

# Journal of the Hellenic Veterinary Medical Society

Vol 75, No 4 (2024)



## Usability of Gazelle Form Beech Tree (*Fagus Orientalis* L.) leaves as an alternative roughage source in dairy cattle: using in vitro gas production method

A (Adem) Kaya , A (Ali) Kaya , H Kaya , M Macit , V Palangi

doi: [10.12681/jhvms.36990](https://doi.org/10.12681/jhvms.36990)

Copyright © 2025, A (Adem) Kaya , A (Ali) Kaya , H Kaya , M Macit , V Palangi



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

### To cite this article:

Kaya , A. (Adem), Kaya , A. (Ali), Kaya , H., Macit , M., & Palangi, V. (2025). Usability of Gazelle Form Beech Tree (*Fagus Orientalis* L.) leaves as an alternative roughage source in dairy cattle: using in vitro gas production method. *Journal of the Hellenic Veterinary Medical Society*, 75(4), 8325–8334. <https://doi.org/10.12681/jhvms.36990>

## Usability of Gazelle Form Beech Tree (*Fagus Orientalis* L.) leaves as an alternative roughage source in dairy cattle: using *in vitro* gas production method

A. Kaya (Adem)<sup>1</sup>, A. Kaya (Ali)<sup>1</sup>, H. Kaya<sup>1</sup>, M. Macit<sup>1</sup>, V. Palangi<sup>2,\*</sup>

<sup>1</sup>Department of Animal Science, Agricultural Faculty, Ataturk University, 25240 Erzurum, Türkiye

<sup>2</sup>Visiting Researcher at Department of Life Sciences, Western Caspian University, Baku, Azerbaijan

**ABSTRACT** :An evaluation was conducted to determine whether beech tree leaves can be used as an alternative forage source for dairy cattle in TMR rations and their anti-methanogenic properties. The TMR rations were altered by substituting 0, 25, 50, 75, and 100% of gazelle from beech leaf for dry meadow grass. As a result, significant differences were found between experimental groups in terms of estimated parameters, total gas, dry matter, organic matter, NDF, ADF, protein digestion values, TUFA, acetic, propionic, and butyric acid values ( $P<0.05$ ). As a result of substituting beech leaves for the entire dry meadow grass, *in vitro* TDMD, MPP, MPSE, and TDD values improved and decreased propionic and butyric acid levels. Using beech leaves as a roughage source reduced *in vitro* methane production numerically. Therefore, it was concluded that tree leaves in the form of gazelle can be used as a source of roughage in dairy cattle TMR instead of the whole dry meadow grass, and further studies are needed to clarify this issue.

**Keywords:** Beech leaf; alternative roughage; *in vitro* gas production; methane; digestibility; volatile fatty acids; microbial protein

Abbreviations: DM: Dry matter; OM: organic matter; CP: crude protein; EE: ether extract; CA: crude ash; TUFA: total volatile fatty acids; OMD: organic matter digestibility; ME: metabolic energy; NEL: net energy lactation; MPP: microbial protein production; TDMD: true dry matter digestion; MPSE: microbial protein synthesis efficiency; TDD: true digestion degree; NDF: neutral detergent fiber; ADF: acid detergent fiber; and ADL: acid detergent lignin

*Corresponding Author:*

Valiollah Palangi, Visiting Researcher at Department of Life Sciences, Western Caspian University, Baku, Azerbaijan;  
E-mail address: valiollah.palangi@yahoo.com

*Date of initial submission: 19-2-2024*  
*Date of acceptance: 6-6-2024*

## INTRODUCTION

Human population growth, climate change, and droughts have resulted in a decrease in the area of land used to grow forage crops for ruminant animals (Yavuz et al., 2020). The forage crops, meadows, and pastures produced do not adequately satisfy ruminant animals' nutritional requirements. As a first step towards achieving sustainable animal production, quality forage must be provided at a lower price (Bıçakçı and Açıkbaş, 2018). Nevertheless, finding alternative food sources has become necessary due to competition between some feed sources and human food (Eslampeivand et al., 2022). Also, semi-arid and arid regions produce the least feed in the summer season because of climate and ecological conditions, and the increasing greenhouse gases in recent years have contributed to global warming and climate change, resulting in an increase in drought periods and areas (Mlambo and Mapiye, 2015). This situation causes livestock grazing restrictions. As trees and shrubs have deep root systems, they can maintain the nutritional quality of their leaves during these periods of drought. In many places where grazing is problematic, the leaves of trees and shrubs play a vital role in meeting the vital activities and nutrients required for the productivity of ruminant animals, especially small ruminants, and are very important for feeding ruminant animals throughout the year (Yusuf et al., 2023; Papachristou and Nastis, 1996; Paterson et al., 1998). In general, tree and shrub leaves are not considered as feed that can completely replace roughage used in ruminant animals' nutrition. This is due to their lower methane production and nutritional value to than normal meadows and pastures. In addition, tree and shrub leaves are used to feed ruminant animals in places and times where pasture is inadequate. Some recent studies have shown that the use of tree and shrub leaves in ruminant ration improves feed consumption, degree of digestion, and rumen fermentation (Raghuvansi et al., 2007), reduces enteric methane production (Soliva et al., 2008), and stabilizes the ecosystem and provides fodder for animals in dry periods and salty soils (El Shaer, 2020). Bhatta et al. (2002) emphasized that tree leaves are less costly than other feeds because they can be used throughout all four seasons, and that they play a crucial role in animal production continuity. Using the nutrient composition of beech tree leaves as a basis for their study, Özdemir and Kaya (2020) found that crude protein, ether extract, NDF, and ADF, and condensed tannin, respectively, were 6.25, 3.85, 38.22, 24.99, and 1.38%. It is reported that

tree leaves reduce methane emissions in ruminants due to their higher nutritional content than some forages (Özkan et al., 2020). Deuri et al. (2020) found that *Bauhinia* leaves supplementation significantly reduced enteric methane production. A study published in 2020 found that sainfoin (*Onobrychis viciifolia* Scop) and/or hazelnut (*Corylus avellana* L.) reduce rumen fermentation, methane production, and protein degradation in ruminants (Niderkorn et al., 2020). The minerals in tree leaves are superior to those in grasses (e.g. hay), which is why tree and shrub leaves are important sources of minerals for animals and rumen microbes (Leng, 1997).

The secondary compounds present in tree and shrub leaves that have antinutritional properties limit their use in ruminant feeding. Several studies have reported that secondary compounds found in tree and shrub leaves have antinutritional effects (Seidavi et al., 2020; Ku-Vera et al., 2020). However, it has been reported that the use of tree and shrub leaves or secondary compounds in appropriate doses in ruminant rations provides benefits similar to those obtained from commercial feed additives. For example, it has been reported that adding tree and shrub leaves containing secondary compounds such as tannins, saponins, flavonoids and essential oils, alone or mixed, to ruminant rations reduces methane production and energy losses, prevents excessive breakdown of proteins, increases microbial protein synthesis and prevents excessive gas formation in the rumen (Palangi and Lackner, 2022; Kamra et al., 2006; Wina et al., 2005).

In Turkey, beech trees make up 11.3% of the forest population, but there is little literature on their nutrient composition. As part of this study, which aimed at reducing the roughage deficit of ruminants and determining whether these tree leaves were usable as an alternative feed to roughages used in ruminant rations as well as their anti-methanogenic properties, we examined the chemical composition, *in vitro* production of total gas (GP) and methane (M) and volatile fatty acids (VFA) of gazelle-shaped beech tree leaves.

## MATERIALS AND METHODS

No animals were involved in this study, nor were they used for experimentation or tissue collection. Therefore, the ethics committee's approval was not required.

## MATERIALS

Atatürk University Food and Livestock Research

and Application Center provided the feed raw materials (dry meadow grass, alfalfa hay, corn silage, and commercial concentrated feed) for the study. The leaves of gazelle from beech trees (*Fagus Orientalis L.*) were collected between September and October from at least 10 trees that are adapted to growing in the same region within Erzurum province's central district. The rumen fluid used in the in vitro gas production technique was taken from the rumen of three animals (7-year-old female Brown Swiss) that had completed their rumen development and were slaughtered in the Erzurum Meat and Milk Institution slaughterhouse, according to the method reported by Palangi et al. (2022) and transported to the laboratory.

## METHODS

After the feed raw materials and beech tree leaves used in dairy cattle TMR were dried, they were

ground to pass through a 1 mm sieve and used in the analyses. Dry matter, organic matter, crude protein, ether extract, crude ash, and nitrogen-free extract analyses of the dried and ground feed samples and the prepared trial group TMR are determined according to the Weende analysis system. Crude cellulose is analyzed according to the method reported by AOAC (1990), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) contents, which constitute the cell wall components of the feeds, according to the method reported by Van Soest. et al. (1991) using an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp. Fairport, NY, USA). Condensed tannin analysis was done with the Butanol-HCl method (Canbolat, 2019). The chemical composition of the feed raw materials used in dairy cattle TMR was determined and beech tree leaves in gazelle form were included in the total mixed rations

**Table 1.** Chemical composition of beech tree leaves and feed raw materials used in dairy cattle TMR (% in DM)

Chemical composition (%)	Alfalfa hay	Dry meadow grass	Factory feed	Corn silage	Beech tree leaves
DM	94.10	93.35	94.08	96.21	93.63
CA	9.28	6.76	6.79	7.43	19.29
CP	17.04	11.70	21.75	13.04	9.64
EE	2.21	1.83	4.98	3.36	6.63
NDF	62.07	72.29	46.39	61.83	76.37
ADF	38.89	40.30	17.67	33.21	33.17
ADL	10.17	15.22	11.80	11.43	13.88
CF	25.09	30.78	7.80	20.50	27.19
CT	-	-	-	-	1.19

DM: Dry matter; CA: Crude ash; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; CF: Crude fiber; CT: Condensed tannin

**Table 2.** Ingredients and chemical composition of the experimental ration

Ingrredient (g/kg)	KO	K25	K50	K75	K100
Alfalfa hay	22.6	94.8	245.4	359.1	410.6
Dry meadow grass	200	150	100	50	0
Corn silage	277.1	226.2	133.0	59.7	20
Factory feed (CP 21%)	500.3	479	421.6	381.2	369.4
<i>Fagus Orientalis</i>	0	50	100	150	200
Total	1000	1000	1000	1000	1000
Chemical composition (g/kg DM)					
DM	953.1	951.2	944.4	942.1	951.5
CA	69.6	77.3	87.1	95.9	102.1
CP	170.8	171.1	172.3	172.8	171.1
EE	38.1	39.3	39.2	39.8	40.9
NDF	557.4	563.4	577.6	586.8	584.4
ADF	267.6	271.7	287.0	296.5	294.4
ADL	122.4	120.9	118.6	116.7	114.1
CT	-	1.1	1.7	2.3	5.0

and were substituted instead of dry meadow grass at the rates of 0% (K0), 25% (K25), 50% (K50), 75% (K75), 100% (K100) and isocaloric and isonitrogenic trial groups were created (Table 1 and 2).

#### Application of *in vitro* gas production technique

Special glass syringes with a volume of 100 ml were used to determine the *in vitro* parameters of the prepared dairy cattle total mixed rations (TMR) with the gas production technique reported by Menke et al. (1979). To determine the *in vitro* gas production amounts (IVGPR) and other parameters of the prepared TMR groups, the samples (based on dry matter) weighing approximately 500 mg and ground to pass through a 1 mm sieve were placed in glass syringes in 4 parallel directions and was placed on them. 40 ml of buffered rumen fluid (1/2 rumen fluid/buffer solution) was prepared according to the method reported by Menke et al. (1979) was transferred. Along with the feed samples, blank (buffered rumen fluid only) and Hohenheim grass standards were also incubated in four replicates. Then, the glass syringes were incubated in a water bath at 39°C. The amount of gas produced in the tubes at the 24th hour of incubation was determined. The gas values obtained from the blind trial were subtracted from the gas measurements determined for the feed groups, the net total gas amounts obtained from the feed samples were calculated, and the correction coefficient according to the Hohenheim weed standard was applied to the net gas values formed in the feed at the end of 24 hours.

An infrared methane analyzer (Sensor Europa GmbH, Erkrath, Germany model) was used to determine methane production (Goel et al., 2008). After reading the total amount of gas obtained for 24 hours of fermentation in the *in vitro* gas production technique, the gas accumulated in the injectors was taken to the methane analyzer through a special pipe (via plastic injectors) and the methane ratio in the total gas was determined.

Organic matter digestion (OMD), metabolic energy (ME), and Net Energy Lactation (NEL) contents were calculated according to the formulas below by taking into account the *in vitro* gas production amount (GP) at the 24th hour, crude protein (CP, g/kg DM), crude ash (CA, g/kg DM) and ether extract (EE, g/kg DM) contents of samples (Menke and Steingass, 1988).

OMD, %:  $14.88 + 0.8893 \cdot GP + 0.448 \cdot CP + 0.651 \cdot CA$

ME, (MJ/kg KM):  $0.72 + 0.1559 \cdot GP + 0.0068 \cdot CP + 0.0249 \cdot EE$

NEL, (MJ/kg KM):  $-0.61 + .1138 \cdot GP + 0.0046 \cdot CP + 0.015 \cdot EE$

#### Determination of ammonia nitrogen (NH<sub>3</sub>), pH and volatile fatty acids

After measuring the pH of the rumen fluid remaining in the syringes at the end of the *in vitro* gas production experiment, NH<sub>3</sub> contents were determined according to Markham's method (Markham, 1942). Total volatile fatty acids (TUFA) as well as individual acetic, propionic, and butyric acids were analyzed by Wiedmeier et al. (1987). It was determined in a gas chromatography device (Agilent Technologies 6890N gas chromatography, Stabilwax-DA, 30 m, 0.25 mm ID, 0.25 μm df. Max. temp: 260 °C. Cat. 11023) according to Wiedmeier et al. (1987) method.

#### Determination of *in vitro* digestant and microbial protein production (MPU)

At the end of the incubation, the total mixed rations of the trial group; The true digested matter amount (TDMA), partition factor (PF), microbial protein production (MP), microbial protein synthesis efficiency (MPSE), and true digestion degree (TDD) values were determined according to the method reported by Blummel et al. (1997). The rumen fluid and feed residue remaining in a 100 ml glass syringe were placed in beakers with a volume of 250 ml, 75 ml of the prepared NDF solution was added and boiled on a hot plate for 1 hour. After 1 hour, water was filtered through the thrombus in gooch crucibles with a porosity of 1 made of tempered glass material. The crucibles were kept in the oven at 750C for 3-4 hours and the weight was determined (Blummel et al., 1997).

TDMA = Incubated dry matter (mg) - Remaining dry matter (mg)

PF = (TDMA / GP)

MP = (TDMA - (2.2 x GP))

MPSE = (MP/TDMA) x 100

TDD= (TDMA/ Incubated dry matter (mg)) x 100

#### Determination of *in vitro* true dry matter, NDF, ADF, protein and organic matter digestibility values

Ankom Daisy II incubator D 220 device was used



to determine the in vitro true nutrient digestibility of the experimental groups by the filter bag method (Van Soest et al., 1991). 0.5 g of feed samples were weighed into tared Ancom F57 bags and incubated for 48 hours in a Daisy II incubator, 4 parallels. After 48 hours, all the bags were removed from the glass jars. They were kept under tap water until clear water flowed, and dried in an oven at 105 °C until they reached a constant weight. After weighing the bags taken out of the oven, in vitro dry matter digestibility (IVDMD) was calculated by applying the following formula. In the bags incubated for 48 hours, NDF and ADF contents were assessed on the ANKOM 2000 Fiber Analyzer device. This was done according to the method reported by Van Soest et al. (1991), and in vitro, true NDF (IVTNDFD) and ADF (IVTADFD) digestibility values were calculated. To determine the in vitro true organic matter digestibility (IVTOMD) values of the TMR groups, after incubation, the bags containing the samples were burned in a muffle furnace at 550°C for 3-4 hours. The weight of the samples delivered at the end of the incineration process was determined. The IVTOMD values were determined using the following formula.

$$\%IVTD=100 - (((D3-D1)/(D2-D1)*100)$$

$$\%IVTNDFD=100 - (((D4-D1)/(D2-D1)*100)$$

$$\%IVTADFD=100-(((D5-D1)/(D2-D1)*100)$$

$$\%IVTOMD=100 - (((D6-D2)/(D7-D8)*100)$$

Here; D1: Tare of F57 bags, D2: Dry weight of the ration content, D3: The amount of ration remaining at the end of incubation, D4: Ration content processed in NDF solution and dried in the oven in the Ankom 200/220 cellulose analyzer, D5: In ADF solution in the Ankom 200/220 cellulose analyzer. ration con-

tent processed and dried in the oven, D6: remaining amount after burning in the 550 °C ash oven, D7: DM content of the ration content, D8: % organic matter content of the ration content.

### Statistical analysis

The raw values obtained during the experiment were analyzed in the SPSS 17.0 (1996) package program. The differences between the groups' averages were determined with the Duncan multiple comparison test.

## RESULTS AND DISCUSSION

### *In vitro* gas and methane production and digestion parameters of the Experimental Groups

The in vitro method developed by Menke et al. (1979) can be used to assess the digestibility of alternative feeds or total mixed rations that can be fed to ruminants in a short period, as well as the final products of fermentation (NH<sub>3</sub>, VFA, CH<sub>4</sub>, etc.) and their estimated energy values. It is stated that it will be possible (Algan et al., 2018). An overview of the experimental groups' parameters for gas and methane production and in vitro digestion is shown in Table 3. At the end of the 24th hour of fermentation on in vitro conditions, the gas production values of the experimental group rations in the syringe at 0.5gr/kg DM feed were determined to be between 106.49 and 112.02 ml, and the differences between the groups were found to be significant (p<0.01). Gas production values of the trial group rations were higher than the values found by Boğa et al. (2020) and Kaya and Kaya (2021). Methane gas, which is defined as a by-product and is released as a result of the fermentation of carbohydrates in the rumen, has a greenhouse gas effect that is 23-25 times greater than CO<sub>2</sub> gas in the atmosphere (Steinfeld et al., 2006). In

**Table 3.** In vitro gas and methane production and digestion parameters of the experimental groups

Trial Groups	Gas Production (ml)	Methane Production (ml)	Methane Production (%)	TDMA (mg)	PF (mg/ml)	MP (mg)	MPSE (%)	TDD (%)
K0	112,02a	20,19	18,02	298,17c	2,66c	51,73c	17,35c	61,66c
K25	111,92a	19,65	17,56	297,10c	2,66c	50,87c	17,03c	61,77c
K50	107,77b	18,54	17,20	314,96b	2,92b	77,86b	24,72b	66,13b
K75	107,91b	17,95	16,65	311,79b	2,89b	74,39b	23,85b	65,97b
K100	106,49b	17,63	16,54	329,89a	3,10a	95,62a	28,96a	68,65a
SEM	2,64	1,56	1,19	13,60	0,01	18,70	5,05	2,98
P	0,001	0,202	0,577	0,000	0,000	0,000	0,000	0,000

a-c: means within the column with unlike superscript differ significantly (P<0.05). TDMA: true digested matter amount, PF: Partition factor, MP: Microbial protein production, MPSE: Microbial protein synthesis efficiency, TDD: true digestion degree, SEM: standard error of means

addition, 2-15% of the energy loss of the gross energy of the feed consumed by ruminants is due to methane gas (Meale et al., 2012). The amount of methane gas released in the trial group TKRs as a result of fermentation was determined to be between 17.63 and 20.19 ml, but the differences between the groups were found to be insignificant ( $p>0.05$ ). According to the classification determined by Lopez et al. (López et al., 2010) for the methane gas values formed in the feed consumed by ruminants, the feeds are low antimethanogenic ( $>11\%$  and  $\leq 14\%$ ), medium antimethanogenic ( $>6\%$  and  $<11\%$ ) and high anti-methanogenic ( $>0\%$  and  $<6\%$ ). They reported that classification could be made as 0 and  $<6\%$ ). They also stated that preparation of TMR by considering these classifications can increase the energy use efficiency in ruminants and reduce methane gas, which causes global warming. Enteric methane released during fermentation is not desired by both environmentalists and nutritionists because it causes both global warming and energy loss of feed. Methane rates on in vitro gas measured from the experimental group ration were found to be between 16.54-18.02% ( $p>0.05$ ). Although methane production detected in the K100 group (16.54%) was 8% less than in the control group (18.02%), this decrease was not statistically significant. Differences between gas, methane, and digestibility values of the trial group rations arise from the fermentation of rumen microorganisms, the amount of degradable substances contained in the feed, and secondary metabolites (Olomonchi et al., 2022). It is emphasized that when evaluating the feed to be given to ruminants, not only gas production values should be taken into account, but also the digestibility parameters should be taken into account and the ration should be prepared (Blummel et al., 1997). The true digested dry matter (TDDM) amounts and digestion degrees (TDD) of the trial group rations were between 297.10-329.89 mg, and 61.66-68.65%, respectively, and the differences between the groups were found to be signifi-

cant ( $p<0.05$ ). According to Blummel et al. (1997), feeding ratios should range from 2.75 to 4.41 and this is the factor that determines microbial protein synthesis efficiency. TF values (2.66-3.10) of the trial group rations were among the values reported by Blummel et al. (1997) and the differences between the groups were found to be significant ( $p < 0.05$ ). Jones and Mangan, (1997) stated that the presence of 10-40 g/kg DM in the rations of ruminant animals of condensed grain, called phenolic content, reduces its breakdown in the rumen by forming a compound with the protein contained in the feed and increases the bypass protein efficiency. Ruminant animals meet their protein needs from bypass protein and microbial protein. In this context, it is reported that the effectiveness of microbial protein production and synthesis should be taken into consideration when determining the value of feed used in the nutrition of ruminant animals (Van Soest, 1994). MP and MPSE values of the trial group rations were found between 50.87-95.62 mg, and 17.03-28.96%, respectively, and the highest MP and MPSE were detected in the K100 group ration and the lowest in the K25 group ration. Differences between groups were found to be significant ( $p<0.01$ ).

#### Metabolic energy, net energy and organic matter digestion degree of the trial groups

As shown in Table 4, the average values and variance analysis were performed on the metabolic energy, net energy lactation values, and organic matter digestion levels of the experimental groups that were fed gazelle-shaped beech leaves rather than dry meadow grass in dairy cattle TMRs.

Metabolic energy contents of the trial group rations varied between 7.58-7.92 MJ/kgDM, net energy lactation contents varied between 4.38-4.63 MJ/kgDM, and organic matter digestibility (OMD) ranged between 66.61-67.39%. Differences between groups in terms of metabolic energy and net energy lactation, excluding organic matter digestion, were significant

**Table 4.** Metabolic energy, net energy, and organic matter digestion degree of the trial group rations

Trial groups	ME( mj/kg DM)	NEL( mj/kg DM)	OMD (%)
K0	7.92 <sup>a</sup>	4.63 <sup>a</sup>	66.91
K25	7.91 <sup>a</sup>	4.62 <sup>a</sup>	67.39
K50	7.66 <sup>b</sup>	4.43 <sup>b</sup>	66.61
K75	7.67 <sup>b</sup>	4.44 <sup>b</sup>	67.25
K100	7.58 <sup>b</sup>	4.38 <sup>b</sup>	67.07
SEM	0.16	0.04	0.54
P	0.001	0.001	0.475

a-b: means within column with unlike superscript differ significantly ( $P<0.05$ ). SEM: standard error of means; ME: metabolizable energy, NEL: net energy lactation, OMD: organic matter digestion

( $p < 0.05$ ). The highest metabolic energy and net energy lactation values were observed in the K0 group. Metabolic and net energy lactation values decreased due to the increase in beech tree leaves in the ration. The lowest metabolic and net energy lactation values were observed in the K100 group, with the increase in beech tree leaves in the diet, NDF, ADF, and CA contents also increased. The fact that these values are high in the total mixed ration caused the amount of degradable substances to decrease and the ME and NEL values to decrease by limiting rumen microorganism fermentation.

#### Ammonia-nitrogen (NH<sub>3</sub>-N), pH and volatile fatty acid amounts of the trial groups

According to Table 5, ammonia-nitrogen, pH, total volatile fatty acids, and individual fatty acids detected in the TMRs of the experimental group at the end of *in vitro* incubation are averaged and variance analyzed. TMRs had pH values between 6.65 and 6.70 at the end of fermentation, and there was no significant difference between groups ( $p > 0.05$ ). For microorganisms to thrive in the rumen environment, the pH level needs to be between 5.5 and 7.0 (Özel and Sarıçiçek, 2009). The pH values of the rations for the

trial groups were determined between these values. We found significant differences between TMRs in terms of acetic acid (AA), propionic acid (PA), butyric acid (BA), and total volatile fatty acids (TVFA) ( $P < 0.01$ ). Based on the TMR trial groups, the highest TVFA values were observed in K0 (127.74 mmol/l) and the lowest in K75 (117.50 mmol/l). According to the results, acetic acid levels ranged from 85.82-94.35 mmol/L, propionic acid levels ranged from 14.36-19.75 mmol/L, and butyric acid levels ranged from 16.05-17.63 mmol/L. TVFA (mmol/l) determination showed that the highest PA and BA production occurred in the K25 group, while the highest AA production occurred in the K100 group. Among volatile fatty acids such as acetic, propionic, and butyric acid, the composition of the total mixed ration may affect their formation (Kılıç and Sarıçiçek, 2006).

#### *In vitro* true DM, NDF, ADF, Protein and OM digestibility values

Since the *in vivo* digestion technique is a labor-intensive, costly, and time-consuming technique, the Dasiy incubator technique was developed as an alternative method that can provide us with information about the real digestion values of feed raw materi-

**Table 5.** Average ammonia-nitrogen, pH, and volatile fatty acid contents of the experimental groups

Trial Groups	pH	NH <sub>3</sub> (mg/l)	TVFA (mmol/l)	AA (mmol/l)	PA (mmol/l)	BA (mmol/l)	AA (%)	PA (%)	BA (%)
K0	6.67	557.67	127.74 <sup>b</sup>	92.52 <sup>ab</sup>	17.69 <sup>b</sup>	17.29 <sup>b</sup>	72.43 <sup>bc</sup>	13.85 <sup>ab</sup>	13.54 <sup>b</sup>
K25	6.66	551.33	130.98 <sup>a</sup>	93.13 <sup>ab</sup>	19.75 <sup>a</sup>	17.63 <sup>a</sup>	71.10 <sup>c</sup>	15.08 <sup>a</sup>	13.46 <sup>b</sup>
K50	6.65	548.33	123.21 <sup>c</sup>	90.02 <sup>b</sup>	16.03 <sup>c</sup>	16.99 <sup>c</sup>	73.05 <sup>b</sup>	13.02 <sup>b</sup>	13.80 <sup>ab</sup>
K75	6.70	559.33	117.50 <sup>d</sup>	85.82 <sup>c</sup>	14.72 <sup>cd</sup>	16.68 <sup>d</sup>	73.02 <sup>b</sup>	12.53 <sup>bc</sup>	14.21 <sup>a</sup>
K100	6.70	563.33	125.06 <sup>bc</sup>	94.35 <sup>a</sup>	14.36 <sup>d</sup>	16.05 <sup>e</sup>	75.44 <sup>a</sup>	11.49 <sup>c</sup>	12.84 <sup>c</sup>
SEM	0.03	10.66	4.92	3.65	2.19	0.57	1.64	1.41	0.51
P	0.195	0.465	0.000	0.006	0.000	0.000	0.000	0.000	0.000

a-e: means within column with unlike superscript differ significantly ( $p < 0.05$ ). SEM: standard error of means;

AA: acetic acid; PA: propionic acid; BA: butyric acid; TVFA: total volatile fatty acids.

**Table 6.** True DM, NDF, ADF, Protein, and Organic matter digestion parameters of the experimental groups

Trial groups	IVTDMD (%)	IVTNDFD (%)	IVTADFD (%)	IVTPD (%)	IVOMD (%)
K0	44,84 <sup>b</sup>	63,46 <sup>c</sup>	78,21 <sup>b</sup>	40,38 <sup>a</sup>	92,79 <sup>c</sup>
K25	44,64 <sup>b</sup>	63,63 <sup>c</sup>	78,72 <sup>b</sup>	13,47 <sup>c</sup>	93,40 <sup>d</sup>
K50	47,72 <sup>a</sup>	68,01 <sup>b</sup>	80,25 <sup>a</sup>	20,08 <sup>b</sup>	94,25 <sup>c</sup>
K75	48,18 <sup>a</sup>	67,94 <sup>b</sup>	78,57 <sup>b</sup>	21,74 <sup>b</sup>	94,65 <sup>b</sup>
K100	46,97 <sup>a</sup>	70,17 <sup>a</sup>	80,57 <sup>a</sup>	21,71 <sup>b</sup>	95,16 <sup>a</sup>
SEM	1,71	2,89	1,17	9,54	0,89
P	0,002	0,000	0,008	0,000	0,000

a-e: means within the column with unlike superscript differ significantly ( $P < 0.01$ ). SEM: standard error of means; IVTDMD: True dry matter digestion, IVTNDFD: true neutral detergent fiber digestion, IVTADFD: true acid detergent fiber digestion, IVTPD: true protein digestion, IVTOMD: true organic matter digestion



als or TMRs in a short time (Tassone et al., 2020). According to Table 6, DM, NDF, ADF, protein, and organic matter digestion parameters were determined for each group. In vitro true dry matter (IVTDMD), NDF (IVTNDFD), ADF (IVTADFD), protein (IVTPD), and organic matter (IVOMD) digestion values of the trial TMRs varied between 44.64-48.18, 63.46-70.17, 78.21-80.57, 13.47-40.38 and 92.79-95.16% respectively. The differences between the groups in terms of the parameters in question were significant ( $p < 0.01$ ).

## CONCLUSIONS

Accordingly, the current study concluded beech tree leaves can be used as an alternative feed for ruminants in arid regions without adequate roughage in ruminant rations. The results obtained should be backed up by in vivo research, since such tree leaves contain phytochemicals, and should be included in the ration

according to their phytochemical content.

## ACKNOWLEDGMENT

The experiment was supported by Atatürk-BAP under the project number of FBA-2022-10422.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## FUNDING

This research was supported by the grant number FBA-2022-10422 from Atatürk University

## REFERENCES

- A.O.A.C. (1990). Official method of analysis. Association of Official Analytical Chemists pp. 66-88. 15th. edition Washington, DC. USA.
- Algan D, Aydın İ, Ocak N. (2018). Economic analysis of fertilization based on the nutritional value of rangeland: A new opinion. *Anadolu J. Agr. Sci.*, 33: 246-253.
- Bhatta, R., Shinde, A. K., Vaithyanathan, S., Sankhyan, S. K., Verma, D. L., (2002). Effect of polyethylene glycol-6000 on nutrient intake, digestion and growth of kids browsing *Prosopis cineraria*. *Animal Feed Science and Technology*, 101(1-4), 45-54.
- Bıçakçı, E., Açıkbaş, S., (2018). Determination of roughage production potential for farm animals in Bitlis province. *BEU Journal of Science*, 7(1): 180-185. <https://doi.org/10.17798/bitlisfen.364336>
- Blummel, M., Steingäß, H., Becker, K., (1997). The relationship between in vitro gas production, in vitro microbial biomass yield and 15N incorporation and its implications for the prediction of voluntary feed intake of roughages. *British Journal of Nutrition*, 77(6), 911-921.
- Boga, M., Kurt, O., Ozkan, C. O., Atalay, A. İ., Kamalak, A., (2020). Evaluation of some commercial dairy rations in terms of chemical composition, methane production, net energy and organic matter digestibility. *Progress in Nutrition*, 22(1), 199-203.
- Canbolat, Ö., (2019). Yemlerin Buldukları Yere Göre Örnek Alma ve Yem Analizleri. *Yem Analiz Yöntemleri ve Yem Değerlendirme*, 1. Bakı, Medyay Kitapevi. 2019.
- Deuri, P., Sood, N., Wadhwa, M., Bakshi, M. P. S., & Salem, A. Z. M. (2020). Screening of tree leaves for bioactive components and their impact on in vitro fermentability and methane production from total mixed ration. *Agroforestry Systems*, 94(4), 1455-1468.
- El Shaer, H. M. (2020). Potential use of halophytes and salt-tolerant forages as animal feed in the Arab Region: an overview. *Handbook of halophytes: from molecules to ecosystems towards biosaline agriculture*, 1-20.
- Eslampeivand, A., Taghizadeh, A., Safamehr, A., Palangi, V., Paya, H., Shirmohammadi, S., ... & Abachi, S. (2022). Nutritive value assessment of orange pulp ensiled with urea using gas production and nylon bag techniques. *Biomass Conversion and Biorefinery*, 1-9. <https://doi.org/10.1007/s13399-022-03053-4>
- Goel, G., Makkar H.P.S., Becker K., (2008). Effect of *Sesbania sesban* and *Carduus pycnocephalus* leaves and Fenugreek (*Trigonella foenum-graecum* L) seeds and their extract on partitioning of nutrients from roughage-and concentrate-based feeds to methane. *Anim Feed Sci Technol*, 147(1-3), 72-89.
- Jones, W. T., Mangan, J. L., (1997). Complexes of the condensed tannins of sainfoin with fraction 1 leaf protein and with sub maxillary mucoprotein and the reversal by polyethylene glycol and pH. *Journal of the Science Food and Agriculture*, 28(2), 126-136.
- Kamra, D. N., Agarwal, N., Chaudhary, L. C., (2006). "Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds" *International Congress Series*, 1293,156- 163.
- Kaya, A., Kaya, A., (2021). The effect of some vegetable oils added to dairy calf rations on in vitro feed value and enteric methane production. *Journal of Agricultural Production*, 2(1), 1-6.
- Kılıç, Ü., Sariçiçek, B.Z., (2006). In vitro gaz üretim tekniğinde sonuçları etkileyen faktörler. *Hayvansal Üretim*, 47(2).
- Ku-Vera, J. C., Jiménez-Ocampo, R., Valencia-Salazar, S. S., Montoya-Flores, M. D., Molina-Botero, I. C., Arango, J., ... & Solorio-Sánchez, F. J. (2020). Role of secondary plant metabolites on enteric methane mitigation in ruminants. *Frontiers in Veterinary Science*, 7, 584.
- Leng, R. A., (1997). Tree foliage in ruminant nutrition: FAO Animal Production and Health Papers 139. FAO, Rome, Italy ISBN 9251040869.
- López, S., Makkar, H. P., Soliva, C. R., (2010). Screening plants and plant products for methane inhibitors. In vitro screening of plant resources for extra-nutritional attributes in ruminants: nuclear and related methodologies (pp. 191-231). Springer, Dordrecht.
- Markham, R., (1942). A steam distillation apparatus suitable for micro-Kjeldahl analysis. *Biochemical Journal*, 36(10-12), 790.
- Meale, S.J., Chaves, A.V., Baah, J., McAllister, T.A., (2012). Methane production of different forages in in vitro ruminal fermentation. *Asian-Australasian Journal of animal sciences*, 25(1), p.86.
- Menke, K. H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., (1979). The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *The Journal of Agricultural Science*, 93(1), 217-222.
- Menke, K.H., Steingass, H., (1988). Estimation of the energetic feed value obtained from chemical analysis and in-vitro gas production using rumen fluid. *Animal Research and Development*, 28: 7-55.
- Mlambo, V., & Mapiye, C. (2015). Towards household food and nutrition security in semi-arid areas: What role for condensed tannin-rich ruminant feedstuffs?. *Food Research International*, 76, 953-961. <https://doi.org/10.1016/j.foodres.2015.04.011>
- Niderkorn, V., Barbier, E., Macheboeuf, D., Torrent, A., Mueller-Harvey, I., & Hoste, H. (2020). In vitro rumen fermentation of diets with different types of condensed tannins derived from sainfoin (*Onobrychis viciifolia* Scop.) pellets and hazelnut (*Corylus avellana* L.) pericarps. *Animal Feed Science and Technology*, 259, 114357.
- Olomonchi, E.A.O., Garipoğlu, A.V., Ocak, N., Kamalak, A., (2022). Nutritional values and in vitro fermentation parameters of some fodder species found in two rangeland areas in the Republic of Benin. *Turkish Journal of Veterinary & Animal Sciences*, 46(1), pp.88-94.
- Özdemir, Ö., Kaya, A. (2020). Determination of Feed Values of Some Tree Leaves by in vitro Gas Production Technique. *Yuzuncu Yıl University Journal of Agricultural Sciences*, 30(3), 454-461.
- Özel, O., Sariçiçek, B., (2009). Ruminantlarda rumen mikroorganizmalarının varlığı ve önemi (derleme). *Tübvav Bilim Dergisi*, 2(3), 277-285.
- Özkan, Ç. Ö., Cengiz, T., Yanık, M., Evlice, S., Selçuk, B., Ceren, B., Kamalak, A., (2020). Determination of In Vitro Gas Production, Methane Production, Digestibility and Microbial Protein Production of Some Forages and Concentrates Used in Ruminant Animal. *Black Sea Journal of Agriculture*, 3(1), 56-60.
- Palangi, V., & Lackner, M. (2022). Management of enteric methane emissions in ruminants using feed additives: A review. *Animals*, 12(24), 3452.
- Palangi, V., Macit, M., Nadaroglu, H., & Taghizadeh, A. (2022). Effects of green-synthesized CuO and ZnO nanoparticles on ruminal mitigation of methane emission to the enhancement of the cleaner environment. *Biomass Conversion and Biorefinery*, 1-9.
- Papachristou, T. G., Nastis, A. S., (1996). Influence of deciduous broad-leaved woody species in goat nutrition during the dry season in northern Greece. *Small Ruminant Research*, 20(1), 15-22.
- Paterson, R. T., Karanja, G. M., Nyaata, O. Z., Kariuki, I. W., Roothaert, R. L., (1998). A review of tree fodder production and utilization within smallholder agroforestry systems in Kenya. *Agroforestry systems*, 41(2), 181-199.
- Raghuvansi, S. K. S., Prasad, R., Mishra, A. S., Chaturvedi, O. H., Tripathi, M. K., Misra, A. K., Saraswat, B. L., Jakhmola, R. C., (2007). "Effect of inclusion of tree leaves in feed on nutrient utilization and rumen fermentation in sheep" *Bioresource Technology*, 98 (3): 511-517.
- Seidavi, A., Tavakoli, M., Rasouli, B., Corazzin, M., & Salem, A. Z. (2020). Application of some trees/shrubs in ruminant feeding: a review. *Agroforestry Systems*, 94, 1353-1364.
- Soliva, C. R., Zeleke, A. B., Clement, C., Hess, H. D., Fievez, V., Kreuzer, M., (2008). In vitro screening of various tropical foliages, seed, fruits and medicinal plants for low methane and high ammonia generating potentials in the rumen. *Animal Feed Science Technology*, 147, 53-71.
- SPSS, (1996). SPSS for windows release 10.0, SPSS Inc. Chicago.
- Steinfeld, H., Gerber, P., Wassenaar, T. D., Castel, V., Rosales, M., Rosales, M., de Haan, C., (2006). *Livestock's long shadow: environmental issues and options*. Food & Agriculture Org.

- Tassone, S., Fortina, R. and Peiretti, P.G., (2020). In Vitro techniques using the DaisyII incubator for the assessment of digestibility: A review. *Animals*, 10(5), p.775.
- Van Soest, P.J., (1994). *Nutritional Ecology of the Ruminant*, second ed. Cornell University Press, Ithaca NY, USA.
- Van Soest, P.J., Robertson J.B. and Lewis, A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci*, 74, 3583-3597.
- Wiedmeier, R.D., Arambell M.J., Walters J.L., (1987). Effect of orally administered pilocarpine on ruminal characteristics and nutrient digestibility in cattle. *J. Dairy Sci.*, 70: 284-289.
- Wina, E., Muetzel, S., Becker, K. 2005. "The impact of saponins or saponin containing plant materials on ruminant production A Review", *Journal of Agriculture and Food Chemistry*, 53,8093-8105.
- Yavuz, T. , Kır, H. Gül, V., (2020). Evaluation of Roughage Production Potential in Turkey: The Case of Kırşehir Province. *Turkish Journal of Agricultural Research*. 7 (3), 345-352. <https://doi.org/10.19159/tutad.728119>
- Yusuf, A., Shehu, B., & Murtala, U. (2023). Role of Browse Tree Legumes as Dietary Supplements for Ruminants: A Review. *Nigerian Journal of Animal Science and Technology (NJAST)*, 6(2), 144-155.