Research Article

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Determination of the *In Vitro* Gas Production and Potential Feed Value of Olive, Mulberry and Sour Orange Tree Leaves

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Abstract: This study was conducted to determine the potential nutritive value and *in vitro* gas production (IVGP) parameters of *Olea europaea L.* (Olive = OL*)*, *Morus alba L.* (Mulberry = ML) and *Citrus aurantium L.* (Sour orange = SOL) tree leaves. Hohenheim gas test was used to determine the *in vitro* gas productions of the leaves. The gas production of samples over time was recorded for 3, 6, 9, 12, 24, 48, 72 and 96 h after incubation. Completely Randomized Design was used to compare gas production, and gas production kinetics of samples. The findings of the present study suggested that there were differences among the tree leaves in terms of crude protein, NDF, *in vitro* gas productions, organic matter digestibility (OMD), metabolisable energy (ME), net energy lactation (NE_L) and relative feed values (RFV) ($P<0.01$). ML had the highest condensed tannin contents ($P<0.05$), *in vitro* gas production (IVGP), OMD and energy values ($P<0.01$). SOL had highest RFV values. OL showed the lowest IVGP when compared to SOL and ML. Low NDF and ADF contents of SOL would probably increase the voluntary intake, digestibility and relative feed values of these leaves by ruminants. In conclusion, it was determined that OL, ML and SOL used in the study have low *in vitro* gas production and can be utilized as alternative roughage feed in ruminants. However, it is recommended that the results obtained from this research should be tested in *in vivo* studies.

Keywords: Tree leaves, *in vitro* gas production, energy value, relative feed value

1 Introduction

Fodder plants and crop residues of field, grass and pastures are mainly traditional forage sources for ruminants in the Mediterranean Region. However, roughage is required during drought seasons and winter months. For this reason, it was reported that some of the shrubs, thorny plants and tree leaves can be used as an alternative roughage feeds [1-5].

Leaves and fruits of trees are important to the nutrition of browsing ruminants such as goats, sheep, cattle and deer in the Mediterranean region. It is also known that the leaves and fruits of some trees are meeting the requirements of ruminant in semi-arid areas like as Mediterranean region [5-7]. As a result of their high nutritive value, and positive effects on rumen function, microbial yields and body metabolism, tree leaves are being increasingly recognized as a potentially high quality feed resource for ruminants, particularly to supply crude protein [8] and are exploited for livestock feeding as an energy source [9]. However, the use of tree leaves by herbivores is often restricted by the plants defence or deterrence mechanisms related to high tannin and phenolic matter contents [10-11]. Potential animal fodder plants include: *Olea europaea L.* (Olive*)*, *Morus alba L.* (Mulberry) and *Citrus aurantium L.* (Sour orange), all trees abundantly found in the Mediterranean Sea region of Turkey (Adana province).

Approximately 25-30 kg of leaves are produced by one olive tree per year (5% of the olive tree mass produced) and it is known that 7.0 to 12.9% dry matter (DM), crude protein contain olive leaves [12-13]. It is reported that olive leaves can be used as a low quality roughage feed in ruminants after being dried, they have similar nutritional values to

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cereal straws, and that olive leaves can be used to meet the maintenance requirement of lambs [14-16].

Mulberry tree leaves are also rich in protein (20% of DM), it has been reported that sheep, goats and dairy cattle like to consume it and the efficiency of milk yield was increased [17-18]. Furthermore, there are studies on the essential oil and secondary metabolite contents of SOL, which is widespread in the Mediterranean region [19-20], but it has not been included in studies on the use of ruminant nutrition. In addition, it is thought that the SOL, OL and ML, which have a certain amount of phenolic contents, will reduce methane production.

Some research was carried out on the chemical composition of olive, mulberry and sour orange tree leaves [3, 22-24], but, there is limited information on *in vitro* gas production, energy contents and organic matter digestibility. The aim of this study was to determine the chemical composition, relative feed value, *in vitro* gas production parameters, energy values (metabolisable energy and net energy lactation) and organic matter digestibility of the leaves of mulberry, sour orange and olive trees growing in Southern Turkey.

2 Materials and Methods

2.1 Collection of leaves and chemical analysis

The leaves of tree were harvested in mid-August from Adana province (located at an altitude of 28 m above sea level) in the south of Turkey. In this study, three different tree leaves (*Olea europaea L.* (Olive*)*, *Morus alba L.* (Mulberry) and *Citrus aurantium L.* (Sour orange)) were used. The leaves were hand harvested from at least 10 different trees for each sample, pooled and oven dried at 60˚C for 48 hours. Then the leaves were milled in a hammer mill through a 1 mm sieve for chemical analysis. Dry matter (DM) was determined by drying samples at 105ºC for 24 hours. Ash content was determined by ashing in a muffle furnace at 550ºC for 8 hours. Nitrogen (N) contents were analysed using the Kjeldahl method according to the AOAC [24] procedure. Crude protein (CP) was calculated as N × 6.25. The ether extracts (EE) content was determined by using Ankom^{XT15} analyser [25]. The analyses of neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents of the leaves were based on the method of Van Soest et al. [26] using an Ankom fibre analyser. Condensed tannin (CT) contents of the leaves were determined according to Makkar et al. [27].

2.2 *In vitro* gas (Hohenheim) production technique

Three infertile Holstein cows with ruminal cannulas (average live weight 650 kg) were used in the *in vitro* gas production technique. Approximately 200 mg dry weight of samples were weighed in triplicate into 100 ml calibrated glass syringes (Model Fortuna from Germany) following the procedures of Menke and Steingass [28]. The syringes were pre-warmed at 39ºC before the injection of 30 ml rumen fluid (10 ml) buffer mixture (20 ml) (1:2) into each syringe and incubated in a water bath at 39 ºC. Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 hours of incubations. Five replications of each leaf were used for the *in vitro* study. Rumen fluid was obtained from the fistulated Holstein cows fed twice daily (08.30-16.30) with a diet containing forage (corn silage, 60%) and concentrates (40%). The data were fitted to the model of Ørskov and McDonald [29] by NEWAY computer package programme.

$$y = a + b\ (1\text{-}exp^{-ct})$$

Where; a: the gas production from the immediately soluble fraction (ml), b: the gas production from the insoluble fraction (ml), a + b: potential gas production (ml), c: the gas production rate constant for the insoluble fraction (ml/h), t: incubation time (h), y: gas produced at a time “t”.

Organic matter digestibility (OMD), metabolizable energy (ME) and net energy lactation (NE_L) contents of the leaves were estimated according to the equations given at below used for forages.

OMD, % = 14.88+ 0.8893GP + 0.448CP + 0.651 ash [30]

ME, MJ/kg DM = 2.20+0.136GP + 0.057CP + 0.002859 EE^2 [30]

NE_L, MJ/kg DM = 0.101GP + 0.051CP + 0.11EE [28]

Where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), EE: Ether extract (%)

2.3 Determining relative feed values of samples

The relative feed value (RFV) of leaves was calculated as follows [31];

DMI = Dry matter intake (Live Weight =LW %) = 120 / (NDF %)

DMD = Dry matter digestibility (%) = 88.9 – (0.779 x ADF %)

RFV = Relative feed value = (DMD x DMI) / 1.29

2.4 Statistical analysis

Completely Randomized Design was used to compare chemical composition, gas production, and gas production kinetics of SOL, ML and OL using General Linear Model of SPSS (SPSS version 10.0) package programmes. Significance between individual means was identified using the Duncan's multiple comparison test. To observe the relations among traits Canonical correlation analysis was performed but the results among variable sets were found statistically insignificant (P>0.05). In this situation, Pearson correlation coefficients were calculated to show relations among traits.

3 Results

Nutrient contents of the tree leaves are given in Table 1. The findings of this study indicated that the ML had the highest CP, EE and NDF and had the lowest DM content (P<0.01). SOL had the highest DM and had the lowest NDF contents (P<0.01). OL had the highest ADF and had the lowest CP contents (P<0.01). In terms of CP contents, all the leaves in the study showed values (6.85-13.68% DM) which were higher than the straws with low quality roughage sources. In the study, it was determined that the CT contents of the leaves were generally low (between 0.46-1.06% DM). The lowest CT content was found in OL where the highest CT content was observed in ML. There was no significant difference in CT between ML and SOL (P> 0.05).

Relative feed values and forage qualities of the tree leaves are given in Table 2. This indicates that all the leaves were significantly different from each other (P <0.01) and they were placed into a low quality roughage class. However, the highest DMD, DMI and RFV values were found to be SOL while the lowest values were ML (P<0.05)

In vitro gas production of the leaves were given in Table 3 and Figure 1. There was no difference in terms of *in vitro* gas production between the leaves at the beginning of incubation (3 hours incubation) (P> 0.05). However, the highest IVGP values for 6, 9, and 12 hours incubation were SOL where the highest IVGP values for 24, 48, 72 and 96 hours incubation were observed in ML (P <0.01). Olive leaves were determined to have lower IVGP values than the other leaves for 24, 48, 72 and 96 hours incubation (P <0.01).

In vitro gas production kinetics, estimated energy values (ME and NE_L) and organic matter digestibility of the tree leaves are given in Table 4. In terms of a+b value, OMD, ME and NEL in the study, the highest values were determined in ML followed by SOL. The lowest values for

Table 1. Chemical compositions and condensed tannin contents of the leaves (as DM%)

	DM	CP	EE	Ash	NDF	ADF	CT
Leaves	%	DM %					
SOL	43.37a	8.00b	1.80b	19.65a	27.84c	26.14b	0.92a
ML	31.17c	13.68a	5.18a	19.61a	47.81a	25.90b	1.06a
OL	42.80b	6.85c	2.43b	5.86b	39.93b	30.32a	0.46b
SEM	0.00	0.11	0.41	0.16	0.99	0.42	0.10
Significant	0.533	0.00	0.00	0.00	0.00	0.00	0.01

a,b,c..: Means with different supercripts in the same column are significantly different. DM: Dry matter, CP: Crude protein, EE: Ether extracts, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, CT: condensed tannin, SEM: Standard error of means

Table 2. Relative feed values and forage qualities of the leaves

Leaves	DMD, %	DMI, % BW	RFV	RFV Quality
SOL	41.95a	1.87a	60.80a	5
ML	24.14c	0.79c	14.71c	5
OL	33.71b	1.29b	33.62b	5
SEM	0.86	0.02	1.07	
Significant	<0.000	<0.000	<0.000	

a,b,c..: Means with different supercripts in the same column are significantly different. SEM: Standard error of means, According to the Quality Grading Standard assigned by The Hay Marketing Task Force of the American Forage and Grassland Council, the RFV were assessed as roughages based on prime >151 , 1 (premium) 151-125, 2 (good). 124-103. 3 (fair). 102-87, 4 (poor). 86-75 , 5(reject).< 75.

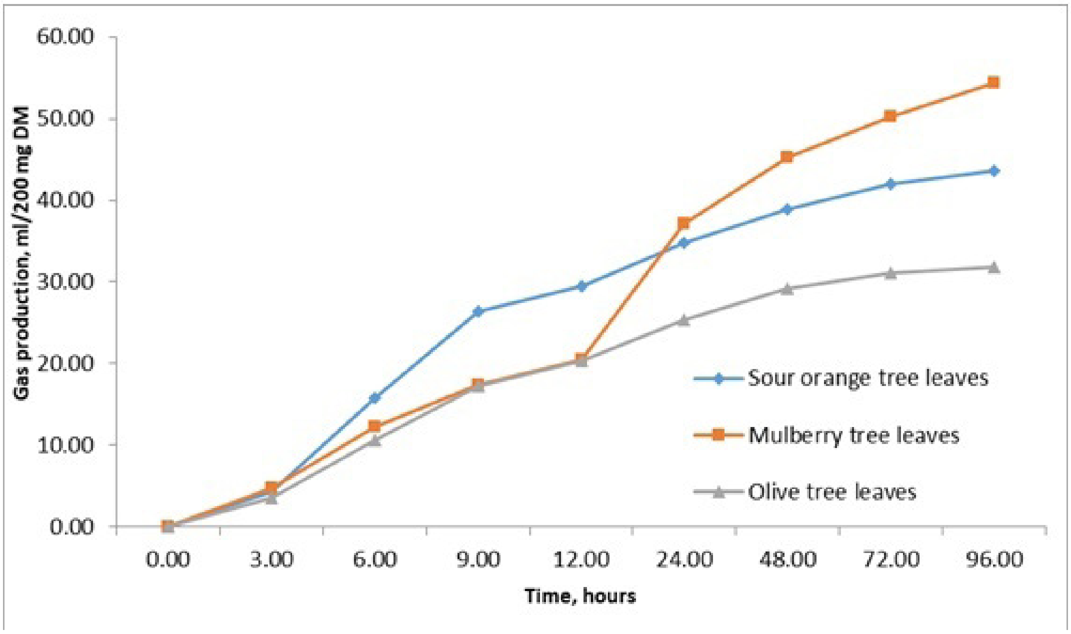

Figure 1. *In vitro* gas productions of the tree leaves

Table 3. In vitro gas production of the leaves (ml/200 mg DM)

	Incubation times, hour							
Leaves	**3**	**6**	**9**	**12**	**24**	**48**	**72**	**96**
SOL	4.38	15.78a	26.33a	29.51a	34.82b	38.87b	42.06b	43.64b
ML	4.82	12.33b	17.40b	20.58b	37.18a	45.20a	50.24a	54.29a
OL	3.64	10.58c	17.34b	20.43b	25.37c	29.25c	31.07c	31.80c
SEM	0.35	0.38	0.32	0.29	0.46	0.42	0.59	0.55
Significant	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00

a, b, c Column means with common superscript do not differ, P<0.05. SEM: Standard error of means

Table 4. in vitro gas production kinetics and pH values after 96.h incubation of the tree leaves

Leaves	pH*	a+b	c	OMD	ME	NEL
SOL	6.84a	40.82b	0.13a	50.73b	7.40b	4.12b
ML	6.69c	53.13a	0.05c	55.38a	8.11a	5.02a
OL	6.75b	30.53c	0.10b	40.91c	6.06c	3.18c
SEM	0.006	0.486	0.002	0.410	0.063	0.047
Significant	0.00	0.00	0.00	0.00	0.00	0.00

a, b, c Column means with common superscript do not differ, a+b: potential gas production (ml), c: the gas production rate constant for the insoluble fraction (ml/h), OMD: organic matter digestibility (%), ME: metabolisable energy (MJ/kg DM), NEL: net energy lactation (MJ/kg DM), P<0.05. *: pH values after 96.h incubation

these parameters were observed in OL (P <0.01). In this study, the highest gas production rate was observed in SOL, but OL and ML were following (P <0.01). In the study, the pH values after 96 hours of incubation indicated whether the buffer solution was sufficient or not for normal rumen conditions. According to this, the highest pH value was observed in SOL (6.84), where OL and ML were succeeding; 6.75 and 6.69 respectively. Therefore, it can be said that the buffer solution was not completely consumed until the end of the incubation period by microorganisms and the amount of buffer did not affect the results of this study.

Pearson correlation coefficients were calculated to show relations among traits, the largest positive correlation was observed between crude protein and EE. The largest negative correlation was observed between NDF and DMI, RFV (Table 5). The largest relations with gas production with nutrient contents were found between 6h and 12h (Table 6). When the Pearson correlation coefficients were examined, it is clear that observations on consecutive time points were highly related (Table 7). The highest positive correlation was observed between crude protein and pH. The highest negative correlation was observed between dry matter and pH (Table 8). The highest correlated traits with ME among b, ab and OMD (Table 9). For the traits given in Table 10 was highly correlated gas production after 24h incubation times than before 24h incubation times (Table 10).

Table 5. Pearson correlation coefficients with NDF, ADF, EE, CT, DMD, DMI and RFV.

	NDF	ADF	EE	Ash	CP	CT	DMD	DMI	RFV
DM	-0,812	0,485	-0,922	-0,461	-0,979	-0,565	0,893	0,862	0,831
NDF		0,094	0,861	-0,117	0,685	0,081	-0,976	-0,991	-0,991
ADF			-0,302	-0,954	-0,641	-0,820	0,040	-0,006	-0,075
EE				0,321	0,888	0,422	-0,906	-0,870	-0,846
Ash					0,628	0,837	-0,043	0,047	0,104
CP						0,673	-0,790	-0,745	-0,703
CT							-0,224	-0,146	-0,100
DMD								0,986	0,985
DMI									0,996

DM: Dry matter, CP: Crude protein, EE: Ether extracts, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, CT: condensed tannin, DMD: Dry matter digestibility, DMI: Dry matter intake, RFV: Relative feed value

Table 6. Pearson correlation coefficients of NDF, ADF, EE, CT, DMD, DMI and RFV with in vitro incubation times.

	3	6	9	12	24	48	72	96
DM	-0,132	-0,928	-0,994	-0,995	-0,364	-0,159	-0,125	-0,070
NDF	0,490	0,901	0,785	0,794	0,808	0,679	0,657	0,613
ADF	0,518	-0,247	-0,524	-0,512	0,570	0,730	0,760	0,785
EE	0,197	0,860	0,906	0,909	0,441	0,274	0,246	0,191
Ash	-0,548	0,174	0,490	0,484	-0,649	-0,797	-0,814	-0,847
CP	0,004	0,853	0,980	0,979	0,172	-0,041	-0,074	-0,130
CT	-0,256	0,377	0,577	0,561	-0,436	-0,576	-0,616	-0,637
DMD	-0,409	-0,928	-0,865	-0,874	-0,702	-0,550	-0,528	-0,477
DMI	-0,462	-0,941	-0,839	-0,846	-0,778	-0,631	-0,605	-0,560
RFV	-0,489	-0,918	-0,802	-0,810	-0,806	-0,671	-0,648	-0,604

DM: Dry matter, CP: Crude protein, EE: Ether extracts, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, CT: condensed tannin, DMD: Dry matter digestibility, DMI: Dry matter intake, RFV: Relative feed value

Table 7. Pearson correlation coefficients with in vitro incubation times.

Incubations	6	9	12	24	48	72	96
3	0,451	0,141	0,130	0,663	0,664	0,665	0,662
6		0,932	0,929	0,609	0,426	0,391	0,346
9			0,999	0,340	0,129	0,095	0,040
12				0,348	0,137	0,105	0,048
24					0,975	0,966	0,952
48						0,998	0,996
72							0,998

Table 8. Pearson correlation coefficients of DM, NDF, ADF, EE, ash, CP CT, DMD, DMI and RFV with pH, b, c and a+b values, OMD, ME and NE_L

	pH	b	c	a+b	OMD	ME	NE_L
DM	-0,902	-0,445	-0,737	0,010	-0,241	-0,212	-0,053
NDF	0,505	0,855	0,216	0,551	0,733	0,713	0,597
ADF	-0,770	0,506	-0,923	0,830	0,670	0,687	0,786
EE	0,771	0,520	0,575	0,120	0,336	0,309	0,166
Ash	0,784	-0,579	0,932	-0,887	-0,744	-0,762	-0,856
CP	0,960	0,258	0,853	-0,208	0,044	0,015	-0,147
CT	0,768	-0,369	0,884	-0,684	-0,526	-0,548	-0,649
DMD	-0,637	-0,763	-0,369	-0,409	-0,613	-0,589	-0,458
DMI	-0,570	-0,829	-0,295	-0,493	-0,694	-0,672	-0,546
RFV	-0,524	-0,854	-0,240	-0,540	-0,729	-0,708	-0,588

DM: Dry matter, CP: Crude protein, EE: Ether extracts, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, CT: condensed tannin, DMD: Dry matter digestibility, DMI: Dry matter intake, RFV: Relative feed value, pH: after for 96 hour incubation, b: potential gas production, c: gas production rate, OMD: organic matter digestibility, ME: Metabolisable energy, NEL: net energy lactation

Table 9. Pearson correlation coefficients of pH, b, c, a+b, OMD, ME with b, c and a+b values, OMD, ME and NE_L

Parameters	b	c	a+b	OMD	ME	N_{EL}
pH	0,043	0,938	-0,419	-0,181	-0,209	-0,362
b		-0,273	0,887	0,973	0,966	0,914
c			-0,682	-0,474	-0,499	-0,631
a+b				0,967	0,973	0,997
OMD					1,000	0,982
ME						0,987

pH: after for 96 hour incubation, b: potential gas production, c: gas production rate, a+b: total gas production, OMD: organic matter digestibility, ME: Metabolisable energy, NEL: net energy lactation

Table 10. Pearson correlation coefficients of in vitro incubation times with pH, b, c and a+b values, OMD, ME and NE_L

İncubations	pH	b	c	a+b	OMD	ME	NE_L
3	-0,233	0,598	-0,341	0,661	0,680	0,680	0,676
6	0,700	0,656	0,502	0,272	0,505	0,481	0,338
9	0,905	0,415	0,754	-0,040	0,214	0,186	0,025
12	0,908	0,425	0,748	-0,032	0,222	0,194	0,033
24	-0,052	0,993	-0,355	0,924	0,991	0,987	0,948
48	-0,260	0,953	-0,548	0,985	0,995	0,997	0,993
72	-0,291	0,941	-0,578	0,990	0,991	0,994	0,995
96	-0,345	0,922	-0,621	0,997	0,984	0,988	0,999

pH: after for 96 hour incubation,b: potential gas production, c: gas production rate, a+b: total gas production, OMD: organic matter digestibility, ME: Metabolisable energy, NEL: net energy lactation

4 Discussion

In this study, it was determined that the crude protein contents of olive leaves were lower than those (7.0-12.9% DM) reported by Martin-Garcia et al. [13] and Shakeri et al. [32] . The NDF, ADF and ash contents of the olive leaves were found to be similar to those reported in the literature (34.9-41.5% DM), (24.5-34.2% DM) and (5.40-6.93% DM) respectively [16, 32-35]. It has been reported in the literature that the amount of CT in olive leaves varies between 0.58-1.11% DM [16, 35-37], the CT value determined in the study was 0.46%, which was lower than those reported in the previous studies. It has reported that the observed differences were due to the origin of the olive leaves, the leaf-branch ratio, storage and climate conditions, the level of the soil or oil contaminations and the drying process applied [38]. Martin-Garcia and Molina-Alcaide [35] reported that the application of different drying processes was an important effect on the CT content of olive leaves, where the highest CT content was determined in the fresh - form of the leaves (1.0% DM) and the lowest CT content was found to be those dried in the oven at 60°C (0.62% DM).

Karabulut et al. [21] determined that CP (12.9%), NDF (29.5%), ADF (25.3%), ash (10.4%) and CT (0.59%) contented in *Citrus aurantium* leaves. The CP content (8.0%) determined in this study was lower; the CT content (0.92%) was higher, and the NDF (27.84%) and ADF (26.14%) were similar to result reported by Karabulut et al. [21]. Yao et al. [39] reported that the nutrient contents of the ML harvested in the spring DM (23.6-24.4%), CP (20.8-21.6% DM) and NDF (37.5-39.5% DM) have changed when compared to those harvested in the autumn (29.6-30.45% DM, 19.6- 21.9% DM, 38.9-43.4% DM respectively); the CP content has decreased as harvesting is delayed while NDF content increased. It was thought that the variations observed were due to the difference in varieties, characteristics of the soil grown, time of harvesting, fertilization, drying process, leaf-branch ratio, species, climate etc. [38, 40, 41].

Karabulut et al. [21] reported the total gas production for SOL as 76.9 ml/200mg DM and the gas production rate "c" as 6.7 ml/hour. These values were determined as 40.82 ml/200mg DM and 0.13 ml/hour in the present study, respectively, and were lower than those reported in the literature.

Karabulut et al. [21] reported the OMD and ME contents of SOL as 73.3% and 10.9 MJ/kg DM respectively. The OMD (50.73%) and ME (7.40 MJ/kg DM) values determined in this study were lower than those reported in the previous studies. The main reason for this is that the 24 hour gas production in the equations used for the calculation was low.

Mulberry tree leaves have high DM and OM digestibility (82-89% DM), high CP (14.0-18.6% DM), low NDF (24.6-27.6% DM) and low ADF (20.8-25.1% DM) contents with high ash (13.3-14.3% DM) content [42, 43]. Guven [41] reported that chemical contents (DM: 25.97-42.20%; CP: 11.75-23.72%; ash: 15.40-22.36%; CT: 0.47-0.76%) can be affected by different species of ML. In this study, the CP, NDF, ADF and ash contents were determined as 13.68, 47.81, 25.90 and 19.61% DM, respectively, and the variations observed were related to the factors such as variety, soil structure, harvest time, season etc. [38, 41]. However, it is thought that ML to be forage feed source that can be used to feed ruminants. It is clear that low CT content will not affect the consumption of animals in the negative direction. It is also known that young ML has higher rumen degradability [42] and it should be taken into consideration that the feeding value of young leaves is higher.

Karabulut et al. [21] reported that there is a negative relationship between 24 hour gas production with CP, NDF and ADF contents of leaves. However, the findings obtained in this study were different from this report. Having high CP and NDF content, ML has the highest 24 hour gas production value. Yao et al. [39] report that *in vitro* gas production of ML showed higher values when harvested in spring (43.9-52.7 ml) than those harvested in autumn (36.7-38.0 ml). When harvested in autumn, it was determined the c value as 6.16-7.01 ml/hour, 24 hour gas production 30.8-31.3 ml/200mg DM and OMD values as 58.4-60.1%; but, when harvested in the spring, they were determined as 7.26-9.5 ml/hour, 39.6-47.6 ml/200mg DM and 65.9-71.9%, respectively. It can be seen, that the harvesting season has also a significant effect on the *in vitro* gas production and gas production parameters. In this study, the c value obtained from ML was lower than those in the literature, but the values of a+b and 24 hour gas production were found to be similar to leaves harvested in the spring. The OMD value determined in the study was also close to that of autumn harvested leaves in the literature reports. It is thought that the differences seen were due to variation in nutrient content (higher amount of ash, lower CP content) [40].

Shakeri et al. [32] reported the *in vitro* gas production of olive leaves as 245-247 ml/g DM (49-49.4 ml/200 mg DM). These values were considerably higher than the total gas production (a + b = 30.53 ml / 200mg DM) and gas production obtained after 96 hours incubation (31.80 ml / 200mg DM). However, Shakeri et al. [32] reported that the use of OL reduced total gas and methane production in ruminants. This suggests that the use of olive leaves in certain ratio in the ruminant feeding will provide significant environmental benefits and that the energy of the feeds will be better utilized.

In this study, all leaves were found to be significantly different in relative feed values (P<0.01), and they fall into a low quality roughage feed class. It was found that the RFV values of citrus leaves were similar to the RFV values of wheat and soybean straws reported by Mohamoud Abdi and Kilic [44] and Kilic and Gulecyuz [45], while the RFV values of other leaves were lower than these straws. According to the wheat and soybean straws, ruminants will have less voluntary feed intake of the ML with the lowest DMI value and the SOL will be consumed more.

The findings of each leaf in this study supported the fact that the anti-nutritive factors like tannins may also contribute to a reduction of *in vitro* gas production. Kamalak et al. [7] reported that in tree leaves a condensed tannins can be found in the bound and soluble form. Karabulut et al. [21] reported a negative correlation between CT with 24 hour gas production and OMD and ME. According to that, it was expected that ML with the highest CT content will have the lowest *in vitro* gas production. As shown in Figure 1, the IVGP value determined in the ML, which has the highest CT content in leaves up to 24 hours, was lower than the IVGP values determined in the SOL; the higher values for OL were thought to be related to the CT release in the rumen environment. The low IVGP value of ML up to 24 hour incubation can be due to the high release of ML contained CT into the rumen during first 24 hours. The reason for this, is that the amount of CT remaining in the ML structure will decrease after 24 hours of incubation and the amount of CT released into the rumen will be low, resulting in an increase of IVGP value.

It was suggested that CT contents of 5-10% may result disgust of feeds, reduces forage consumption and live weight gain, decreases digestibility and absorption, reduce performance and lead toxicity related effects [46]. Since the CT contents of leaves used in this study were between 0.46-1.06%, it is believed that this will not have an adverse effect on the feed consumption. As a result, it was concluded that the sour orange, the mulberry and the olive tree leaves used in the study can be utilized as alternative roughage source for ruminants if it is used within certain limits in the feeding (adding 5-10% into the ration) after being dried in a condition that the loss of leaves is reduced. Thus, this would have economic benefits and advantages for the closure of the forage feed gaps.

It has reported that ammonia and urea treatment have positive effects on improving the nutritive value of olive leaves [47, 48]. When the olive leaves (treated with ammonia) were compared with the alfalfa hay as roughage in lactating sheep, there was no difference among the groups in terms of milk yield, but the group fed with olive leaf was reported to contain more oleic acid and linoleic acid [49]. In terms of milk yield, milk fat and protein content, no adverse effect was observed in sheep and goats consumed olive leaves freely [50]. In addition, it seems that supplement of olive leaves has no negative effect on feed intake and utilization. In general, it is advisable to use ML and SOL in animal feeding after being processed like OL through above mentioned procedures.

5 Conclusions

It is concluded that there were significant differences among tree leaves in terms of chemical composition and potential gas production. Fermentation characteristics of OL *in vitro* were identified as having the potential to modulate rumen fermentation. It was also found that OL reduced *in vitro* gas production in comparing to ML and SOL. The leaves had beneficial effects on rumen fermentation. However, these results need to be confirmed further with *in vivo* studying, before these products can be advanced further in goats and sheep nutrition. CT contents should be taken into account and used in limited amounts in the ruminant rations.

Conflict of interest: Authors state no conflict of interest

Abbreviation List

a+b: Potential gas production (ml),
ADF: Acid detergent fibre
c: The gas production rate constant for the insoluble fraction (ml/h),
CF: Crude fibre
CP: Crude protein
CT: Condensed tannin
DM: Dry matter
EE: Ether extract
ME: Metabolisable energy (MJ/kg DM),
ML:Mulberry tree leaves
NDF: Neutral detergent fibre
NE_L: Net energy lactation (MJ/kg DM),
NFE: Nitrogen free extracts
OL: Olive tree leaves
OMD: Organic matter digestibility (%),
SOL: Sour orange tree leaves.

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