximately 200±10 mg of feed sample was placed in triplicate in special glass tubes (volume: 100 mL). Then, an RS/buffer solution prepared according to the method reported by Menke et al. (1979) was added. The tubes were incubated in a 39°C water bath, and the gas produced by fermentation was measured at different time intervals (3, 6, 12, 24, 48, 72, and 96 hours).

Metabolic energy (ME) and organic matter digestibility (OMD) of feed raw materials were determined by the following equations reported by Menke and Steingass (1988).

$$\boldsymbol{OMD}\% = \mathbf{15.38} + \mathbf{0.8453}\,\boldsymbol{x}\,\boldsymbol{GP} + \mathbf{0.0595}\,\boldsymbol{x}\,\boldsymbol{CP} + \mathbf{0.0675}\,\boldsymbol{x}\,\boldsymbol{CA} \qquad \text{(Eq. 1)}$$

$$\boldsymbol{ME}\left(\frac{\boldsymbol{MJ}}{\boldsymbol{kg}}\boldsymbol{KM}\right) = \mathbf{2.20} + \mathbf{0.1357}\,\boldsymbol{x}\,\boldsymbol{GP} + \mathbf{0.0057}\,\boldsymbol{x}\,\boldsymbol{CP} + \mathbf{0.0002859}\,\boldsymbol{x}\,\boldsymbol{EE}^2 \qquad \text{(Eq. 2)}$$

(GP: net gas production at the end of the 24-hour incubation period of 200 mg dry feed sample, CP: crude protein%, EE: ether extract%, and CA: crude ash%).

Determination of relative feed value (RFV): The relative feed value of Rohweder et al. (1978) was calculated by the following equations.

First, dry matter digestion (DMD%) was calculated from the ADF value to calculate the relative feed value. (ADF: acid detergent fiber)

$$\boldsymbol{DMD}\% = \mathbf{88.9} - (\mathbf{0.779}\,\boldsymbol{x}\,\boldsymbol{ADF}\%) \qquad \text{(Eq. 3)}$$

Depending on the animal's live weight, dry matter intake (DMI%) was calculated from the NDF value.
(NDF: neutral detergent fiber)

$$\boldsymbol{DMI}\% = \frac{\mathbf{120}}{\boldsymbol{NDF}} \qquad \text{(Eq. 4)}$$

To calculate the relative feed value (RFV), DMD% and DMI% values are substituted in the formula.

$$\boldsymbol{RFV} = \boldsymbol{DMD}\%\,\boldsymbol{x}\,\boldsymbol{DMI}\%\,\boldsymbol{x}\,\mathbf{0.775} \qquad \text{(Eq. 5)}$$

When the relative feed value is 41% ADF and 53% NDF in the composition of the feeds, 100 is considered standard (Rohweder et al., 1978). If this value is higher than 100, it is used as an indicator of high-quality feed; if it is low, it is used as an indicator of low-quality feed.

Statistical analysis: The data obtained from the study were statistically analyzed using analysis of variance (ANOVA) and Duncan’s test to compare the significant differences (Snedecor and Cochran, 1967).

3. Results and Discussion

3.1. Nutrient Contents of Different Fruit Tree Leaves

In this study, the nutrient contents of fruit tree leaves collected from the Niğde region were determined and are presented in *Table 1*.

Table 1. Nutrient content of leaves (%)

Different Fruit Tree Leaves	DM	CP	CA	EE	NDF	ADF	ADL	TANNIN
Grape	89.72^{c}	12.83^{b}	9.47^{a}	3.79^{c}	47.84^{a}	32.04^{c}	10.20^{c}	9.89^{b}
Mulberry	80.43^{e}	13.09^{a}	8.67^{b}	4.74^{ab}	42.93^{c}	29.81^{d}	8.89^{d}	7.89^{d}
Apple	91.13^{a}	11.25^{c}	5.70^{d}	5.03^{a}	47.85^{a}	36.52^{a}	12.47^{a}	10.99^{a}
Apricot	87.813^{d}	10.99^{d}	6.78^{c}	4.41^{b}	44.47^{b}	34.07^{b}	11.74^{b}	8.25^{c}
Cherry	90.32^{b}	10.33^{e}	6.91^{c}	3.39^{c}	47.21^{a}	35.48^{ab}	9.34^{d}	10.11^{b}
SEM	0.031	0.068	0.122	0.154	0.416	0.521	0.223	0.085
SIG	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

a-d: Differences between averages shown with different letters in the same column are significant ($P<0.05$),
DM: dry matter, CP: crude protein, CA: crude ash, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, SEM: standard error of mean, SIG: significance level

Among the fruit tree leaves, apple leaves exhibited the highest dry matter (DM) content at 91.13%, while mulberry leaves had the lowest DM content at 80.43%. The crude protein (CP) ratio was highest in mulberry leaves at 13.09% and lowest in cherry leaves at 10.33%. Apple leaves had the highest ether extract (EE) content at 5.03%, while cherry and grape leaves had the lowest at 3.39% and 3.79%. The highest tannin content was observed in apple leaves at 10.99%, whereas mulberry leaves had the lowest at 7.89%. Furthermore, the crude ash (CA) ratio ranged from 5.70 to 9.47, the neutral detergent fiber (NDF) ratio ranged from 42.93 to 47.85, the acid detergent fiber (ADF) ratio ranged from 29.81 to 36.52, and the acid detergent lignin (ADL) ratio ranged from 8.89 to 12.47 among different fruit tree leaves. Grape leaves had the highest CA content (9.47%), while apple leaves had the lowest (5.70%). It is thought that raw ash contents may vary depending on the feed type, applied agricultural techniques and weather conditions. It has been stated that in cases where the raw ash rate is above 17%, the feed may be contaminated for any reason (Kılıç ve Sarıçiçek, 2006). Although apple leaves had the highest NDF ratio (47.85%), cherry and grape leaves showed similar values, which were statistically insignificant, and mulberry leaves had the lowest NDF ratio (42.93%). Apple leaves also exhibited the highest ADF ratio (36.52%), whereas mulberry leaves had the lowest ADF ratio (29.81%). The ADL content was highest in apple leaves (12.47%), followed by cherry leaves (9.34%), and mulberry leaves had the lowest ADL ratio (8.89%).

The findings of this study revealed that apple tree leaves had the highest DM content at 91.13%. Similarly, Belewu and Alokomaro (2007) reported a rate of 93.65%, and Sheikh et al. (2011) found it to be 88.67%. In contrast, Kazemi and Mokhtarpour (2021) reported a DM rate of 41.73% for apple leaves, and Gourlie (2016) found it to be 9.6%. In our study, mulberry leaves had the lowest DM content (80.43%), while Kandylis et al. (2009) reported 89%, and Kamalak et al. (2004) found 93.17% for mulberry leaves. However, Güven (2012) reported a range of 42-46%, Olfaz et al. (2018) reported 31.17%, and Yao et al. (2000) reported 20.9-21.1% for the DM content of mulberry leaves. Malgaz and Atalay (2022) determined the DM values as 43.43%, 29.67, 38.07, 26.79 and 28.38% in peach, plum, cherry, apricot and apple trees, respectively, which are lower than the values obtained in our study. Özdemir and Kaya (2020) found DM values between 79.21 and 95.40% in their study with different tree leaves. The dry matter values obtained from this study are in similar proportions. It is thought that the reason for these differences in dry matter content is directly affected by factors such as tree type, shedding time, climate and rainfall in the leaf samples collected.

Mulberry leaves exhibited the highest CP content at 13.09%. Similarly, Olfaz et al. (2018) reported 13.68%, Kamalak et al. (2004) reported 14%, and Hassan et al. (2020) reported 14-34.2% for mulberry leaves. Güven (2012) reported a range of 16-18%, Neto et al. (2018) reported 17% CP content in mulberry leaves, and Kaya (2019) reported 8.5%. On the other hand, Kandylis et al. (2009) found 20.1%, Cheema et al. (2011) found 23%, and Habib et al. (2016) reported 24% CP content in mulberry leaves. In our study, cherry tree leaves had the lowest CP content at 10.33%. Similarly, Emile et al. (2018) reported 12.6%, and Kazemi and Bezdi (2022) reported 12.8% for cherry leaves. In contrast, Nahand et al. (2012) found the CP content of cherry tree leaves to be 2.76%. Liu et al. (2021) reported that mulberry leaves are consumed with pleasure by dairy breed heifers because they contain approximately 20% protein in the dry matter, and therefore have a higher milk production potential. In the study, the lowest ADF content was found in mulberry leaves. Therefore, it seems that animal nutritional value is more important than others. Fibrous feeds with an NDF content of less than 45% (DM) are in the daily roughage class, and therefore, the tree leaves examined in this study can be considered high-quality roughage sources. A diet rich in NDF can reduce dry matter intake (DM). However, tree leaves with low NDF content in the rumen break down, take in, and digest the feed as quickly as grass. While the crude protein (CP) in these tree leaves satisfies certain ruminant needs, it falls short during production periods. Therefore, animal diets should be supplemented with an additional protein source (Yavuz and Öztürk, 2023). The variations in outcomes are likely attributed to the presence of different tree species within the same genus, each with distinct harvest times and chemical compositions. Additionally, the diversity in chemical composition among tree leaves may stem from variations in geographical distribution, climate, and plant species maturity levels (Kılıç, 2010). Consistent with prior research, these species offer promising alternative sources of fibrous feed for small ruminants due to their nutritive values. Furthermore, these species can be provided to ruminants as substitute feed during feed scarcity periods, with careful attention paid to the quantity supplied.

Grape leaves exhibited the highest CA content at 9.47%. Similarly, Gürbüz (2007) reported 11-12%, and Kaya (2019) reported 13% CA content in grape leaves. In contrast, Denek et al. (2014) found the CA rate to be 18.14% in grape leaves. In our study, apple tree leaves had a CA content of 5.70%, and Sheikh et al. (2011) reported 7.50% for apple leaves. However, Nahand et al. (2011) reported an 8.60% CA content for apple leaves.

The maximum EE content in fruit tree leaves was found to be 5.03% in apple leaves. Similarly, Belewu and Alokomaro (2007) reported a 3.9% EE content. The NDF content in fruit tree leaves was found to be 47.85% in apple leaves, and Sheikh et al. (2011) reported 49.76%. However, Nahand et al. (2011) found 23%, Kazemi and Mokhtarpour (2021) found 36.52%, and Belewu and Alokomaro (2007) reported that 70.69% of apple tree leaves had NDF content. The lowest NDF content was found in mulberry tree leaves at a rate of 42.93%. Similarly, Kamalak et al. (2004) reported 42.33%, Kaya (2019) reported 41.46%, and Yao et al. (2000) reported 38.8-41.1% NDF content in mulberry leaves. Ayaşan and Baylan (2016) reported a 33-46 range%, and Olfaz et al. (2018) found 47.81% NDF content in mulberry leaves. However, Güven (2012) reported 19-22%, Habib et al. (2016) reported 26.4%, Kandylis et al. (2009) reported 26.8%, and Neto et al. (2018) reported 34% NDF content in mulberry leaves.

The highest ADF content was found in apple leaves at 36.52%. In contrast, Nahand et al. (2011) reported 15.4%, Kazemi and Mokhtarpour (2021) reported 18.65%, and Sheikh et al. (2011) reported 57.87% ADF content in apple tree leaves. In our study, the lowest ADF content was 29.81% in mulberry tree leaves. Similarly, Ayaşan and Baylan (2016) reported 28-35%, Neto et al. (2018) reported 26.06%, and Olfaz et al. (2018) reported 25.9% ADF content in mulberry leaves. Kamalak et al. (2004) found the ADF content to be 25.35% in mulberry leaves. However, Kandylis et al. (2009) reported 14.8%, Habib et al. (2016) reported 17.4%, Güven (2012) reported 17-19%, and Kaya (2019) reported 22.91% ADF content in mulberry leaves.

The highest rate of ADL in fruit tree leaves was found in apple leaves at 12.47%. In contrast, Kazemi and Mokhtarpour (2021) reported ADL content (6.75%) in apple leaves. The tannin content in fruit tree leaves was 10.99% in apple leaves. In contrast, Kazemi and Mokhtarpour (2021) reported tannin content as 0.32%. The tannin content for other tree leaves was as follows: cherry (10.11%), grape (9.89%), apricot (8.25%), and mulberry (7.89%). Denek et al. (2014) reported a tannin ratio of 17.40% for grape leaves. In our study, the lowest tannin content was found in mulberry tree leaves (7.89%). On the other hand, Güven (2012) reported 0.74%-0.76%, Olfaz et al. (2018) reported 1.06%, and Kamalak et al. (2004) reported 1.42% tannin content in mulberry leaves.

3.2. In Vitro Gas Production of Different Fruit Tree Leaves

Figure 1 and *Table 2* demonstrate that *in vitro* gas production increases over time. The results indicate that mulberry leaves exhibited the highest *in vitro* gas production at 3, 6, 12, 24, 48, 72, and 96 hours.

The *in vitro* gas production of various fruit tree leaves differed from one another. Mulberry leaves had the highest gas production at 3, 6, 24, 48, 72, and 96 hours, while apple leaves had the lowest. Mulberry leaves also exhibited the highest gas production at the 12th hour, whereas grape leaves had the lowest. These findings align with the positive correlation between gas production and crude protein (CP) reported by Canbolat et al. (2013), confirming our current research.

In our study, the 24-hour gas production value of mulberry leaves was 50.13 mL/200 mg DM. Kaya (2019) reported a value of 67.25 mL, and Ngamsaeng et al. (2006) reported 63.2 mL/g DM. The 24-hour gas production value for apricot leaves was 48.37 mL/200 mg DM, while Kaya (2019) determined it to be 91 mL. Regarding grape leaves, our study found a 24-hour gas production value of 47.20 mL/200 mg DM, while Kaya (2019) reported a value of 77.50 mL. For apple leaves, the gas production values for 12, 24, 48, 72, and 96 hours were 29.50, 44.80, 48.00, 53.67, and 55.33 mL/200 mg DM, respectively. Similarly, Kazemi and Mokhtarpour (2021) reported 33.70, 46.16, 51.93, 56.97 and 57.50 mL values.

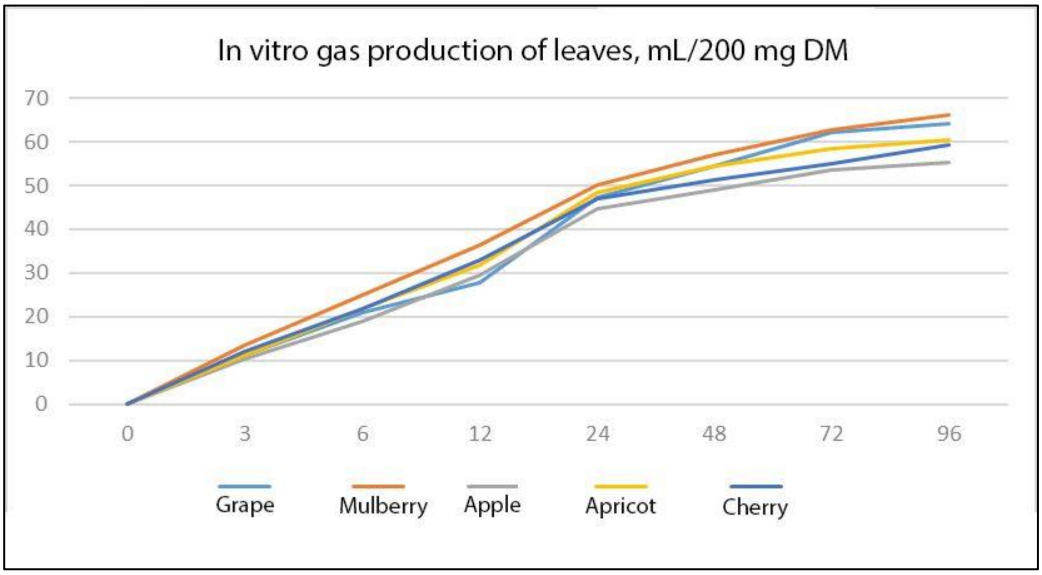

Figure 1. In vitro gas production of leaves, mL/200 mg DM

Table 2. In vitro gas production of leaves, mL/200 mg DM (Hour)

Different Fruit Tree Leaves	3	6	12	24	48	72	96
Grape	11.10^{bc}	20.83^{b}	27.93^{d}	47.20^{b}	54.37^{b}	62.13^{a}	64.10^{b}
Mulberry	13.50^{a}	25.00^{a}	36.33^{a}	50.13^{a}	57.00^{a}	62.83^{a}	66.13^{a}
Apple	10.33^{c}	18.83^{c}	29.50^{c}	44.83^{c}	49.00^{d}	53.67^{c}	55.33^{d}
Apricot	11.27^{bc}	21.67^{b}	31.70^{b}	48.37^{b}	54.50^{b}	58.37^{b}	60.57^{c}
Cherry	11.93^{b}	21.87^{b}	32.83^{b}	47.13^{b}	51.37^{c}	55.00^{c}	59.27^{c}
SEM	0.296	0.476	0.416	0.476	0.426	0.456	0.575
SIG	0.000	0.000	0.000	0.000	0.000	0.000	0.000

a-d: Differences between averages shown with different letters in the same column are significant (P<0.05),
SE: Standard Error of Mean, Sig: Significance Level

This study's findings of individual leaves supported the idea that non-nutritional factors such as tannins may also contribute to reduced gas production *in vitro*. Kamalak et al. (2004) reported that condensed tannins in tree leaves can be found in bound and soluble form. Karabulut et al. (2007) noted a negative correlation between tannin content and 24-hour gas production and OMD and ME. Accordingly, mulberry, with the highest tannin content, was expected to have the lowest *in vitro* gas production. The fact that the *in vitro* gas production value determined in mulberry, which has the highest tannin content in the leaves up to 24 hours, was lower than the *in vitro* gas production values determined in apple, apricot, grape, and cherry, was thought to be related to the release of tannin in the rumen environment. The low *in vitro* gas production value of mulberry up to 24 hours of incubation may be due to the high release of tannin-containing mulberry into the rumen during the first 24 hours. This is because the amount of tannin remaining in the mulberry structure decreases after 24 hours of incubation, and the amount of tannin released into the rumen is low, increasing in the *in vitro* gas production value.

Post-incubation pH values indicate whether the buffer solution is sufficient for normal rumen conditions. Therefore, it can be said that the buffer solution was not completely consumed by the microorganisms until the end of the incubation period and the amount of buffer did not affect the results of this study.

3.3. Metabolic Energy, Organic Matter Digestion, and Relative Feed Value of Different Fruit Tree Leaves

Table 3 presents various fruit tree leaf metabolic energy, organic matter digestion, and relative feed value properties.

Table 3. Metabolic energy, organic matter digestion, relative feed value characteristics

Different Fruit Tree Leaves	ME (MJ/kg)	OMD (%)	DDM (%)	DMI (%)	RFV (%)
Grape	9.24^{d}	56.49^{c}	63.94^{b}	2.51^{c}	124.33^{c}
Mulberry	10.69^{a}	58.98^{a}	65.68^{a}	2.80^{a}	142.36^{a}
Apple	10.07^{c}	54.46^{d}	60.46^{d}	2.51^{c}	117.56^{d}
Apricot	10.24^{b}	57.34^{bc}	62.36^{c}	2.70^{b}	130.47^{b}
Cherry	9.33^{d}	58.31^{ab}	61.26^{cd}	2.54^{c}	120.72^{cd}
SEM	0.050	0.377	0.407	0.025	1.694
SIG	0.000	0.000	0.000	0.000	0.000

a-d: Differences between averages shown with different letters in the same column are significant ($P<0.05$).
SEM: Standard error of Mean. SIG: Significance Level, ME: Metabolic energy, OMD: Organic matter digestibility, DDM: Digestible dry matter, DMI: Dry matter intake, RFV: Relative feed values

In our study, mulberry leaves exhibited the highest metabolic energy (ME) value at 10.69 MJ/kg. Similarly, Cheema et al. (2011) reported a value of 10.5 MJ/kg. In contrast, Kaya (2019) reported 7.60 MJ/kg, Ayaşan and Baylan (2016) reported 4.73-9.38 MJ/kg, Hassan et al. (2020) reported 4.73-9.38 MJ/kg, and Güven (2012) reported 9.77 MJ/kg. This pattern followed for apricot, apple, cherry, and grape leaves, with the ME value of grape leaves being 9.24 MJ/kg, while Kaya (2019) reported 8.33 MJ/kg.

The highest organic matter digestibility (OMD) was found in mulberry leaves at 58.98%. Cheema et al. (2011) reported a value of 94%, while Güven (2012) found 66.83-71.28% values for two different mulberry varieties. The OMD values for cherry, apricot, grape, and apple leaves were 58.31%, 57.34%, 56.49%, and 54.46%, respectively.

Regarding digestible dry matter (DDM), mulberry leaves exhibited the highest value at 65.68%. Similarly, Kaya (2019) reported a value of 70.25%. In contrast, Hassan et al. (2020) reported values of 75-85%, Habib et al. (2016) reported 84.6%, and Cheema et al. (2011) reported 90.2%. In our study, grape, apricot, cherry, and apple leaves followed mulberry leaves in terms of DDM. The DDM value for apple leaves was 60.46%. However, Karan and Başbağ (2022) reported a value of 71.24% in their study.

Regarding dry matter intake (DMI), mulberry leaves had the highest value at 2.80%. Apricot, cherry, grape, and apple leaves followed with values of 2.70, 2.54, 2.51, and 2.51%, respectively. In contrast, Karan and Başbağ (2022) reported DMI values of 3.95% for grape leaves, 4.97% for apple leaves, and 6.25% for cherry leaves in their study.

The relative feed value (RFV) was highest for mulberry leaves at 142.36. Apricot, grape, cherry, and apple leaves followed with values of 130.47, 124.33, 120.72, and 117.56, respectively. However, Karan and Başbağ (2022) reported RFV values of 274.29 for apple leaves, 207.91 for grape leaves, and 372.39 for cherry leaves in their study.

The higher nutritional value of mulberry leaves can explain why they exhibit higher values in terms of metabolic energy, organic matter digestion, and relative feed value than other groups.

The use of fruit tree leaves in animal feeding is among the issues that attract attention today. It is estimated that as such feeds become widespread in animal nutrition globally, they can be used as alternative feed raw materials for ruminant animals. In the literature, the nutrient composition, gas production, methane production, degree of digestion, and ME values of different tree leaves vary depending on the plant type and period. It should be considered that enteric methane production from ruminants will also be reduced if tree leaves are given together with low-quality roughage. Factors to be regarded in animal feeding generally include the tree's capacity to renew its leaves, plant type, growing period, nutritional value, and the amount of tannin it contains. Feeds obtained from fruit trees may be recommended in the nutrition of ruminant animals, compared to low-quality grasses and grasses. When feeding tree leaves to animals, they should not be considered a source of roughage alone, and it is recommended to give them together with other roughage and not to exceed a specific rate.

3.4. Essential Fatty Acids in Rumen Fluid after In Vitro Gas Production of Different Fruit Tree Leaves

Table 4 presents the volatile fatty acid (VFA) concentrations in rumen fluid after *in vitro* gas production of different fruit tree leaves in mmol/L, while *Table 5* provides the same information as percentages.

Table 4. Rumen fluid volatile fatty acids after in vitro gas production of leaves (mmol/L)

Different fruit tree leaves	**TVFA**	**AA**	**PA**	**IBTA**	**BA**	**IVA**	**VA**	**AA/PA**
Grape	69.16^{b}	44.90^{c}	13.07^{bc}	0.87^{a}	7.46^{ab}	1.50^{ab}	1.36^{ab}	3.45^{b}
Mulberry	76.25^{a}	48.52^{a}	14.91^{a}	0.93^{a}	9.18^{a}	1.43^{ab}	1.28^{b}	3.26^{b}
Apple	66.04^{c}	41.93^{d}	13.21^{b}	1.09^{a}	7.70^{ab}	0.96^{b}	1.14^{b}	3.18^{b}
Apricot	68.63^{b}	46.82^{b}	12.18^{cd}	0.42^{b}	6.96^{b}	0.58^{c}	1.66^{a}	3.84^{a}
Cherry	65.24^{c}	43.62^{c}	11.66^{d}	0.98^{a}	7.21^{ab}	0.51^{c}	1.27^{b}	3.74^{a}
SEM	0.454	0.442	0.28	0.125	0.626	0.12	0.101	0.092
SIG	0.000	0.000	0.000	0.031	0.18	0.000	0.044	0.002

a-d: Differences between averages shown with different letters in the same column are significant ($P<0.05$),
SEM: Standard error of mean, SIG: Significance level, TVFA: Total volatile fatty acid, AA: Acetic acid, PA: Propionic acid, IBTA: Isobutyric acid, BA: Butyric acid, VA: Valeric acid, IVA: Isovaleric acid, AA/PA: Acetic acid/propionic acid

The rumen fluid displayed the following ranges of VFA content: total volatile fatty acids (TVFA) between 65.24-76.25, acetic acid (AA) between 41.93-48.52, propionic acid (PA) between 11.66-14.91, isobutyric acid (IBT) between 0.42-1.09, butyric acid (BA) between 6.96-9.18, isovaleric acid (IVA) between 0.51-1.50, and valeric acid (VA) between 1.14-1.66. Analyzing the VFA concentrations in rumen fluid after *in vitro* gas production of the leaves, it was observed that mulberry leaves exhibited the highest levels of TVFA, AA, PA, and BA content.

Table 5. The ratio of volatile fatty acids in rumen fluid after in vitro gas production of leaves (%)

Different Fruit Tree Leaves	**AAR**	**PAR**	**IBTAR**	**BAR**	**IVAR**	**VAR**
Grape	64.95^{bc}	18.89^{ab}	1.26^{a}	10.773	2.17^{a}	1.96^{b}
Mulberry	63.66^{c}	19.56^{a}	1.22^{a}	12.017	1.87^{ab}	1.67^{b}
Apple	63.50^{c}	20.01^{a}	1.65^{a}	11.66	1.46^{b}	1.73^{b}
Apricot	68.23^{a}	17.75^{b}	0.61^{b}	10.14	0.84^{c}	2.42^{a}
Cherry	66.85^{ab}	17.87^{b}	1.50^{a}	11.05	0.78^{c}	1.95^{b}
SE	0.855	0.37	0.182	0.79	0.164	0.136
SIG	0.011	0.005	0.021	0.512	0.000	0.022

a-d: Differences between averages shown with different letters in the same column are significant ($P<0.05$),
SEM: Standard error of Mean, SIG: Significance Level, AAR: Acetic acid ratio, PAR: Propionic acid ratio, IBTAR: Isobutyric acid ratio, BAR: Butyric acid ratio, IVAR: Isovaleric acid ratio, VAR: Valeric acid ratio

Regarding the VFA percentages in rumen fluid after *in vitro* gas production of leaves, the following ranges were determined: acetic acid ratio (AAR) between 63.50-68.23%, propionic acid ratio (PAR) between 17.75-20.01%, isobutyric acid ratio (IBTAR) between 0.61-1.50%, butyric acid ratio (BAR) between 10.14% and -

12.02%, isovaleric acid ratio (IVAR) between 0.78-2.17%, and valeric acid ratio (VAR) between 1.67%-2.42%. Apricot and cherry leaves exhibited the highest acetic acid content, while apple, mulberry, and grape leaves showed the highest propionic acid content. Apricot leaves had the lowest isobutyric acid ratio, while apricot leaves had the highest valeric acid ratio, and grape leaves had the highest isovaleric acid ratio. The butyric acid ratio was statistically insignificant across tree leaves.

Volatile fatty acids (VFAs) are formed through microbial fermentation of cellulose in the rumen, providing 70 % of the total metabolic energy in ruminants (Tekce and Gül, 2014). The concentration of VFA in the rumen is influenced by various factors, such as the roughage-to-concentrate ratio, which increases the acetic acid content with higher roughage ratios and the propionic acid content with higher concentrate ratios (Garipoğlu et al., 2015). Other factors affecting VFA concentration in the rumen include ration composition, rumen pH, animal species, measurement time, feed maturity period and harvest time, and applied feed treatments (Özel and Sarıçiçek, 2009). Reducing the diet's acetic acid/propionic acid ratio can decrease methane production and associated greenhouse gas emissions. However, a high acetic acid ratio in rumen microflora is often accompanied by a high propionic acid ratio, thus reducing the acetic acid/propionic acid ratio and resulting in decreased methane emissions (Kılıç and Boğa, 2021). The addition of essential oils to rumen fluid significantly reduces total volatile fatty acid, acetic acid, propionic acid, butyric acid, AA/PA ratio, and ammonia (NH3) levels while increasing rumen pH (Canbolat et al., 2013). Suppose the acetic acid/propionic acid ratio in the rumen is 0.5. In that case, no energy from the feed will be released as methane, whereas fermenting all carbohydrates in the feed to acetic acid in the rumen without producing propionic acid is estimated to result in 33% energy loss as methane (Baytok et al., 2015). In our study, the lowest AA/PA ratio was found in apple tree leaves (3.17%), followed by mulberry (3.25%), grape (3.44%), cherry (3.74%), and apricot (3.84%) tree leaves.

Table 6. Effects of leaves on carbon dioxide (CO_2) and methane (CH_4) gas production (mmol/L)

Different Fruit Tree Leaves	**CO_2**	**CH_4**
Grape	36.91^b	22.91^c
Mulberry	41.76^a	25.12^a
Apple	35.83^b	21.52^d
Apricot	36.89^b	23.84^b
Cherry	35.54^b	22.49^c
SEM	0.796	0.238
SIG	0.002	0.000

a-d: Differences between averages shown with different letters in the same column are significant ($P<0.05$), SE: Standard error of Mean,
SIG: Significance Level

CO_2 is formed as a result of VFA reacting with the buffer solution due to carbohydrate digestion in the feed. *In vitro* gas production is measured by the release of CO_2 gas (Malgaz and Atalay, 2022). Our study determined that mulberry leaves exhibited the highest CO_2 values among fruit tree leaves, with 41.76 mmol/L, while cherry leaves had the lowest at 35.54 mmol/L (Table 6). Similarly, Canbolat (2012b), in his study on cherry leaves, reported that CO2 gas production varied between 30.3 and 42.0 mmol/L, and methane gas production varied between 17.1 and 21.1 mmol/L. Kazemi and Mokhtarpour (2021) noted methane gas production from apple leaves at 18.17 mL, while Şimşek and Kamalak (2019) reported methane gas production from tree leaves ranging from 4.03 to 5.59 mL. Başer and Kamalak (2020) stated that methane production from the 24-hour incubation of trees varied between 5.78 and 10.47 mL. In our study, mulberry leaves exhibited the highest methane gas production at 25.12 mmol/L, while apple leaves had the lowest methane gas production at 21.51 mmol/L.

4. Conclusions

Our study determined that mulberry tree leaves have a higher relative feed value than apricot, grape, cherry, and apple leaves in terms of metabolic energy, digestible dry matter, and organic matter digestibility. Additionally, it was observed that the *in vitro* production of carbon dioxide and methane gases from fruit tree leaves was highest in mulberry leaves. The *in vitro* method was employed in the study to determine the nutrient content and digestibility of tree leaves in a shorter time and with more cost-effectiveness. The results suggest that fruit tree leaves can be used as a source of roughage, although further *in vivo* studies are recommended to support these findings.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

The idea for the original draft belongs to MB. FK and OE wrote the introduction, material, and methods sections, and MB and FK wrote the research findings and conclusion sections. FK collected data. MB and FK constructed the research area section in the study together. MB and OE reviewed, language editing and edited the manuscript. The authors read and approved the final manuscript.

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